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Addiction-Related Alterations in D_1 and D_2 Dopamine Receptor Behavioral Responses Following Chronic Cocaine Self-Administration

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The cocaine-addicted phenotype can be modeled in rats based on individual differences in preferred levels of cocaine intake and a propensity for relapse in withdrawal. These cocaine-taking and -seeking behaviors are strongly but differentially regulated by postsynaptic D_1 and D_2 receptors in the mesolimbic dopamine system. Thus, we determined whether addiction-related differences in cocaine self-administration would be related to differential sensitivity in functional D_1 and D_2 receptor responses. Using a population of 40 outbred Sprague–Dawley rats trained to self-administer cocaine for 3 weeks, we found that animals with higher preferred levels of cocaine intake exhibited a vertical and rightward shift in the self-administration dose–response function, and were more resistant to extinction from cocaine self-administration, similar to phenotypic changes reported in other models of cocaine addiction. After 3 weeks of withdrawal from cocaine self-administration, high intake rats were subsensitive to the ability of the D_1 agonist SKF 81297 to inhibit cocaine-seeking behavior elicited by cocaine priming, but supersensitive to cocaine seeking triggered by the D_2 agonist quinpirole, when compared to low intake rats. Additionally, high intake rats developed profound increases in locomotor responses to D_2 receptor challenge from early to late withdrawal times, whereas low intake rats developed increased responsiveness to D_1 receptor challenge. In a second experiment, responses to the mixed D_1/D_2 agonist apomorphine and the NMDA glutamate receptor antagonist MK-801 failed to differ between low and high intake rats. These findings suggest that cocaine addiction is related specifically to differential alterations in functional D_1 and D_2 receptors and their ability to modulate cocaine-seeking behavior.
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INTRODUCTION

Drug addiction can be modeled in rodents based on phenotypic differences in drug consumption and drug-seeking behavior in an attempt to delineate 'addicted' from 'nonaddicted' biological states (Ahmed and Koob, 1998; Ahmed *et al*, 2000; Piazza *et al*, 2000; Sutton *et al*, 2000; Deroche-Gamonet *et al*, 2004). In one approach, the addicted phenotype is selected from outbred rat populations based on higher preferred levels of drug intake. Previous studies have shown that rats with higher preferred levels of cocaine intake also exhibit a propensity for cocaine-seeking behavior when reinforcement is withheld during self-administration or after a period of withdrawal, thereby encompassing both addictive traits in a single subpopulation (Piazza *et al*, 2000; Sutton *et al*, 2000).

Cocaine-taking and -seeking behaviors are strongly regulated by D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4) classes of dopamine receptors (hereafter referred to as D_1 and D_2 receptors). Systemic pretreatment with either D_1 or D_2 receptor agonists reduces cocaine self-administration in rats, whereas pretreatment with either D_1 or D_2 receptor antagonists increases intake when access to cocaine is relatively unrestricted (eg, Koob *et al*, 1987; Corrigall and Coen, 1991; Caine *et al*, 1999), suggesting that both receptors provide inhibitory feedback regulation of cocaine intake during self-administration. Relapse to cocaine-seeking behavior is also strongly regulated by both D_1 and D_2 dopamine receptor classes, but here D_1 and D_2 receptors mediate differential effects. Selective stimulation of D_2 receptors strongly induces, or reinstates, cocaine-seeking behavior after cocaine-seeking responses have been extinguished in withdrawal, whereas selective D_1 receptor stimulation produces relatively weak reinstating effects

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and attenuates cocaine seeking elicited by cocaine-related environmental cues when agonists are administered systemically (Wise *et al*, 1990; Self *et al*, 1996; De Vries *et al*, 1999; Alleweireldt *et al*, 2002; Dias *et al*, 2004). In addition, pretreatment with D_1 and D_2 agonists produces opposite effects on cocaine's ability to reinstate this behavior, since D_1 agonists attenuate and D_2 agonists facilitate cocaine seeking induced by cocaine priming injections (Self *et al*, 1996; Alleweireldt *et al*, 2003). A similar D_1/D_2 dichotomy regulates cocaine seeking in non-human primates (Khroyan *et al*, 2000), and also may suppress and stimulate craving responses in humans, respectively (Haney *et al*, 1998, 1999). Together, these studies suggest that D_2 receptors could play a major role in eliciting relapse to cocaine seeking when environmental stimuli such as cocaine-related cues or stress activate the mesolimbic dopamine system (Phillips *et al*, 2003; Pruessner *et al*, 2004), while D_1 receptor tone may provide inhibitory regulation over cocaine seeking. In contrast to agonists, both D_1 and D_2 receptor antagonists block reinstatement of cocaine seeking in rats (Weissenborn *et al*, 1996; Ciccocioppo *et al*, 2001; Norman *et al*, 2002; Vorel *et al*, 2002; Schenk and Gittings, 2003; Gilbert *et al*, 2005), and indiscriminately block the reinstating effects of nucleus accumbens agonist administration (Bachtell *et al*, 2005), consistent with well-characterized enabling and synergistic interactions between D_1 and D_2 receptors on behavioral responses (Waddington and Daly, 1993).

The transition from nonaddicted to addicted states could reflect differential adaptations in the sensitivity of these D_1 and D_2 receptor-mediated responses, whether sensitization in D_2-mediated responses that trigger cocaine seeking, or tolerance in D_1-mediated responses that inhibit drug seeking elicited by cues or cocaine priming. Moreover, higher preferred levels of cocaine intake in addicted animals could reflect a compensatory response to reduced D_1 receptor function. Furthermore, such differences could emerge or be exacerbated during withdrawal from cocaine, since the propensity for cocaine seeking increases in a time-dependent manner from early to late withdrawal times (Tran-Nguyen *et al*, 1998; Grimm *et al*, 2001).

In this study, we tracked changes in postsynaptic D_1 and D_2 receptor-mediated locomotor responses from before the onset of cocaine self-administration to early (2–3 days) and late (4 weeks) withdrawal times. We compared these changes with individual differences in preferred levels of cocaine intake to identify neuroadaptive changes that could contribute to the development of addiction. Importantly, we also compared the ability of D_1 receptor stimulation to attenuate cocaine seeking elicited by cocaine priming injections, and the ability of D_2 receptor stimulation alone to trigger relapse, after a period of withdrawal to identify potential differences associated with a cocaine-addicted phenotype. In contrast to locomotor tests, D_1 and D_2 receptors were challenged under different conditions in reinstatement tests (with or without cocaine) since systemically administered D_1 agonists have little effect on low baseline cocaine seeking after extinction. We chose agonist rather than antagonist challenge to determine receptor sensitivity, since cross-blockade of one receptor subtype by antagonists acting at the other subtype could obscure subtype-specific changes in sensitivity, or prevent meaningful interpretation of observed effects.

MATERIALS AND METHODS

Subjects

Sixty-four outbred male Sprague–Dawley rats (Charles River, Kingston, NY), weighing 300–325 g on arrival, were individually housed in a climate-controlled environment (21°C) on a 12-h light–dark cycle (lights on at 0700 hours) in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Animals were allowed free access to lab chow and water, except during initial lever-press training for sucrose pellets and during initial acquisition of cocaine self-administration (see below).

Surgery

Animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) supplemented with atropine sulfate (0.10 mg, s.c.), prior to surgical implantation of a chronic indwelling intravenous catheter. The catheters consisted of Silastic tubing (0.02 inch i.d. $\times$ 0.037 inch 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 cannula (Plastics One, Roanoke, VA) embedded in dental cement on a 1 inch Marlex surgical mesh base (Bard Inc., Cranston, RI). Following surgery, animals received a prophylactic injection of penicillin (60 000IU/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 clotting and curb infection.

Locomotor and Self-Administration Apparati

Locomotor activity was recorded in the dark in circular test chambers with a 12 cm wide runway, equipped with four pairs of photocells located at 90-degree intervals around the 1.95 m perimeter. The operant test chambers (Med Associates, East Fairfield, VT) used for self-administration, extinction, and reinstatement testing were contextually distinct from the locomotor test chambers, and located in a different room. Each chamber was enclosed in a ventilated, sound-attenuating box and was equipped with an injection pump assembly consisting of a Razel Model A injection pump (Stamford, CT) and a 10-ml glass syringe connected to a fluid swivel (Instech, Plymouth, PA) with Teflon® tubing. Tygon® tubing connected the swivel to the animal's catheter exit port and was enclosed by a metal spring secured to Teflon® threads on the catheter assembly. Each operant chamber contained two response levers (4×2 cm), located 2 cm off the floor; during self-administration testing, a 20 g lever-press response at the active lever delivered an intravenous cocaine injection, and produced no programmed consequence at the inactive lever. Each cocaine injection was delivered over 2.5 s in a 50-µl volume. During the injection period, a cue light (above the lever) was illuminated and the house light was extinguished, followed by an additional 12.5 s time-out interval when the house lights remained off and responding at the active lever had no programmed consequences. The illumination of the

house light signaled the end of the 15 s injection/time-out interval.

Serial Testing Procedures

To facilitate acquisition of cocaine self-administration, animals were maintained on a restricted diet ($\sim$15 g of chow/day) to prevent weight gain and trained to press the active lever for 45-mg sucrose pellets until they reached acquisition criterion (100 correct responses) on 3 successive days. All animals reached criterion in the first overnight session (12 h) and on two subsequent sessions ($<$20 min). Animals were fed *ad libitum* at least 1 day prior to surgical catheterization and allowed to recover for 1 week before the onset of testing. All rats subsequently underwent an identical sequence of serial testing in locomotor and self-administration procedures beginning always at 0800 hours (see Figure 1). On the first 2 days, locomotor pre-tests were conducted with either the D_1 agonist SKF 81297 (((+/−)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide)) or the D_2 agonist quinpirole (((4a*R*-*trans*)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-g] quinoline hydrochloride)) (saline, 0.1, 0.3, 1.0 mg/kg, s.c.) in counterbalanced order in a within-session (6 h) dose–response procedure. In this procedure, rats were habituated to the testing environment for 2 h, and then removed from the locomotor apparatus and given an injection of saline, replaced in the test chamber and locomotor activity was recorded for 1 h. Rats were removed again and injected hourly with ascending doses of either the D_1 or D_2 agonist for the final 3 h of testing, and locomotor activity following each dose was recorded.

Self-administration testing commenced 3 days after the second locomotor pre-test, and rats were allowed to acquire cocaine self-administration (0.5 mg/kg/injection, i.v.) on a fixed-ratio 1: time-out 15 s reinforcement schedule in daily 4 h test sessions for 3 weeks (6 days/week). Animals were maintained at a constant body weight during the first week of cocaine self-administration to facilitate acquisition, but fed *ad libitum* for the remainder of testing. Catheter patency was verified on nonexperimental days by intravenous infusion of the short-acting barbiturate sodium methohexital (0.1 mg/0.1 ml); a positive test was indicated by rapid onset of brief anesthesia. Under these conditions, 40 of 40 rats acquired cocaine self-administration, as indicated by self-administering more than 50 cocaine injections in each of the final 6 days of acquisition training. Another five rats lost catheter patency during training; these rats were excluded from the analysis. Following the 3-week acquisition phase, animals were tested in a between-session self-administration dose–response procedure with decreasing cocaine doses available each day for 5 days (1.0, 0.3, 0.1, 0.03, and 0 mg/kg, i.v.). After determination of self-administration dose–response curves, a second locomotor test was conducted with the D_1 and D_2 agonists identical to the initial test and corresponding to days 2–3 of early cocaine withdrawal (including the final 0 mg/kg dose in self-administration testing).

On days 11–15 of cocaine withdrawal, animals returned to the operant test chambers for extinction testing in the absence of cocaine reinforcement for 4 h/day. Responses at the drug-paired lever were recorded but had no programmed consequence. During the following week (days 18–25 of cocaine withdrawal), the ability of the D_2 agonist quinpirole (saline, 0.3, 1.0, and 3.0 mg/kg, s.c.) to reinstate cocaine-seeking behavior, and the ability of the D_1 agonist SKF 81297 (saline, 1.0 and 3.0 mg/kg, s.c.) to attenuate reinstatement of cocaine seeking induced by a cocaine priming injection (15 mg/kg, i.p.), was assessed under extinction conditions. Reinstatement test sessions were 4 h in duration; the first 3 h served to extinguish residual responding to low levels prior to pharmacological challenge with cocaine or quinpirole (SKF 81297 or saline was given as a pretreatment 30 min prior to cocaine challenge). Both drug-paired and inactive lever responses were recorded during the final 1 h of the test session immediately following challenge with cocaine or quinpirole. The order of dose presentation was counterbalanced for the D_1 and D_2 agonists across test days, except for the high dose of quinpirole that was always tested last due to potential sensitizing influences. After reinstatement testing, a third and final locomotor test was conducted with the D_1 and D_2 agonists over 2 days in late cocaine withdrawal (days 28–29) in a manner identical to earlier tests (the order of drug testing for each animal was maintained throughout the experiment).

A second experiment was conducted under identical serial testing conditions except that the nonselective dopamine receptor agonist apomorphine ((6-methyl-5,6,6a,7-tetrahydro-4*H*-dibenzo[de,g]quinoline-10,11-diol)) (saline, 0.1, 0.3, and 1.0 mg/kg/ml, s.c.) and the non-dopaminergic NMDA glutamate receptor antagonist MK-801 ((5*H*-dibenzo[a,d]cyclohepten-5,10-imine, 10,11-dihydro-5-methyl-(5*S*)-)) (saline, 0.05, 0.10, and 0.15 mg/kg/ml, s.c.) were used as positive controls in locomotor and reinstatement challenge experiments instead of the D_1 and D_2 agonists. In this experiment, 24 out of 25 rats met acquisition criterion, and were included in the final analysis. Both drugs were tested for their locomotor activating effects, whereas only MK-801 was tested in reinstatement since pilot data found that apomorphine was generally ineffective as a reinstating stimulus, consistent with a previous study (De Vries *et al*, 1999).

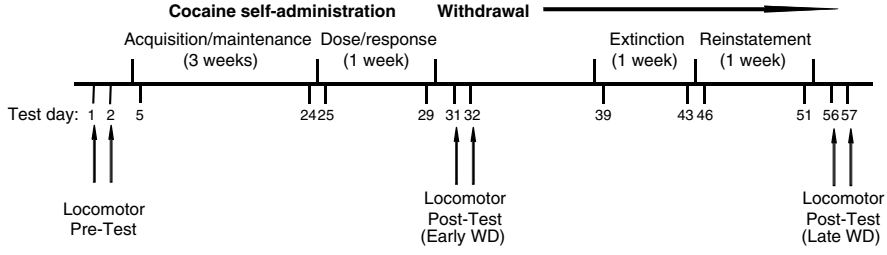

Figure 1 Serial behavioral testing procedure. The first and last day of each test are indicated below in chronological order.

Since D1 agonist challenges were conducted with and without cocaine present in reinstatement and locomotor tests, respectively, we determined whether SKF 81297 would alter the psychomotor profile of cocaine using a dose combination that emulated the highest dose of SKF 81297 tested in reinstatement. Twelve drug-naïve animals were tested in 2 h test sessions consisting of 30 min habituation immediately followed by subcutaneous pretreatment with either saline or SKF 81297 (3.0 mg/kg), and 30 min later animals were challenged with intraperitoneal saline or cocaine (15 mg/kg). Locomotor activity was measured over the last 60 min of the test session, and the four treatment combinations were tested on separate days in counterbalanced order.

Measurement of Brain Cocaine Levels

At 7 days after completion of all behavioral studies, 12 self-administering animals were given an i.v. cocaine challenge (2 mg/kg) and killed by decapitation exactly 10 min later. Whole brains were dissected, rapidly frozen in isopentane, and stored at -80°C until assay. On the day of assay, brains were homogenized in deionized water (1 : 4 by brain weight). In total, 1 ml of the 1 : 4 diluted homogenate (0.25 g tissue) from each brain was assayed as follows: 200 ng of deuterated cocaine was added as an internal standard, and the pH was adjusted to 9.3 with ammonium chloride buffer. Cocaine was extracted into 4 ml of *n*-butyl chloride, and subsequently back extracted into 0.5 ml of 0.1 N sulfuric acid. The pH was adjusted back to 9.3 with ammonium chloride buffer and the cocaine was extracted into 2 ml of *n*-butyl chloride and dried. The residue was reconstituted with 30 µl of *n*-butyl chloride. After calibration with a standard curve, the extracts were quantified on an Agilent 5973N GC-MS in selected ion mode, and results corrected for deuterated cocaine recovery.

Post Hoc Analysis of Low and High Cocaine Intake Subpopulations

Animals in the first ($n = 40$) and second ($n = 24$) experiment were grouped into low and high cocaine intake subgroups determined by the median of average cocaine intake over the final six of 18 test sessions during acquisition training in each experiment. Cumulative locomotor dose–response data for each test drug (not including saline) were analyzed by two-factor ANOVA with repeated measures on test (pre-test, early withdrawal, and late withdrawal). Self-administration and locomotor dose–response curves, active/inactive lever response ratios, and extinction and reinstatement data were analyzed separately by two- or three-factor ANOVA with repeated measures on dose/test session and lever (D_1 and D_2 receptor challenge in reinstatement were analyzed separately). Normalized reinstatement data were analyzed by nonparametric Kruskal–Wallis tests. Significant interactive effects were followed by tests for simple effects between low and high subgroups at each dose/test session. Locomotor activity totals for saline, SKF 81297, cocaine, and combined treatment were analyzed by one-factor ANOVA with repeated measures followed by Tukey's pairwise comparisons. Brain cocaine levels and extinction latencies were compared by Student's *t*-tests.

Drugs

SKF 81297, quinpirole, apomorphine, and MK-801 were purchased from Sigma (St Louis, MO). All drugs were dissolved in a physiological saline vehicle (2 mg/ml). Ascorbic acid was added to apomorphine test vehicles (0.1%) to prevent oxidation of the drug. Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC), and was dissolved in sterile-filtered physiological saline for i.v. self-administration.

RESULTS

Animals self-administering cocaine (0.5 mg/kg/injection in 18 daily 4-h sessions) were divided into low and high intake groups ($n = 20$/group) based on a median split of the average cocaine intake for their last 6 days of self-administration training (Figure 2a). Low and high intake groups averaged 42.5 ± 2.1 and 54.7 ± 3.6 mg/kg/day of cocaine intake, respectively, in days 13–18 of training. Self-administration data in this acquisition phase were not

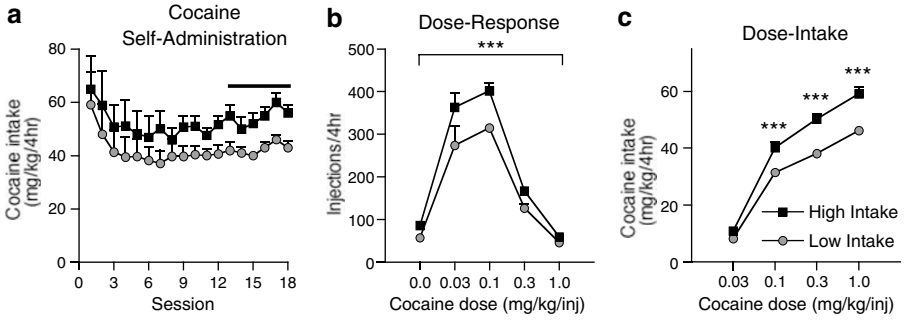

Figure 2 Cocaine self-administration in 40 outbred Sprague–Dawley rats. (a) Low and high intake groups ($n = 20$/group) were determined by the median of average daily cocaine self-administration (0.5 mg/kg/injection) during the last 6 of 18 4-h training sessions (solid line). (b) High intake animals exhibit an upward shift in peak self-administration rates, and a rightward shift in the descending limb of the self-administration dose–response curve. (c) Cocaine self-administration data converted to dose-intake curves shows that high intake animals take greater daily amounts of cocaine at doses on the descending limb of the dose–response curve shown in panel (b). Data reflect the mean ± SEM for low and high intake groups in each test session. Asterisks indicate (b) a main effect of group by ANOVA, or (c) that high differ from low intake animals by tests for simple effects following significant group by test session interaction (***$p < 0.001$).

statistically analyzed since they reflect the independent variable used to define study groups. There were no significant differences in daily active/inactive lever response ratios between low and high intake groups, and both groups effectively discriminated reinforced responding throughout training (not shown). Cocaine self-administration produces an inverted U-shaped dose–response curve on low fixed-ratio schedules, spanning dose thresholds for maintaining self-administration, and a descending limb where increasing the injection dose prolongs the duration of cocaine effects, resulting in fewer self-injections over time. Cocaine self-administration dose–response curves were determined across five test sessions (Figure 2b). High intake animals exhibited a vertical shift in peak self-administration rates when compared to the low intake group (main effect of group, $F_{1,38} = 15.775$, $p < 0.001$), indicative of greater cocaine reinforcement in these animals, and consistent with phenotypic differences observed in a previous study (Piazza *et al*, 2000). High intake animals also self-administered substantially greater amounts of cocaine on the descending limb of the curve (main effect across four doses, $F_{1,38} = 42.118$, $p < 0.001$), an effect that is more apparent when dose–response data are transformed into dose-intake data based on the total amount of cocaine consumed in the 4 h test sessions (Figure 2c). This difference in preferred levels of cocaine intake was exacerbated by increasing the injection dose available for self-administration (group × dose interaction, $F_{3,114} = 7.076$, $p < 0.001$).

Following an 11-day withdrawal period, animals returned to the self-administration test chambers, and resistance to extinction of cocaine-seeking behavior was determined by the number of nonreinforced drug-paired lever responses recorded in daily 4-h test sessions for 5 days. Figure 3a shows that high intake animals engaged in greater drug-paired lever responding than low intake animals (main effect of group, $F_{1,38} = 8.368$, $p = 0.006$), but responding declined to equivalent levels by the final extinction test session (group × test day interaction, $F_{4,152} = 9.784$, $p < 0.001$). Thus, after a period of withdrawal, cocaine seeking elicited by initial exposure to the cocaine-paired environmental context was two-fold greater in high than in low intake animals. Moreover, the latency to extinguish self-administration behavior, defined as the number of test sessions required to achieve <60 lever presses in 4 h, was also significantly longer in the high than in low intake groups ($p = 0.039$). Taken together, high intake animals exhibited differences in cocaine self-administration and cocaine-seeking behavior that are consistent with addiction-related changes reported in previous studies.

Given that systemic administration of D_1 and D_2 receptor agonists differentially modulates cocaine-seeking behavior in reinstatement tests, we determined whether the ability of D_1 and D_2 agonists to modulate this behavior would differ in low and high intake groups. Figure 4a shows that high intake animals were less sensitive, or refractory, to the ability of the full efficacy D_1 agonist SKF 81297 to attenuate drug-paired lever responding elicited by a cocaine priming injection in reinstatement tests that were conducted from days 18 to 23 of withdrawal from cocaine self-administration (main effect of group, $F_{1,38} = 5.717$, $p = 0.022$; group × lever interaction, $F_{1,38} = 7.982$, $p = 0.007$). Although D_1 agonist treatment dose-dependently attenuated cocaine-induced cocaine-seeking behavior in both groups (main

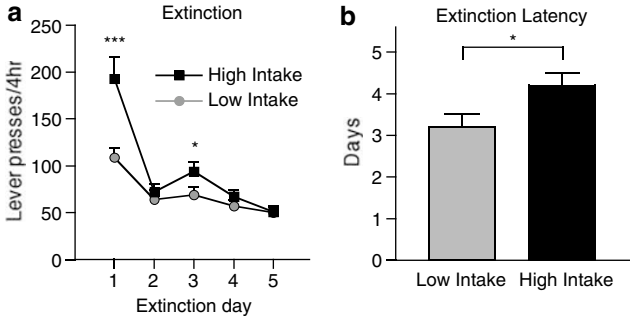

Figure 3 Extinction of cocaine self-administration in low and high intake animals. (a) High intake animals exhibit two-fold greater nonreinforced, drug-paired lever responses during the initial 4-h extinction test when compared to low intake animals. (b) High intake animals also show greater resistance to extinction of cocaine self-administration compared to low intake animals, achieving extinction criteria (<60 drug-paired lever responses/session) with longer latencies. Data reflect the mean ± SEM for low and high intake groups ($n = 20$ group). Asterisks indicate that high differ from low intake animals by tests for simple effects following significant group by test session interaction or by *t*-test for extinction latencies (*$p < 0.05$, ***$p < 0.001$).

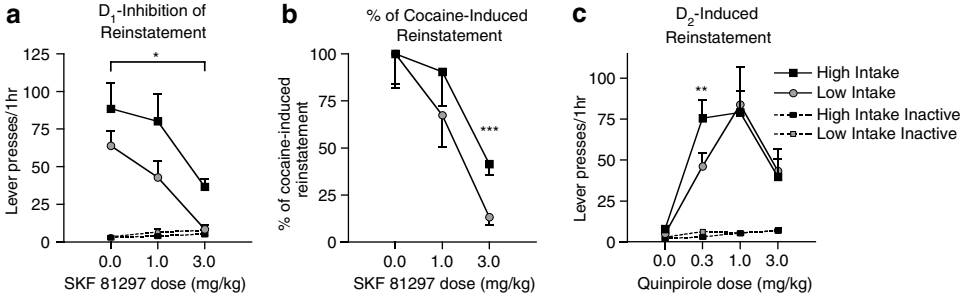

Figure 4 Differential sensitivity to opposite modulation of cocaine-seeking behavior by D_1 and D_2 receptor agonists in low and high intake animals. (a) Reduced ability of the D_1 receptor agonist SKF 81297 (given as a 30 min pretreatment) to inhibit cocaine seeking elicited by an i.p. priming injection of cocaine (15 mg/kg) in high relative to low intake animals ($n = 20$/group). (b) Resistance to D_1-mediated inhibition of reinstatement in high intake animals after normalization of data shown in (a) to individual group baselines (100%) for cocaine-primed reinstatement (saline pretreatment). (c) Conversely, high intake animals are more sensitive to the ability of the D_2 receptor agonist quinpirole to induce cocaine-seeking behavior. Data reflect the mean ± SEM for low and high intake groups responding at the drug-paired (solid line) and inactive levers (dotted lines). Asterisks indicate (a) *$p < 0.05$, group effect; (b) ***$p < 0.001$ by nonparametric Mann–Whitney *U*-test; (c) **$p < 0.01$ by test for simple effects.

effect of dose, $F_{2,76} = 13.113$, $p < 0.001$), high intake animals showed a trend for greater cocaine seeking in the absence of D_1 agonist treatment (control condition), and so the attenuation of cocaine seeking by D_1 agonist treatments was normalized to cocaine alone (100%). Despite normalization to mean within-group baselines, high intake animals displayed subsensitivity to D_1 receptor-mediated inhibition of reinstatement at the highest dose tested (Figure 4b, $U1 = 343$, $p < 0.001$). In contrast, this dose of SKF 81297 failed to alter the temporal profile or total amount of cocaine-induced horizontal locomotor activity compared to cocaine alone when a similar SKF 81297/cocaine combination was tested in separate animals (Figure 5), suggesting that inhibition of reinstatement occurs without psychomotor impairment. However, when given alone, the 3.0 mg/kg dose of SKF 81297 induced less locomotor activity than lower doses (see below), reflecting an inverted U-shaped dose–response function on locomotor activity.

Figure 4c shows that treatment with the full efficacy D_2 receptor agonist quinpirole dose-dependently reinstated cocaine seeking, resulting in an inverted U-shaped dose–response curve at the drug-paired lever (dose × lever interaction, $F_{3,114} = 21.367$, $p < 0.001$). In contrast to D_1 receptor-mediated attenuation of cocaine seeking, high intake animals were more sensitive to reinstatement of cocaine seeking induced by a low dose of quinpirole compared to the low intake group (dose × group interaction on ascending limb, $F_{1,38} = 7.208$, $p = 0.011$), although there were no differences in response to priming with the saline vehicle. Thus, high intake animals exhibit opposing alterations in sensitivity to D_1 and D_2 receptor regulation of cocaine-seeking behavior when compared to low intake animals. However, neither the maximal reinstating efficacy of quinpirole, or responding on the descending limb of the curve differed between the two groups. An even lower dose of quinpirole (0.1 mg/kg) was tested in a small number of animals ($n = 4$/group), and no differences in drug-paired lever responding between low intake (25.5 ± 5.9) and high intake (21.8 ± 3.9) groups were found. Table 1 shows that mean drug-paired lever responding in the 1 h period preceding each reinstatement test failed to differ, ranging from 2.8 ± 1.4 to 6.4 ± 1.5 responses for low intake animals, and from 4.3 ± 1.3 to 7.7 ± 2.4 for high intake animals. Inactive lever responding also failed to differ between low and high intake animals, with responding ranging from 2.1 ± 0.8 to 8.1 ± 1.9 lever presses in low intake animals, and 2.6 ± 0.9 to 5.7 ± 2.1 in high intake animals, across all treatment conditions (Figure 4).

Reinstatement of preconditioned behaviors cannot be determined prior to conditioning (self-administration), and so we utilized the unconditioned psychomotor response to dopamine receptor challenge to track the development of changes in sensitivity to D_1 or D_2 receptor stimulation from before the onset of cocaine self-administration to early and

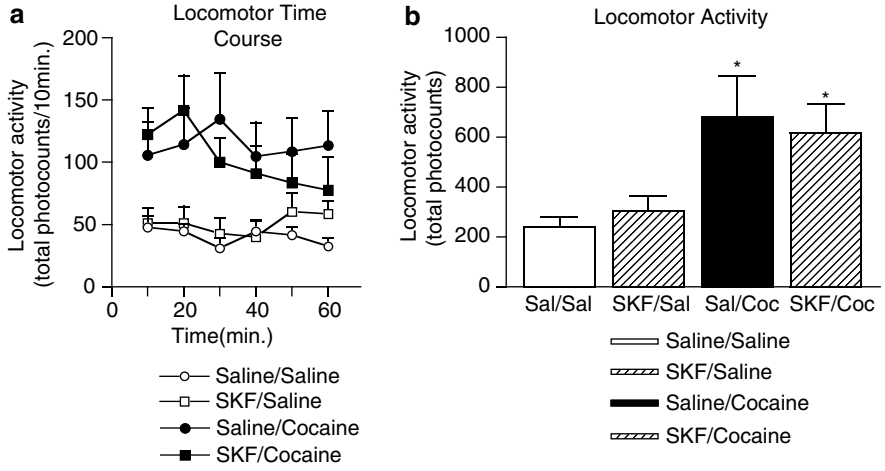

Figure 5 Pretreatment with the 3.0 mg/kg dose of SKF 81297 fails to alter cocaine-induced locomotor activity (15 mg/kg, i.p.) when coadministered in drug-naive animals similar to reinstatement tests shown in Figure 4. (a) Time course showing similar elevations in locomotor activity following cocaine challenge in saline- and SKF 81297-treated groups compared with saline challenge in a 1 h test. (b) Total cocaine-induced locomotor activity fails to differ between saline- and SKF 81297-treated groups over the 1 h test. This dose of SKF 81297 fails to increase horizontal locomotion when given alone reflecting an inverted U-shaped dose–response for SKF 81297 (compare with lower doses shown in Figure 6). Asterisks indicate $^*p < 0.05$ by Tukey's pairwise comparison with saline/saline treatment following one-factor ANOVA ($F_{3,33} = 5.119$, $p < 0.005$).

Table 1 Baseline Drug-Paired Lever Responses Prior to Reinstatement Tests

			Reinstatement stimulus				
Subgroup	**Saline**	**Cocaine**	**Cocaine +D1 (low)**	**Cocaine +D1 (high)**	**D2 (low)**	**D2 (mid)**	**D2 (high)**
High intake group	4.4 (1.9)	5.1 (0.9)	6.7 (1.5)	4.3 (1.3)	4.8 (1.3)	7.7 (2.4)	5.9 (1.9)
Low intake group	2.8 (1.4)	3.8 (1.1)	6.4 (1.5)	4.1 (1.3)	4.9 (1.5)	5.3 (1.1)	6.1 (3.4)

Standard error of means in parentheses.

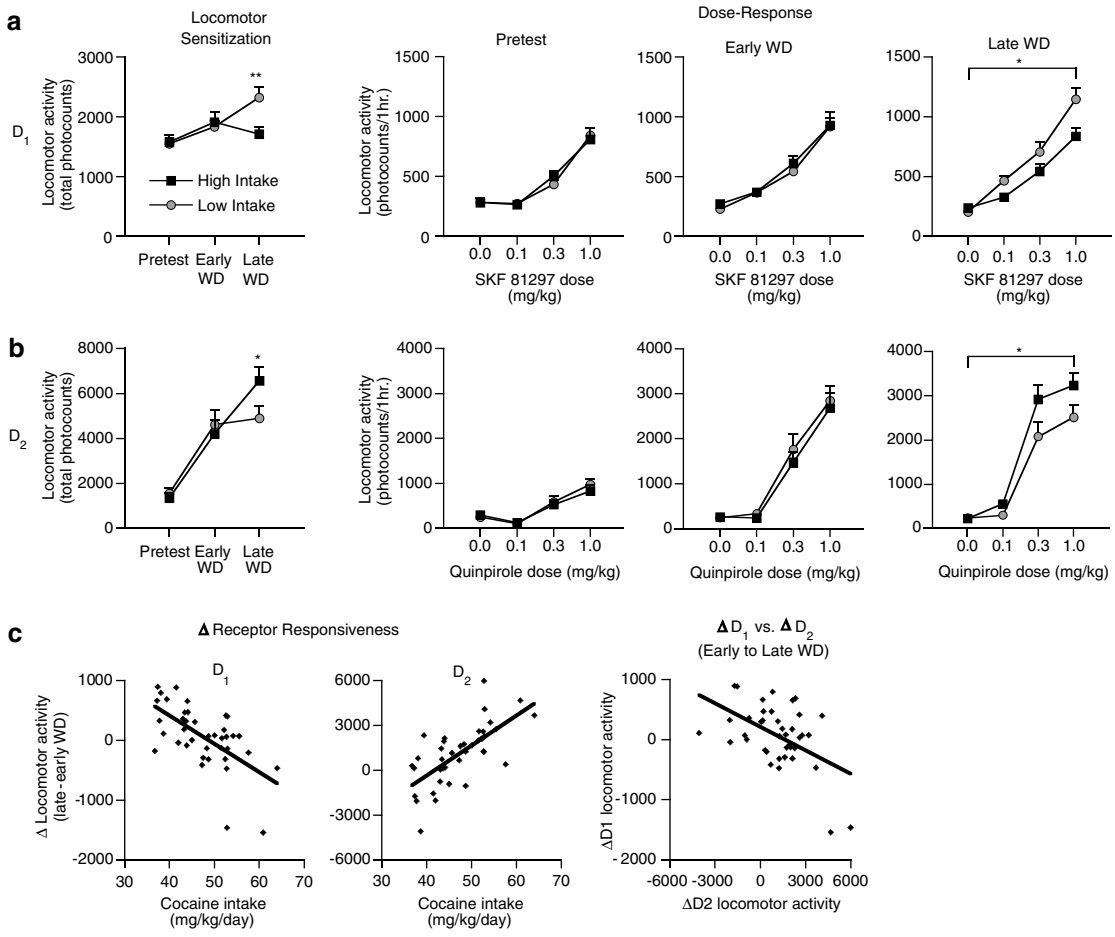

Figure 6 Differential development of D_1 and D_2 receptor sensitization in low and high intake animals during withdrawal from chronic cocaine self-administration. (a) Low intake animals develop D_1 receptor sensitization from early to late withdrawal, (b) whereas high intake animals develop greater D_2 receptor sensitization ($n = 20$ group). Data in left panels show changes in cumulative locomotor responses for dose–response tests (0.1–1.0 mg/kg) with the D_1 agonist SKF 81297 and the D_2 agonist quinpirole conducted before self-administration (pretest) and at early (2–3 days) and late (28–29 days) withdrawal (WD) times. (right panels) Locomotor dose–response functions are similar in low and high intake animals challenged with the D_1 and D_2 agonists prior to and early after cocaine self-administration, but differences emerge at the late withdrawal time. (c) Average daily cocaine intake during the last 6 days of acquisition training is negatively correlated with the change (Δ) in D_1 responsiveness from early to late withdrawal ($r = -0.507$, $p = 0.001$), but positively correlated with the change in D_2 responsiveness ($r = 0.686$, $p < 0.001$). Individual locomotor data reflect difference scores (late–early WD) for cumulative locomotor responses in dose–response tests. (right panel) The development of D_1 sensitization is negatively correlated with the development of D_2 sensitization from early to late withdrawal ($r = -0.412$, $p = 0.001$). Asterisks indicate that high differ from low intake animals by main group effect or tests for simple effects following significant group × time interaction (*$p < 0.05$, **$p < 0.01$).

late withdrawal times. In addition, since responses to the D_1 agonist were conducted in the presence of cocaine in reinstatement tests, it was important to study responses to D_1 receptor stimulation alone. Figure 6a and b (left panels) show cumulative horizontal locomotor responses to D_1 and D_2 receptor challenge in a within-session dose–response determination conducted before and after cocaine self-administration. There was no difference in D_1 and D_2 receptor-mediated locomotor responses between low and high intake groups before cocaine self-administration, and in early cocaine withdrawal (2–3 days), but a significant divergence in D_1 and D_2 receptor responsiveness emerged at the late withdrawal time (28–29 days). Thus, neither group developed significant sensitization to D_1 receptor challenge early after withdrawal from chronic cocaine self-administration, but low intake animals ultimately developed ~50% sensitization to D_1 receptor challenge at late cocaine withdrawal (Figure 6a, left panel), and showed a significantly greater response than high intake animals that failed to sensitize to D_1 challenge (group × time interaction, $F_{2,76} = 8.026$, $p = 0.001$). Conversely, although both groups developed a profound D_2 receptor sensitization (~400%) when challenged early after cocaine self-administration, this D_2 receptor sensitization intensified from early to late withdrawal only in high, but not in low, intake animals (Figure 6b, left panel), resulting in a six-fold increase when compared to pre-self-administration baselines (group × time interaction, $F_{2,76} = 6.670$, $p = 0.002$).

Similarly, the locomotor dose–response curves for D_1 and D_2 agonists in low and high intake groups show no differences when challenged before and early after cocaine self-administration, but opposite changes in D1 and D2 receptor responsiveness are reflected in the dose–response curves obtained at the late withdrawal time (Figure 6a and b, right panels). Thus, while neither group differed in their locomotor response to saline, low intake animals were more

responsive to all doses of the D_1 agonist SKF 81297 compared to high intake animals (main effect of group, $F_{1,38} = 6.631$, $p = 0.014$). Conversely, high intake animals were more responsive to all doses of the D_2 agonist quinpirole compared to low intake animals (main effect of group, $F_{1,38} = 5.599$, $p = 0.023$). Thus, differential sensitization in D_1 and D_2 receptor responses developed in low and high intake rats as a consequence of cocaine self-administration, and these changes paralleled differences found in reinstatement tests.

We also compared a series of behavioral measures reflecting these differences by linear regression analysis of continuous data in the entire population of 40 self-administering rats (Table 2). Individual levels of cocaine intake during the final 6 days of self-administration training were negatively correlated with the change (Δ) in D_1-stimulated locomotion from early to late withdrawal ($r = -0.507$, $p = 0.001$), but positively correlated with the change in D_2-stimulated locomotion ($r = 0.686$, $p < 0.001$), and these relationships are illustrated graphically in Figure 6c. As shown in the right panel, the capacity of animals to develop locomotor sensitization to the D_1 agonist from early to late withdrawal was negatively correlated with their capacity to develop further sensitization to the D_2 agonist ($r = -0.412$, $p < 0.01$), indicating that the two phenomena may be mutually exclusive. Resistance to D_1-mediated inhibition of cocaine seeking (high dose) in reinstatement tests also correlated positively with levels of prior cocaine intake (Table 2), consistent with differences exhibited by low and high intake groups. Similarly, resistance to D_1-mediated inhibition of cocaine seeking (high dose) correlated positively with D_2-mediated reinstatement of cocaine seeking (low dose), consistent with the differential capacity to develop D_1 or D_2 locomotor sensitization when behavioral responses were directionally similar. Other differences between low and high intake groups, such as reinstatement induced by the D_2 agonist (low dose), failed to correlate significantly with cocaine intake, possibly due to greater within-subject variance in these behavioral measures.

To determine whether preferred levels of cocaine intake would relate to responses produced by simultaneous D_1/D_2 dopamine receptor activation, or whether differences would generalize to similar behavior induced by non-dopaminergic pharmacological challenge, separate groups of low and high intake animals ($n = 12$/group) were tested with the mixed D_1/D_2 dopamine receptor agonist apomorphine and the NMDA glutamate receptor antagonist MK-801 in locomotor and reinstatement tests. High intake animals in this experiment exhibited similar upward shifts in self-administration dose–response (main effect of group, $F_{1,22} = 24.150$, $p < 0.001$) and dose-intake (main effect of group, $F_{1,22} = 36.709$, $p < 0.001$) curves, along with greater drug-paired lever responding in the initial extinction test session (group $\times$ test day interaction, $F_{4,88} = 5.719$, $p < 0.001$) and a longer latency to extinguish self-administration behavior ($p = 0.025$) when compared to low intake animals. This replication of earlier results is summarized in Table 3. However, there were no significant group differences in the ability of the NMDA antagonist MK-801 to reinstate cocaine seeking, even at a low dose where sensitivity to the D_2 agonist quinpirole differed (Figure 7). In addition, MK-801-mediated locomotor responses failed to differ between low and high intake groups, despite a profound sensitization to MK-801 following chronic cocaine self-administration, similar to the degree of sensitization to D_2 agonist challenge. There were also no group differences in the locomotor response to the mixed D_1/D_2 agonist apomorphine, and no evidence for sensitization based on cumulative dose–response curves compared to pre-self-administration baselines (not shown).

Brain cocaine levels were determined 10 min following an intravenous cocaine injection (2 mg/kg) in 12 rats that completed the second experiment. At this time, about 50% of cocaine is metabolized based on a previous study in rats (Piazza *et al*, 2000). Low and high intake groups ($n = 6$/group) for this determination averaged 43.7 ± 3.3 and 59.5 ± 4.1 mg/kg/day of cocaine intake, respectively, over the final 6 days of self-administration training. Figure 8 shows that brain cocaine levels were indistinguishable in low and high intake groups, averaging about 4 μg/g of brain tissue. These results indicate that preferred levels of cocaine intake do not originate from individual differences in cocaine metabolism or other factors influencing brain cocaine concentrations.

DISCUSSION

The study shows that within an outbred rat population, animals with higher preferred (self-regulated) levels of cocaine intake become less sensitive to D_1, but more sensitive to D_2, receptor-mediated responses when compared to animals with lower preferred levels of cocaine intake, and after an extended period of withdrawal from chronic cocaine self-administration. High intake animals also exhibited an increase in peak self-administration rates, and greater cocaine intake when self-administering higher doses on the descending limb of the dose–response curve. Increases in peak self-administration rates are thought to reflect enhanced motivation for cocaine (reinforcing efficacy), a view supported by the fact that high intake animals exert greater effort to maintain self-administration when response requirements are increased (Piazza *et al*, 2000). In contrast, increased self-administration when higher doses of cocaine are freely available reflects a reduction in inhibitory feedback regulation of drug intake, because reducing the unit dose/injection on the descending limb produces a similar increase in self-administration rates. Similar phenotypic differences in the dose–response for cocaine self-administration are produced by prolonging daily access to the drug, as animals are thought to transit from nonaddicted to addicted states (Ahmed and Koob, 1998). These differences cannot be explained by differential metabolism or bioavailability of cocaine in the present study, since brain cocaine levels were identical 10 min after cocaine challenge. In addition, high intake animals exhibited greater cocaine-seeking behavior and resistance to extinction when reinforcement was withheld. These findings support the relationship between higher cocaine intake and a propensity for drug- and cue-induced relapse to cocaine seeking using reinstatement procedures in other studies (Sutton *et al*, 2000; Deroche-Gamonet *et al*, 2004), and suggest that animals with higher preferred levels of cocaine

Table 2 Correlation of Individual Self-Administration, Extinction, Reinstatement and Locomotor Responses

						Reinstatement stimulus			Locomotor sensitization		
Variable	**Intake average**	**Cocaine SA (1.0 mg/kg)**	**Cocaine SA (0.1 mg/kg)**	**Extinction day 1**	**Extinction latency**	**Cocaine RSTMT**	**Cocaine+D1 (low)**	**Cocaine+D1 (high)**	**D2 RSTMT (low)**	**ΔD1 (late–early WD)**	**ΔD2 (late–early WD)**
Intake average	—	**0.807*** **	**0.635*** **	**0.450** **	0.172	0.163	0.232	**0.559*** **	0.154	**−0.507*** **	**0.686*** **
Cocaine SA—1.0 mg/kg	—	—	**0.556*** **	0.282	0.128	0.247	0.250	**0.415** **	0.228	**−0.420** **	**0.677*** **
Cocaine SA—0.1 mg/kg	—	—	—	**0.441** **	0.067	**0.341***	**0.557*** **	**0.571*** **	0.379	−0.301	**0.420***
Extinction day 1	—	—	—	—	**0.532*** **	**0.371***	**0.377***	**0.346***	0.204	**−0.325***	0.389*
Extinction latency	—	—	—	—	—	0.074	0.170	0.098	**0.387***	−0.148	0.183
Cocaine RSTMT	—	—	—	—	—	—	0.304	0.149	0.316	−0.059	0.159
Cocaine RSTMT + D1 (low)	—	—	—	—	—	—	—	**0.399***	0.216	−0.098	0.176
Cocaine RSTMT + D1 (high)	—	—	—	—	—	—	—	—	**0.441***	−0.282	**0.436** **
D2 RSTMT (low)	—	—	—	—	—	—	—	—	—	−0.045	0.194
ΔD1 (late-early WD)	—	—	—	—	—	—	—	—	—	—	**−0.412** **
ΔD2 (late-early WD)	—	—	—	—	—	—	—	—	—	—	—

Correlation coefficients (Pearson's Product Moment) for comparisons of 11 test variables. Correlation coefficients with p-values <0.05 are indicated in bold ($n=40$ rats).
Asterisks indicate $*p \leqslant 0.05$, $**p \leqslant 0.01$, and $***p \leqslant 0.001$.
Abbreviations: SA, self-administration; RSTMT, reinstatement stimulus; WD, cocaine withdrawal.

Table 3 Cocaine Self-Administration and Extinction Responses for MK-801 Experiment

	Intake (mg/kg/day)	Cocaine SA dose–response (injections/4 h)			Extinction response (lever presses/4 h)		
Subgroup	**Average last 6 days**	**1.0 mg/kg**	**0.1 mg/kg**	**0.0 mg/kg**	**Test day 1**	**Test day 5**	**Extinction latency days**
High intake group	58.8 (4.8)	62.4 (2.9)	429.1 (23.8)	76.6 (8.2)	224.3 (26.0)	55.4 (10.7)	4.3 (0.5)
Low intake group	44.8 (3.5)	48.8 (2.1)	324.0 (18.4)	52.1 (8.9)	132.1 (15.6)	57.9 (11.2)	2.8 (0.6)

Standard error of means in parentheses.

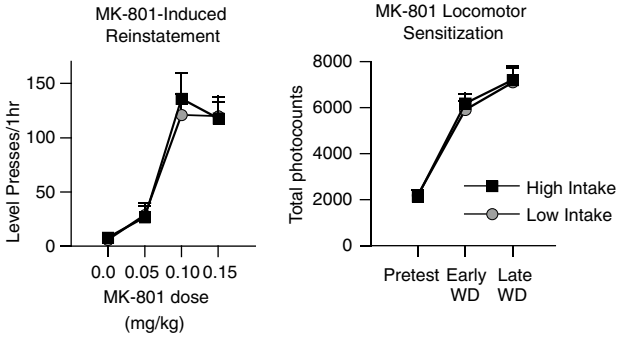

Figure 7 Lack of differential responsiveness in low and high intake animals following challenge with the non-dopaminergic NMDA glutamate receptor antagonist MK-801 in locomotor sensitization and reinstatement tests ($n = 12$ group). Low intake and high intake animals in this experiment exhibited differences in cocaine self-administration dose–response, dose intake, and extinction responding similar to the previous experiment (Table 3).

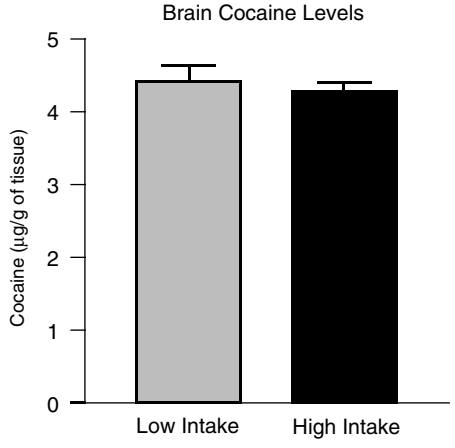

Figure 8 Brain cocaine levels are similar in low and high intake animals. Brain tissue was collected 10 min after a 2 mg/kg intravenous cocaine injection and cocaine levels were analyzed by gas chromatography/mass spectrophotometry methods. Low and high intake groups ($n = 6$/group) averaged 43.7 ± 3.3 and 59.5 ± 4.1 mg/kg/day of cocaine intake, respectively, over the final 6 days of self-administration training.

intake encompass changes in drug-taking and -seeking behaviors indicative of a cocaine-addicted phenotype (Ahmed and Koob, 1998; Piazza *et al*, 2000; Sutton *et al*, 2000; Deroche-Gamonet *et al*, 2004; Self, 2004).

There were no differences in the locomotor response to D_1 and D_2 receptor challenge before the onset of cocaine self-administration or initially after cocaine withdrawal, indicating that differential sensitivity in these postsynaptic responses was not due to pre-existing individual differences, but emerged as a consequence of chronic cocaine self-administration and longer withdrawal times. Differential sensitivity to D_1 and D_2 receptor regulation of cocaine seeking paralleled changes in locomotor responsiveness after 4 weeks of withdrawal, despite the fact that D_1 and D_2 receptors mediate opposing effects on cocaine-seeking behavior (with and without cocaine priming, respectively), but directionally similar effects on locomotor behavior in the absence of cocaine. Thus, high intake animals were less sensitive to the ability of D_1 receptor agonist SKF 81297 to inhibit cocaine seeking induced by a cocaine priming injection, and sensitivity to this response also was negatively correlated with cocaine intake across all animals. This difference probably reflects the development of sensitization to postsynaptic D_1 responses in low intake animals, rather than tolerance in high intake animals, since low intake animals developed sensitized locomotor responses relative to pre-self-administration baselines in the absence of cocaine. Indeed, the emergence of D_1 receptor-mediated locomotor sensitization in withdrawal was negatively correlated with prior cocaine intake during self-administration. Therefore, D_1 receptor sensitization could reflect a neuroadaptation to cocaine that protects against a propensity for relapse in withdrawal. Furthermore, it is unlikely that inhibition of cocaine seeking by the D_1 agonist is related to interfering stereotypies, since it blocked cocaine seeking at a dose that failed to affect horizontal locomotor responses to cocaine. In addition, a previous study showed that an identical combined dose of cocaine and SKF 81297 attenuated cocaine seeking without altering stereotypic behaviors that normally occur during cocaine-induced reinstatement (Alleweireldt *et al*, 2003).

Changes in D_1 receptor sensitivity are probably not caused by D_1 receptor upregulation, since other studies have found a downregulation of striatal D_1 receptor binding and D_1-stimulated adenylate cyclase activity in the nucleus accumbens of rats and non-human primates following chronic cocaine self-administration (Graziella De Montis *et al*, 1998; Moore *et al*, 1998a). Similarly, escalating cocaine intake induced by prolonged daily access has been associated with a decreased capacity for rate-increasing effects of a mixed D_1/D_2 dopamine receptor antagonist, and increased sensitivity to the rate suppressing effects of higher doses, suggesting a decrease in the amount of dopamine receptors available for blockade (Ahmed and Koob, 2004). Thus, in both individual difference and extended access models of cocaine-addicted rats, animals with higher preferred levels of cocaine intake exhibit evidence for

reduced dopamine receptor function when compared to low intake animals. Our results suggest that this effect may be attributable entirely to lower D_1 receptor function, since both low and high intake animals developed a profound sensitization to D_2 receptor-mediated responses after chronic cocaine self-administration, although D_2 responses have not been studied in escalation models. Moreover, while lower D_1 receptor responsiveness could underlie higher cocaine intake in animals with prolonged daily access, this effect cannot account for increased cocaine self-administration based on individual differences in our study because D_1 receptor subsensitivity in high intake animals was not evident until later in cocaine withdrawal.

However, other studies have reported increases in D_1 receptor binding in several striatal regions 1 day following a shorter period of cocaine self-administration in monkeys (Nader *et al*, 2002), and functional increases in D_1 receptor-mediated electrophysiological responses in nucleus accumbens neurons that develop several days after withdrawal from passive cocaine administration in rats (Henry and White, 1991). This latter finding could relate to sensitization of D_1-mediated behavioral responses in low intake animals, since this effect also required a longer withdrawal period to develop. In addition, levels of cocaine exposure that produce supersensitive D_1-mediated physiological responses in nucleus accumbens neurons are more similar to levels in low than high intake animals in our study. However, it should be noted that nucleus accumbens infusions of D_1 agonists induce rather than inhibit reinstatement of cocaine seeking, suggesting that widespread D_1 receptor activation in multiple or other brain regions is required for satiation of cocaine seeking by systemic D_1 agonist administration (Bachtell *et al*, 2005).

In contrast to D_1 agonists that produce only minor reinstating effects when given alone, and inhibit cocaine- and cue-induced relapse to cocaine-seeking behavior, systemic administration of D_2 agonists strongly induce relapse to cocaine seeking (Wise *et al*, 1990; Self *et al*, 1996; De Vries *et al*, 1999; Alleweireldt *et al*, 2002, 2003; Dias *et al*, 2004). We found that high intake animals were more sensitive to the ability of D_2 receptor stimulation with quinpirole to trigger cocaine-seeking responses at the drug-paired lever. This effect was paralleled by enhanced locomotor responses to D_2 receptor stimulation in late withdrawal, despite the fact that low intake animals also developed substantial locomotor sensitization to D_2 receptor challenge. The development of further sensitization to D_2 receptor responses from early to late withdrawal was positively correlated with higher preferred levels of cocaine intake across all animals, and negatively correlated with the capacity to develop sensitization to D_1 receptor challenge. Thus, the capacity to develop D_1 receptor sensitization within individual rats could occlude the capacity to develop further D_2 sensitization. It is important to note that locomotor testing was conducted in an environment distinct from the cocaine self-administration test chambers, and so changes in D_1 and D_2 receptor responsiveness occurred in the absence of cocaine-conditioned locomotor effects. However, the negative correlation between D_1 and D_2 receptor sensitization in locomotor tests was mirrored by a positive correlation between resistance to D_1-mediated attenuation of cocaine seeking and sensitivity to D_2-mediated reinstatement in the cocaine-associated environment. Together, these findings suggest that differential sensitivity to D_1 and D_2 receptor regulation of cocaine seeking in high intake rats is not due to an indirect influence of one receptor on the response mediated by the other in reinstatement tests. Furthermore, while it is possible that drug challenges in reinstatement testing influenced locomotor responses in late withdrawal, all animals were exposed to identical testing conditions, and this caveat cannot account for differential sensitivity to D_1 and D_2 receptor challenge in reinstatement.

Although cocaine self-administration produced functional increases in postsynaptic D_2 receptor responses, several studies have found reduced D_2 receptor binding in striatal and other brain regions following chronic psychostimulant use in monkeys and humans (Volkow *et al*, 1990, 1993, 2001; Moore *et al*, 1998b; Nader *et al*, 2002; Martinez *et al*, 2004), and binge cocaine administration in rats (Maggos *et al*, 1998). Given that prominent D_2 receptor sensitization develops in both low and high intake groups, and this overall effect was found in a previous study comparing cocaine to saline self-administration (De Vries *et al*, 2002), it is possible that D_2 receptor sensitization reflects intracellular alterations in D_2 receptor coupling, or an indirect enhancement via perturbations in neural input to D_2-containing neurons, rather than an increase in D_2 receptor surface expression. Further investigation is needed to determine whether these changes are reflected in differential sensitivity to dopamine receptor-regulated biochemical or electrophysiological responses.

In a separate experiment, low and high intake animals were challenged with the mixed D_1/D_2 receptor agonist apomorphine and the noncompetitive NMDA glutamate receptor antagonist MK-801 in identical locomotor and reinstatement (MK-801 only) tests. The psychomotor and reinstating effects of MK-801 are thought to occur independent of dopamine release and dopamine receptor activation (Druhan *et al*, 1996; Wise *et al*, 1996; De Vries *et al*, 1998; Chartoff *et al*, 2005). Similar to D_2 receptor challenge, animals in both low and high intake groups displayed robust locomotor sensitization to MK-801 challenge following chronic cocaine self-administration. However, neither group differed in this response over the course of testing, nor were there any differences in the ability of the NMDA antagonist to reinstate cocaine-seeking behavior, despite similar phenotypic differences in cocaine self-administration and extinction responding in low and high intake animals. These findings indicate that differential sensitivity to D_2 receptor challenge in low and high intake groups is not due to generalized changes in mechanisms regulating sensitization and reinstatement, but instead reflect a specific enhancement of D_2 receptor responsiveness in high intake animals.

Furthermore, there was virtually no sensitization to generalized dopamine receptor activation with the mixed D_1/D_2 receptor agonist apomorphine following chronic cocaine self-administration, similar to D_1 receptor challenge, but also no emergent differences in sensitivity between low and high intake groups in withdrawal. Thus, differential alterations in postsynaptic D_1 and D_2 receptor responsiveness are revealed only when each receptor is stimulated independently, possibly due to competing D_1

and D_2 receptor effects in low and high intake animals. Given that high intake animals tended to show greater cocaine-induced reinstatement of cocaine seeking, the lack of difference with apomorphine challenge may be related to direct rather than indirect mechanisms of dopamine receptor stimulation. In addition, these apomorphine data agree with a previous study that found that the ability of drugs to reinstate cocaine-seeking behavior is related to their ability to cross-sensitize with cocaine (De Vries *et al*, 1999). However, as in the case of MK-801, our results suggest that the degree of cross-sensitization and reinstatement is not necessarily related to addicted phenotypes with non-dopaminergic ligands.

Addiction-related changes in cocaine self-administration dose–response curves may reflect increased motivation for drugs (incentive sensitization), but reduced pharmacological impact of drugs on reward processes (tolerance) leading to compensatory increases in drug intake (Emmett-Oglesby *et al*, 1993; Piazza *et al*, 2000). Our results suggest that such changes, whether due to higher cocaine exposure or an inherent disposition, could result in enhancing D_2 and reducing D_1 receptor responsiveness, respectively. Furthermore, the emergence of these features from early to late withdrawal parallels time-dependent increases in cocaine-seeking behaviors that have been shown to persist from weeks to months following chronic cocaine self-administration (Tran-Nguyen *et al*, 1998; Grimm *et al*, 2001). The further intensification of increased D_2 receptor function is particularly troublesome, since it could exacerbate the behavioral response to conditioned stimuli (cues) and stressful situations that activate dopamine release in rats and humans (Rouge-Pont *et al*, 1998; Phillips *et al*, 2003; Pruessner *et al*, 2004), and, thus, facilitate relapse to cocaine use despite efforts to abstain. While our results suggest that reduced D_1 receptor function could exacerbate this situation, they also suggest that restoring the balance between functional D_1 and D_2 receptor responses, whether through increasing D_1, decreasing D_2, or both should be considered as a potential therapeutic approach for reversing alterations associated with cocaine addiction.

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