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Administration of the D2 Dopamine Receptor Antagonist Sulpiride into the Shell, but not the Core, of the Nucleus Accumbens Attenuates Cocaine Priming-Induced Reinstatement of Drug Seeking

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Enhanced dopamine transmission in the nucleus accumbens plays an important role in cocaine priming-induced reinstatement of drug-seeking behavior. However, the contribution of each dopamine receptor subtype to this behavior remains unclear. The present experiments were designed to assess the role of D2-like dopamine receptors in the nucleus accumbens core and shell subregions in cocaine priming-induced reinstatement of drug seeking. Rats were trained to lever press for cocaine using a fixed ratio (FR) 5 schedule of reinforcement. After approximately 18 days of cocaine self-administration, the animals underwent an extinction phase during which cocaine was replaced with saline. Daily extinction sessions were conducted until responding was less than 10% of the response rate maintained by cocaine self-administration. Following the extinction phase, priming-induced reinstatement of cocaine-seeking behavior was assessed. A range of doses of antagonists selective for D2- (sulpiride, 0.2 or 2.0 μg), D3- (U99194A, 3.9 or 7.8 μg), or D4- (L-750,667, 5.5 or 11 μg) dopamine receptors were microinjected into either the nucleus accumbens core, shell or lateral septum prior to a priming injection of cocaine (10 mg/kg, i.p.). Following administration into the shell, but not core or lateral septum, sulpiride dose-dependently attenuated reinstatement induced by a cocaine priming injection. In contrast, U99194A and L-750,667 failed to influence cocaine seeking at any of the doses tested in either accumbal subregion. Collectively, these findings indicate that activation of D2 dopamine receptors mediates cocaine priming-induced reinstatement of cocaine seeking in a region-specific manner within the nucleus accumbens.

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INTRODUCTION

Drug-induced relapse of cocaine self-administration among human addicts is modeled in laboratory animals by administering noncontingent priming drug injections following the extinction of cocaine self-administration behavior. Using this paradigm, intraperitoneal or intravenous injections of cocaine result in robust reinstatement of cocaine seeking (Spealman *et al*, 1999). Interestingly, systemically administered dopamine reuptake inhibitors mimic cocaine's ability to reinstate drug-seeking behavior (De Vries *et al*, 1999; Schenk, 2002), which indicates that enhanced dopamine transmission contributes to the reinstatement of cocaine-seeking behavior induced by a drug priming injection.

Five dopamine receptor subtypes have been identified, and categorized as either D1-like (D1 and D5) or D2-like (D2, D3, and D4) based on sequence homology and pharmacology (Sibley *et al*, 1993; Meador-Woodruff, 1994). Both D1- and D2-like receptor mechanisms are known to mediate the reinstatement of cocaine-seeking behavior. Peripheral administration of D1-like antagonists attenuate drug-seeking behavior induced by a priming injection of cocaine (Norman *et al*, 1999; Spealman *et al*, 1999; Khroyan *et al*, 2000; Alleweireldt *et al*, 2003; Khroyan *et al*, 2003; Schenk and Gittings, 2003). Similarly, administration of D2 dopamine receptor antagonists blocks cocaine priming-induced drug-seeking behavior (Spealman *et al*, 1999; Khroyan *et al*, 2000; Schenk and Gittings, 2003). While D1- and D2-like receptor mechanisms in general have

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been widely investigated in cocaine reinforcement and reinstatement, the paucity of selective drugs for the D2-like receptor subtypes has made it difficult to define a definitive role for each subtype in the reinstatement of cocaine seeking. Recent evidence indicates that systemic administration of selective D3 antagonists decreases cocaine priming-induced (Vorel *et al*, 2002) and cue-induced (Gilbert *et al*, 2005) reinstatement of cocaine seeking in rats, but does not influence the priming effects of cocaine in non-human primates (Khroyan *et al*, 2000). Studies performed in squirrel monkeys also demonstrate that pretreatment with a D4/D3 antagonist attenuates cocaine-seeking behavior as effectively as D2-selective receptor antagonists (Spealman *et al*, 1999; Khroyan *et al*, 2000). These data, taken together, indicate that there is at least some evidence that each D2-like dopamine receptor subtype plays a role in drug priming-induced reinstatement; the precise mechanisms, however, remain unclear.

Although the distribution of dopamine receptors in the rodent brain is varied, there is considerable overlap in the expression of all subtypes within limbic areas. In particular, the nucleus accumbens, a locus of demonstrated importance in cocaine reinforcement, expresses each of the five dopamine receptor subtypes (Di Chiara *et al*, 2004). The nucleus accumbens is a heterogeneous structure consisting of two functionally and anatomically distinct subregions, the central, basal ganglia-oriented core and the surrounding, limbic-associated shell (Heimer *et al*, 1997; Groenewegen *et al*, 1999; Zahm, 2000). While several studies have sought to elucidate the contribution of the core and shell subregions to cue- and stress-induced cocaine seeking (Di Ciano and Everitt, 2001; Fuchs *et al*, 2004; Ito *et al*, 2004; McFarland *et al*, 2004), relatively few investigations have examined the role of the core and shell in cocaine priming-induced reinstatement. Previous findings from our laboratory have shown that D1/D5 dopamine receptors in the nucleus accumbens shell, but not core, promote reinstatement of cocaine-seeking behavior (Anderson *et al*, 2003).

To date there has been no systemic evaluation of the extent to which D2-like dopamine receptors in the core and shell of the nucleus accumbens influence cocaine priming induced reinstatement of cocaine-seeking behavior. The present experiments were designed to address this issue by selectively blocking D2, D3, and D4 receptors in the nucleus accumbens core and shell prior to a priming injection of cocaine. The current results indicate a regional specificity to the dopaminergic mechanisms underlying cocaine priming-induced reinstatement, with a preferential contribution from the shell subregion. Furthermore, accumbal shell D2 dopamine receptors, and apparently not D3 or D4, appear to play an important role the reinstatement of cocaine-seeking behavior.

MATERIALS AND METHODS

Animals and Housing

Male Sprague–Dawley rats (*Rattus norvegicus*) weighing 250–300 g were obtained from Taconic Laboratories (Germantown, NY). Animals were individually housed, with food and water available ad libitum in the home cage (rats undergoing food reinstatement were placed on restricted diets, as outlined below). A 12/12 h light/dark cycle was used with the lights on at 0700. All experimental procedures were performed during the light cycle. The experimental protocols were all consistent with the guidelines issued by the US National Institutes of Health and were approved by the Boston University School of Medicine Institutional Animal Care and Use Committee.

Materials

All experiments used Med-Associates (Georgia, VT) modular testing instrumentation enclosed within ventilated, sound attenuating chambers. The apparatus was equipped with response levers, stimulus lights, food pellet dispensers, and injection pumps for infusing drugs intravenously.

Surgery

Prior to surgery, the rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter (inner diameter 0.33 mm, outer diameter 0.64 mm) was placed into the right jugular vein (side opposite the heart) and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade, and was either routed to a screw-on mount (Plastics One, Roanoke, VA) that was cemented to the skull or to a mesh backmount platform (St John's Innovation Centre, Cambridge, UK) that secured the placement. Catheters were flushed daily with 0.3 ml of the antibiotic Timentin (ticarcillin disodium/potassium clavulanate, 0.93 mg/ml) dissolved in heparinized saline. The catheters were sealed with plastic obturators when not in use.

Following catheter insertion, animals were mounted in a stereotaxic apparatus and guide cannulae (12 mm, 24 gauge) for microinjections were implanted bilaterally dorsal to the nucleus accumbens core and lateral septum/nucleus accumbens shell (that is, the same guide cannulae were used for both lateral septum and shell microinjections; a separate set of cannulae were used for the core microinjections). The coordinates for the ventral ends of the guide cannulae, relative to bregma according to the atlas of Paxinos and Watson (1997), were as follows: lateral septum/nucleus accumbens shell: +1.0 mm A/P, $\pm$0.8 mm M/L, −4.0 mm D/V; nucleus accumbens core: +1.0 mm A/P, $\pm$2.5 mm M/L, −5.0 mm D/V. The stereotaxic arm was set at 0° (ie the cannluae were not implanted at an angle). Cannulae were cemented in place by affixing dental acrylic to stainless steel screws secured in the skull. Stainless steel obturators (14 mm, 33 gauge) were inserted into the guide cannulae after surgery.

Cocaine Self-Administration and Extinction Training

Following a 7-day recovery period from surgery, rats initially were trained using a fixed ratio (FR) 1 schedule of reinforcement, with each daily self-administration session initiated by an intravenous priming injection of cocaine (0.25 mg). Under the FR1 schedule, each active lever response resulted in an infusion of cocaine. When the animals achieved stable responding with the FR1 schedule (ie less than 15% variation in response rates over 3 consecutive days), they were switched to an FR5 schedule.

This schedule required that the rat respond five times on the active lever before receiving a contingent cocaine infusion. With both the FR1 and FR5 schedules, a 60-s timeout period (during which responses had no scheduled consequences) followed each cocaine infusion. The rats were limited to a maximum of 30 cocaine infusions per daily 2-h self-administration session.

After 2 weeks of responding under the FR5 schedule (approximately 18 days of cocaine self-administration total), the animals underwent an extinction phase during which cocaine was replaced with saline. Daily 2-h extinction sessions were conducted until responding was less than 10% of the response rate maintained by cocaine self-administration for 2 consecutive days. This initial extinction criterion typically was met in 4–5 days.

Reinstatement Testing

For all reinstatement testing, satisfaction of the response requirements for each component resulted in saline rather than cocaine infusion. Each reinstatement session was followed by extinction sessions until responding was again less than 10% of the response rate maintained by cocaine self-administration. Generally, only a single day was necessary in order to reach extinction criterion between reinstatement test sessions. The FR5 schedule was used throughout the extinction and reinstatement phases of these experiments.

During the reinstatement phase, selective antagonists for each dopamine receptor subtype were administered directly into the nucleus accumbens shell, core, or lateral septum 10 min prior to an injection of cocaine (10 mg/kg, i.p.). Animals received either the selective D2 antagonist S-(−)sulpiride (nucleus accumbens shell: 0.2 or 2.0 μg/0.5 μl, nucleus accumbens core and lateral septum: 2.0 μg/0.5 μl), the D3-preferring antagonist U99194A (nucleus accumbens shell and core: 3.9 or 7.8 μg/0.5 μl), the high-affinity D4 dopamine receptor antagonist L-750,667 (nucleus accumbens shell and core: 5.5 or 11 μg/0.5 μl) or vehicle control. The administration of the antagonists and vehicles were counterbalanced across reinstatement sessions. Animals were placed in the modular testing chambers immediately following the cocaine injection.

Microinjection Procedures

The obturators were removed from the microinjection guide cannulae and replaced by 33 gauge stainless steel injectors, which extended 1 mm (lateral septum), 2 mm (core) or 3 mm (shell) below the ends of the guide cannulae into the structure of interest. The same guide cannulae were used for both lateral septum and shell microinjections; a separate set of guide cannulae were used for core microinjections (see stereotaxic coordinates listed above). Bilateral infusions were made over 120 s in a volume of 0.5 μl/side. The guide cannulae were left in place for 60 s (to allow the compound to diffuse away from the tips of the cannulae) and then removed. Each animal served as its own control and, typically, two drugs were tested per animal per neuronal site (ie two doses plus vehicle for two drugs for a maximum of six microinjections per brain region). We deviated from this design only when it was unavoidable (eg when technical difficulties, such as clogged microinjection cannulae or loss of intravenous catheter patency, made it impossible to test all doses of a test compound and respective vehicle on a specific animal). In order to control for potential order effects of drug and vehicle administrations, all drug and vehicle treatments were counterbalanced across reinstatement sessions.

Using this experimental design, the rats underwent a series of extinction and reinstatement sessions that lasted approximately 20 days. During this period, extinction of the ability of cocaine to induced reinstatement is a concern. However, we have previously shown that reinstatement of cocaine seeking persists for at least 20 day after the initial extinction of cocaine self-administration (Park *et al*, 2002; Anderson *et al*, 2003). Moreover, since the drug treatments were counterbalanced across reinstatement days, in the current experiments we were able to assess the magnitude of reinstatement to a cocaine priming injection following vehicle administration into the brain. All subjects demonstrated stable drug seeking throughout the reinstatement phase of these experiments.

Food Reinstatement

Potential nonspecific rate-suppressing effects of microinjection of the tested compounds were evaluated by assessing the influence of sulpiride on reinstatement of food-reinforced responding. Rats were trained initially to press a lever under an FR1 schedule of sucrose pellet (Research Diets, Inc., New Brunswick, NJ) delivery in daily 1-h sessions. The animals were restricted to three pellets of lab chow (Harlan Teklad, Wilmington, DE) for the duration of these experiments. Once animals had acquired self-administration on the FR1 schedule (less than 15% variation in responding on 2 consecutive days), the rats were switched to an FR5 schedule of reinforcement. Animals were limited to 30 pellets within a 1-h session.

After 3 weeks of food-maintained responding on the FR5 schedule, rats underwent an extinction phase where the sucrose pellets were removed from the dispenser, and responding no longer resulted in food delivery. After lever pressing decreased to 10% or less of the responding maintained by contingent food reinforcement, animals began reinstatement testing the following day with bilateral microinjections of 2.0 μg/0.5 μl sulpiride or saline into the nucleus accumbens shell. At 10 min following the microinjection, the animals were placed in the modular testing chambers, and the session began with the issue of a noncontingent sucrose pellet prime. The experimenter remotely administered one sucrose pellet every 2 min thereafter for the first 10 min of the reinstatement session. Each 1-h reinstatement session was followed by extinction sessions until responding was again less than 10% of the response rate maintained by food.

Verification of Cannulae Placements

Following the completion of all microinjection experiments, the animals were given an overdose of pentobarbital (100 mg/kg) and perfused intracardially with 0.9% saline followed by 10% formalin. The brain was removed and coronal sections (100 μm) were taken at the level of the

nucleus accumbens with a Vibratome (Technical Products International, St Louis, MO). The sections were mounted on gelatin-coated slides and stained with Cresyl violet. An individual unaware of the animals' behavioral response determined cannula placements as well as potential drug- or cannula-induced neuronal damage. Cellular death and associated gliosis also were assessed. Animals with cannulae placements outside of the areas of interest, or with excessive mechanical damage, were excluded from subsequent data analysis.

Data Analysis

The total mean active and inactive lever responses were analyzed with one-way analyses of variance (ANOVAs). Pairwise comparisons for all ANOVAs were made using Tukey's HSD. The active and inactive lever responses obtained from the sulpiride food-reinforced groups were analyzed with paired *t*-tests, whereas total and inactive responses for accumbal core and lateral septum sulpiride microinjections were analyzed with unpaired *t*-tests.

Drugs

Cocaine was obtained from the National Institute on Drug Abuse (Rockville, MD) and dissolved in bacteriostatic 0.9% saline. Sulpiride (Sigma/RBI, St Louis, MO) and U99194A (Sigma-Aldrich, St Louis, MO) were dissolved in sterile 0.9% saline. L-750,667 (Sigma-Aldrich, St Louis, MO) was dissolved in distilled water.

The doses of sulpiride (Phillips *et al*, 1994a, c; Baker *et al*, 1996) and U99194A (Kling-Petersen *et al*, 1995) were chosen based on previous investigations of intra-accumbal injections in comparable behavioral paradigms, as well as on the basis of optimizing efficacy at the D3 *vs* D2 receptor. D3 receptors have a 70-fold greater affinity for dopamine than any other dopamine receptor (Richtand *et al*, 2001). Sulpiride is approximately 100-fold more selective for the D2 *vs* D3 dopamine receptor subtype (Vallone *et al*, 2000), while the D3 antagonist used in these experiments, U99194A, has 30-fold preference for D3 *vs* D2 dopamine receptors (Kling-Petersen *et al*, 1995; Vallone *et al*, 2000). In contrast, little is known about the extent of L-750,667's behavioral effects. However, the doses used in this study were selected based on the initial characterization of the drug's pharmacological profile *in vitro* (Patel *et al*, 1996).

RESULTS

Reinstatement of Cocaine Seeking

The data summarized in Figure 1 are the mean (±SEM) number of active lever responses from 10 representative subjects on the last day of self-administration, the last day of extinction and following the administration of 10 mg/kg cocaine (i.p.) during the reinstatement phase. Note that extinction was less than 10% of the rate of responding during self-administration and that cocaine administration during the reinstatement phase produced a level of responding substantially higher than during extinction. These rats self-administered cocaine for 18.4±1.4 days; extinction required 4.1±0.35 days to reach criterion.

Figure 1 The mean (±SEM) number of active lever responses from 10 representative subjects on the last day of self-administration, the last day of extinction, and following the administration of 10 mg/kg cocaine (i.p.) during the reinstatement phase. Note that extinction was less than 10% of the rate of responding during self-administration and that cocaine administration during the reinstatement phase produced a level of responding substantially higher than during extinction.

Histology

The cannula placements from the experiments summarized in Figures 3–5 included the medial nucleus accumbens shell, encompassing the medial portion of the shell and the border between the medial shell and the core, the nucleus accumbens core and the lateral septum. The microinjections aimed at the lateral septum were approximately 2 mm above the medial nucleus accumbens shell. The locations of the cannulae placements in these three brain regions are detailed in Figure 2. Any animals with placements outside the target region were discarded from further analysis. Repeated microinjection did not cause excessive mechanical damage or cell death in any of these targeted brain regions.

Microinjection of the D2 Antagonist Sulpiride into the Nucleus Accumbens Shell, but not Core or Lateral Septum, Attenuated Drug Seeking Induced by 10 mg/kg Cocaine

Animals were administered saline ($n=9$), 0.2 ($n=5$) or 2.0 μg ($n=9$) sulpiride into the nucleus accumbens shell prior to a priming injection of cocaine (10 mg/kg, i.p.). The total active lever presses during reinstatement (Figure 3a) were analyzed with a one-way ANOVA, which revealed a significant main effect of treatment ($F(2,20)=6.328$, $p<0.007$). Subsequent pairwise analyses (Tukey's HSD) showed total active responses were significantly different between the saline and 2.0 μg sulpiride treatments. The active lever responses made during each of the 10 min blocks in the intra-accumbal shell saline plus cocaine (10 mg/kg, i.p.) and the intra-accumbal shell 2.0 μg sulpiride plus cocaine (10 mg/kg, i.p.) treatments are shown in Figure 3b. These data were analyzed with a mixed-factors ANOVA (repeated measures over time). This analysis revealed significant main effects of treatment ($F(1,16)=18.308$, $p<0.0006$) and time ($F(11,176)=7.472$, $p<0.0001$) as well as a significant treatment × time interaction ($F(1,11)=2.336$, $p<0.011$). Subsequent pairwise analyses (Tukey's

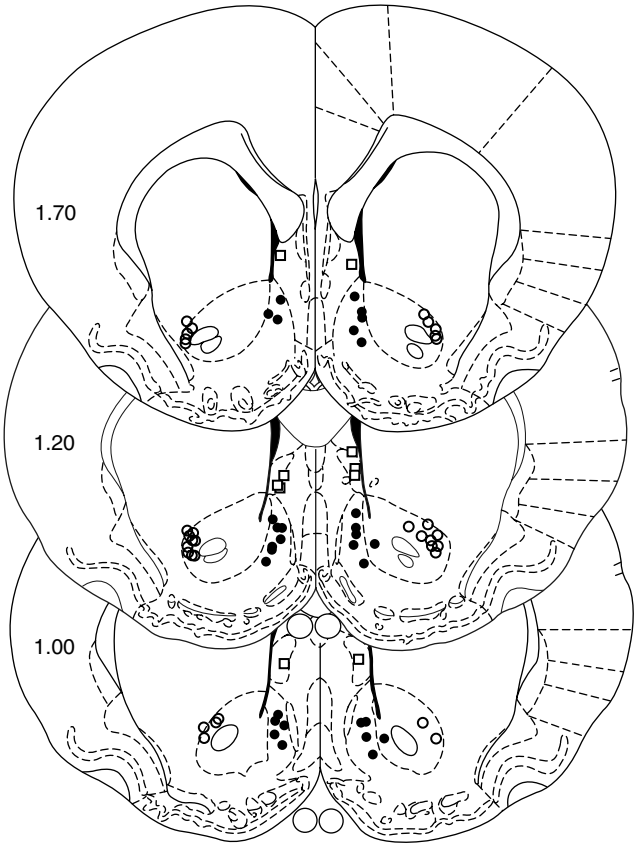

Figure 2 Placements of bilateral guide cannula (ventral tip) are shown for the treatments summarized in Figures 3–5. Open circles depict placements in the core subregion, closed circles are those in the shell, and boxes represent the lateral septum.

HSD) showed that the response rate (number of active lever responses/10 min) of the 2.0 μg sulpiride plus cocaine treatment significantly differed from saline plus cocaine treatment during the 20-min time period. Following microinjection of sulpiride into the nucleus accumbens shell, inactive lever presses for each treatment group were also recorded (Figure 3a). These data were analyzed with a one-way ANOVA, the results of which revealed no significant main effect of treatment ($F(2,20) = 1.181$, $p < 0.328$).

As a control for potential diffusion up the cannulae following microinjection, animals also were administered saline ($n = 5$) or 2.0 μg sulpiride ($n = 4$) into the lateral septum prior to a priming injection of cocaine (10 mg/kg, i.p.). There was no significant difference in either active ($t(8) = 1.44$, $p < 0.194$) or inactive ($t(8) = 0.57$, $p < 0.586$) lever responses between these treatments, as analyzed with unpaired *t*-tests (Figure 3c).

An additional cohort of animals received saline ($n = 8$) or 2.0 μg ($n = 8$) sulpiride microinjections into the nucleus accumbens core prior to a priming injection of cocaine (10 mg/kg, i.p.). The total active ($t(15) = 1.38$, $p < 0.188$) and inactive ($t(15) = 0.34$, $p < 0.74$) lever presses during reinstatement were analyzed with unpaired *t*-tests, which revealed no significant difference between the saline and sulpiride treatments (Figure 3c). We also evaluated the effect of a higher dose of sulpiride (10 μg) when administered into the accumbens core. Although this higher dose did attenuate reinstatement of cocaine seeking, observation of these animals indicated that they were clearly behaviorally impaired (ie movement slowed to the point of catalepsy).

Intra-Accumbal Shell Sulpiride did not Influence Reinstatement of Food-Seeking Behavior

Rats were administered 2.0 μg sulpiride ($n = 6$) or saline ($n = 6$) into the medial limb of the nucleus accumbens shell 10 min before a 1 h reinstatement test session where renewal of lever pressing maintained by food reinforcement was initiated by noncontingently administered sucrose pellets. The active lever responses obtained following microinjections into the nucleus accumbens shell are depicted in Figure 3d. These data were analyzed with an unpaired *t*-test, which revealed no significant difference between treatments ($t(11) = 0.338$, $p < 0.742$).

Microinfusion of U99194A into Either Accumbal Subregion had no Influence Reinstatement of Cocaine-Seeking Behavior

In another set of experiments, the D3-selective antagonist U99194A or saline vehicle was bilaterally microinjected into the nucleus accumbens shell or core. There were a total of 10 animals in each vehicle treatment cohort, and six animals received the 3.9 or 7.8 μg/0.5 μl dose in both subregions. The total active lever responses following bilateral microinjection of 3.9 or 7.8 μg/0.5 μl U99194A or saline into the nucleus accumbens shell (Figure 4a) and core (Figure 4b) subregions were each analyzed with a one-way ANOVA. No significant differences in responding induced by a 10 mg/kg priming injection of cocaine were revealed between saline and U99194A treatments in either the shell ($F(2,19) = 0.248$, $p < 0.783$) or core ($F(2,19) = 1.129$, $p < 0.344$). Following microinjection into the nucleus accumbens subregions, inactive lever presses for each treatment were also obtained (Figure 4a, b). These data were analyzed with a one-way ANOVA, the results of which revealed no significant main effect of treatment in either the shell ($F(2,19) = 0.647$, $p < 0.535$) or the core ($F(2,19) = 3.074$, $p < 0.07$).

Administration of Multiple Doses of L-750,667 into Either Accumbal Subregion Failed to Influence the Reinstatement of Cocaine-Seeking Behavior

In order to investigate the involvement of accumbal D4 dopamine receptors, the selective antagonist L-750,667 or vehicle was bilaterally microinjected into the nucleus accumbens shell and core. There were a total of seven animals in all treatments, except for the 11 μg/0.5 μl dose of L-750,667 in the shell, which totaled eight animals. The active lever responses for the 120 min session following bilateral microinjection of 5.5 or 11 μg/0.5 μl L-750,667 or vehicle into the nucleus accumbens shell (Figure 5a) and core (Figure 5b) subregions were analyzed with a one-way ANOVA. No significant differences between vehicle and L-750,667 treatments on responding induced by a 10 mg/kg priming injection of cocaine were revealed in either the shell ($F(2,19) = 0.217$, $p < 0.807$) or core ($F(2,18) = 0.113$,

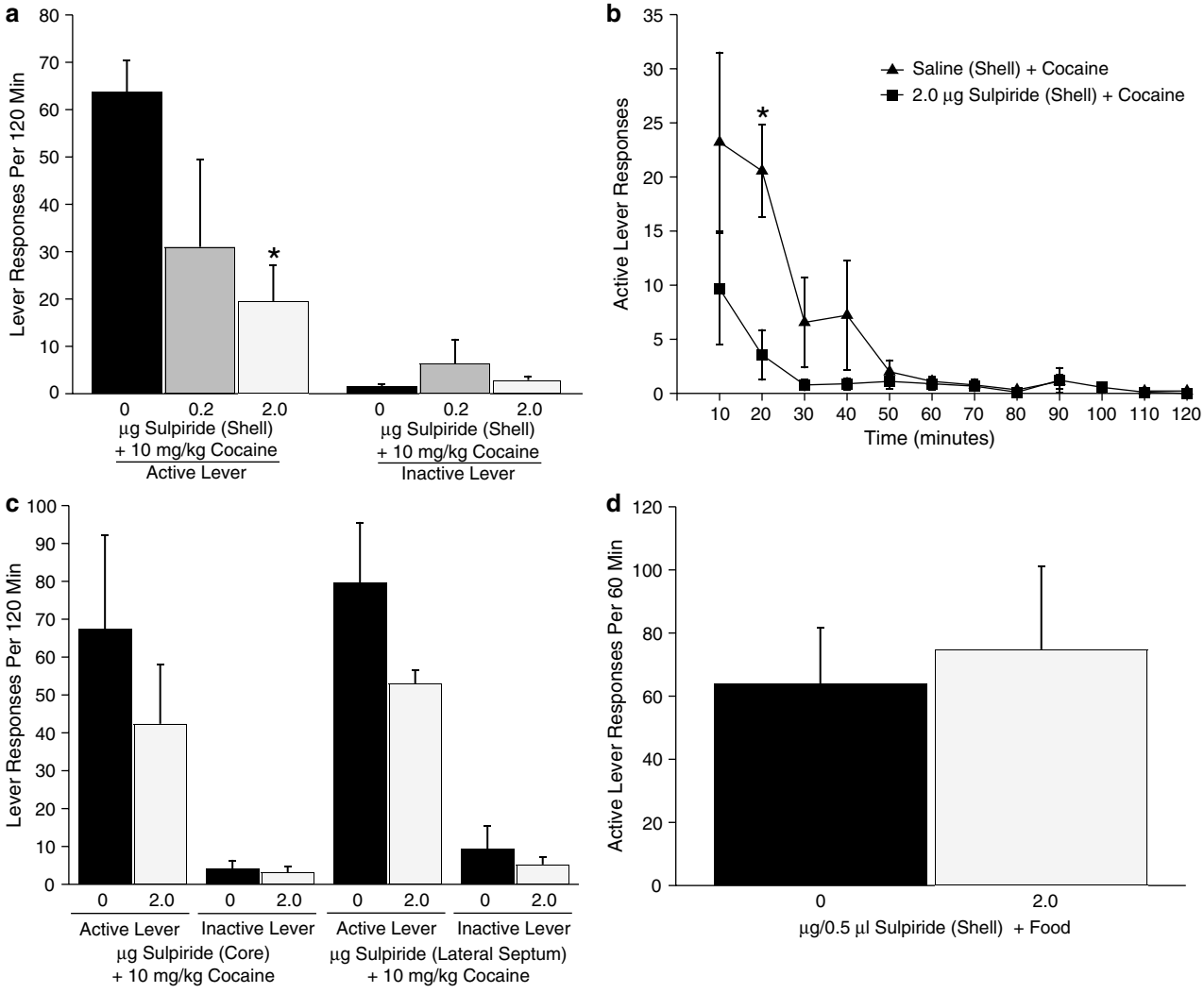

Figure 3 Microinjection of the D2 dopamine receptor antagonist sulpiride into the nucleus accumbens shell attenuates cocaine-priming induced reinstatement. (a) The total number of responses on the active and inactive levers during the reinstatement phase of the experiment, when saline, 0.2, or 2.0 µg sulpiride were administered into the nucleus accumbens shell 10 min prior to the systemic injection of 10 mg/kg cocaine (i.p.). The asterisk represents a significant difference between the saline and 2.0 sulpiride treatments (Tukey's HSD, $p<0.05$). In contrast, 2.0 µg sulpiride, when microinjected into the core or lateral septum, failed to influence cocaine seeking (c). (b) The time course of active lever responses for the saline plus cocaine (10 mg/kg, i.p.) and the 2.0 µg sulpiride plus cocaine (10 mg/kg, i.p.) treatments in the shell (from panel a), shown in 10-min intervals. The 2.0 µg sulpiride plus cocaine treatment significantly differed from saline controls during the 20 min time period (Tukey's HSD, $p<0.05$), as indicated by the asterisk. (d) Animals microinjected with saline or 2.0 µg sulpiride directly into the nucleus accumbens shell prior to the reinstatement of food seeking displayed no difference in total active lever presses between the treatments. Number of animals per treatment: shell cocaine = 5–9, shell food = 6, core cocaine = 8, lateral septum cocaine = 4–5.

$p<0.893$). Following microinjection into the nucleus accumbens shell and core, inactive lever presses for each treatment group were also obtained (Figure 5a, b). These data were analyzed with a one-way ANOVA, the results of which revealed no significant main effect of treatment in either the shell ($F(2,19)=0.363$, $p<0.70$) or the core ($F(2,18)=0.545$, $p<0.589$).

DISCUSSION

The present results indicate that D2, but apparently not D3 or D4, dopamine receptors in the nucleus accumbens shell play an important role in the reinstatement of drug-seeking behavior initiated by a priming injection of cocaine. These results are consistent with a growing body of evidence implicating both D1-like and D2 dopamine receptors mechanisms in the reinstatement of drug seeking induced by a cocaine priming injection (Wise *et al*, 1990; Self *et al*, 1996; De Vries *et al*, 1999; Spealman *et al*, 1999; Khroyan *et al*, 2000; Anderson *et al*, 2003), and the first to specifically identify D2-like dopamine receptor subtype involvement within the subregions of the nucleus accumbens. Furthermore, these results contribute to the understanding of nucleus accumbens subregion functionality by demonstrating dissociation between dopaminergic mechanisms in the core and shell.

When using D2 dopamine receptor antagonists such as sulpiride general behavioral suppression is a concern. In the present experiments, several measures were used to evaluate potential nonspecific rate suppressing effects of intra-accumbal shell administration of sulpiride. The modular

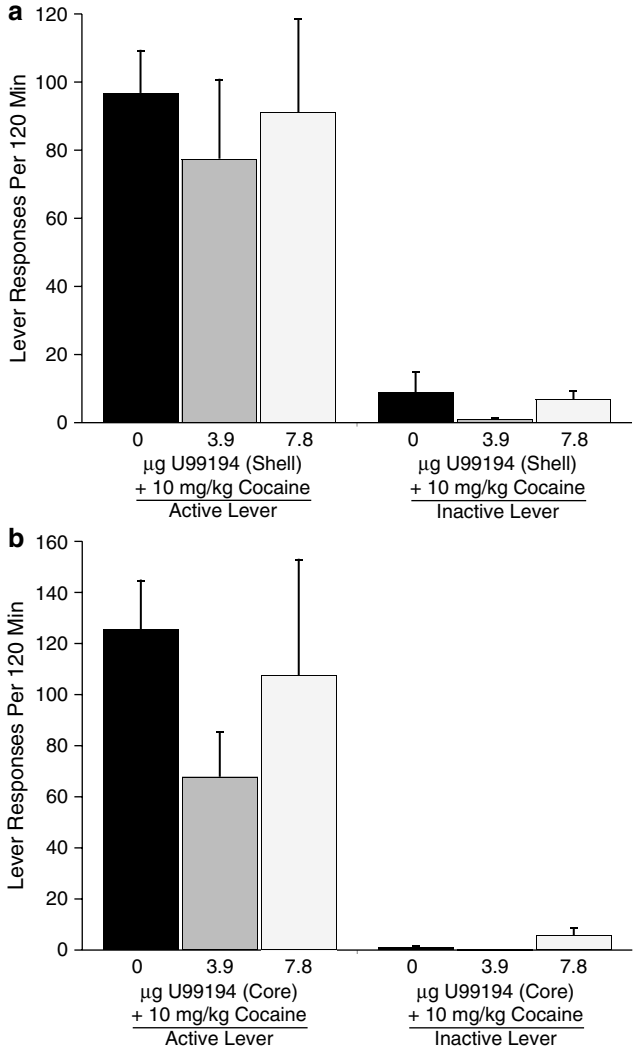

Figure 4 Intra-accumbal administration of the D3 dopamine receptor antagonist U99194A does not influence cocaine-seeking behavior. The data summarized here depict the total number of responses (mean ± SEM) on the active and inactive levers during the 120-min reinstatement session. Saline, 3.9 or 7.8 μg/0.5 μl U99194A was microinjected into the nucleus accumbens shell (a) or core (b) 10 min prior to a priming injection of cocaine (10 mg/kg, i.p.). There were no significant differences between these treatments in either region. Number of animals per treatment: shell and core = 6–10.

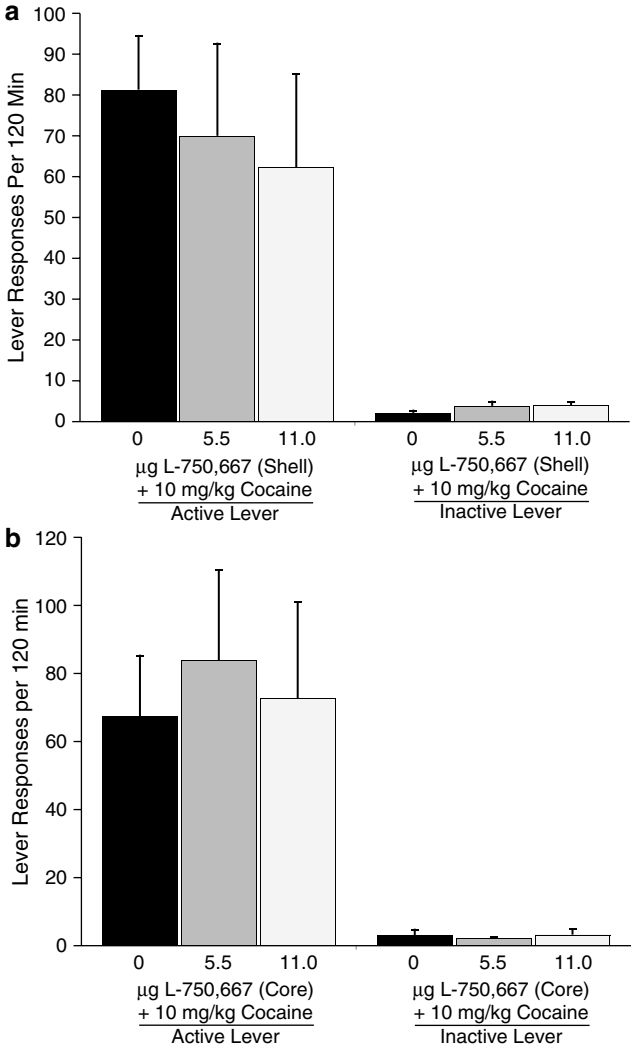

Figure 5 Intra-accumbal administration of the D4 dopamine receptor antagonist L-750,667 does not influence cocaine-seeking behavior. These data depict the total number of responses (mean ± SEM) on the active and inactive levers during the 120-min reinstatement session. Saline, 5.5, or 11 μg L-750,667 was administered into the nucleus accumbens shell (a) or core (b) 10 min prior to a priming injection of cocaine (10 mg/kg, i.p.). There were no significant differences between these treatments in either region. Number of animals per treatment: shell = 7–8, core = 7.

testing chambers were equipped with an inactive lever, responses on which often are used as a measure of nonspecific alterations in lever pressing during the reinstatement phase. Although sulpiride had no significant influence on responding on the inactive lever, the number of inactive lever presses was uniformly low, which limits the utility of this measure to meaningfully assess potential rate suppressant drug effects. Therefore, we also assessed the effect of intra-accumbal shell sulpiride on food reinstatement, where noncontingent administration of food reinstates responding previously maintained by food reinforcement. Using this paradigm, intra-accumbal infusions of sulpiride at the same dose that attenuated cocaine seeking did not alter food seeking. The fact that sulpiride failed to have an effect on reinstatement responding induced by food suggests that the attenuation of cocaine seeking by intra-accumbal shell administration of this drug was not due to general motor impairment. However, it is possible that the behavioral effects of sulpiride in cocaine pretreatment animals may be substantially different than animals without a history of cocaine exposure (such as those in our food reinstatement experiment). Previous work indicates that sulpiride, when administered into the nucleus accumbens, decreases cocaine-induced behavioral hyperactivity (Baker *et al*, 1996) but nonetheless has no effect on cocaine-induced conditioned place preference (Baker *et al*, 1996). In addition, intra-accumbal shell administration of a dose of sulpiride similar to the higher dose used in the present study actually increases cocaine self-administration (Phillips *et al*, 1994b). Taken together, these data suggest that it is unlikely that the attenuation drug priming-induced reinstatement of cocaine-seeking behavior by intra-accum-

bal shell sulpiride was due to nonspecific behavioral disruption.

Given the importance of dopaminergic mechanisms in the reinforcing effects of cocaine (Di Chiara *et al*, 2004; Wise, 2004), a role for dopamine in the reinstatement of cocaine-seeking behavior is not surprising. Accordingly, administration of dopamine reuptake inhibitors (De Vries *et al*, 1999; Schenk, 2002) or D2-like dopamine receptor agonists (Wise *et al*, 1990; Self *et al*, 1996; De Vries *et al*, 1999, 2002; Spealman *et al*, 1999; Khroyan *et al*, 2000; Fuchs *et al*, 2002) reinstates cocaine seeking, whereas D2-like dopamine receptor antagonists attenuate cocaine priming-induced reinstatement of drug-seeking behavior (Spealman *et al*, 1999; Khroyan *et al*, 2000; Vorel *et al*, 2002).

The present finding that a D2 antagonist administered into the shell of the nucleus accumbens attenuates cocaine priming-induced reinstatement is consistent with a previous report that microinjection of a D1-like dopamine receptor antagonist into the accumbens shell blocks cocaine priming-induced reinstatement of drug seeking (Anderson *et al*, 2003). These results coupled with evidence that intra-accumbal administration of cocaine (Park *et al*, 2002) or dopamine (Cornish and Kalivas, 2000) reinstate cocaine seeking indicate that enhanced dopamine transmission in the nucleus accumbens plays a critical role in cocaine priming-induced reinstatement of cocaine seeking. In contrast, intra-accumbal administration of a nonselective dopamine receptor antagonist had no influence on cocaine seeking (Cornish and Kalivas, 2000). However, these microinjections were administered into both the core and shell subregions of the nucleus accumbens (Cornish and Kalivas, 2000). Since the current and previous work from our laboratory (Anderson *et al*, 2003) indicates that augmented dopamine transmission in the shell rather than the core of the nucleus accumbens contributes to cocaine reinstatement, the lack of subregion anatomical specificity likely accounts for the lack of effect of a dopamine antagonist reported by Cornish and Kalivas (2000).

There is growing evidence that the nucleus accumbens core contributes significantly to cue- and stress-induced reinstatement of cocaine seeking (Di Ciano and Everitt, 2001; Fuchs *et al*, 2004; Ito *et al*, 2004; McFarland *et al*, 2004). In terms of cocaine priming induced reinstatement of drug seeking, reversible inactivation of the core, but not the shell, of the nucleus accumbens with a GABA agonist cocktail attenuates cocaine-seeking behavior induced by a cocaine priming injection (McFarland and Kalivas, 2001). However, the present and recent evidence indicates that increased dopamine transmission in the shell, and not the core, of the nucleus accumbens promotes cocaine seeking (Anderson *et al*, 2003). Although experimental differences may account for these apparently conflicting findings, it seems more likely that the neuronal circuits including the core and the shell of the nucleus accumbens each contribute to the reinstatement of cocaine seeking. Since the flow of information through the striatal complex is governed by parallel and integrated hierarchical subcircuits (Haber, 2003), it is possible that the sequence of events that promotes cocaine seeking behavior initially engages the accumbens shell followed by heightened activity in circuits that ultimately organize motor ouput including the accumbens core and neostriatum.

Among dopaminoreceptive nuclei, expression of D3 dopamine receptors in the rat nucleus accumbens is particularly high (Landwehrmeyer *et al*, 1993; Booze and Wallace, 1995). Indeed, the density of D3 receptors in the nucleus accumbens is only slightly less than D2 dopamine receptors (Landwehrmeyer *et al*, 1993; Booze and Wallace, 1995). The potential role of D3 dopamine receptors in the reinstatement of cocaine seeking elicited by a drug priming injection remains unclear. Recent studies have shown that the systemic administration of the D3 dopamine receptor antagonist SB-277011 attenuated drug seeking maintained by cocaine-associated cues (Di Ciano *et al*, 2003) as well as cocaine priming-induced reinstatement of cocaine seeking (Vorel *et al*, 2002). Moreover, following at least 22 days of withdrawal from cocaine self-administration, there is an increase in the expression of D3 dopamine receptors in the nucleus accumbens (Neisewander *et al*, 2004). In contrast, the preferential D3 receptor agonist PD 128,907 neither mimicked nor enhanced reinstatement of cocaine seeking induced by a cocaine priming injection (Spealman *et al*, 1999; Khroyan *et al*, 2000).

The present results indicated that intra-accumbal shell administration of the selective D3 dopamine receptor antagonist, U99194A, had no influence on cocaine priming-induced reinstatement of drug seeking. One possible explanation for the discrepancy between these findings and those of Vorel *et al* (2002) could reside with the higher selectivity of SB-277011 as compared to U99194A for D3 *vs* D2 dopamine receptors (Kling-Petersen *et al*, 1995; Reavill *et al*, 2000; Vallone *et al*, 2000). Another possibility is that D3 receptor mechanisms acting at neuronal sites outside of the nucleus accumbens contribute to cocaine reinstatement. This explanation is particularly attractive since past studies have employed a peripheral route of administration, rather than intracerebral injections aimed at a specific structure. Potential regions other than the nucleus accumbens with a relatively high density of D3 receptors (Bouthenet *et al*, 1991) and a demonstrated role in reinstatement of cocaine seeking include the basolateral amygdala, ventral tegmental area, prefrontal cortex, and hippocampus (Vorel *et al*, 2001; Shalev *et al*, 2002; Kalivas and McFarland, 2003; See *et al*, 2003; Kalivas *et al*, 2005).

The fact that intra-accumbal administration of L-750,667, a potent and highly selective D4 dopamine receptor antagonist (Patel *et al*, 1996), did not alter cocaine-seeking behavior is consistent with the relatively low level of D4 dopamine receptor expression in the nucleus accumbens (Khan *et al*, 1998). In addition to the nucleus accumbens, D4 dopamine receptors are also found on pyramidal neurons in the prefrontal cortex, where they are known to regulate both GABAergic signaling and NMDA receptor surface expression (Wang *et al*, 2002, 2003). Therefore, it is possible that non-accumbal D4 dopamine receptors play a more prominent role in cocaine reinstatement. Alternatively, this dopamine receptor subtype may participate marginally, or be insufficient alone, to significantly regulate cocaine-seeking behavior. Previously, a nonselective D4/D3 antagonist, but not a D4 selective antagonist, was shown to attenuate drug-primed reinstatement (Spealman *et al*, 1999; Khroyan *et al*, 2000), which suggests that the D4 receptor may require coactivation of additional D2-like dopamine

receptor subtypes in order to influence drug priming-induced reinstatement.

In summary, the current results indicate that activation of D2-like dopamine receptors in the nucleus accumbens shell, and not the accumbens core, plays a critical role in the reinstatement of drug-seeking behavior elicited by a cocaine priming injection. Thus, intra-accumbal shell administration of the D2/D3 antagonist sulpiride significantly attenuated the reinstatement of cocaine seeking. In contrast, intra-accumbal shell microinjection of the D3/D2 dopamine receptor antagonist U99194A or the highly selective D4 antagonist L-750,667 had no influence on cocaine reinstatement, which suggests that accumbal shell D2 dopamine receptors are primarily involved in the reinstatement of cocaine seeking. However, since U99194A is only a moderately selective D3 dopamine receptor antagonist, it is premature to rule out a role for accumbal shell D3 dopamine receptors in the reinstatement of drug seeking induced by a cocaine priming injection.

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