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Distinct Contributions of Dopamine in the Dorsolateral Striatum and Nucleus Accumbens Shell to the Reinforcing Properties of Cocaine

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Dopaminergic neurotransmission in the dorsal and ventral striatum is thought to be involved in distinct aspects of cocaine addiction. Ventral striatal dopamine mediates the acute reinforcing properties of cocaine, whereas dopamine in the dorsolateral striatum (DLS) is thought to become involved in later stages of the addiction process to mediate well-established cue-controlled drug seeking. However, it is unclear whether the DLS also has a role in the reinforcing properties of cocaine itself. Therefore, we systematically investigated the involvement of dopamine in dorsal and ventral striatal regions in cocaine self-administration, using various schedules of reinforcement in animals with limited drug taking experience. Intra-DLS infusion of the dopamine receptor antagonist α-flupenthixol did not affect the acquisition of cocaine self-administration, increased cocaine self-administration under a fixed ratio-1 (FR-1) schedule of reinforcement, caused a rightward and downward shift of the dose–response curve of cocaine under an FR-1 schedule of reinforcement and decreased responding for cocaine under a progressive ratio (PR) schedule of reinforcement. Infusion of α-flupenthixol into the ventral nucleus accumbens (NAcc) shell inhibited the acquisition of cocaine self-administration, reduced responding for the drug under FR-1 and PR schedules of reinforcement, and caused a downward shift of the dose–response curve of cocaine self-administration under an FR-1 schedule of reinforcement. These data show that dopamine in both the DLS and NAcc shell is involved in cocaine reinforcement. We suggest that the DLS and the NAcc shell mediate somewhat distinct facets of the reinforcing properties of cocaine, related to its rewarding and motivational aspects, respectively.
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INTRODUCTION

Cocaine addiction is a devastating neuropsychiatric disorder characterized by a loss of control over drug intake. The descent from casual, recreational cocaine use into the compulsive patterns of cocaine taking that characterize addiction, is thought to result from a multitude of drug-induced functional changes in the neural circuits involved in positive and negative emotions, incentive motivation, habit formation and cognitive control over behavior (Robinson and Berridge, 2003; Koob *et al*, 2004; Volkow and Li, 2004; Bechara, 2005; Everitt and Robbins, 2005; Vanderschuren and Everitt, 2005; Perry and Carroll, 2008; Pierce and Vanderschuren, 2010). It is thought that one of the critical neural changes that mediates the transition from casual to compulsive cocaine use is a change in the striatal regions in control of drug seeking and taking, that is, a progressive involvement of dorsal striatal regions in cocaine use (Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010).

The dopaminergic innervation of the ventral striatum has been widely implicated in the reinforcing properties of cocaine (Wise, 2004; Pierce and Kumaresan, 2006). Drug-naive rats self-administer cocaine into the most ventromedial regions of the striatum, that is, the nucleus accumbens (NAcc) shell and the olfactory tubercle (Rodd-Henricks *et al*, 2002; Ikemoto, 2003), and the reinforcing properties of cocaine are reduced after infusion of dopamine receptor antagonists into the NAcc (Maldonado *et al*, 1993; McGregor and Roberts, 1993; Caine *et al*, 1995; Bachtell *et al*, 2005; Bari and Pierce, 2005; Suto *et al*, 2009), or dopamine depletion of the NAcc (Roberts *et al*, 1977, 1980; Pettit *et al*, 1984; Gerrits and van Ree, 1996).

The dorsolateral striatum (DLS) is known to mediate stimulus-response habit learning (Packard and Knowlton, 2002; Yin and Knowlton, 2006). This region is thought to

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become involved in drug seeking after lengthy drug taking experience, when drug use becomes habitual and stimulus driven (Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010). Evidence to support this gradual involvement of dorsal striatal regions in cocaine use comes from self-administration studies in primates. These experiments have shown that with increasing cocaine self-administration experience, changes in the density of the dopamine D2 receptor and the dopamine transporter spread from ventral to dorsal striatal regions (Moore *et al*, 1998; Letchworth *et al*, 2001; Nader *et al*, 2002; Porrino *et al*, 2004). Behavioral studies have associated dopamine in the DLS with well-established, perhaps habitual, cue-controlled cocaine seeking in rats. During cue-controlled cocaine seeking under a second-order schedule of reinforcement, dopamine overflow increased in the DLS, but not in the ventral striatum, and infusion of a dopamine receptor antagonist into the DLS reduced cue-controlled cocaine seeking (Ito *et al*, 2000, 2002; Vanderschuren *et al*, 2005; Belin and Everitt, 2008). Further support for a role of the DLS in habitual cocaine seeking comes from a recent study that showed that functional inactivation of the DLS restored the sensitivity of cocaine seeking to devaluation of the drug outcome, that is, extinction of the cocaine taking response (Zapata *et al*, 2010). Moreover, exposure to drug cues in cocaine addicts increases dopamine activity in the dorsal striatum, and this increase in dopamine activity is correlated with the intensity of cue-induced craving (Volkow *et al*, 2006; Wong *et al*, 2006). However, while the DLS gains an important role in cocaine seeking after prolonged drug self-administration, ventral striatal regions remain involved. For example, AMPA glutamate receptors in the NAcc core mediate well-established cue-controlled drug seeking (Di Ciano and Everitt, 2001), and the development of compulsive, addiction-like cocaine self-administration has been shown to be associated with impaired long-term depression in NAcc core neurons (Kasanetz *et al*, 2010).

Based on the studies discussed above, it has been hypothesized that ventral striatal mechanisms have an important role in primary drug reinforcement, while the dorsal striatum becomes involved in cue-controlled forms of addictive behavior after extensive drug taking experience (Everitt and Robbins, 2005; Pierce and Vanderschuren, 2010). However, as its role in primary drug reinforcement is largely unexplored (Maldonado *et al*, 1993; Caine *et al*, 1995; Vanderschuren *et al*, 2005; Suto *et al*, 2011), it is not clear whether the dorsal striatum is only involved in habitual cue-controlled drug seeking in highly drug-experienced subjects. Therefore, we examined the involvement of dopamine neurotransmission in different regions of the ventral and dorsal striatum in cocaine self-administration in rats with limited drug taking experience.

MATERIALS AND METHODS

Subjects

Male Wistar rats (Charles River) weighing 250 ± 15 g at arrival were singly housed in Macrolon cages (40 × 26 × 20 cm) in climate-controlled rooms (temperature: 21 ± 2 °C, 60–65% relative humidity) under a reversed 12 h day/night cycle (lights on 1900 h). Animals were allowed to habituate to the housing conditions for at least 9 days before surgery. Rats received 20 g chow (SDS) per day, which is sufficient to maintain bodyweight and growth. Water was available *ad libitum*. Self-administration sessions were carried out between 0900 and 1800 h for 5–7 days a week. Experiments were approved by the Animal Ethics Committee of Utrecht University, and were conducted in agreement with Dutch (Wet op de dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Surgery

Rats were anaesthetized with ketamine-HCl (75 mg/kg i.m.) and medetomidine (0.4 mg/kg s.c.) and a catheter (Cam-Caths) was placed into the right jugular vein. Next, the rats were positioned in a stereotaxic apparatus and 26 G guide cannulas (Plastics One) were implanted bilaterally, 1 mm above target structures. Coordinates relative to bregma (Paxinos and Watson, 1998) were as follows: NAcc shell: anteroposterior (AP) +1.2 mm, mediolateral (ML) ±2.8 mm, dorsoventral (DV) −7.5 mm at an angle of 10°; NAcc core: +1.2 mm AP, ±2.8 mm ML, −6.4 mm DV at an angle of 10°; dorsomedial striatum (DMS): +1.2 mm AP, ±3.6 mm ML, −4.1 mm DV at an angle of 22°; DLS: +1.2 mm AP, ±4.2 mm ML, −3.7 mm DV at an angle of 10°. Cannulas were fixed using stainless steel screws and dental acrylic and a stylet was inserted into each cannula. Carprofen (5 mg/kg) was administrated once before and twice after surgery. Gentamycin (5 mg/kg) was administered before surgery and for 5 days post-surgery. Animals were allowed at least 9 days to recover from surgery.

Apparatus

Operant conditioning chambers (29.5 × 24 × 25 cm; l × w × h; Med Associates) situated in light- and sound-attenuating cubicles equipped with a ventilation fan were used. Each chamber contained two retractable levers. A cue light was present above each lever and a house light was located on the opposite wall. Sucrose pellets (45 mg, formula F, Research Diets) could be delivered at the wall opposite to the levers via a dispenser. Priming infusions of cocaine were never given. After each session, catheters were flushed with 0.15 ml heparinized saline. Experimental events and data recording were controlled using MED-PC for Windows.

Microinfusions

Microinfusions were made through 33 G injector cannulas (Plastics One) that extended 1.0 mm below the guide cannulas. Using a syringe pump (Harvard Apparatus), bilateral infusions (0.3 μl/side/60 s) were made, and the injectors were left in place for another 60 s to allow for diffusion. Self-administration sessions began 7 min after the start of the microinfusion.

Self-Administration

Fixed ratio-1 schedule of reinforcement. Rats were trained to self-administer cocaine under a fixed ratio-1 (FR-1) schedule of reinforcement. During 2 h sessions, two levers

were present, one of which was designated as active. The position of the active and inactive levers was counterbalanced between animals. Pressing the active lever resulted in the infusion of 0.25 mg cocaine in 0.1 ml saline over 5.6 s, retraction of the levers, switching off of the house light, followed by a 20-s time-out period. During the infusion, the cue light above the lever was illuminated. When rats showed stable cocaine intake, defined as $<10\%$ variation over three sessions, they received a habituation infusion with saline into the NAcc shell, NAcc core, DMS or DLS. After at least 2 more days of stable responding, rats received infusions of α-flupenthixol (0, 3.75, 7.5 and 15.0 μg/side). Each animal received all doses of α-flupenthixol in a counterbalanced manner. Test sessions were separated by at least one session without treatment. In separate experiments, rats were trained to self-administer sucrose under an FR-1 schedule during 30 min sessions. Pressing the active lever produced one sucrose pellet, followed by a 5.6-s time-out. Animals were fed *ad libitum* once they acquired sucrose self-administration to avoid that hunger influenced the results. After responding had stabilized, rats received a habituation infusion, followed by counterbalanced infusions with 0 and 15.0 μg α-flupenthixol/side into the NAcc shell or the DLS.

FR-1 dose–response curve for cocaine. Rats were trained to self-administer cocaine under an FR-1 schedule of reinforcement as above. After stabilization of cocaine intake, rats received a habituation infusion into the NAcc shell or DLS. After at least 2 more days of stable responding, rats received counterbalanced infusions with 0 and 15.0 μg α-flupenthixol/side before testing for a within-session dose–response curve for cocaine. Dose–response sessions were separated by at least two FR-1 sessions without treatment. To circumvent effects of the initial loading phase of cocaine on the results, dose–response curve sessions started with 30 min of self-administration of 0.25 mg/infusion cocaine. Subsequently, the animals were allowed to respond for descending doses of cocaine (0.5, 0.25, 0.125, 0.063 and 0.031 mg/infusion). Each dose was available for 1 h and the introduction of a new dose was preceded by a 10-min time-out period.

Progressive ratio schedule of reinforcement. A seeking–taking (ST) chain schedule of reinforcement (Olmstead *et al*, 2000) was used, with a progressive ratio (PR) requirement on the seeking link and an FR-1 requirement on the taking link. The assignment of the left and right lever as the seeking and taking levers was counterbalanced between animals. Training started with acquisition of cocaine taking under an FR-1 schedule of reinforcement, as above, except that only one lever was present. Next, the ST-PR schedule of reinforcement was introduced. The ST-PR sessions started with the illumination of the house light and insertion of the seeking lever. Under this schedule, animals had to meet a response requirement on the seeking lever that progressively increased after every earned reward (1, 2, 4, 6, 9, 12, 15, 20, 25, etc; Richardson and Roberts, 1996), to get access to the taking lever. When rats met the response requirement on the seeking lever, the lever retracted and the taking lever was inserted. One press on this taking lever led to a cocaine infusion (0.25 mg/infusion), retraction of the lever, the switching off of the house light, illumination of the cue light above the taking lever for the duration of the infusion, followed by a 10-min time-out period during which both levers remained retracted. After the time-out period, the cycle re-started with the insertion of the seeking lever and illumination of the house light. Sessions continued until rats failed to obtain a reward within 1 h. The highest number of seeking responses an animal performed for one single reward was defined as the breakpoint. When rats showed stable cocaine intake, defined as <2 rewards variation over the last three sessions, they received a habituation infusion into the NAcc shell or DLS. After at least 2 more days of stable responding, rats received bilateral infusions of α-flupenthixol (0, 3.75, 7.5 and 15.0 μg/side). Each animal received all doses of α-flupenthixol in a counterbalanced manner. Test sessions were separated by at least one session without treatment.

Acquisition of cocaine self-administration. Rats received a habituation infusion with saline into the NAcc shell or DLS. Starting the next day, rats were allowed to self-administer cocaine for 10 consecutive sessions under an FR-1 schedule of reinforcement as above. Preceding the first five sessions, rats received 0 or 15 μg α-flupenthixol/side into the NAcc shell or DLS, followed by five sessions without treatment.

Histology

Rats were killed using an overdose of pentobarbital. The brains were removed, immediately fresh frozen on dry ice and stored at $-80\,^{\circ}$C. Coronal sections (20 μm) were sliced on a cryostat and every fifth section was stained with haemaluin and eosin. Cannula placements were assessed under a light microscope.

Solutions

Cocaine-HCl (Bufa BV) and *cis*-(Z)-α-flupenthixol-diHCl (Sigma) were dissolved in sterile physiological saline (0.9% NaCl).

Statistics

Cocaine self-administration under an FR-1 schedule of reinforcement was analyzed using a repeated measures ANOVA and *post hoc* comparisons were made using paired *t*-tests. Response patterns during a session were analyzed using a two-way repeated measures ANOVA with time as within-subjects factor and α-flupenthixol dose as between-subjects factor. Sucrose self-administration under an FR-1 schedule of reinforcement was analyzed using paired *t*-tests. The cocaine dose–response self-administration experiments were analyzed using a two-way repeated measures ANOVA with cocaine dose as within-subjects factor and α-flupenthixol as between-subjects factor. *Post hoc* comparisons were made using paired *t*-tests. Breakpoints in the ST-PR experiments are derived from an escalating curve, which violates the homogeneity of variance. Therefore, we analyzed breakpoints using the non-parametric Friedman test, followed by a *post hoc* Wilcoxon signed ranks test. Acquisition of cocaine self-administration was analyzed using a repeated measures ANOVA with session as within-subjects factor and α-flupenthixol as between-subjects

factor. For the acquisition of cocaine self-administration experiments, two separate analyses were performed: sessions 1–5 (acquisition of self-administration during treatment) and sessions 6–10 (self-administration without treatment). Next, we performed a planned comparison within and between session 5 and 6 (ie, the last session with treatment and the first session without treatment) using paired and unpaired *t*-tests, respectively.

RESULTS

Dopamine in Striatal Regions and Cocaine Self-Administration: FR-1 Schedule of Reinforcement

We first assessed in which striatal areas dopamine has a role in cocaine self-administration after limited cocaine use under an FR-1 schedule of reinforcement. We bilaterally infused different concentrations of the dopamine receptor antagonist α-flupenthixol into the NAcc shell, NAcc core, DMS and the DLS (Figure 1) once animals had acquired cocaine self-administration under an FR-1 schedule of reinforcement. The unit dose of cocaine used (0.25 mg/infusion) is on the descending limb of the dose–response curve of cocaine self-administration (see Figure 3). Acquisition of stable levels of responding under this schedule took 11.5 ± 0.5 sessions. Infusion of α-flupenthixol into the NAcc shell decreased cocaine self-administration ($F_{3,27} = 3.71$, $p = 0.02$). *Post hoc* analysis showed that responding for cocaine was significantly reduced after intra-NAcc shell infusion of 15 μg α-flupenthixol (Figure 2a). Analysis of the response patterns showed that, after an initial 'loading phase' of ~20 min, animals displayed stable levels of cocaine intake during the remainder of the 2-h self-administration session. α-Flupenthixol reduced cocaine intake throughout the session (time, $F_{5,180} = 94.11$, $p < 0.001$; treatment, $F_{3,36} = 3.06$, $p = 0.04$; time × treatment, $F_{15,180} = 0.82$, $p = 0.66$; Figure 2e). Infusion of α-flupenthixol into the NAcc core and DMS did not affect cocaine self-administration (NAcc core, $F_{3,15} = 0.42$, $p = 0.75$; DMS, $F_{3,24} = 2.44$, $p = 0.09$; Figure 2b and c). Infusion of α-flupenthixol into the DLS resulted in a dose-dependent increase in cocaine self-administration ($F_{3,27} = 12.70$, $p < 0.001$). *Post hoc* analysis showed that responding for cocaine was significantly enhanced after intra-DLS infusion of 7.5 and 15 μg α-flupenthixol (Figure 2d). Analysis of the response patterns showed that α-flupenthixol increased cocaine intake throughout the session (time, $F_{5,180} = 28.22$, $p < 0.001$; treatment, $F_{3,36} = 7.99$, $p < 0.001$; time × treatment $F_{15,180} = 0.21$, $p = 0.99$; Figure 2f). The absence of an interaction between time and treatment for the effects of α-flupenthixol in the NAcc shell and DLS shows that the effects of the dopamine receptor antagonist did not change as the session progressed. This indicates that the effects of α-flupenthixol on cocaine self-administration were exerted

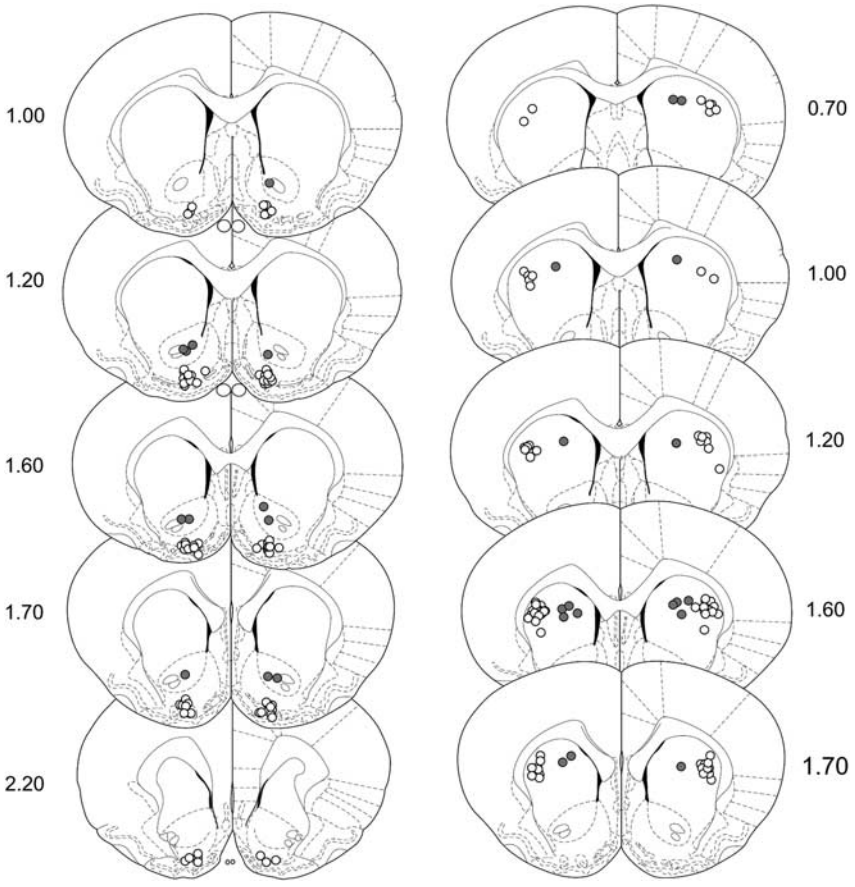

Figure 1 Schematic representation of the bilateral flupenthixol injection sites in coronal sections. On the left the NAcc shell (open circles) and the NAcc core (gray circles) infusion sites, and on the right the DLS (open circles) and the DMS (gray circles) infusion sites. Numbers indicate the distances anterior to bregma in millimeters (adapted from Paxinos and Watson, 1998).

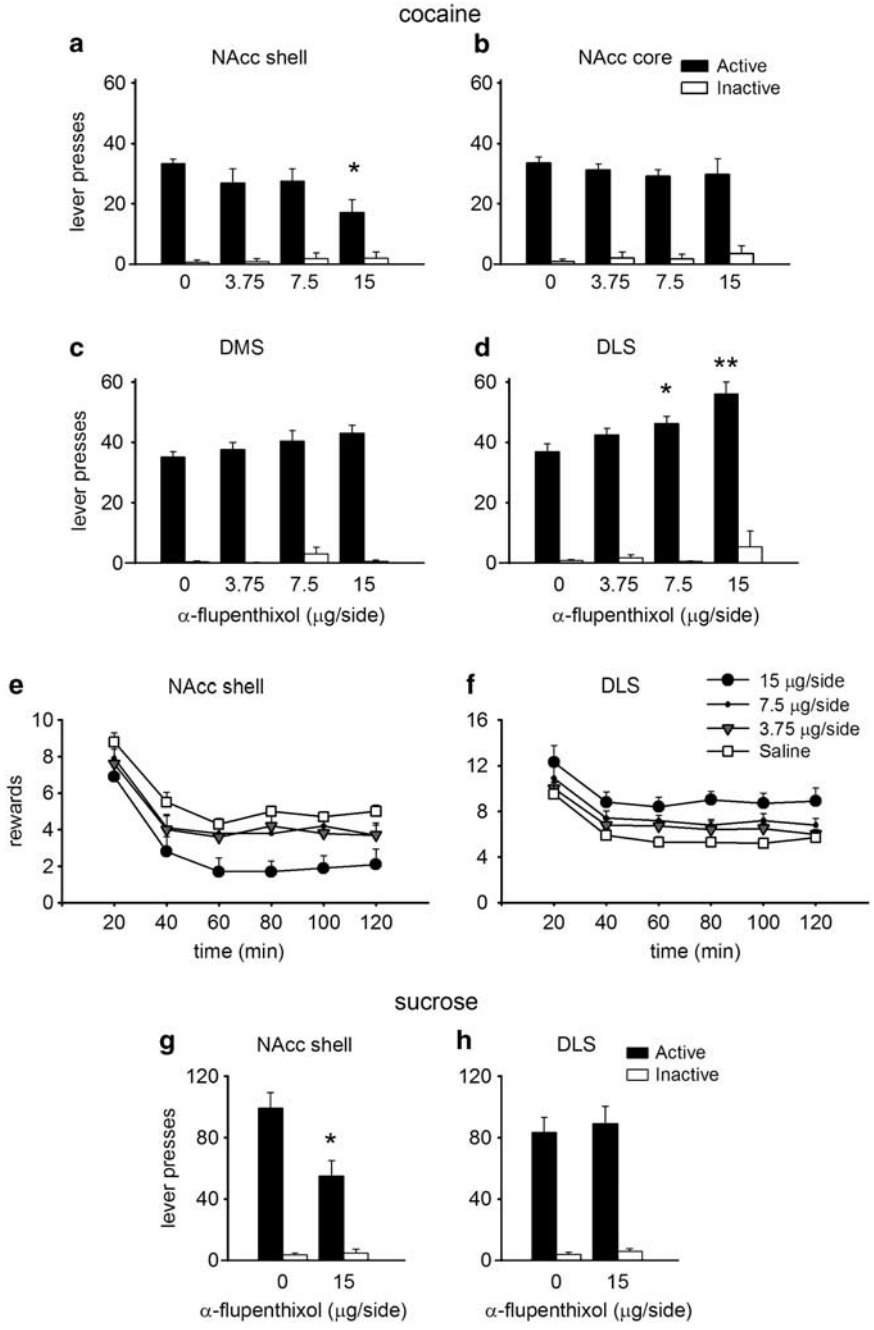

Figure 2 Effects of the intracerebral infusions of α-flupenthixol on intravenous cocaine self-administration under an FR-1 schedule of reinforcement. Graphs express the total number of active (black bars) and inactive (white bars) lever presses during the 2-h self-administration session following infusion of saline or α-flupenthixol into (a) the NAcc shell ($n = 10$), (b) the NAcc core ($n = 6$), (c) the DMS ($n = 9$) or (d) the DLS ($n = 10$). Number of rewards (ie, cocaine infusions) per 20 min of the 2-h self-administration session following infusion of saline or α-flupenthixol into either (e) the NAcc shell or (f) the DLS. Effects of intracerebral infusions of α-flupenthixol on sucrose self-administration under an FR-1 schedule. Graphs express the total number of active (black bars) and inactive (white bars) lever presses during the 30-min self-administration session following infusion of saline or α-flupenthixol into either (g) the NAcc shell ($n = 8$) or (h) the DLS ($n = 7$). Data are presented as mean ± SEM, $^*p < 0.05$, different from saline (paired *t*-test).

in the NAcc shell and DLS, respectively, rather than being the result of diffusion of the dopamine receptor antagonist into adjacent areas. Inactive lever presses were not affected by α-flupenthixol in any of the regions (NAcc shell, $F_{3,27} = 2.50$, $p = 0.08$; NAcc core, $F_{3,15} = 2.23$, $p = 0.13$; DMS, $F_{3,24} = 1.32$, $p = 0.29$; DLS, $F_{3,27} = 0.70$, $p = 0.56$; Figure 2a–d). α-Flupenthixol infusion into the NAcc shell decreased responding for sucrose under an FR-1 schedule of reinforcement (rewards, $t_7 = 4.74$, $p = 0.002$; inactive lever presses, $t_7 = -0.42$, $p = 0.68$; Figure 2g), but infusion of α-flupenthixol into the DLS did not affect sucrose self-administration (rewards, $t_6 = -1.93$, $p = 0.10$; inactive lever presses, $t_6 = -1.80$, $p = 0.12$; Figure 2h). These experiments indicate an important role for dopamine in the NAcc shell and the DLS in responding for cocaine under an FR-1 schedule of reinforcement.

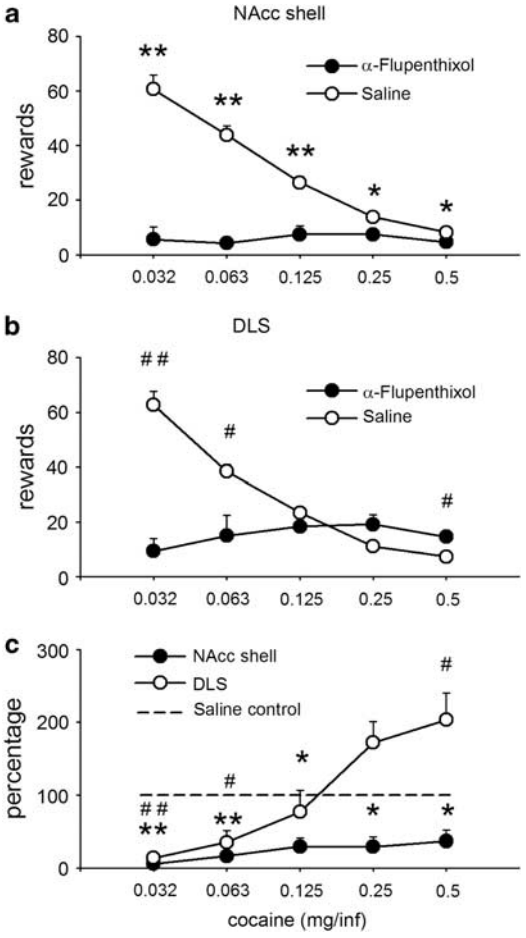

Figure 3 Dose–response curve functions of cocaine self-administration. Graphs express (a, b) the number of rewards (ie, cocaine infusions) obtained after infusion of saline or 15 μg α-flupenthixol into (a) the NAcc shell ($n=5$) or (b) the DLS ($n=5$) during intravenous cocaine self-administration in a within-session dose–response curve under an FR-1 schedule of reinforcement, where each dose of cocaine was available for 1 h. (c) The relative effect of α-flupenthixol into the NAcc shell and DLS on intravenous cocaine self-administration, as a percentage of responding after saline infusion (ie, responding under saline was set at 100%). Data are presented as mean ± SEM, $^{*}p<0.05$, $^{**}p<0.01$; $^{\#}p<0.05$, $^{\#\#}p<0.01$, different from saline for NAcc shell and DLS, respectively (paired *t*-test).

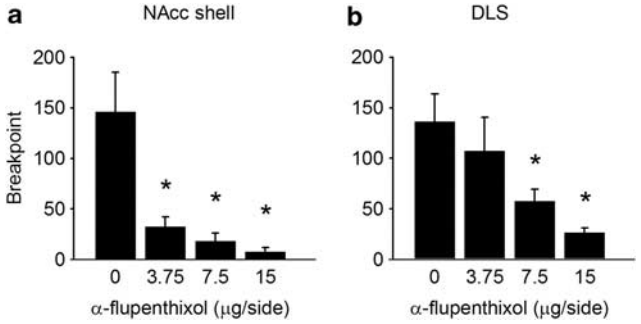

Figure 4 Breakpoints under the ST(PR) schedule of cocaine self-administration after infusions of saline or α-flupenthixol in either (a) the NAcc shell ($n=8$) or (b) the DLS ($n=7$). Data are presented as mean ± SEM, $^{*}p<0.05$, different from saline (Wilcoxon signed ranks test).

Dopamine in NAcc Shell and DLS and Cocaine Self-Administration: Dose–Response Curve Under an FR-1 Schedule of Reinforcement

We next examined whether the effects of α-flupenthixol infusions into the NAcc shell and the DLS on cocaine self-administration under the FR-1 schedule of reinforcement were the result of a change in the sensitivity to the reinforcing properties of the drug. To that aim, we assessed the effects of α-flupenthixol infusions into the NAcc shell and DLS on a within-session dose–response curve of cocaine self-administration under an FR-1 schedule of reinforcement. In animals responding for descending unit doses of cocaine under an FR-1 schedule of reinforcement, infusion of α-flupenthixol into the NAcc shell suppressed responding for cocaine for all unit doses of cocaine (treatment, $F_{1,8}=59.66$, $p<0.001$; treatment × dose, $F_{4,32}=58.46$, $p<0.001$; Figure 3a and c), resulting in a downward shift of the dose–response curve. Infusion of α-flupenthixol into the DLS increased cocaine self-administration for doses >0.125 mg/infusion. However, for cocaine doses up to 0.063 mg/infusion, responding for cocaine was reduced (treatment, $F_{1,8}=11.06$, $p=0.01$; treatment × dose, $F_{4,32}=26.38$, $p<0.001$; Figure 3b and c). Thus, after infusion of α-flupenthixol into the DLS, the dose–response curve for cocaine shifted rightward and downward.

Dopamine in NAcc Shell and DLS and Cocaine Self-Administration: PR Schedule of Reinforcement

To test whether α-flupenthixol affected the motivation for cocaine, we evaluated the effects of α-flupenthixol infusions into the NAcc shell and DLS on responding for cocaine under a PR schedule of reinforcement (Richardson and Roberts, 1996), using an ST chain (ie, ST-PR) schedule of self-administration (Olmstead *et al*, 2000). In animals responding for cocaine under an ST-PR schedule of reinforcement, α-flupenthixol infusions into the NAcc shell decreased breakpoints, which was significant for all doses of α-flupenthixol tested (3.75, 7.5 and 15 μg; $\chi^2_3=15.96$ $p=0.001$; Figure 4a). Infusion of α-flupenthixol into the DLS also reduced responding for cocaine under an ST-PR schedule of reinforcement, which was significant for the two highest doses of α-flupenthixol (7.5 and 15 μg; $\chi^2_3=12.30$, $p=0.006$; Figure 4b).

Dopamine in NAcc Shell and DLS and Cocaine Self-Administration: Acquisition of Self-Administration Under an FR-1 Schedule of Reinforcement

The findings that dopamine in the DLS was involved in cocaine self-administration in animals with limited drug experience raised the question whether this region is involved from the very first stages of cocaine self-administration onwards. Therefore, we infused α-flupenthixol into the NAcc shell or the DLS of cocaine-naive rats during the acquisition of cocaine self-administration under an FR-1 schedule of reinforcement. α-Flupenthixol administered into the NAcc shell during the first five self-administration sessions suppressed the acquisition of cocaine self-administration (α-flupenthixol, $F_{1,11}=11.24$, $P=0.006$; α-flupenthixol × session, $F_{4,44}=3.18$, $p=0.02$; Figure 5a). As soon as α-flupenthixol treatment was

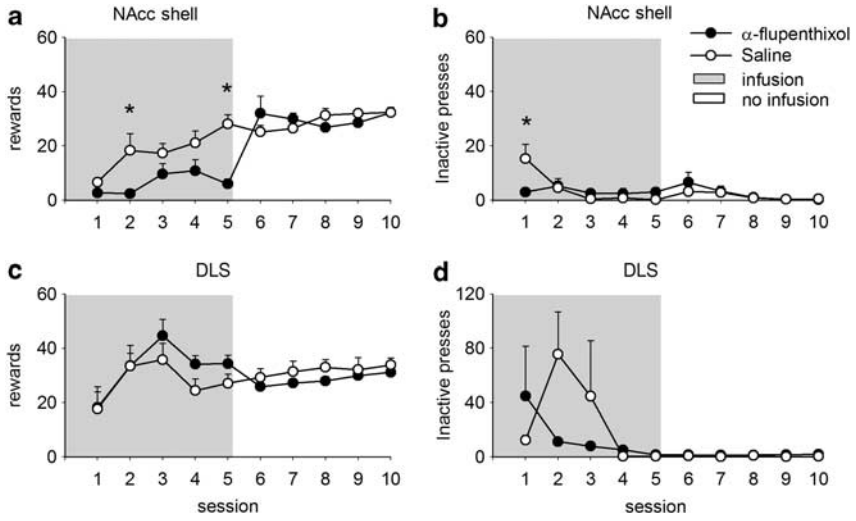

Figure 5 Effects of intracerebral infusions of α-flupenthixol on the acquisition of intravenous cocaine self-administration under an FR-1 schedule of reinforcement. Graphs express (a, c) the number of rewards (ie, cocaine infusions) obtained or (b, d) the number of inactive lever presses during the 2-h self-administration session following infusion of saline or α-flupenthixol into either (a, b) the NAcc shell (saline: $n=6$; α-flupenthixol: $n=7$) or (c, d) the DLS (saline: $n=7$; α-flupenthixol: $n=10$). Data are presented as mean ± SEM, $^{*}p<0.05$, different from saline (paired *t*-test).

discontinued, rats responded at the same level as control rats (session 5 saline *vs* α-flupenthixol $t_{11}=5.81$, $p<0.0001$; session 6 saline *vs* α-flupenthixol $t_{11}=-0.93$ $p=0.37$; α-flupenthixol group session 5 *vs* session 6, $t_6=-3.97$, $p=0.007$). Moreover, there was no effect of previous α-flupenthixol treatment in the subsequent five self-administration sessions (sessions 6–10; α-flupenthixol, $F_{1,11}=0.02$, $p=0.89$) and there was no further change in self-administration during these sessions in either group (sessions 6–10; α-flupenthixol × session, $F_{4,44}=1.87$, $p=0.13$). There was a general effect of acquisition on the inactive lever presses (sessions 1–5, $F_{4,44}=4.26$, $p=0.005$; sessions 6–10, $F_{4,44}=3.09$, $p=0.03$; Figure 5b) and an interaction between session and treatment for the first five sessions ($F_{4,44}=3.87$, $p=0.009$). Further analysis showed that this was the result of the saline group responding more on the inactive lever than the animals treated with α-flupenthixol in the first sessions. From the second session onwards, both groups showed comparable low levels of responding on the inactive lever. Infusion of α-flupenthixol into the DLS during the first five self-administration sessions had no effect on the acquisition of cocaine self-administration (sessions 1–5; α-flupenthixol, $F_{1,15}=1.00$, $p=0.33$; α-flupenthixol × sessions 1–5, $F_{4,60}=0.44$, $p=0.78$; sessions 6–10; α-flupenthixol, $F_{1,15}=1.16$, $p=0.30$; α-flupenthixol × session, $F_{4,60}=0.79$, $p=0.54$; Figure 5c). Discontinuation of α-flupenthixol treatment reduced cocaine taking on day 6 (α-flupenthixol group session 5 *vs* session 6, $t_9=3.34$, $p=0.009$), although there were no differences between the α-flupenthixol and the saline group during sessions 5 and 6 (session 5, $t_{15}=-1.55$, $p=0.14$; session 6, $t_{15}=1.03$, $p=0.32$). This suggests that α-flupenthixol already has a mild effect on cocaine self-administration after five sessions. There was no general effect of acquisition on the inactive lever presses (sessions 1–5; α-flupenthixol, $F_{4,60}=1.67$, $p=0.17$; α-flupenthixol × session, $F_{4,60}=1.84$, $p=0.13$; sessions 6–10; α-flupenthixol, $F_{4,60}=0.12$, $p=0.98$; α-flupenthixol × session, $F_{4,60}=0.12$, $p=0.97$; Figure 5d). During the second five sessions, responding on the inactive lever was somewhat increased in the α-flupenthixol group, most likely a result of the very low levels of responding on the inactive lever in the saline group (average number of inactive presses in sessions 6–10: saline 0.2 ± 0.1 presses/session; α-flupenthixol 1.4 ± 1.0 presses/session; $F_{1,15}=5.89$, $p=0.03$).

DISCUSSION

The present experiments show that suppression of dopaminergic neurotransmission in the DLS increased cocaine self-administration under an FR-1 schedule of reinforcement, caused a rightward and downward shift of the dose–response curve of cocaine, decreased the reinforcing efficacy of cocaine tested under a PR schedule of reinforcement, but did not affect the acquisition of cocaine self-administration. Suppression of dopaminergic neurotransmission in the NAcc shell inhibited the acquisition of cocaine self-administration, reduced responding for the drug under FR-1 and PR schedules of reinforcement, and caused a downward shift of the dose–response curve of cocaine self-administration. Infusion of α-flupenthixol into the DLS did not affect sucrose self-administration under an FR-1 schedule of reinforcement, whereas intra-NAcc shell infusion of α-flupenthixol reduced it. Suppression of dopaminergic neurotransmission in the NAcc core or the DMS did not affect responding for cocaine under an FR-1 schedule of reinforcement. These data show that dopamine in both ventromedial (ie, NAcc shell) and dorsolateral regions of the striatum is involved in the reinforcing properties of cocaine.

In this study, we systematically characterized the role of the DLS in the reinforcing properties of cocaine. This adds to our understanding of DLS mechanisms in addictive behavior, since previous studies have for the most part focused on its role in cue-induced drug seeking, rather than drug taking (Ito *et al*, 2002; Vanderschuren *et al*, 2005;

Fuchs *et al*, 2006; See *et al*, 2007; Belin and Everitt, 2008; Bossert *et al*, 2009). Studies investigating the role of the dorsal striatum (although not the DLS studied here) have reported mixed effects of infusion of the dopamine D1 receptor antagonist SCH23390 into a caudal portion of the dorsal striatum on cocaine self-administration under an FR-5 schedule of reinforcement (Maldonado *et al*, 1993; Caine *et al*, 1995). One study found no effect (Maldonado *et al*, 1993), while the delayed increase in cocaine self-administration in another study (Caine *et al*, 1995) was interpreted as being the result of diffusion of the antagonist into adjacent regions, perhaps the more rostral parts of the DLS targeted in the present study. These studies suggest that caudal regions of the dorsal striatum only have a limited role in cocaine self-administration. Given the heterogeneity of the dorsal striatum, with different functions being subserved by its medial *vs* lateral and rostral *vs* caudal regions (Voorn *et al*, 2004; Yin *et al*, 2004, 2005; Corbit and Janak, 2007; Corbit and Janak, 2010), these results are not necessarily at odds with our findings. Rather, they indicate that different subregions of the dorsal striatum have distinct roles in food and drug reinforcement (Everitt and Robbins, 2005; Yin *et al*, 2008). Indeed, studies investigating rostral portions of the dorsal striatum have shown that infusion of α-flupenthixol into the DLS increased cocaine self-administration under an FR-1 schedule of reinforcement in rats previously trained and tested under a second-order schedule of reinforcement (Vanderschuren *et al*, 2005). Recently, electrolytic lesions of central (ie, inbetween medial and lateral) regions of the rostral dorsal striatum were shown to reduce self-administration of cocaine and morphine under a PR schedule of reinforcement (Suto *et al*, 2011). Our present results suggest that suppressing dopaminergic neurotransmission in the DLS reduces the rewarding properties of cocaine. Animals increased their drug intake under an FR-1 schedule of reinforcement, most likely to compensate for the decreased rewarding effects of cocaine after infusion of the dopamine receptor antagonist (De Wit and Wise, 1977; Ettenberg *et al*, 1982; Caine and Koob, 1994). The PR and dose–response experiments are consistent with this explanation. The reduction of breakpoints under the PR schedule of reinforcement, which occurs at the same doses of α-flupenthixol that increase responding for cocaine under the FR-1 schedule, may be the result of decreased rewarding properties of cocaine. Under a PR schedule of reinforcement, compensation for decreased drug reward by taking more drug infusions is not likely to happen, because the response requirement increases after every drug infusion. Thus, when the rewarding properties of cocaine were reduced after intra-DLS infusion of α-flupenthixol, the rats would consequently be less motivated to respond for the drug, resulting in lower breakpoints. Moreover, the rightward shift of the dose–response curve for higher unit doses of cocaine indicates that dopamine receptor blockade in the DLS decreases the rewarding properties of the drug (Altman *et al*, 1996; Ahmed and Koob, 1998; Piazza *et al*, 2000). The downward shift in the dose–response curve for the lower unit doses of cocaine is consistent with this notion. By suppressing dopaminergic neurotransmission in the DLS, the rewarding properties of low doses of cocaine could become so low that animals no longer compensate for the lower cocaine reward by taking more drug infusions and therefore ceased responding for the drug.

Suppressing dopaminergic neurotransmission in the DLS did not affect the acquisition of cocaine self-administration. This is in agreement with previous findings that cocaine-naive rats do not self-administer cocaine into dorsal regions of the striatum (Ikemoto, 2003) and that the dorsal striatum is not required for the acquisition of instrumental learning (Hernandez *et al*, 2002; Atallah *et al*, 2007). However, cocaine self-administration significantly decreased as soon as α-flupenthixol treatment was discontinued, that is, after only five self-administration sessions. This suggests that DLS dopamine already becomes involved in cocaine taking after very limited experience with the drug. The involvement of the DLS in cocaine reinforcement became more pronounced as soon as self-administration had stabilized. This involvement of DLS dopamine in instrumental responding was specific for cocaine, since intra-DLS α-flupenthixol did not alter responding for sucrose.

Before concluding that dopamine in the DLS is involved in the rewarding properties of cocaine, alternative explanations must be considered, however. The DLS has a prominent role in stimulus-response habit learning (Packard and Knowlton, 2002; Yin and Knowlton, 2006), and Tiffany (1990) proposed that both seeking and taking drugs can become automatic and habitual. Therefore, one could argue that the changes in cocaine self-administration after infusion of α-flupenthixol into the DLS were the result of a suppression of habitual aspects of cocaine use, rather than cocaine reward. However, we do not think that our results can be explained by dopamine in the DLS mediating habitual aspects of drug taking. If dopamine receptor blockade in the DLS suppresses habitual behavior (Faure *et al*, 2005), intra-DLS infusion of α-flupenthixol should have decreased, rather than increased, cocaine self-administration under the FR-1 schedule of reinforcement. Thus, although we did not strictly test whether cocaine self-administration was habitual using devaluation or extinction procedures (Dickinson, 1985; Dickinson *et al*, 2002; Miles *et al*, 2003; Zapata *et al*, 2010), our results are not consistent with a habit-like pattern of responding for cocaine. In fact, the limited operant experience of our animals under FR-1 or PR schedules of reinforcement is not likely to be sufficient to engage a habitual form of self-administration (Zapata *et al*, 2010), in contrast to variable interval schedules that more readily promote the development of habitual behavior (Dickinson, 1985). Alternatively, habitual forms of behavior or stereotyped behavior induced by cocaine mediated by the DLS may compete for behavioral output with ventral striatal mechanisms (Joyce and Iversen, 1984). In this scheme, habits or stereotyped behavior mediated by the DLS may serve as a functional brake on behaviors mediated by the ventral striatum, such as goal-directed cocaine self-administration. Releasing this brake on ventral striatal mechanisms by suppressing dopaminergic neurotransmission in the DLS would then result in an increase in cocaine taking. However, our dose–response data do not support the notion that suppression of dopaminergic neurotransmission in the DLS facilitates goal-directed cocaine taking, since responding did not increase for all cocaine unit doses tested. In fact, responding for the lowest cocaine unit doses actually

decreased. Furthermore, if suppression of DLS dopaminergic neurotransmission facilitates goal-directed forms of cocaine self-administration, then breakpoints under the PR schedule of reinforcement should have increased after intra-DLS α-flupenthixol infusion, whereas we observed decreased breakpoints. Taken together, our data indicate that DLS dopamine mediates the rewarding properties of cocaine in animals with limited drug experience, expanding the role of the DLS in drug use beyond habitual aspects of cue-controlled drug seeking after extended drug taking experience.

Blockade of dopamine receptors in the ventral NAcc shell attenuated responding for cocaine both during the acquisition and maintenance of cocaine self-administration. The dose–response analysis showed a suppression of cocaine taking for all unit doses of cocaine, resulting in a pronounced downward shift of the dose–response curve. Infusion of α-flupenthixol into the NAcc shell markedly reduced responding for cocaine under a PR schedule of reinforcement. This pattern of effects indicates that dopamine receptor blockade in the NAcc shell decreases the motivation to respond for cocaine, the rewarding properties of the drug, or both. In the acquisition experiment, responding was profoundly decreased during intra-NAcc shell treatment with α-flupenthixol, but immediately increased to control levels when dopaminergic neurotransmission in the NAcc shell was no longer suppressed. This suggests that during the first five self-administration sessions, the animals did learn some rewarding effects of cocaine, but since these were much reduced, they only consumed small quantities of the drug. In addition, the motivation of the animals to respond for the drug could also be inhibited by α-flupenthixol. As soon as dopaminergic neurotransmission was no longer blocked, the rewarding and motivational properties of cocaine were experienced in full, leading to levels of responding comparable to those of saline-treated rats. In animals trained to respond for cocaine under an FR-1 schedule of reinforcement, intra-NAcc shell α-flupenthixol reduced self-administration. FR-1 schedules of reinforcement assess reward consumption, which is the result of a variety of processes, including the rewarding and motivational properties of rewards. Therefore, reductions in responding for cocaine could indicate a lower rewarding effect of cocaine, or a lower motivation to respond. After systemic or intra-NAcc treatment with dopamine receptor antagonists, animals usually compensate for the decreased rewarding effects of cocaine by consuming more of the drug (De Wit and Wise, 1977; Ettenberg *et al*, 1982; Maldonado *et al*, 1993; McGregor and Roberts, 1993; Caine and Koob, 1994; Caine *et al*, 1995; Bachtell *et al*, 2005; Suto *et al*, 2009). When the rewarding effects of cocaine are reduced further, animals will show decreased levels of self-administration, resembling an extinction-like pattern of responding (De Wit and Wise, 1977; Ettenberg *et al*, 1982; Caine and Koob, 1994). Thus, the effect of intra-NAcc shell α-flupenthixol on responding for cocaine under an FR-1 schedule of reinforcement indicates that either the rewarding effects of cocaine were much reduced, or the motivation to respond for the drug was inhibited. We investigated these possibilities further using a dose–response analysis of cocaine self-administration. Reduced cocaine reward by intra-NAcc shell treatment with α-flupenthixol would then be expected to result in a rightward shift of the dose–response curve, whereas a downward shift indicates that the motivation for the drug was affected (Altman *et al*, 1996; Ahmed and Koob, 1998; Piazza *et al*, 2000). We found that intra-NAcc treatment with α-flupenthixol induced a marked downward shift of the dose–response curve of cocaine, suggesting that the motivation to respond for cocaine was decreased. Motivational influences on behavior can be more directly addressed using a PR schedule of reinforcement (Hodos, 1961; Richardson and Roberts, 1996). Therefore, we also investigated the effect of intra-NAcc shell α-flupenthixol on responding for cocaine under a PR schedule of reinforcement, and we found a marked decrease in breakpoints. Interestingly, responding under the PR schedule was more sensitive to α-flupenthixol than responding under an FR-1 schedule, since a four-fold lower dose of α-flupenthixol reduced responding for cocaine under the PR schedule than under the FR-1 schedule of reinforcement. Together, these data suggest that infusion of α-flupenthixol into the ventral NAcc shell reduced the motivation to respond for cocaine, although an effect on the rewarding effects of the drug cannot be ruled out.

Interestingly, in previous studies, infusions of dopamine receptor antagonists into more medial portions of the NAcc shell resulted in increased cocaine self-administration under an FR-1 schedule of reinforcement, indicating reduced cocaine reward, rather than reduced motivation (Caine *et al*, 1995; Bachtell *et al*, 2005). In the present study, we aimed our microinfusions at the most ventral portion of the NAcc shell to cover the entire ventromedial to dorsolateral extent of the striatum in our experiments (Voorn *et al*, 2004). These infusion sites in the NAcc shell bordered on the olfactory tubercle (see Figure 1), which has been implicated in the initiation of cocaine self-administration (Ikemoto, 2003). Therefore, it is likely that the effects of α-flupenthixol infusions into the ventral NAcc shell were in part mediated within the olfactory tubercle. Together, this suggests that the rewarding and motivational properties of cocaine are mediated by dopamine in distinct subregions of the ventral striatum, consistent with the reported functional heterogeneity of this structure along its mediolateral as well as rostrocaudal axis (Reynolds and Berridge, 2002; Ikemoto *et al*, 2005; Smith and Berridge, 2007; Lammel *et al*, 2011).

Administration of α-flupenthixol into the NAcc shell also attenuated sucrose self-administration under an FR-1 schedule of reinforcement. Dopamine in the NAcc shell has been implicated in the vigor of responding for food, rather than in its positive subjective, rewarding properties (Cardinal *et al*, 2002; Baldo and Kelley, 2007; Salamone *et al*, 2007). The motivation for food, tested under a PR schedule of reinforcement, has been shown to depend on dopamine neurotransmission in the NAcc (Aberman *et al*, 1998; Zhang *et al*, 2003, but see Bari and Pierce, 2005). Remarkably, however, the effects of dopaminergic manipulations on responding for food are usually observed under demanding schedules of reinforcement, whereas intra-shell administration of α-flupenthixol in our study reduced responding for sucrose under an FR-1 schedule of reinforcement. This indicates that dopamine in the ventral region of the NAcc shell targeted in the present study may mediate the

motivation for both drugs and natural rewards, again reflecting the functional heterogeneity of the most ventral regions of the striatum (Reynolds and Berridge, 2002; Ikemoto *et al*, 2005; Smith and Berridge, 2007; Lammel *et al*, 2011).

A limitation of the present study was the use of a non-selective dopamine receptor antagonist. This drug was chosen in order to investigate the role of dopaminergic innervation of different subregions of the striatum in the reinforcing properties of cocaine, rather than studying the different dopamine receptor subtypes involved. Previous studies have shown that within the NAcc, both dopamine D1 and D2 (but not D3 or D4) receptors mediate the reinforcing properties of cocaine (Bachtell *et al*, 2005; Bari and Pierce, 2005; Suto *et al*, 2009). The type of dopamine receptor that mediates the rewarding properties of cocaine within the DLS remains to be tested in future studies.

The absence of an effect of dopamine receptor blockade in the NAcc core and the DMS on responding under an FR-1 schedule suggests that these regions are not critical for cocaine taking. However, given that different subregions of the striatum have complementary roles in instrumental responding (Everitt and Robbins, 2005; Yin *et al*, 2008), it is likely that these structures do mediate other aspects of the reinforcing and addictive properties of cocaine (Bari and Pierce, 2005; McFarland and Kalivas, 2001; Di Ciano and Everitt, 2001; Suto *et al*, 2009).

It is likely that the NAcc shell and DLS mediate cocaine reinforcement in concert, and that ventral striatal mechanisms allow for the involvement of the DLS in cocaine self-administration to develop. This may occur through a spiraling pathway from ventral to dorsal striatum via the striatal projections to the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) (Nauta *et al*, 1978; Haber *et al*, 2000). Alternatively, shorter pathways may be responsible for the connection between the NAcc shell and DLS, such as the projection from the NAcc shell to the medial SNc that in turn innervates the DLS (van Dongen *et al*, 2009), or projections within the VTA-nigral complex from VTA to the medial SNc (Ferreira *et al*, 2008). These possibilities remain to be tested in future studies.

In sum, our study sheds new light on the role of different striatal regions in the early stages of cocaine use. We show that dopamine in both the DLS and NAcc shell is involved in cocaine reinforcement. The DLS and the NAcc shell likely mediate somewhat distinct facets of the reinforcing properties of cocaine, related to its rewarding and motivational aspects, respectively.

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DISCLOSURE

The authors declare that, except for income received from their primary employers, no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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