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Experience-Dependent Effects of Cocaine Self-Administration/ Conditioning on Prefrontal and Accumbens Dopamine Responses

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Abstract

Experiments were performed to examine the effects of cocaine self-administration and conditioning experience on operant behavior, locomotor activity, and nucleus accumbens (NAcc) and prefrontal cortex (PFC) dopamine (DA) responses. Sensory cues were paired with alternating cocaine and nonreinforcement during 12 (limited training) or 40 (long-term training) daily operant sessions. After limited training, NAcc DA responses to cocaine were significantly enhanced in the presence of cocaine-associated cues compared with nonreward cues and significantly depressed after cocaine-paired cues accompanied a nonreinforced lever response. PFC DA levels were generally nonresponsive to cues after the same training duration. However, after long-term training, cocaine-associated cues increased the magnitude of cocaine-stimulated PFC DA levels significantly over levels observed with nonreinforcement cues. Conversely, conditioned cues no longer influenced NAcc DA levels after long-term training. In addition, cocaine-stimulated locomotor activity was enhanced by cocaine-paired cues after long-term, but not after limited, training. Findings demonstrate that cue-induced cocaine expectation exerts a significant impact on dopaminergic and behavioral systems, progressing from mesolimbic to mesocortical regions and from latent to patent behaviors as cocaine and associative experiences escalate.

Keywords

microdialysis; drug abuse; operant; reinforcement; reward

The prefrontal cortex (PFC) and nucleus accumbens (NAcc) dopamine (DA) terminals are functionally significant components of short- and long-term cocaine use; both show immediate DA enhancement after self-administered cocaine (Ikegami & Duvauchelle, 2004) and modified neural activation after cocaine and conditioning experiences (Ciccocioppo, Sanna, & Weiss, 2001; Grimm & See, 2000; McLaughlin & See, 2003). These regions, which receive dopaminergic projections from the ventral tegmental area (Emson & Koob, 1978; Van Bockstaele, Wright, Cestari, & Pickel, 1994), are also involved in diverse learning and memory processes. The PFC is known to be active during attentional processes and working memory (Bouret & Sara, 2004; Goldman-Rakic, 1990; Granon et al., 2000; Mizoguchi et al., 2000; Romanides, Duffy, & Kalivas, 1999), whereas both the PFC and NAcc are involved in various aspects of associative learning and incentive motivational tasks (Bassareo & Di Chiara, 1997; Cheng, de Bruin, & Feenstra, 2003; Richardson & Gratton, 1996; Young, Ahier, Upton, Joseph, & Gray, 1998).

Associative learning with natural rewards can produce experience-dependent expectations that can alter DA neuronal firing activity. For example, *prediction error* is a term used to describe a scenario in which reward outcomes deviate from learned cue–reward associations (Schultz & Dickinson, 2000; Tobler, Dickinson, & Schultz, 2003; Waelti, Dickinson, & Schultz, 2001). In behaving primates, DA firing activity is depressed when anticipated reinforcement is not received (negative prediction error) and enhanced when unexpected reinforcement is presented (positive prediction error; Schultz & Dickinson, 2000; Tobler et al., 2003; Waelti et al., 2001). These findings reveal that precise relationships between learned expectations and outcomes are uniquely reflected through variations in DA neuronal activity (Schultz, 2004, 2005).

Conditioning and learning are primary factors in the process of addiction and drug use relapse (Childress, McLellan, Ehrman, & O'Brien, 1988; Childress et al., 1999). Therefore, because drug and intense natural rewards activate the same brain systems (Kelley & Berridge, 2002), DA changes observed during the course of natural reward associative learning may provide key insight to drug-induced alterations. The current experiments were conducted to test the hypothesis that, similar to what is seen in natural reward-associative learning, variations in cocaine experience and cognitive expectations of cocaine reward have specific effects on DA responses to cocaine- and nonrewarded conditions. Because the PFC and NAcc DA regions are activated during associative learning, drug reward (Bardo, 1998; P. E. Phillips, Stuber, Heien, Wightman, & Carelli, 2003; See, Grimm, Kruzich, & Rustay, 1999; Sun & Rebec, 2005; Tzschenke & Schmidt, 1999; Weiss, Ciccocioppo, et al., 2001; Weiss et al., 2000) and cue-induced cocaine expectation (Grant et al., 1996; Hotsenpiller, Horak, & Wolf, 2002; Mattson & Morrell, 2005), these areas were targets of the current investigation.

Conditioned place preference and intravenous self-administration procedures are two of the most widely used techniques to study the rewarding properties of cocaine. These procedures make use of two outcomes of cocaine administration: primary reward and conditioned rewarding stimuli (A. G. Phillips & Fibiger, 1990). Primary reward is demonstrated by cocaine self-administration behavior, which is rapidly acquired by rats and maintains relatively stable response rates over extended periods (Depoortere, Li, Lane, & Emmett-Oglesby, 1993; Emmett-Oglesby et al., 1993; Koeltzow & Vezina, 2005). Conditioned place preferences are established for cocaine-paired environments after limited exposure to alternating cocaine and saline treatments, indicating acquisition of both conditioned reward and discriminative learning (Duvauchelle, Ikegami, & Castaneda, 2000; O'Dell, Khroyan, & Neisewander, 1996). The current study combined conditioned place preference and self-administration methodology as a means to associate specific sensory cues with either cocaine or nonreinforced self-administration sessions. For example, rats were trained to expect cocaine delivery following a lever press in the presence of one sensory cue and saline delivery in the presence of another sensory cue. At the completion of training, PFC or NAcc extracellular DA was assessed after a single lever press for saline or cocaine in the presence of either saline or cocaine cues.

In previous work, we observed that during initial exposure, NAcc DA was more responsive to cocaine reinforcement, compared with PFC DA (Ikegami & Duvauchelle, 2004). However, during initial responses to stressful or aversive stimuli (Bland et al., 2003; Di Chiara, Loddo, & Tanda, 1999; Pani, Porcella, & Gessa, 2000) or anxiogenic drugs (Bassareo, Tanda, Petromilli, Giua, & Di Chiara, 1996) PFC DA is preferentially activated. Because anatomical sites within which DA elevations have been observed may change over the course of learning (Di Chiara et al., 1999), it was therefore also of interest to examine NAcc and PFC DA responses during early (limited) and late (long-term) stages of training under this operant task.

Method

Subjects

Male albino Sprague–Dawley rats (Animal Resource Center, Austin, TX) weighing approximately 250–300 g at the beginning of the experiments were used. The animals were group housed and maintained on a 12-hr reversed light–dark cycle (lights on 7 p.m. to 7 a.m.). Animals were handled daily at least 1 week prior to experiments and continuously handled throughout experiments. Food and water were available ad libitum in the home cage except during the food-training phase.

Apparatus

Food training, in vivo microdialysis test sessions, and self-administration sessions were conducted in identical one-lever operant chambers (28 × 22 × 21 cm) located within sound-attenuating compartments. In all chambers, the ceiling, front, and back walls were constructed of Plexiglas. The two sidewalls were constructed of metal with a single retractable operant lever on one of the walls. A stimulus light was located above the retractable lever, and a house light was located on the opposite metal wall. Three sets of photocells were located on the front and back walls of the chamber, one in the center and the two others at 5 cm from each end. Photobeam breakages within the operant chamber were compiled as locomotor activity units. The injector system was connected to a swivel mounted on a counterbalanced arm at the top of each chamber. One end of the swivel was connected, via polyethylene tubing (Tygon Microbore, Akron, OH, 1.5 mm o.d.), to a 10 ml syringe mounted on a syringe pump (Razel, Princeton, IN, Model A, 33.3 rpm). A spring-covered catheter (Plastics One, Roanoke, VA) connected the other side of the swivel to the catheter termination mounted on the top of the animal's head. The experimental programs were controlled and operant and locomotor data were collected by a Med Pentium 100 MHz computer and Med-PC (Med Associates, St. Albans, VT) software.

Food Training

Animals were food restricted ($\approx$ 6 g of standard Rat Chow [Ralston Purina, St. Louis, MO] per day, adjusted as needed to maintain, not decrease, body weight) and trained to lever press for food on a food-reinforced operant schedule of reinforcement. Each lever response resulted in dispensing one sucrose pellet (45 mg; P.J. Noyes, Lancaster, NH). After the lever-press response for food was acquired, 10-min food-reinforced operant sessions were conducted for the next 6 days without food restriction.

Surgery

After the completion of food-training sessions, animals were implanted with a chronic Silastic intravenous jugular catheter (0.6 mm o.d.) under pentobarbital sodium (Nembutal, 50 mg/kg ip) anesthesia. Atropine sulfate (250 μ g sc) was given prophylactically to prevent respiratory tract secretions. Supplemental chloral hydrate (80 mg/kg ip) was given, if necessary, to prolong anesthesia. Catheters were implanted such that the free end of the catheter with a cannula termination (Plastics One) passed subcutaneously on the side of the neck, out an incision in the animal's head, and mounted on the skull. Animals were also stereotactically implanted with a unilateral guide cannula (21 g) either (a) aimed 3.55 mm above the medial PFC (mPFC) according to Paxinos and Watson, 1997 (AP: +3.8 mm; ML: $\pm$ 0.6 mm; DV: $-$ 1.5 mm) or (b) aimed 6.3 mm above the NAcc (tooth bar: +5.0 mm; AP: +3.0 mm; ML: $\pm$ 1.7 mm; DV: 2.5 mm). The catheter cannula and the guide cannula were affixed to the skull with four stainless steel screws and dental acrylic cement. Animals underwent a minimum of 1-week recovery prior to the beginning of the experiments. After the surgery, animals received 0.1 ml of saline solution consisting of one U/ml streptokinase, 67.0 mg/ml of the antibiotic Timentin, and 30

U/ml heparin through their intravenous catheters daily for the next week. Animals continued receiving the same solution daily without the Timentin component through the duration of the experiment to maintain catheter patency (Emmett-Oglesby et al., 1993).

In Vitro Recovery Calibration

Microdialysis probes were constructed as previously described (Duvauchelle, Ikegami, Asami, et al., 2000), with an active membrane length of 2.5 mm at the probe tip. Prior to probe recovery, all probes were flushed with nanopure water. On the day of probe calibration, 1.0 ml gastight Hamilton (Reno, NV) 1000 series syringes were filled with freshly prepared filtered Ringer's solution (128.3 mM NaCl, 1.35 mM CaCl₂, 2.68 mM KCl, and 2.0 mM MgCl₂ and pumped through the probe at 1.63 μ l/min, with the probe tips in a beaker containing the Ringer's solution, ascorbate (0.001%), and 20 nM DA, and maintained at 37 °C. Perchloric acid solution (1M Na Bisulfite and 0.2 M EDTA in 0.05 N HClO₄) was added into the collecting tubes to prevent DA degradation. Ten-min samples from each probe were collected and assayed by high-performance liquid chromatography with electrochemical detection. We calculated recovery values for each probe by comparing the peak heights of samples with those from a standard (1.25 nM DA). The mean ($\pm$ SEM) recovery of probes used in the experiment was $16.12 \pm 0.43\%$ for PFC probes and $15.17 \pm 0.35\%$ for NAcc probes.

Training

Conditioning/self-administration sessions—Training consisted of alternating days of cocaine and saline availability during 1-hr conditioning/self-administration sessions. During the first 30 min of each session, the chamber was darkened, and the lever was retracted while animals habituated to the neutral environment. After 30 min, the houselight was illuminated, sensory cues (see below) and the lever were presented, and cocaine or saline was made available for the remaining 30 min. Animals were weighed daily, and cocaine concentrations were altered accordingly for cocaine sessions so that each lever press resulted in the delivery of 0.5 mg/kg cocaine hydrochloride in a volume of 0.1 ml of isotonic saline. During saline sessions, an equal volume of saline was infused. After each infusion, there was a 20-sec time-out period during which time the lever was retracted, the stimulus light turned off, and no infusions delivered.

Sensory cues—Visual and olfactory environmental cues were introduced into the operant chamber immediately following the 30-min darkened habituation period. Visual cues consisted of either black or white felt walls attached to the sides of the clear Plexiglas operant chamber. Olfactory cues consisted of an oil-based scent (e.g., cinnamon or rose), saturated on a cotton ball located under the grid floor of the operant chamber.

Group assignments—Animals with PFC or NAcc-implanted cannulae were assigned to undergo either limited or long-term training sessions. The duration of training for the limited training groups was 12 days (e.g., 6 sessions each of the cocaine and nonreinforced [saline] self-administration) and 40 days for the long-term training groups (20 sessions each of cocaine and saline self-administration). During training, certain cue combinations (e.g., visual + olfactory) were associated with either cocaine administration (conditioned stimulus [CS] + = cocaine-associated) or nonreinforced (CS- = saline- or nonreinforcement-associated) operant responses and were consistent throughout the duration of the training period.

Microdialysis Probe Implantation

Within 12 hr after completion of all self-administration sessions, animals were briefly anesthetized with ultrashort-acting barbiturate, methohexital sodium (Brevital, approximately 6.0 mg/iv) and implanted with a microdialysis probe through the previously implanted guide cannula. Each microdialysis probe was connected to a 1.0 ml gastight Hamilton 1000 series

syringe mounted on a syringe pump (Razel, Model A), and freshly prepared Ringer's solution was pumped through the probe. Animals implanted with the probe remained in a holding chamber overnight with the syringe pump speed set at 0.261 $\mu\text{l}/\text{min}$. Bedding, food, and water were available in the holding chamber. Thirty min prior to the test session, the pump speed was changed to 1.63 $\mu\text{l}/\text{min}$.

Test Conditions

Animals were tested 24 hr after the completion of training (at least 12 hr postprobe implantation) under a variety of experimental conditions. These conditions were defined by the particular cues (e.g., CS+ and CS-) and available reinforcement (e.g., a single self-administered cocaine or saline injection) presented during the test session. Therefore, animals implanted with dialysis probes within the PFC or NAcc and given the CS+ or CS- cue during the test session comprised PFC/CS+, PFC/CS-, NAcc/CS+, and NAcc/CS- groups. These groups were further subdivided into animals that self-administered a single cocaine injection or a single saline injection during the test session. (Data from animals tested with cocaine are depicted in Figure 1, and those tested with saline are depicted in Figure 2.)

Test Session

Animals were placed in the operant chamber with the lever retracted for the first 30 min (baseline). After 30 min, the house-light was illuminated, the lever was extended into the chamber, and the assigned cues were introduced into the chamber. Animals were allowed to respond once on the lever and received either a single self-administered injection of cocaine (3.0 mg/kg) or saline (approx 0.1 ml) infused over a 6-s interval. The lever was then retracted for the remainder of the session. The intravenous cocaine dose of 3.0 mg/kg was chosen because the NAcc DA peak effects are comparable with intraperitoneal doses (10–15 mg/kg) commonly used to determine cocaine sensitization effects (Kalivas & Duffy, 1993; Sabeti, Gerhardt, & Zahner, 2003; Zavala, Nazarian, Crawford, & McDougall, 2000). The 3.0 mg/kg iv dosage also enables a comparison of DA response magnitude between NAcc and PFC regions, as it has been shown to enhance extracellular levels of both NAcc and PFC DA in cocaine-naïve rats (Ikegami & Duvauchelle, 2004). In vivo microdialysis samples were collected at 10 min intervals across the entire test session, comprising 3 10-min baseline and 3 10-min test (e.g., postcocaine or saline injection) samples. Locomotor activity units (photobeam breakages) were assessed in correspondence with dialysis sampling.

Assay of Dialysate

For PFC DA detection, we analyzed the dialysates for DA using high-performance liquid chromatography equipped with Shizeido capcell C-18 narrow bore column, ESA (Chelmsford, MA) Model 5200A Coulochem II Detector, a Model 5020 Guard Cell, and a Model 5041 amperometric analytical cell. The mobile phase contained 150 mM $\text{Na}_2\text{H}_2\text{PO}_4$, 50 μM EDTA, 4.5 mM ~ 6.0 mM sodium dodecyl sulfate, 4.76 mM citric acid, 10%–15% (v/v) acetonitrile, 10%–15% methanol, pH 5.6. The analytical cell potential was set at +200 mV (oxidation). The detection limit for PFC DA was calculated at 0.050 nM with a signal:noise ratio of 2:1. For NAcc DA detection, a Rainin (Emeryville, CA) Microsorb-MV C-18 column with ESA 5014B Coulometric analytical cell was used. Mobile phase contained 82.4 mM $\text{Na}_2\text{H}_2\text{PO}_4$, 2.23 mM EDTA, 7.5%–8.0% (v/v) acetonitrile, 0.46–2.08 mM 1-octanesulfonic acid, pH 5.5. The oxidizing potential was set at +200 mV, and the reducing potential was set at -150 mV. The detection limit for NAcc DA was calculated at 0.126 nM with a signal:noise ratio of 2:1. For both sites, the flow rate was set at 0.2 ml/min, and 15 μl samples were injected immediately following collection into the column and compared with fresh standards of DA HCl (Sigma Chemical, St. Louis, MO). The amount of DA within each sample was determined by comparison with standards prepared and analyzed on the day of sample analysis. After

correcting for probe recovery, we calculated mean ($\pm$ SEM) basal DA concentrations (first baseline sample) in the PFC and NAcc at 0.99 ± 0.18 and 4.40 ± 0.66 nM, respectively.

Data were collected and analyzed with an ESA Model 500 Data station.

Histological Analysis

After the experiment, animals were killed by an overdose of pentobarbital sodium. Brains were removed and stored in 10% formaldehyde/30% sucrose solution. We verified the probe placements within the PFC and NAcc (see Figure 3) from coronal sections (48 μ m) stained with cresyl violet.

Statistical Analyses

We compared data obtained from the limited and long-term training conditions separately using two- and three-way analyses of variance (ANOVAs). Cocaine- and nonreinforced operant responses during conditioning/self-administration sessions from PFC- and NAcc-implanted animals were combined and analyzed with two-way repeated measures ANOVAs (Treatment $\times$ Session). To compare the magnitude of PFC and NAcc DA responses with cues and either cocaine or nonreinforcement at test, we corrected dopamine data in nM according to probe recovery rates and converted them to percentage of baseline for data analyses (overall baseline averaged from within-subject means of three baseline measurements). For these data, three-way repeated measures ANOVAs (Brain Region $\times$ Cue Condition $\times$ Time) were used. Locomotor activity units during sessions were tabulated as breakages in any of the three sets of photobeams in the operant chamber (see *Apparatus*). To compare locomotor activity responses with cues and cocaine or saline at test, we used two-way repeated measures ANOVAs (Cue Condition $\times$ Time). We used post hoc analyses (Fisher's least significant difference) to detect any significant group differences (e.g., at least $p < .05$) at specific time points when main effects and/or interaction effects were indicated by overall analyses.

Results

Operant Responding: Cocaine and Nonreinforced Conditioning/Self-Administration Sessions

Two-way repeated measures ANOVAs (Brain region $\times$ Treatment) revealed no significant group differences in operant response rates during conditioning/self-administration sessions between animals implanted with PFC or NAcc cannulae during cocaine, limited, $F(1, 49) = 1.65$, *ns*, and long-term, $F(1, 36) = 0.0006$, *ns*; and nonreinforced, limited, $F(1, 49) = 0.065$, *ns*, and long-term, $F(1, 36) = 2.88$, *ns*, training sessions. Therefore, we combined operant response data from PFC and NA-implanted animals to compare the number of cocaine and nonreinforced (saline) operant responses during limited and long-term training sessions.

Limited training—A two-way repeated measures ANOVA performed on lever responses for the six cocaine and six saline self-administration sessions (Treatment $\times$ Session Day) showed significant treatment, session and Treatment $\times$ Session Day interaction effects, $F(1, 100) = 8.25$, $p = .005$; $F(5, 500) = 8.96$, $p < .0001$; $F(5, 500) = 3.01$, $p = .01$, respectively. Post hoc tests revealed responses during the first four nonreinforced (saline) sessions were significantly greater than those during corresponding cocaine self-administration sessions (see Figure 4A).

Long-term training—A two-way repeated measures ANOVA performed on the number of lever responses for the 20 cocaine self-administration and 20 nonreinforced operant sessions (Treatment $\times$ Session Day) showed significant session day and Treatment $\times$ Session Day interaction effects, $F(19, 1406) = 15.79$ and $F(19, 1406) = 5.33$, respectively, $p < .0001$ for both, but no significant effects of treatment alone, $F(1, 74) = 0.09$, *ns*. As in the limited training

data, post hoc tests revealed significantly greater responding during initial nonreinforced sessions. As sessions progressed, cocaine responding gradually increased such that by the last few sessions, responding for cocaine was significantly greater than for nonrewarded lever presses during corresponding session days (see Figure 4B).

PFC and NAcc DA: Effects of Associated Cues, Cocaine Self-Administration, and Nonreinforced Operant Responses

Three-way repeated measures ANOVAs (Brain region $\times$ Cue Condition $\times$ Time) were performed for limited and long-term training groups on PFC and NAcc DA responses tested with self-administered cocaine or saline in the presence of cocaine- or nonreinforcement-associated cues.

Limited training/cocaine—A three-way ANOVA revealed significant brain region, $F(1, 24) = 4.77, p = .039$, and time, $F(5, 120) = 24.91, p < .0001$, but no overall cue effects, $F(1, 24) = 3.33, ns$. Significant interaction effects were detected between brain region and time, $F(5, 120) = 4.41, p = .001$, and between conditioned cues and time, $F(5, 120) = 2.36, p = .044$. Post hoc comparisons revealed that cocaine-stimulated DA was significantly enhanced from baseline levels in both NAcc DA cue groups (NAcc/CS+ and NAcc/CS-) and in the PFC group tested with cocaine-paired cues (PFC/CS+), but not in the PFC/CS- condition. Also, the magnitude of NAcc DA increase was significantly greater in the NAcc-implanted animals self-administering cocaine in the presence of cocaine-associated cues (NAcc/CS+), compared with DA responses to cocaine in the presence of saline-associated cues (NAcc/CS-) and with both PFC groups (PFC/CS+ and PFC/CS-; see Figure 1, top).

Long-term training/cocaine—A three-way ANOVA revealed significant time, $F(5, 85) = 18.73, p < .0001$, and Brain Region $\times$ Time interaction effects, $F(5, 85) = 2.64, p = .029$. Post hoc tests revealed that all groups showed significant enhancement of DA after cocaine self-administration. In contrast with findings after limited training, cocaine- and saline-associated cues did not differentially alter cocaine-stimulated NAcc DA levels, but cocaine-associated cues enhanced cocaine-stimulated PFC DA (PFC/CS+) significantly more than what was observed in the PFC/CS- group, and also to a greater proportion than the NAcc DA responses (see Figure 1, bottom).

Limited training/nonreinforcement—A three-way ANOVA (Brain Region $\times$ Cue Condition $\times$ Time) with repeated measures on the time factor was performed on DA levels of all limited training groups in response to a nonreinforced operant response. This analysis revealed no overall significant brain region or cue condition effects, $F(1, 22) = 0.14$ and $F(1, 22) = 1.78$, both *ns*, but significant effects of time, $F(5, 110) = 7.81, p < .0001$, and an all-factor interaction effect (Brain Region $\times$ Cue Condition $\times$ Time), $F(5, 110) = 3.18, p = .01$. Post hoc tests revealed that NAcc DA levels dipped significantly lower in animals after a nonreinforced operant response was received in the presence of a cocaine-associated cue (NAcc/CS+), compared with congruent cues with nonreinforcement (NAcc/CS-). In contrast, after a nonreinforced operant response with corresponding cues (PFC/CS-) PFC DA levels were significantly lower than when cocaine-associated cues accompanied nonrewarded responses (PFC/CS+; see Figure 2, top).

Long-term training/nonreinforcement—A three-way ANOVA (Brain Region $\times$ Cue Condition $\times$ Time) with repeated measures on the time factor was performed on DA levels of all long-term training groups. This analysis revealed no overall significant brain region or cue condition effects, $F(1, 13) = 4.02$ and $F(1, 13) = 0.31$, both *ns*, but significant effects of time, $F(5, 65) = 11.51, p < .0001$. Post hoc tests revealed that PFC DA levels were significantly lower after a nonreinforced response in the CS+ condition compared with the CS- group. Also,

PFC and NAcc DA levels decreased below at least one baseline measurement during at least one test interval after a nonrewarded operant response in all groups except PFC/CS− (see Figure 2, bottom).

Locomotor Activation: Effects of Associated Cues, Cocaine Self-Administration, and Nonreinforced Operant Responses

Two-way ANOVAs (Brain Region $\times$ Time) performed on each cue and treatment condition in both limited and long-term training groups (e.g., PFC/CS+ vs. NAcc/CS+ receiving cocaine, or PFC/CS− vs. NAcc/CS− receiving saline) showed no group differences in locomotor activity when animals were given the same cue and treatment at test. Therefore, locomotor data from PFC- and NAcc-implanted animals within each cue (e.g., CS+ and CS−) and testing condition (e.g., cocaine or nonreinforced lever response) were combined for overall analyses.

Limited training—A three-way ANOVA (Test Condition $\times$ Cue Condition $\times$ Time) with repeated measures on the time factor was performed on the locomotor activity measures of all tested groups. This analysis showed significant test, $F(1, 47) = 18.77, p < .0001$, time, $F(5, 235) = 11.04, p < .0001$, and Test $\times$ Time Interaction effects, $F(5, 235) = 10.2, p < .0001$, but no significant effects of cue condition, $F(1, 47) = 0.0002, ns$. Post hoc analyses revealed that the groups receiving cocaine at test (e.g., CS+/cocaine and CS−/cocaine) showed significantly greater activity than did nonreinforced animals (see Figure 5, top).

Long-term training—A three-way ANOVA (Test Condition $\times$ Cue Condition $\times$ Time) with repeated measures on the Time factor was performed on the locomotor activity measures of all combined groups. This analysis showed significant test, $F(1, 34) = 8.59, p = .006$, time, $F(5, 170) = 15.9, p < .0001$, and Test $\times$ Time interaction effects, $F(5, 170) = 14.22, p < .0001$. Post hoc analyses revealed significantly increased locomotor activity after cocaine self-administration in both cue conditions, but cocaine-stimulated locomotor activity was significantly greater in the CS+/cocaine animals compared with the CS−/cocaine group immediately after cocaine self-administration ($p = .01$). No cue-associated differences in locomotor activity after a nonreinforced operant response were detected (see Figure 5, bottom).

Discussion

In this study, the relative responsiveness of NAcc and PFC DA to cocaine self-administration and associated cues differed according to the number of self-administration/conditioning training sessions. After short-term (limited) training, the NAcc DA response to cocaine-associated cues during cocaine self-administration was significantly higher in magnitude, relative to PFC DA responses. However, after long-term training, the reverse was true. In addition, when rats with limited training were presented with cocaine-associated cues but received a saline injection, a significant decrease in NAcc (but not PFC) DA occurred. After long-term training, however, PFC DA was significantly lower under these cue/reinforcement conditions. It may be of some relevance that when PFC DA showed the greater relative response to cues, animals had established preferential responding for cocaine, compared with saline. On the other hand, NAcc DA appeared to be most responsive to cue conditions during a period before this relative preference had been fully established.

Mesolimbic/Mesocortical Dopamine Involvement in Cocaine Self-Administration and Conditioning

Findings after long-term training are consistent with the notion that increased cocaine and conditioning experience leads to diminished NAcc involvement in cocaine responses and cue recognition. Indeed, primates with extensive cocaine self-administration experience have shown no effect of cocaine-paired cues on mesolimbic DA responses to cocaine (Bradberry,

Barrett-Larimore, Jatlow, & Rubino, 2000). Such findings are also in agreement with studies showing the dissociation of NAcc DA activity from well-established reinforced behaviors (Choi & Horvitz, 2001; Choi, Balsam, & Horvitz, 2005; Horvitz, 2001) and correspond with human fMRI work showing decreased striatum activation in response to well-learned reward-associated cues (Delgado, Miller, Inati, & Phelps, 2005).

In contrast, incomplete learning of cocaine/saline cue associations (as evidenced by higher response rates for saline over cocaine) was accompanied by NAcc DA levels that were particularly responsive to cues. In these limited training groups, the short-term exposures to cocaine and saline-associated environments were similar to a conditioned place preference paradigm (Duvauchelle, Ikegami, Asami, et al., 2000; O'Dell et al., 1996; Weiss et al., 2000; Weiss, Martin-Fardon, et al., 2001) and therefore directly support evidence of a crucial role of NAcc DA during cocaine/cue associations (Duvauchelle, Ikegami, Asami, et al., 2000; O'Dell et al., 1996; Weiss et al., 2000; Weiss, Martin-Fardon, et al., 2001). However, as noted above, the findings of experience-dependent changes in NAcc DA responses to cues also support work showing dissociations between mesolimbic DA involvement and cocaine-conditioned effects (Bradberry et al., 2000; Brown & Fibiger, 1992; Spyraki, Fibiger, & Phillips, 1982).

Both the core and shell subterritories within the NAcc DA have been touted as the primary site mediating pharmacological and conditioned effects of drugs of abuse. For instance, the NAcc shell region has been reported to be most responsive to the pharmacological effects of cocaine as well as the conditioned effects of morphine and nicotine (Di Chiara et al., 2004). However, other work has shown that self-administered cocaine increases NAcc DA within the shell and core at comparable levels, whereas cocaine-conditioned stimuli increases NAcc DA in the core but not the shell region (Ito, Dalley, Howes, Robbins, & Everitt, 2000). As can be seen in Figure 3, many of the NAcc-implanted dialysis probes in the present study span both core and shell subregions. Therefore, if significantly greater DA responsiveness is localized within either the core or shell, it is possible that the overall NAcc DA values reported here undervalue maximal DA responses occurring within specific NAcc subregions.

The medial PFC (mPFC) has lower DA density (Javitch, Strittmatter, & Snyder, 1985), higher DA release capacity, and slower DA clearance and metabolism rates than does the NAcc (Cass & Gerhardt, 1995; Garris, Collins, Jones, & Wightman, 1993; Garris & Wightman, 1994; Sharp, Zetterstrom, & Ungerstedt, 1986). These distinct characteristics of the mPFC may allow for idiosyncratic responses to cocaine self-administration. Indeed, we have previously reported mPFC DA levels significantly less elevated than NAcc DA after a single self-administered cocaine injection in naïve rats (Ikegami & Duvauchelle, 2004) that were similar in magnitude with the limited training CS+ groups in the current report. In the present study, PFC DA levels were relatively unresponsive to cocaine-associated cues after limited training but sensitive to these cues after long-term training. Indeed, compared with saline-associated cues, the presence of cocaine-paired cues resulted in significantly greater PFC DA responses when cocaine was self-administered and significantly lower PFC DA levels when saline was received. As indicated above, the enhanced PFC DA responsiveness to cues corresponded with the establishment of preferential responding for cocaine compared with saline. These findings are consistent with evidence showing PFC involvement in learning-related plasticity (Bouret & Sara, 2004; Mulder, Nordquist, Orgut, & Pennartz, 2003) and preferential PFC activation in cocaine-experienced animals (Ciccocioppo et al., 2001; McLaughlin & See, 2003). These data additionally support previous findings of opposed DA neurotransmission in the PFC and NAcc after varied levels of reward experience. For instance, mPFC DA release increases after repeated VTA electrical stimulation whereas NAcc DA decreases under these conditions (Garris et al., 1993). Similarly, repeated food presentation attenuates food-induced NAcc DA release, whereas PFC DA levels remain constant under the same conditions (Bassareo & Di Chiara, 1997).

Effects of Cocaine Self-Administration and Conditioning on Locomotor Activation

The current study also observed that locomotor activity was not influenced by cues after limited training. However, after long-term training, cocaine-stimulated locomotor activity was enhanced in the presence of cocaine-paired cues. The findings from the long-term groups are consistent with previous reports of behavioral sensitization following repeated cocaine administration (Duvauchelle, Ikegami, Asami, et al., 2000; Kalivas & Duffy, 1993; Post, Lockfeld, Squillace, & Contel, 1981). In the present study, however, cocaine-associated cues did not enhance nondrug activity levels (e.g., CS+/saline groups). These findings may appear incompatible with work reporting drug-free enhancement of locomotor activity in cocaine-paired environments (Brown & Fibiger, 1992; Brown, Robertson, & Fibiger, 1992; Burechailo & Martin-Iverson, 1996). However, this apparent disparity may be due to differences between associations produced through Pavlovian-type conditioning (e.g., conditioned place preferences) and associative learning through instrumental conditioning. For example, during Pavlovian-type conditioning procedures, the experimenter commonly administers the cocaine treatment. Therefore, cocaine-conditioned animals may anticipate cocaine but have no control over its delivery. In the absence of cocaine and in the uncertainty about receiving it, cue-induced anticipatory processes can induce excitation and produce increased mesolimbic DA and/or locomotor activity (Brown & Fibiger, 1992; Brown et al., 1992; Burechailo & Martin-Iverson, 1996; Duvauchelle, Ikegami, & Castaneda, 2000; Fontana, Post, & Pert, 1993; Weiss et al., 2000). In contrast, cocaine/cue associations in the current study were produced as the result of instrumental conditioning. Because these circumstances require the animal to press a lever to receive reinforcement, the timing and reward delivery is precisely determined (Schultz & Dickinson, 2000). In relation to the present experiment, it is likely that a lever press followed by saline injection would halt any cue-associated anticipatory responses that may arise after long-term training because the absence of cocaine reward would be immediately detected.

Cocaine Self-Administration/Conditioning, in Vivo Microdialysis, and Neuronal Learning Theory

In vivo voltammetry studies have shown that cue-evoked changes in NAcc DA (Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Stuber, Roitman, Phillips, Carelli, & Wightman, 2005; Stuber, Wightman, & Carelli, 2005) correspond in time with cue-influenced DA neuronal firing (Waelti et al., 2001). Therefore, a relationship between DA neuronal firing rates and NAcc DA responses after limited training in the present study is conceivable. For instance, after limited training, a nonrewarded operant response made in the presence of cues associated with cocaine resulted in a below-basal suppression of NAcc DA levels. This finding is consistent in principle with neuronal learning theory and studies showing decreased DA neuronal firing in response to the absence of a predicted reward (Schultz & Dickinson, 2000; Waelti et al., 2001). This decrease in NAcc DA levels after the unfulfilled expectation of cocaine reward (e.g., animals expecting cocaine but receiving saline; the NAcc/CS+ saline condition) signifies that (a) the conditioned effect detected by in vivo microdialysis is of an enduring nature and (b) cues exert a powerful influence over DA basal levels because DA levels remain suppressed over the several-minute dialysate collection interval. However, complementary effects between in vivo microdialysis and electrochemical and electrophysiological measurements do not necessarily indicate that all methodologies reflect the same aspects of DA responses to conditioned stimuli. For instance, the time resolution of in vivo microdialysis measurements is substantially different from data collected with electrochemical or electrophysiological methods (e.g., minutes versus milliseconds). In fact, the topography of subsecond variations in DA that can be detected through voltammetry is not readily detectable through in vivo microdialysis sampling across minutes. Thus, it could be argued that the long-term changes (e.g., over minutes) revealed by microdialysis and short-term changes (e.g., lasting subsecond-seconds) measured by electrochemical techniques may

reflect two distinct functional aspects of the dopamine system in modulating the conditioned drug effects in the NAcc.

Neuronal learning theory also posits that increased DA firing occurs when positive reinforcement is received in a context predicting its absence (e.g., positive prediction error). By extension, then, cocaine reward received in the presence of cues associated with nonreinforcement corresponds with a positive prediction error, and enhanced DA activity would be expected. However, in the present study, this scenario (e.g., cues associated with saline followed by self-administered cocaine in the NAcc/CS– condition) led to significantly less NAcc DA enhancement, compared with cocaine-associated cues followed by self-administered cocaine (e.g., NAcc/CS+). In other words, a cue-induced expectation of nonreward (or no cocaine) appears to have dampened rather than enhanced the dopaminergic response to cocaine. This finding is consistent in theory with other work indicating that cocaine and natural rewards yield unequal impact and outcomes on behavioral and neural systems (Ciccocioppo, Martin-Fardon, & Weiss, 2004; Robinson, Gorny, Mitton, & Kolb, 2001). Though these particular current findings do not correspond with neuronal learning theory, it may be the case that, as indicated above, in vivo microdialysis measurements in the NAcc reflect a distinctly different aspect of cue-induced DA modulation than what is revealed through neural firing responses to reward prediction errors.

Conclusions

The data presented here show experience-dependent effects of cocaine and associative learning experiences on regional DA activation and behavioral activity. These results resemble human fMRI findings showing dissociations between subcortical and cortical functioning during reward and nonreward anticipation, outcome, and prediction errors (Knutson & Cooper, 2005; Knutson, Fong, Adams, Varner, & Hommer, 2001), and parallel clinical work revealing preferential caudate/ventral striatum activation during motor learning and short-term reward prediction and cortical system activation during cognitive tasks and long-term reward prediction (Koechlin, Danek, Burnod, & Grafman, 2002; O'Doherty, Deichmann, Critchley, & Dolan, 2002; Tanaka et al., 2004). Current findings are also consistent with a recent compilation of data indicating the time course of neural activation during learning initially involves subcortical regions and progresses to prefrontal areas (Laubach, 2005).

As PFC involvement is associated with long-term learning and higher level cognition, it is conceivable that judgment and decision-making errors common to addicts may directly reflect chronic cocaine-induced learning effects on cortical function. Because neurochemical and behavioral responses to self-administered cocaine show a relationship to learning-related neural plasticity, a goal of producing unlearning in patterns of drug-taking and addictive behaviors could be a target of future investigations.

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Figure 1.

NAcc and PFC DA response to self-administered cocaine in the presence of cues associated with cocaine or nonreward. Time line represents dopamine (DA) levels (% of baseline mean $\pm$ SEM) at 10-min intervals, from 30 min before to 30 min after a single self-administered cocaine injection (3.0 mg/kg). Cocaine significantly increased DA levels from baseline under all conditions except limited training PFC/CS-. ++, + = significant difference between different cue conditions (e.g., CS+ vs. CS-) in same brain region at $p < .01$ and .05; **, * = significant difference between brain regions (e.g., NAcc vs. PFC) tested with same cue condition at $p < .01$ and .05. Limited training: The magnitude of cocaine-stimulated NAcc response was significantly greater in the presence of cocaine-associated cues (NAcc/CS+; $n =$

9) compared with nonreinforcement paired cues (NAcc/CS⁻; $n = 9$) and significantly greater than PFC DA responses regardless of presented cues (PFC/CS⁺; $n = 5$ and PFC/CS⁻; $n = 5$). Long-term training: The PFC DA response to a self-administered cocaine injection was significantly greater in magnitude in the presence of cocaine-associated cues (PFC/CS⁺; $n = 5$) compared with cues associated with nonreward (PFC/CS⁻; $n = 4$), and compared with NAcc DA responses under the same cue conditions (e.g., NAcc/CS⁺; $n = 6$). DA responses in the NAcc/CS⁺ group were comparable with those in the NAcc/CS⁻ group ($n = 6$).

Figure 2.

NAcc and PFC DA response to nonreinforced operant responses in the presence of cues associated with cocaine or nonreward. Time line represents DA levels (% of baseline mean $\pm$ SEM) at 10-min intervals, from 30 min before to 30 min after a single nonreinforced operant response (e.g., saline, 0.1 ml). ++, + = significant difference between different cue conditions (e.g., CS+ vs. CS-) tested at same brain region at $p < .01$ and $.05$. Limited training: The NAcc DA response to a nonreinforced operant response in the presence of cocaine-associated cues was significantly depressed (NAcc/CS+; $n = 6$) compared with nonreinforcement in the presence of saline-associated cues (NAcc/CS-; $n = 6$) for the entire 30-min postinjection period. A transient depression in PFC DA levels in the PFC/CS- group ($n = 5$) 20 min

postinjection was significantly different from PFC/CS + group ($n = 6$) at the same time point. Observed DA depressions were significantly different from the first two intervals of respective within-group baseline measurements. Long-term Training: PFC DA in the presence of cocaine-associated cues (PFC/CS+; $n = 6$) was significantly lower than PFC DA levels in the presence of saline-associated cues (PFC/CS-; $n = 5$). There were no significant differences between NAcc DA levels in either cue condition (NAcc/CS-; $n = 5$ and NAcc/CS+; $n = 5$) and both groups dropped significantly below baseline DA levels 20 min after a nonreinforced operant response.

Figure 3.

Histological diagram. Tracings represent active membrane of dialysis probe in the prefrontal cortex (PFC) and nucleus accumbens (NAcc) regions. Solid lines represent traces from animals receiving cocaine on test day, and dashed lines represent those receiving saline at test. Illustrations drawn with assistance from *The Rat Brain in Stereotaxic Coordinates* (3rd ed.), G. Paxinos and C. Watson, 1997, pp. 6–16, with permission from Elsevier. A. PFC: Coronal sections of probe traces in the PFC experiment ranged from +4.20 mm through +3.20 mm anterior to bregma. Active membrane region of dialysis probes were located primarily in prelimbic subterritories of the PFC. B. NAcc: Coronal sections of probe traces in the NAcc experiment ranged from +2.70 mm through +0.48 mm anterior to bregma. Active membrane regions of dialysis probes were located in core and shell subterritories of the NAcc.

Figure 4.

Lever presses during cocaine- and non-reinforced operant sessions for limited (A) and long-term training (B) groups. A. Limited training animals ($n = 51$) elicited fewer lever responses during cocaine-reinforced sessions compared with order-matched sessions when lever presses resulted in saline infusions. B. Animals in long-term training ($n = 38$) showed response patterns similar to those of limited training groups during initial sessions, but increased cocaine-reinforced responding over nonreinforced (saline) levels by the end of the training period. *, ** = significantly greater number of lever presses than order-matched session of alternate reinforcement at $p < .01$ and $.05$, respectively (Fisher's least significant difference).

Figure 5.

Locomotor activity before and after cocaine self-administration and nonreinforced operant responses in the presence of associated cues. Combined locomotor data from animals from NAcc and PFC groups were assessed concurrently with in vivo microdialysis measures. Data points represent photobeam breakage means $\pm$ SEM. Limited training: Cocaine (3.0 mg/kg) resulted in locomotor activity levels significantly greater than baseline in the presence of either cocaine- (CS+/cocaine; $n = 14$) or saline-associated (CS+/cocaine; $n = 14$) cues. A nonreinforced lever press did not alter activity levels from baseline for either cued group (e.g., CS+/saline; $n = 11$ and CS-/saline; $n = 12$). No significant effects of paired cues were detected within the cocaine- or nonreinforced groups. Long-term training: In the presence of cocaine-associated cues, a self-administered cocaine infusion (CS+/cocaine; $n = 11$) resulted in significantly greater locomotor activity than when cocaine was self-administered in the presence of cues associated with nonreward (CS-/cocaine; $n = 10$). ++ = significant difference between cocaine effects under different cue conditions at $p < 0.01$. No significant effects of cues on locomotor activity were observed after a nonreinforced lever response (locomotor data for 2 animals in each cued group were lost during the procedure; CS+/saline; $n = 9$ and CS-/saline; $n = 8$).