

The Persistence of Behavioral Sensitization to Cocaine Parallels Enhanced Inhibition of Nucleus Accumbens Neurons

Douglas J. Henry¹ and Francis J. White²

¹Department of Pharmacology, University of Washington, School of Medicine, Seattle, Washington 98195 and ²Neuropsychopharmacology Laboratory, Department of Neuroscience, Finch University of Health Sciences, The Chicago Medical School, North Chicago, Illinois 60664-3095

The mesoaccumbens dopamine system is intricately involved in the locomotor stimulation produced by cocaine and sensitization of this effect following repeated cocaine administration. The mechanisms responsible for the expression of sensitized locomotion appear to involve alterations in both presynaptic (increased dopamine release) and postsynaptic (increased responsiveness of dopamine D1 receptors) aspects of dopamine neurotransmission within the nucleus accumbens. The present experiments used behavioral and single-cell electrophysiological techniques to determine the persistence of sensitization and of enhanced postsynaptic responses to cocaine within the nucleus accumbens following various periods of withdrawal from repeated cocaine treatment (10 mg/kg i.p., twice daily, 14 d). Behavioral sensitization to the locomotor stimulant effects of cocaine was evident after 1 d, 1 week, and 1 month, but not 2 months of withdrawal. A similar time course was observed for the enhanced efficacy of cocaine-induced inhibition of nucleus accumbens neurons, whether cocaine was administered systemically or locally by microiontophoresis. Nucleus accumbens neurons also exhibited sensitized inhibitory responses to iontophoretically applied GABA after 1 d of withdrawal, but not later times. These findings suggest that cocaine sensitization is relatively persistent, but not necessarily permanent, and support the hypothesis that expression of behavioral sensitization to cocaine involves actions within the NAc, particularly those mediated by dopamine D1 receptors.

[Key words: cocaine, dopamine, locomotor behavior, nucleus accumbens, sensitization, psychomotor stimulant, drug withdrawal, dopamine D1 receptor]

Both the locomotor activating and positive reinforcing effects of cocaine are mediated, in large part, by midbrain ventral tegmental area (VTA) dopamine (DA) neurons projecting to the nucleus accumbens (NAc) (see Kalivas and Stewart, 1991; White and Wolf, 1991; Koob, 1992; Roberts, 1992, for recent reviews). Repeated intermittent administration of cocaine augments its locomotor activating effects (Post and Rose, 1976; Kilbey and

Ellinwood, 1977; Kalivas et al., 1988) and, under certain conditions, its reinforcing efficacy (Woolverton et al., 1984; Lett, 1989; Horger et al., 1990). This behavioral sensitization also involves the mesoaccumbens DA pathway, and specific aspects of sensitization result from alterations in different components of the system. The development of sensitization is apparently initiated by processes occurring within the VTA. Although psychomotor stimulants injected into the VTA fail to induce locomotion, repeated intra-VTA injections induce sensitization to either systemic or intra-NAc injections of such drugs (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992; Perugini and Vezina, 1994), including cocaine (DuMars et al., 1988; Hooks et al., 1992). In contrast, intra-NAc injections of psychomotor stimulants cause hyperactivity, but sensitization of this effect does not occur with repeated administration (Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Hooks et al., 1992). However, the NAc is essential for expression of sensitization. Rats that receive repeated systemic or intra-VTA injections of psychomotor stimulants exhibit sensitization to intra-NAc administration of such drugs (Hitzemann et al., 1980; Kolta et al., 1989; Paulson and Robinson, 1991; Perugini and Vezina, 1994).

The most widely accepted mechanism of sensitization is an enhanced ability of psychomotor stimulants to increase extracellular levels of DA within the NAc (see Robinson and Becker, 1986; Kalivas and Stewart, 1991; White and Wolf, 1991, for reviews). However, sensitized behavior occurs when there is no enhanced DA response, or even a diminished DA response (Segal and Kuczenski, 1992a,b; Wolf et al., 1992; Kalivas and Duffy, 1993a; Maisonneuve and Kreek, 1994), indicating that this mechanism is not solely responsible for sensitization. Another neuroadaptation which may contribute to the expression of sensitization is supersensitivity of DA D1 receptor-mediated responses within the NAc (Henry and White, 1992; White et al., 1992, 1995a). Repeated administration of cocaine (Henry et al., 1989; Henry and White, 1991) or amphetamine (Higashi et al., 1989; Wolf et al., 1994) increases the inhibitory efficacy of DA and DA D1 receptor-selective agonists on NAc neurons. The extent to which upregulation of DA D1 receptor-mediated neurophysiological effects occurs as a result of increased DA D1 receptor density is a matter of debate (Kleven et al., 1990; Peris et al., 1990; Mayfield et al., 1992; Alburges et al., 1993; Unterwald et al., 1994), but even in its absence, alterations in intracellular signal transduction (Nestler et al., 1990; Terwilliger et al., 1991; Striplin and Kalivas, 1993) and gene transcription (Hope et al., 1992; Hope et al., 1994) within the NAc could lead to long-term alterations in DA D1 receptor-mediated signaling.

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Correspondence should be addressed to Francis J. White, Ph.D., Department of Neuroscience, FUHS/CMS, 3333 Green Bay Road, North Chicago, IL 60064-3095.

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Tests of neuronal adaptations at various times during and after repeated administration of psychomotor stimulants have provided important information regarding the sequence of mechanisms that is responsible for the induction and maintenance of behavioral sensitization (Ackerman and White, 1990; Peris et al., 1990; Paulson et al., 1991; Wolf et al., 1992; Kalivas and Duffy, 1993a,b). Our previous studies have demonstrated that the enhanced sensitivity of NAc DA D1 receptors was evident within 24 hr of the last cocaine injection and persisted for 1 month, but not two months (Henry and White, 1991). Because behavioral sensitization is considered to be an enduring, if not permanent, phenomenon (Robinson and Becker, 1986; Post et al., 1992), the return of DA D1 receptors to a normosensitive state suggests that other mechanisms might also be responsible for behavioral sensitization at later withdrawal times. However, the persistence of cocaine sensitization (Post et al., 1992), and of enhanced basal and cocaine-stimulated interstitial DA levels (Weiss et al., 1992), is a function of treatment regimen and test conditions. Therefore, the present study determined the withdrawal time course for enhanced behavioral and neuronal responses to cocaine following repeated cocaine administration.

Materials and Methods

Subjects. Male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) weighing 225–275 gm at the start of experiments were used in all studies. The animals were housed 2/cage in metal hanging cages. Food and water were available ad libitum. The colony room was maintained at constant temperature (21–23°C) and humidity (40–50%) with a 12 hr light/12 hr dark cycle (07:00 on, 19:00 off). All experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23).

Repeated cocaine treatment protocol. Rats were randomly assigned to either a cocaine treatment or control group, housed in like-treated pairs and acclimated to the home environment for at least one week. All cocaine-treated rats received twice-daily (10:00 and 18:00) injections of cocaine HCl (10.0 mg/kg, i.p.) for 14 consecutive days. Control subjects received equivalent volume injections (1.0 ml/kg) of the cocaine vehicle, deionized water, under an identical treatment regimen. Rats were given either behavioral or electrophysiological tests at various times after the last cocaine injection. For behavioral tests, the withdrawal periods were 20–24 hr, 7 d, 30 d, or 60 d. Due to technical reasons, the withdrawal periods for electrophysiological tests were not as precise, but they were always conducted within 18–24, 7–8 d, 30–32 d, and 60–64 d of the last treatment injection. Hereafter, these groups are referred to as the 1 d, 1 week, 1 month, and 2 months groups. Separate groups of rats were tested at each of these four periods of withdrawal. In the behavioral studies, the first injection of cocaine was administered in the activity cages. All other injections were administered in the animal's home cage within a separate colony room.

Behavioral testing. Twelve photobeam horizontal locomotion detection devices (PAS monitoring system, San Diego Instruments, San Diego, CA) were mounted around standard rectangular transparent rodent cages (30 × 50 cm). Each frame was equipped with three photoelectric beams which divided the long axis of the cage into four quadrants (12.5 × 30 cm each). Interruption of photobeams generated digital pulses which were computer analyzed to determine both the total number of photobeam interruptions as well as interruptions of consecutive photobeams (ambulations). All behavioral testing occurred between 16:00 and 19:00 hr with the overhead lights on within a separate and environmentally distinct room located in the animal colony. Each exposure to the chamber consisted of a 30 min habituation period, followed by injection and a 90 min test period. Subjects were handled daily by the experimenter for 1 week prior to exposure to the testing boxes.

Acute behavioral effects of cocaine. To obtain baseline data regarding the locomotor stimulant effects of cocaine using our activity system, five separate groups of 12 rats each, weighing 250–275 gm, were injected with either 0, 5, 10, 20, or 40 mg/kg cocaine HCl (i.p.) and tested for locomotor activity for 90 min, as described above. In addition to automated measures, qualitative indices of the behavioral effects of cocaine were obtained by trained observers unaware of the treatment con-

ditions (dose). Each rat was observed for 10 sec during each 5 min interval and the presence or absence of seven behaviors was noted: rearing (lifting the forepaws from the floor), sniffing upwards/outwards, sniffing downwards, "head-bobbing" (repetitive up/down or sideways movements of the head), licking, gnawing, and grooming.

Sensitization tests. All groups received an initial exposure to the test chambers in which the 30 min habituation period was followed by an intraperitoneal injection of drug vehicle and a 90 min test. The following day, rats in each group ($n \geq 12$ /group) received the first injection of their treatment regimen (either cocaine or vehicle) in the behavioral chambers. After these two initial exposures to the testing apparatus, all treatment injections occurred in the colony room. For sensitization tests, both control and cocaine-treated rats were challenged with cocaine (10 mg/kg i.p.). For the 1 d withdrawal groups, rats were injected 20–24 hr after the last treatment injection. The next day, these rats were tested after vehicle injections to determine the possible existence of conditioned locomotion as a result of exposure to the test apparatus. After extended withdrawal periods (1 week to 2 months), rats were tested with vehicle the day before the cocaine challenge to rehabilitate them to the chambers. All animals were also tested with vehicle on the day after the sensitization test to determine whether any conditioning to the locomotor-stimulant effects of cocaine was evident from the previous cocaine test day. By using these testing parameters, we were able to ascertain both between-groups sensitization and within-group sensitization.

Single-cell recording. The details for extracellular single cell recordings from NAc neurons have been detailed previously (Henry and White, 1991). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic frame. Body temperature was maintained at 36–37°C by a thermostatically controlled heating pad. A lateral tail vein was catheterized for administration of cocaine and other drugs or additional anesthetic (as needed). A burr hole was drilled in the skull and the dura was retracted from the area overlying the NAc (0.8–2.0 mm lateral to the midline, 10.5–10.9 mm anterior to lambda), with the aid of stereotaxic coordinates (Paxinos and Watson, 1986). Micromanipulators were used to place the electrode 6.0 mm ventral to the cortical surface and a hydraulic microdrive was used for fine movements of the electrode tip within the limits of the NAc (6.0–7.5 mm ventral to the cortical surface). Electrode signals were passed through a high impedance amplifier/filter and displayed on an oscilloscope. Individual action potentials (>3:1 signal:noise ratio) were detected with a window discriminator and monitored with an audio amplifier. A polygraph recorder was used to plot the integrated rate histograms generated by the output of the window discriminator. Digital counts were obtained for off-line analysis.

Microiontophoresis. Five-barrel glass electrodes were pulled and beveled (tip diameter of 4–7 μ m). The center (recording) barrel was filled with 2 M NaCl saturated with 1% (w/v) fast green dye, resulting in an *in vitro* impedance of 2–6 M Ω , measured at 135 Hz. One side barrel was filled with 2 M NaCl and used for automatic current-balancing. A second side barrel was filled with L-glutamic acid monosodium salt (0.05 M, pH 8) to stimulate quiescent NAc neurons. Glutamate was ejected in 20 sec on/20 sec off pulses (5–30 nA as anion). The ejection current was adjusted until stable activity of 4–6 spikes/sec was observed for at least 4–6 min. A positive retaining current of 10 nA was applied to the glutamate barrel during nonejection periods to minimize passive diffusion. The two remaining side barrels were filled with cocaine (0.01 M, pH 4) and γ -aminobutyric acid (GABA, 0.01 M, pH 4).

After recording stable basal activity for 4–6 min, cocaine or GABA was applied iontophoretically using continuous current which was doubled in amplitude every 80 sec. Each NAc neuron was tested sequentially with the two compounds, and each cell was allowed to recover from the inhibitory effects of the first drug to a new stable 4–6 min baseline before application of the second drug. The order of drug application was reversed from cell to cell within each animal. Data were expressed as the percent basal activity, which was determined by comparing the mean firing rate during the 80 sec drug ejection period to the 4–6 min predrug period. During measurement of the basal glutamate-driven firing of NAc neurons and their responses to drugs, data were collected only during glutamate ejection periods. Cocaine and GABA were ejected as cations, and a negative retaining current of 10 nA was applied during nonejection periods. Ejection currents for cocaine ranged from 2–32 nA, below those at which obvious local anesthetic effects are apparent (White, 1987; White et al., 1993). GABA was applied in currents of 1–16 nA. Only Type I NAc neurons (White

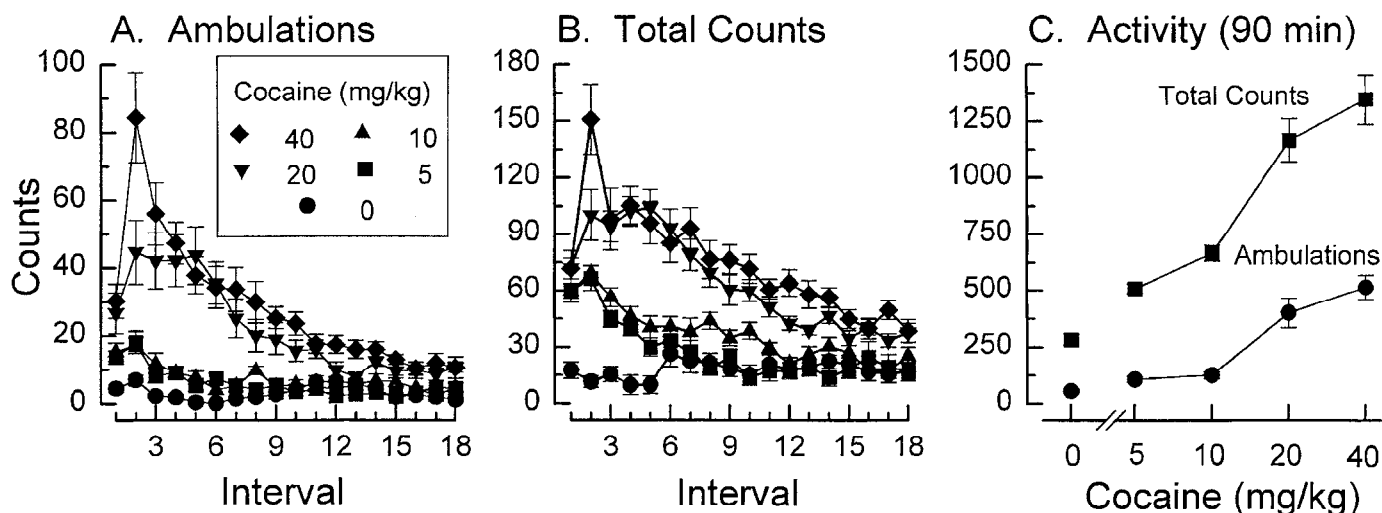


Figure 1. Locomotor response to acute cocaine administration. Dose-dependent increases in ambulations (A) and total activity (B) illustrated for each 5 min interval of the 90 min test ($n = 12$ rats/dose). C presents the cumulative ambulation and total counts for the 90 min test at each dose of cocaine. In each panel, data points represent the mean \pm SEM from separate groups of rats.

et al., 1987, 1993), which comprise 90% of the population under our experimental conditions, were included in this study.

Intravenous cocaine challenge. For some experiments conducted in the 1 d and 2 month withdrawal groups, rats were tested with systemic (intravenous) cocaine. After monitoring stable spontaneous or glutamate-driven activity for 4–6 min, NAc neurons were challenged with intravenous cocaine at a cumulative dose range of 0.1–6.4 mg/kg, with the dose doubled every 80–90 sec. Cocaine-induced inhibition of NAc neuronal activity was reversed by administration of the DA D1 receptor antagonist SCH 23390 (0.05 mg/kg, i.v.). Data from intravenous challenge experiments were expressed as percent basal or glutamate-driven activity.

Histology. After recording sessions, the position of the electrode tip was marked by passing a 25 μ A cathodal current through the recording barrel for 15 min, which deposited a discrete spot of green dye. Rats were then deeply anesthetized with additional chloral hydrate (100–200 mg/kg, i.v.), and transcardially perfused with 0.9% NaCl followed by 10% formalin. The brains were stored in 10% formalin until serial frozen sections (40 μ m thickness) were cut and subsequently stained with cresyl violet. Recording sites were then verified with the use of routine light microscopy. In intravenous studies, only one cell was recorded per rat. In iontophoretic experiments, 1–5 NAc neurons were recorded per rat and the dye spot marked the position of the last neuron recorded, which served as a reference point for the other neurons studied within that animal. When more than one NAc neuron was recorded in a given animal, the cells under study were separated by a minimum distance of 200 μ m.

Statistics. The results of intravenous and microiontophoretic experiments were compared using a two-way (groups \times dose) analysis of variance (ANOVA) with repeated measures on one variable (dose or current). Subsequent planned comparisons between specific control and treatment means were conducted using Dunnett's test. The results of acute dose/time response tests for behavioral activity were analyzed with a two-way ANOVA with repeated measures (time). For sensitization tests, comparisons of locomotor activity counts, either between (repeated cocaine vs vehicle for each withdrawal time) or within-groups, were made with unpaired or paired Student's two-tailed t tests, respectively. Dose–response comparisons for observed behaviors were made with one-way ANOVA, followed by Newman-Keuls tests. Differences between groups with respect to observed behaviors were evaluated using Student's t tests.

Materials. Cocaine HCl, chloral hydrate, and L-glutamic acid monosodium salt were obtained from Sigma Chemical Co. (St. Louis, MO). GABA HCl was obtained from Calbiochem (La Jolla, CA). SCH 23390 HCl was purchased from Research Biochemicals, Inc. (Natick, MA). All compounds were dissolved in deionized water. All drugs were prepared fresh on the day of testing. All concentrations refer to the weights of the respective salts.

Results

Acute behavioral effects of cocaine

Because this was our initial study using the behavioral testing apparatus to assess cocaine sensitization, we first determined the dose and temporal effects of cocaine on rat activity. As shown in Figure 1, acute cocaine administration caused significant ($p < 0.00001$) dose-dependent increases in both ambulation ($F_{4,55} = 27.6$) and total counts ($F_{4,55} = 32.4$). Temporal analysis indicated a significant ($p < 0.00001$) effect of time and a significant time \times dose interaction for both ambulation ($F_{17,935} = 29.6$ and $F_{68,935} = 7.3$, respectively) and total counts ($F_{17,935} = 31.4$ and $F_{68,935} = 8.3$, respectively). For the two lowest doses of cocaine (5 and 10 mg/kg), there was approximately three to four times more activity during the first 10 min, as compared to the vehicle control. The effect gradually subsided to control values by 45 min postinjection. For the two higher cocaine doses (20 and 40 mg/kg), the activation was markedly greater and more prolonged. When the data were combined and presented for the entire 90 min test session, a clear parallel increase in ambulation and total counts was observed (Fig. 1C). Because this parallel relationship was observed in all experiments, only the ambulation results are depicted in subsequent figures.

The results shown in Figure 1 clearly show that, under the present experimental conditions, cocaine-induced increases in horizontal locomotion (ambulation) were monophasic. Thus, there was no apparent switch to more focused, nonambulatory types of stereotyped behaviors, such as stationary sniffing, licking, and gnawing, which would cause a decrease in ambulation counts at higher doses. Direct observation confirmed this interpretation. As shown in Figure 2, there were significant dose-dependent (all $F_{4,55}$ values with $p < 0.00001$) increases in four specific behaviors: rearing (31.4), sniffing upward/outward (18.1) sniffing downward (55.8), and head-bobbing (60.2). Cocaine did not elicit focused oral stereotyped behaviors (sniffing, gnawing) at any of the doses tested. Grooming responses were inconsistent and variable and did not show dose-dependent effects. For the four prevalent behaviors, the pattern of behavioral change increased dose-dependently except that at the largest dose tested (40 mg/kg), rearing and upward sniffing decreased

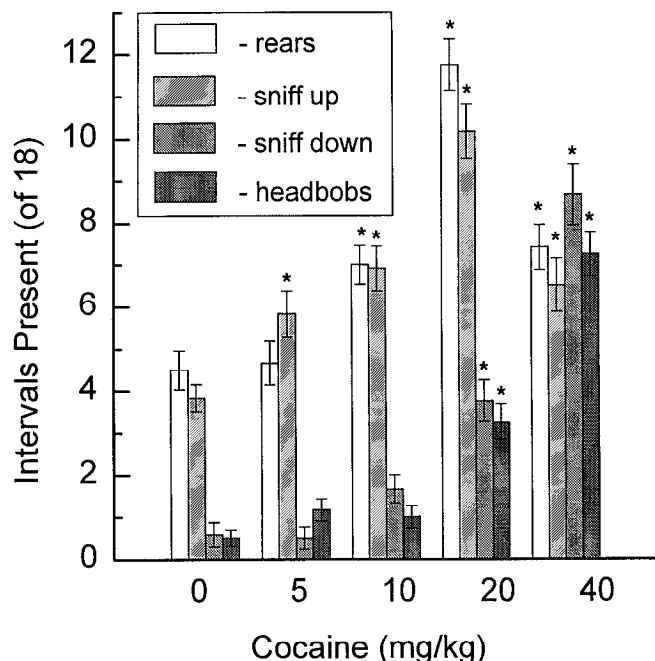


Figure 2. Increases in the presence of repetitive “stereotyped” behaviors following cocaine injections. Cocaine caused a dose-dependent monophasic increase in downward sniffing and “head bobbing.” In contrast, rearing and upward sniffing reached a plateau at the 20 mg/kg dose and declined somewhat at the 40 mg/kg dose. Despite the presence of these stereotyped behaviors, ambulations continued to increase.

as compared to the 20 mg/kg dose whereas downward sniffing and head bobbing increased. Despite the presence of this form of stereotyped behavior, the rats showed further increases in ambulation, indicating that they were not engaged in highly focused stereotyped behaviors that are incompatible with ambulation.

Behavioral effects of cocaine after repeated cocaine administration

As shown in Figure 3A, rats that received repeated cocaine injections exhibited significantly enhanced locomotor responses relative to vehicle-treated controls ($n = 18/\text{group}$) when challenged with cocaine after a one day withdrawal period ($t_{34} = 3.28$, $p = 0.0024$). Sensitization within the cocaine treatment group was also evident since the rats exhibited a greater locomotor response to cocaine in their final exposure to the drug relative to their first exposure ($t_{17} = 3.42$, $p = 0.0033$). When challenged with vehicle on the day after cocaine testing, both the vehicle ($t_{17} = 4.17$, $p = 0.0006$) and cocaine groups ($t_{17} = 9.87$, $p = 0.0001$) exhibited responses that were significantly greater than the corresponding vehicle response observed during the habituation test, and from the Day 1 vehicle test for the control group ($t_{17} = 5.13$, $p = 0.0001$, Fig. 3A). In fact, the degree of ambulation was indistinguishable from the locomotor response to an acute 10.0 mg/kg cocaine challenge (Fig. 1), suggesting that a single exposure to the combination of drug injection and the test environment resulted in a conditioned locomotor response to the test environment.

Following a 1 week withdrawal from cocaine or vehicle injections, a separate group of rats also exhibited sensitization to the locomotor-stimulant effects of cocaine (Fig. 3B), whether the comparison was made within the cocaine-treated group ($t_{11} = 3.21$, $p = 0.008$), or between groups ($t_{22} = 2.77$, $p = 0.011$, n

$= 12/\text{group}$). Unlike the 1 d withdrawal group, but similar to groups tested at all other withdrawal times, there appeared to be no conditioned response to the test environment in the 1 week withdrawal group, because cocaine-treated and control rats exhibited similar responses to vehicle challenge when tested on the habituation day and on the vehicle challenge day (day 8, Fig. 3B).

After a 1 month withdrawal from cocaine or vehicle, a separate group of cocaine-treated rats also displayed significantly enhanced locomotor responses ($t_{22} = 3.50$, $p = 0.002$) to cocaine challenge as compared to their matched control group (Fig. 3C). However, sensitization within the cocaine-treated group ($n = 12$) was not evident at this withdrawal time. Thus, the response to cocaine following a 1 month withdrawal was not significantly higher than the response to the initial injection of cocaine ($t_{11} = 1.19$, $p = 0.258$).

When tested following a 2 month withdrawal from cocaine or vehicle (Fig. 3D), separate groups of rats showed no evidence of sensitized locomotion to cocaine. Thus, neither the between-groups comparison ($t_{22} = 0.48$, $p = 0.644$) nor the within-group comparison was statistically significant ($t_{11} = 0.47$, $p = 0.651$). Although the response of the control group to cocaine at this time point was not significantly different from that of the cocaine-treated group, it was higher than that observed in the other control groups. Thus, there did not appear to be a significant age-related decline in sensitivity to cocaine.

Temporal analysis of the effects of cocaine at each of the four withdrawal times indicated that sensitization consisted of a heightened locomotor response primarily during the first 30 min after cocaine injection (Fig. 4). There was no evidence for an altered temporal pattern of behavior during the 90 min test period as a function of repeated cocaine administration (Fig. 4), except perhaps a greater duration of heightened activity in the 1 d withdrawal group (Fig. 4A). Viewed together, Figures 3 and 4 indicate that sensitization produced by our treatment regimen was most evident immediately after the cessation of treatment (up to 1 week) and waned gradually until it was no longer apparent after a 2 month withdrawal period.

Individual animal analyses

Because the within-group and between-groups comparisons yielded different conclusions with respect to the presence of sensitization in the 1 month withdrawal group, we decided to analyze our data for the presence of sensitization in individual animals. This required setting a criterion to judge a rat as sensitized. To this end, we first ascertained the normal amount of session-to-session variability of cocaine responsiveness in nonsensitized rats. We used a separate group of 12 rats and tested their response to 10 mg/kg of cocaine on two occasions 14 d apart. We chose the 2 week intertest interval based upon published results indicating that context-dependent sensitization that occurs following a single administration of cocaine persists only for several days (Post et al., 1992). Our findings support this conclusion because there was no evidence for sensitization across the two tests: first test, 134 ± 24 ; second test, 116 ± 20 (mean ambulation counts \pm SEM). The average percent change, regardless of direction, was 30.2% with a SD of 18.1%. Accordingly, we chose as our criterion for a sensitized rat a percent increase in ambulation counts of at least 50%, a value that was greater than 1 SD above the mean of the average session-to-session variability. Based upon this criterion, we found the following percentages of sensitized rats in the various cocaine treat-

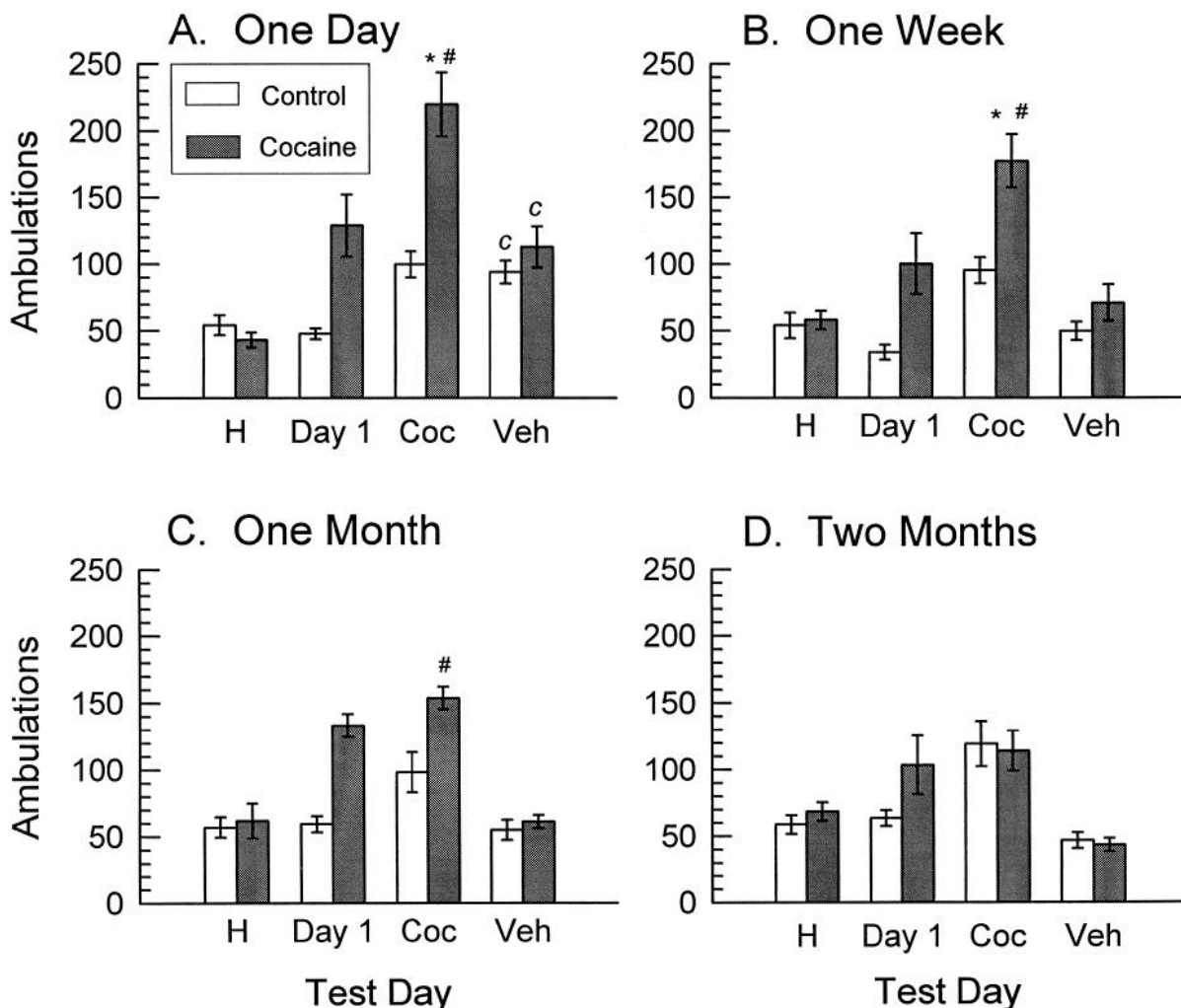


Figure 3. Effects of cocaine and vehicle injections on locomotor activity prior to and subsequent to repeated cocaine administration in the four different withdrawal groups: *A*, 1 d ($n = 18$); *B*, 1 week ($n = 12$); *C*, 1 month ($n = 12$); and *D*, 2 months ($n = 12$). All animals were injected with the cocaine vehicle and habituated to the testing apparatus the day prior to the start of repeated treatment (*H*). *Day 1* represents the response to the first vehicle (control groups) or cocaine injection (cocaine groups) of the repeated treatment regimen. The *Coc* bars represent the cocaine challenge (10 mg/kg, i.p.) given to both the control and cocaine groups at the specified withdrawal times. The *Veh* bars represent the response to vehicle challenge on the day after the cocaine challenge. This protocol provided both a within-group and between-groups assessment of the presence of sensitization. Between-groups sensitization (#) is evident at all but the 2 month withdrawal time whereas within-group sensitization (*) is evident only at the 1 d and 1 week withdrawal times. There was a significant increase in locomotor activity in response to the vehicle on the day after the cocaine challenge (*Veh* bars) only in the 1 d withdrawal group (*c*). Each bar represents the mean \pm SEM. See text for statistical significance information.

ment groups: 1 d, 78%; 1 week, 75%; 1 month, 50%; and 2 months, 25%. Of particular interest was the finding that four of the six nonsensitized rats in the 1 month group showed decreases in ambulation counts during the challenge test as compared to their first cocaine injection. This accounts for the lack of a significant within-subject sensitization in the 1 month withdrawal group.

Additional tests after 2 months of withdrawal

Because the lack of persistence of behavioral sensitization to cocaine is somewhat at odds with reports describing primarily amphetamine sensitization (see Discussion for details), we conducted additional experiments to determine other features of cocaine-induced behavior in similarly treated rats. Because the rats were tested for sensitization only at the 2 month withdrawal time, we could not be sure that they had been sensitized at earlier time points. It is also possible that sensitization was being ex-

pressed as a qualitative alteration in the form of behavior rather than as a quantitative increase in locomotion. It is known that amphetamine sensitization can be expressed as a reduction in locomotion due to the elicitation of more focused stereotyped behaviors (see Discussion for details and references).

Two groups of randomly assigned rats ($n = 16$ /group) received repeated cocaine or vehicle injections as described above. Half of the rats in each group were tested with cocaine (10 mg/kg, i.p.) 20 hr after the last (28th) cocaine injection. All rats were then tested following a 2 month withdrawal, as described above. The rats were tested in the locomotor activity cages and were scored, as above, by an observer who was blind to group assignments. As shown in Table 1, rats that were tested following a 1 d withdrawal not only showed greater ambulation and total counts as recorded automatically, but also engaged in significantly more rearing, sniffing and head bobbing than did their corresponding control group. However, following a 2 month

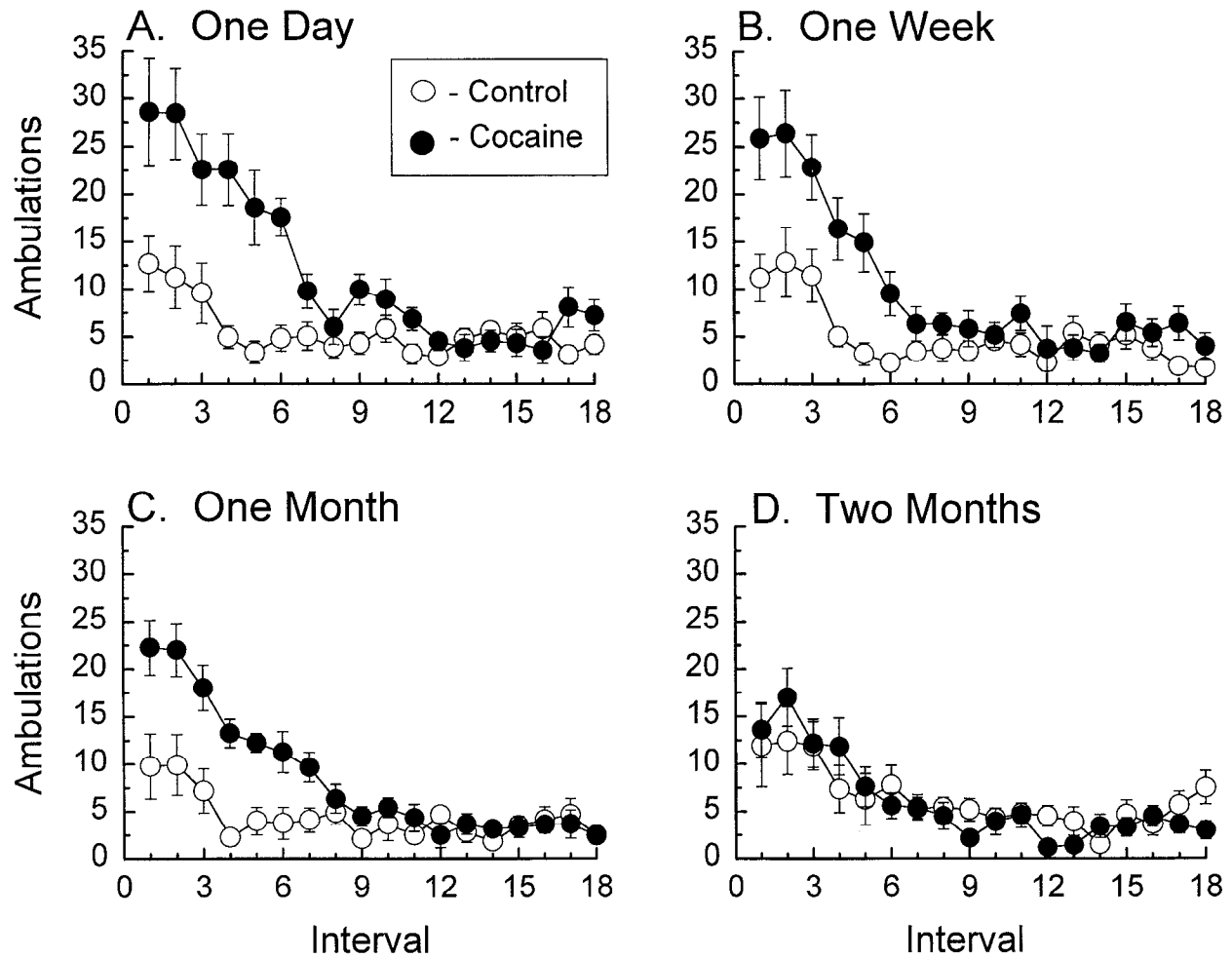


Figure 4. Time course for the locomotor responses to cocaine challenge (10 mg/kg, i.p.) at various withdrawal times after cessation of repeated treatment with either cocaine or vehicle (control). Cocaine sensitization was manifest as an increase in ambulatory responses primarily during the first 30–35 min after cocaine injection with no evidence for increased duration of the behavioral effect. Each point represents the mean \pm SEM.

withdrawal, the control groups as well both cocaine groups were essentially equivalent in all measures of behavior. Thus, the failure to observe sensitization following a two month withdrawal from our cocaine treatment regimen was probably not due to the failure to sensitize the rats at earlier time points or to the induction of qualitatively different, more intense forms of stereotyped behavior.

Electrophysiological experiments following repeated cocaine administration

Iontophoretic administration of cocaine inhibits the firing of most NAc neurons by preventing the reuptake of both endogenous DA and 5-HT (White, 1990; White et al., 1992, 1993). In the present experiments, we determined whether such inhibitory effects were enhanced at various withdrawal times following

Table 1. Lack of cocaine sensitization (10.0 mg/kg challenge) following a 2 month withdrawal

	1 d withdrawal		2 month withdrawal			
	Control	Cocaine	Retested ^a		First test ^b	
			Control	Cocaine	Control	Cocaine
Ambulations	111 \pm 15	203 \pm 18**	92 \pm 14	104 \pm 16	121 \pm 22	116 \pm 18
Total counts	631 \pm 61	1182 \pm 97**	586 \pm 62	615 \pm 48	671 \pm 72	712 \pm 95
Rears ^c	6.8 \pm 0.5	9.2 \pm 0.6**	7.4 \pm 0.4	6.6 \pm 0.6	6.9 \pm 0.7	6.5 \pm 0.5
Sniff-up ^c	6.1 \pm 0.9	10.6 \pm 0.7**	5.4 \pm 0.5	6.1 \pm 0.6	5.7 \pm 0.7	6.1 \pm 0.7
Sniff-down ^c	1.5 \pm 0.3	2.8 \pm 0.4*	1.3 \pm 0.3	1.4 \pm 0.3	1.7 \pm 0.5	1.4 \pm 0.4
Head bobs ^c	0.4 \pm 0.3	3.1 \pm 0.6**	0.3 \pm 0.2	0.2 \pm 0.1	0.4 \pm 0.3	0.3 \pm 0.2

* $p < 0.05$, ** $p < 0.01$, t test.

^a These are the same rats that were tested following a 1 d withdrawal ($n = 8$ /group).

^b These rats had not previously been tested ($n = 8$ /group).

^c Data indicate the number of 5 min intervals (of 18) that this behavior was present.

repeated cocaine administration. To determine the specificity of such changes to cocaine-induced inhibition, we examined the inhibitory effects of GABA. In none of these experiments did we observe a significant difference between the effects of cocaine or GABA on spontaneously active and glutamate-driven neurons; thus, statistical comparisons are made with these two groups of cells pooled. As published previously, we saw no differences in the pharmacological responses of neurons within the shell and core regions of the NAc (White et al., 1993).

The effects of cocaine and GABA on the activity of glutamate-driven NAc neurons are illustrated in Figure 5. Both agents consistently inhibited NAc neurons in a current-dependent manner. Because these effects in control rats were highly similar at the various withdrawal times (see current-response curves in Figs. 6 and 7), only one example is provided (Fig. 5A). Cocaine-induced inhibition was enhanced in rats following a 1 d withdrawal from repeated cocaine treatment (Fig. 5B) as compared to control rats (Fig. 5A). When the results from all cells in these two groups were averaged and plotted (Fig. 6A), it is apparent that repeated cocaine treatment significantly enhanced ($F_{1,27} = 9.02$, $p = 0.0058$) the inhibitory efficacy of cocaine. Following a 1 week withdrawal, NAc neurons in cocaine-treated rats were also significantly ($F_{1,24} = 14.71$, $p \leq 0.0011$) sensitized to the inhibitory effects of cocaine (see Figs. 5C, 6B). Supersensitivity of NAc neurons to cocaine was also clearly evident ($F_{1,25} = 9.86$, $p = 0.0045$) after a one month withdrawal (see Figs. 5D, 6C), but not after a 2 month withdrawal (Figs. 5E, 6D; $F_{1,24} = 1.21$, $p = 0.28$).

Surprisingly, the results indicated that at the one day withdrawal time, NAc neurons in cocaine-treated rats were also sensitized to the inhibitory effects of GABA (compare Fig. 5A and 5B). When these results were plotted (see Fig. 7A) and analyzed, the enhanced response to GABA proved to be statistically significant ($F_{1,30} = 8.286$, $p = 0.0073$). However, this effect was not observed at any other time point (1 week: $F_{1,24} = 0.250$, $p = 0.624$; 1 month: $F_{1,26} = 0.476$, $p = 0.503$; and 2 months: $F_{1,25} = 0.013$, $p = 0.877$, see Figs. 5C–E and 7B–D).

Intravenous administration of cocaine also inhibits the majority of NAc neurons in our preparation (White et al., 1987, 1992; White, 1990). Because this route of administration would mimic our behavioral studies more closely, we also determined whether repeated cocaine administration would alter the responsiveness of NAc neurons to intravenous cocaine. We studied the effects of intravenous cocaine on NAc cells at both the one day and two month withdrawal times (14 rats/group) and compared them to a control group which consisted of eight rats withdrawn for 1 d and six rats withdrawn for 2 months from repeated vehicle injections. The results from these rats were pooled when it was determined that there was no significant difference between the effects of cocaine on NAc cells at these two withdrawal times ($F_{1,12} = 0.65$, $p = 0.43$). In each of these three groups, nine of the cells were glutamate driven and the other five were spontaneously active. As in the iontophoretic studies described above, there were no significant differences (all $F_{1,12}$, $p > 0.28$) between spontaneously active and glutamate-driven neurons in any of the three groups (control: 1.26; 1 d cocaine: 0.67; 2 month cocaine: 0.81). Therefore, the results were combined for the purpose of statistical comparisons between the various treatment groups.

When tested 1 d after the last cocaine or vehicle injection, the inhibitory response of NAc neurons to intravenous cocaine was enhanced in cocaine-treated rats (Fig. 8B) as compared to control rats (Fig. 8A). No such effect was observed in rats with-

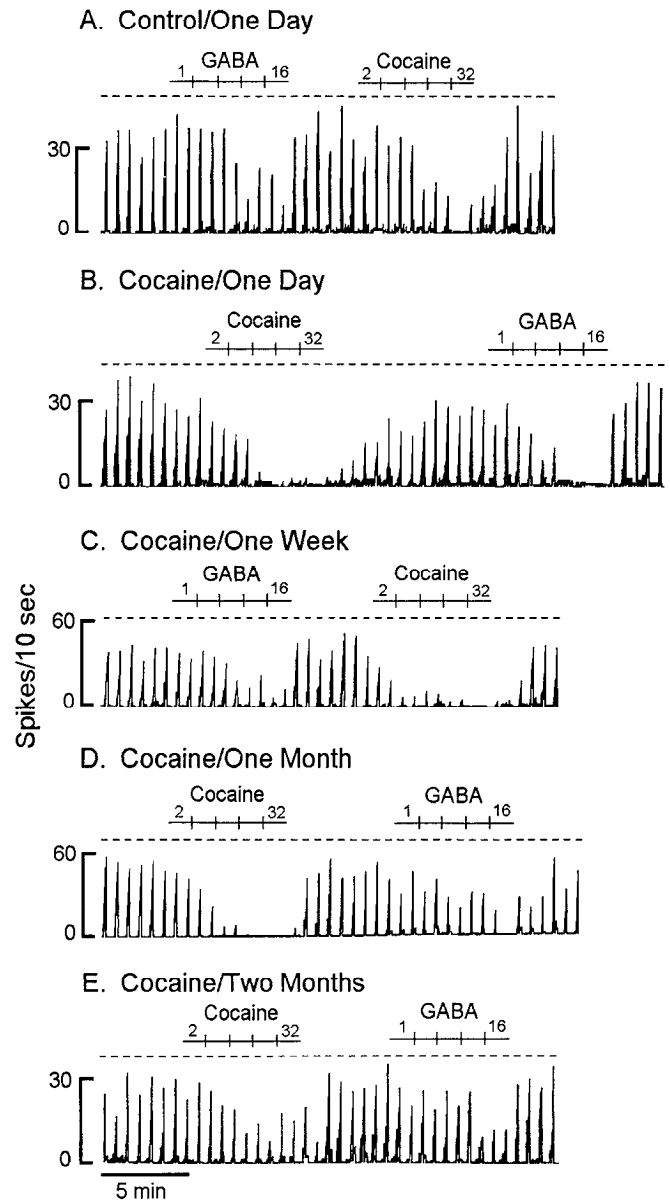


Figure 5. Cumulative rate histograms showing examples of repeated cocaine-induced alterations in the responses of NAc neurons to iontophoretically applied cocaine and GABA. *A*, NAc neurons from a control rat tested 18–24 hr after the final injection of vehicle, illustrating the normal current-dependent inhibitory effects of GABA and cocaine. *B–E*, Effects of GABA and cocaine in rats that received repeated cocaine and were tested at various withdrawal times. Note the enhanced inhibitory efficacy of cocaine at all but the 2 month withdrawal time and the enhanced inhibitory efficacy of GABA only at the 1 d withdrawal time. All neurons pictured were driven to fire using 20 sec pulses of glutamate (dashed lines). Horizontal bars indicate ejection duration and numbers represent current in nanoamperes (nA).

drawn for 2 months (Fig. 8C). When all NAc cells tested in these groups were averaged and plotted (Fig. 9), it was apparent that those recorded from cocaine-treated rats withdrawn for 1 d were significantly ($F_{2,39} = 4.11$, $p = 0.024$) more sensitive to systemic cocaine than those tested in either control rats or in the cocaine-treated rats withdrawn for 2 months.

Discussion

Based upon our observations, behavioral sensitization following repeated cocaine administration appears to be a relatively per-

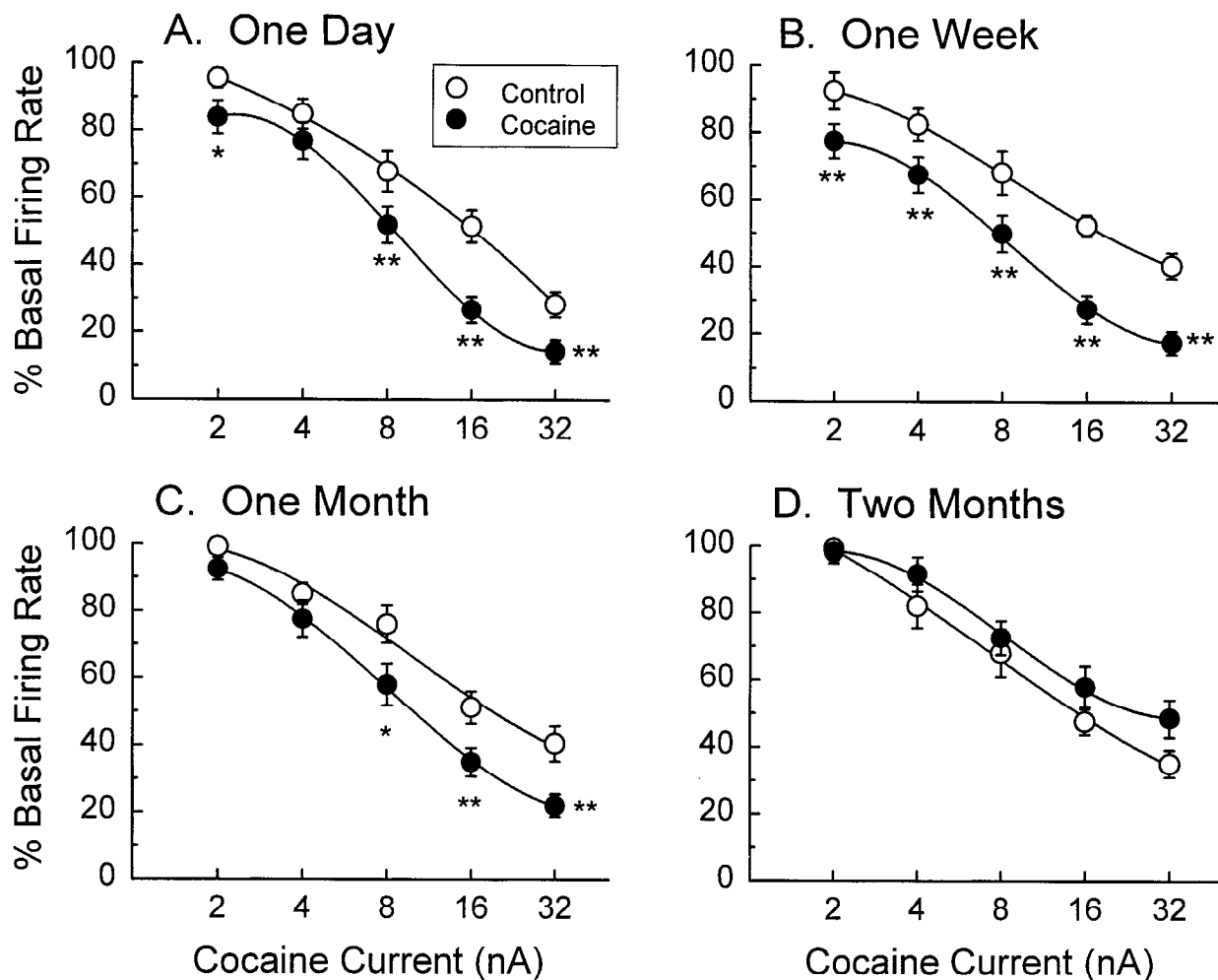


Figure 6. Effects of repeated cocaine on the sensitivity of NAc neurons to iontophoretically applied cocaine at various withdrawal times. These current–response curves show that NAc neurons were more sensitive to cocaine at all but the 2 month withdrawal time. Each point represents the mean \pm SEM (*, $p < 0.05$; **, $p < 0.01$, Dunnett's test). Sample sizes were as follows. *A*: Control—17 cells, 6 spontaneously active (s.a) recorded from 7 rats; cocaine—18 cells, 5 s.a. from 7 rats. *B*: Control (14, 3, 6) and cocaine (13, 4, 5). *C*: Control (14, 4, 6) and cocaine (14, 4, 6). *D*: Control (13, 5, 5) and cocaine (14, 5, 6).

sistent, but not permanent effect. Using a treatment protocol designed to minimize context-dependent sensitization, we observed that sensitization was greatest at early withdrawal times (1 d, 1 week) but waned with extended withdrawal (1 month, 2 months), suggesting a gradual decay in the mechanisms underlying cocaine sensitization. We also demonstrated enhanced sensitivity of NAc neurons to cocaine at early and intermediate, but not long, withdrawal times, an effect that is similar to our previous findings of supersensitive DA D1 receptor-mediated responses (Henry and White, 1991; White et al., 1995a). When viewed along with other work regarding cellular mechanisms underlying cocaine-induced locomotion and alterations in NAc neuronal activity (White et al., 1993; Xu et al., 1994), our findings suggest that increased DA D1 receptor sensitivity caused by repeated cocaine administration amplifies the essential role of this receptor system in cocaine-induced locomotion, leading to sensitization.

Persistence of cocaine sensitization

Our finding of a gradually waning sensitization was obtained from rats that were challenged with cocaine at only one withdrawal time. To our knowledge, the only other study that tested

separate groups of rats at different cocaine withdrawal times revealed evidence for sensitization 23 d after the last cocaine injection (Kalivas and Duffy, 1993a). However, cocaine sensitization has been observed to last 54 to 98 d following treatment cessation when rodents are tested repeatedly during the treatment regimen and/or at various withdrawal times (Kilbey and Ellinwood, 1977; Shuster et al., 1977; Post et al., 1992). Given that transient sensitization can occur after only one exposure to cocaine (Weiss et al., 1989; Post et al., 1992; Jackson and Nutt, 1993), and that repeated association of cocaine with the test environment causes context-dependent sensitization (Post et al., 1992), it is possible that repeated challenges with cocaine at various withdrawal times were sufficient to maintain sensitization in the prior studies. Taken together, these findings are consistent with the notion that context-dependent sensitization is more persistent than context-independent sensitization (Post et al., 1992).

A conditioned increase in locomotor activity resulting from pairing of the test environment with cocaine is a well characterized aspect of cocaine sensitization (see Stewart and Vezina, 1988; Kalivas and Stewart, 1991; Post et al., 1992 for reviews). Such conditioned activity, like context-dependent sensitization,

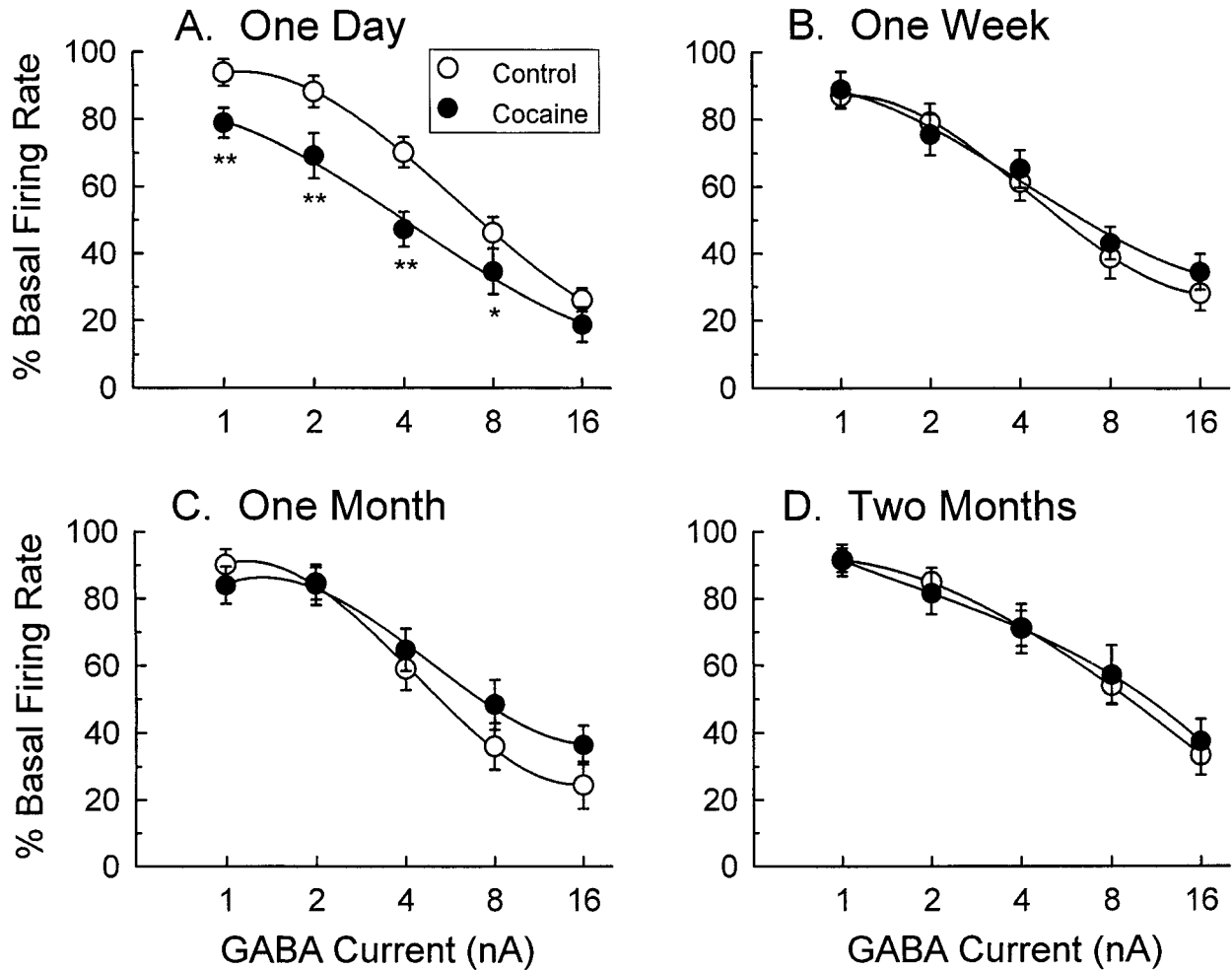


Figure 7. Effects of repeated cocaine on the sensitivity of NAc neurons to iontophoretically applied GABA at various withdrawal times. These are the same cells that were tested with iontophoretic cocaine (Fig. 6). The current–response curves show that NAc neurons were more sensitive to GABA only at the 1 d withdrawal time. Each point represents the mean \pm SEM (*, $p < 0.05$; **, $p < 0.01$, Dunnett's test). See Figure 6 caption for sample sizes.

depends upon the degree of similarity between the injection and test environments (Weiss et al., 1989; Hinson and Poulos, 1981). Because these environments were markedly different in the present study (see Materials and Methods for details), it is not surprising that we found little evidence of conditioned increases in locomotor activity. In fact, only the 1 d withdrawal group exhibited heightened activity during saline tests on the day following cocaine challenges. Given that this group is the only one that did not receive a saline challenge test on the day before the cocaine challenge, it is possible that conditioned locomotion had been suppressed in all other cocaine groups by the explicit pairing of the test environment with the absence of cocaine. Whatever the case, our finding suggests that the sensitization observed with our treatment protocol was largely context-independent. However, traditional Pavlovian paradigms with appropriate controls would be required to confirm this suggestion.

In the animals tested for sensitization following the 1 month withdrawal period, enhanced locomotion was evident only in the between-groups comparison. This appeared to result from a smaller percentage of rats (50%) showing sensitization, and from the decrease in activity in four of the six nonsensitized rats during the cocaine challenge as compared to their first test with cocaine. Although individual variation in the susceptibility to

amphetamine sensitization has long been recognized (Leith and Kuczenski, 1982; Segal and Kuczenski, 1987a; Hooks et al., 1992), this factor has received relatively little attention in cocaine studies. Based upon estimates of session-to-session variability of cocaine responsiveness in nonsensitized rats, we only classified rats as sensitized when they exhibited at least a 50% increase in locomotion during the cocaine challenge as compared to the first cocaine test. Sensitization was evident in 75–78% of the rats at early withdrawal times, a percentage that waned with longer withdrawal. Such individual animal analysis may prove useful in future studies of neuronal alterations associated with cocaine sensitization, inasmuch as nonsensitized, cocaine-exposed rats could serve as an additional control group.

Although our results indicate a lack of sensitization after two months of withdrawal from cocaine, at least two alternative interpretations of these data could be advanced. Because we tested these animals for sensitization only after the long withdrawal, when only 3 of the 12 rats were sensitized, it is possible that the other nine rats had never sensitized. Therefore, we tested another group of rats after both 1 d and 2 month withdrawals and found that the sensitization present after a 1 d withdrawal was no longer evident at 2 months. A second alternative interpretation is that the 2 month withdrawal group was actually

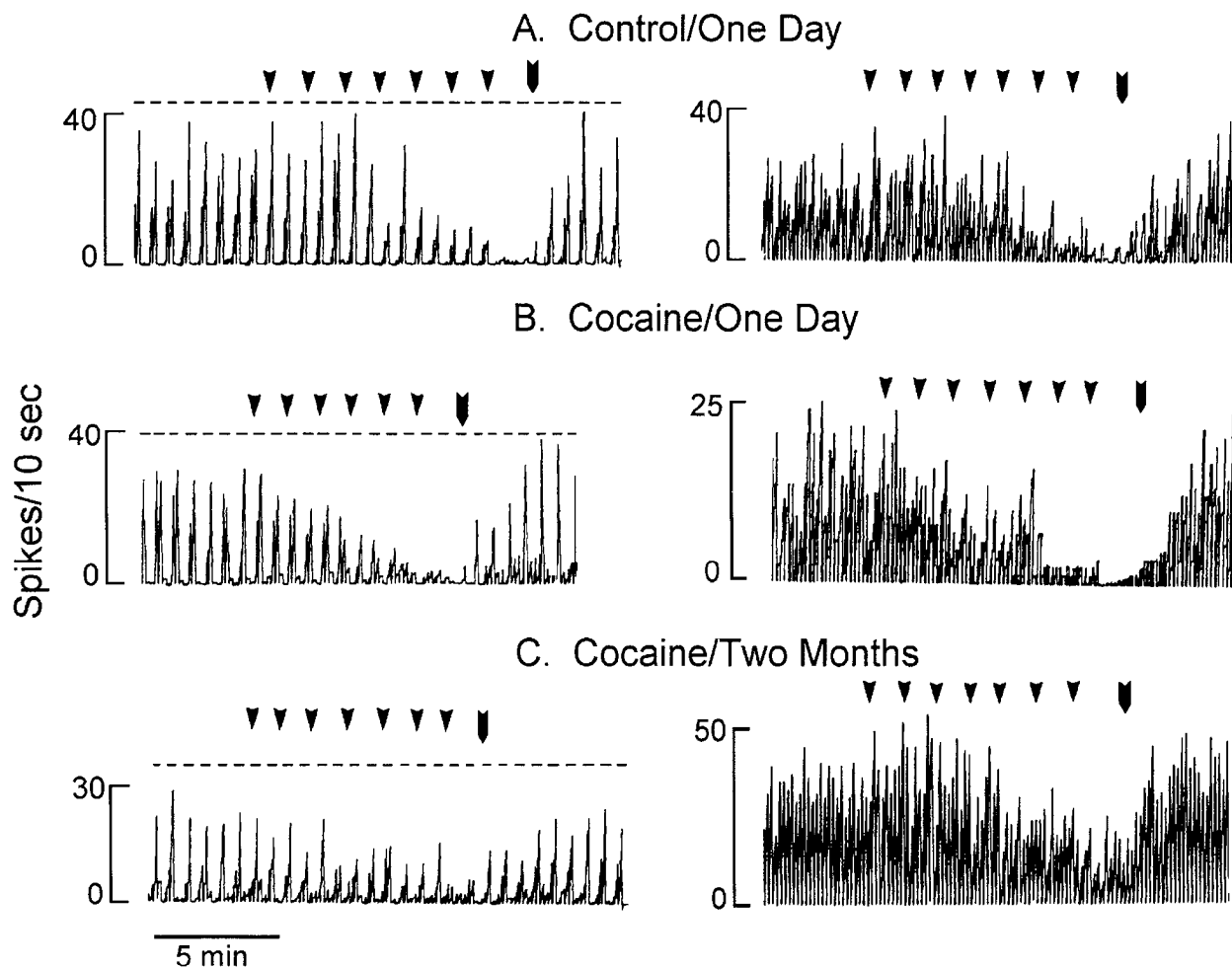


Figure 8. Examples illustrating the effect of repeated cocaine on the sensitivity of NAc neurons to intravenous (i.v.) cocaine following repeated administration of the cocaine vehicle (*A*), a 1 d withdrawal from repeated cocaine administration (*B*), or a 2 month withdrawal from repeated cocaine administration (*C*). The *left panels* illustrate the effects of glutamate-driven neurons ($n = 9$ cells and rats/group, *dashed lines* indicate glutamate pulses) whereas the *right panels* illustrate the effects on spontaneously active neurons ($n = 5$ cells and rats/group). *Arrowheads* indicate points where cocaine was administered, starting at an initial dose of 0.1 mg/kg and doubling at each *arrow* up to a cumulative dose of either 3.2 or 6.4 mg/kg. Cocaine-induced inhibition was reversed by the DA D1 receptor antagonist SCH 23390 (50 μ g/kg i.v., at *large arrowheads*).

more sensitized. Amphetamine sensitization is often expressed as a reduction in locomotion due to the presence of more focused stereotyped behaviors (Segal et al., 1980; Robinson and Becker, 1986; Segal and Kuczenski, 1987a,b), and repeated cocaine treatment, at high doses, can induce more focused stereotyped behaviors (Post and Rose, 1976; Kilbey and Ellinwood, 1977a,b; Roy et al., 1978; Gale, 1984; Dow-Edwards et al., 1989). However, such a transition in the form of behavior could not explain our results because an increase in all behaviors was observed in sensitized rats after a 1 d withdrawal, whereas no behavior was enhanced after 2 months of withdrawal.

Electrophysiological correlates of cocaine sensitization

Repeated cocaine treatment rendered NAc neurons more sensitive to the inhibitory effects of cocaine, whether the drug was administered systemically or locally. Several factors might play a role in the enhanced inhibitory efficacy of cocaine, including alterations in the DA transporter (Izenwasser and Cox, 1990; Sharpe et al., 1991; Pilotte et al., 1994; Wilson et al., 1994) and in cocaine disposition (Ho et al., 1977; Estevez et al., 1979; Reith et al., 1987; Pettit et al., 1990; Pan et al., 1991). However, the time course of DA transporter alterations does not match

that of sensitized electrophysiological responses in the NAc. Similarly, pharmacokinetic changes cannot explain the enhanced neuronal response to iontophoretically administered cocaine or to the DA D1 receptor agonist SKF 38393 (Henry and White, 1991). The similarity of the time courses for supersensitive cocaine and SKF 38393 effects within the NAc suggests that the enhanced inhibitory efficacy of cocaine results from synaptic DA stimulating supersensitive DA D1 receptors. The fact that the DA D1 receptor antagonist SCH 23390 reversed the inhibition produced by systemic cocaine supports this suggestion. However, the inhibition may not be mediated solely by DA D1 receptors. 5-HT receptor activation also plays a significant role in cocaine's effects on NAc neurons (White et al., 1993; Xu et al., 1994). In addition, DA D2-like receptor stimulation may also participate in the inhibitory effect, an action that would also be antagonized by SCH 23390 through prevention of the necessary "enabling" effect of DA D1 receptor stimulation for DA D2-like receptor-mediated effects within the NAc (White, 1987; Wachtel et al., 1989; Xu et al., 1994).

Surprisingly, NAc neurons in cocaine-treated rats were also supersensitive to the inhibitory effects of GABA. However, unlike the supersensitivity to cocaine and SKF 38393 (Henry and

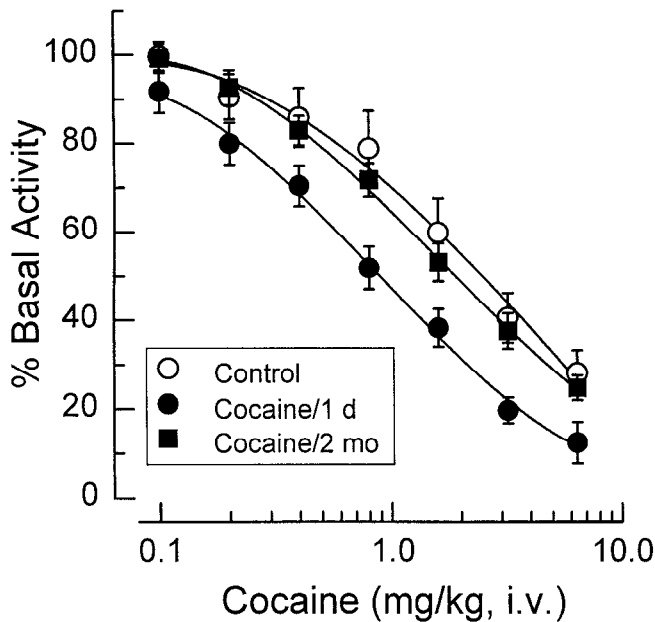


Figure 9. Effects of repeated cocaine on the sensitivity of NAc neurons to intravenous (i.v.) cocaine at 1 d and 2 month withdrawal times. These cumulative dose–response curves illustrate that NAc neurons were more sensitive to intravenous cocaine following a 1 d, but not a 2 month withdrawal. Each point represents the mean \pm SEM. The 1 d withdrawal group was significantly more sensitive than the control group at all cocaine doses above 0.2 mg/kg ($p < 0.01$, Dunnett's).

White, 1991), the enhanced sensitivity to GABA was evident only after a 1 d withdrawal. Two potential explanations for this effect are: (1) enhanced GABA receptor sensitivity resulting from collateral inhibition between GABAergic neurons, and (2) a general decrease in membrane excitability. The latter explanation receives support from recent studies indicating reduced glutamate-induced excitation of NAc neurons in cocaine-sensitized rats (White et al. 1995b). Whatever the mechanism, it is apparent that the transient GABA supersensitivity did not play a significant role in the relatively persistent cocaine sensitization.

Possible mechanisms of cocaine sensitization

When viewed along with our previous electrophysiological studies (Henry and White, 1991, 1992; White et al., 1995a), as well as recent receptor binding (Alburges et al., 1993; Unterwald et al., 1994) and signal transduction results (Nestler et al., 1990; Terwilliger et al., 1991; Striplin and Kalivas, 1993), the present findings suggest that the enhanced behavioral and electrophysiological effects of cocaine following its repeated administration may result from a relatively persistent increase in the functional activity of DA D1 receptor-mediated processes within the NAc. These receptors play an essential role in the locomotor stimulant effects of a variety of drugs (see Waddington and Daly, 1993; White and Hu, 1993 for recent reviews). In fact, mice lacking DA D1 receptors fail to exhibit hyperactivity following cocaine administration (Xu et al., 1994). Accordingly, enhanced transmission through DA D1 receptors seems certain to participate in the expression of sensitized locomotor stimulation (Henry and White, 1991; Henry and White, 1992; Unterwald et al., 1994), particularly during early periods of withdrawal when there is no enhancement of cocaine-induced increases in extracellular DA (Kalivas and Duffy, 1993a; Segal and Kuczenski, 1992b; Maisonneuve and Kreek, 1994). Indeed, the latter effect increases

with withdrawal (Kalivas and Duffy, 1993a) whereas DA D1 receptor supersensitivity declines. Taken together, these findings support the hypothesis that alterations in dopamine release and postsynaptic responsiveness may participate to differing degrees in the expression of cocaine sensitization at different points in time (Kalivas and Stewart, 1991; Henry and White, 1992; Kalivas and Duffy, 1993a; Maisonneuve and Kreek, 1994). Although DA D1 receptors have also been suggested to participate in the development of behavioral sensitization (Kalivas and Stewart, 1991), this may not be true for cocaine because coadministration of SCH 23390 prevents expression (McCreary and Marsden, 1993) but not development of cocaine sensitization (Mattingly et al., 1994).

Conclusions

The findings that neither cocaine sensitization, when induced in a manner that minimized context dependency, nor certain physiological correlates of that sensitization were permanent effects suggest that the mechanisms underlying this form of plasticity are reversible. Available results suggest that context-dependent cocaine sensitization, engendered by repeated pairings of cocaine and the test environment, is more persistent than context-independent sensitization (Post et al., 1992). It may be that context-dependent and context-independent aspects of cocaine sensitization are mediated by different, yet related, neuroadaptations occurring in converging neural networks. Alternatively, contextual cues might render the same neural alterations more persistent, or might engender neuronal events that trigger the expression of relevant neuroadaptations. Given that human cocaine abuse typically occurs in environments with distinct contextual cues, further analysis of cellular mechanisms underlying context-dependent and context-independent aspects of cocaine sensitization may yield important insights into the neurobiology of drug seeking behavior (Robinson and Berridge, 1993).

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