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Extracellular fluctuations of dopamine and glutamate in the nucleus accumbens core and shell associated with lever-pressing during cocaine self-administration, extinction, and yoked cocaine administration

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Abstract

Rationale—Dopamine and glutamate in the nucleus accumbens (NAS) are differentially implicated in cocaine-directed behavior.

Objectives—We sought to compare extracellular fluctuations of dopamine and glutamate in core and shell of NAS associated with operant responding during cocaine self-administration, extinction, and yoked cocaine administration.

Methods—Rats were trained to lever-press for cocaine or saline under FR1 before undergoing microdialysis testing during cocaine self-administration, extinction, or yoked cocaine administration. Microdialysis samples were collected every 20 min and were analyzed for dopamine and glutamate with high-performance liquid chromatography.

Result—Rats actively lever-pressed during cocaine self-administration and extinction. However, lever-pressing was minimal during yoked cocaine administration in both cocaine-trained and saline-trained rats. Dopamine was elevated throughout cocaine self-administration and yoked cocaine administration. The extent of cocaine-evoked dopamine was greater in shell than in core, greater in cocaine-trained than in saline-trained rats, and greater during self-administration than during yoked administration. Dopamine was also elevated in core (first 60 min) and in shell (first 40 min) during extinction. Basal concentration of glutamate, but not dopamine, was lower in cocaine-trained than in saline-trained rats. In cocaine-trained rats, glutamate was elevated during cocaine self-administration and extinction but was depressed below baseline during yoked cocaine administration. The extent and direction of glutamate fluctuation was similar between core and shell. In saline-trained rats, glutamate was not affected by yoked cocaine.

Conclusion—Distinct patterns of dopamine and glutamate fluctuations in core and shell appear to underlie characteristic patterns of lever-pressing associated with cocaine self-administration, extinction, and yoked cocaine administration.

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Keywords

Accumbens; Cocaine; Dopamine; Glutamate; Self-administration; Extinction

Introduction

Dopamine in the nucleus accumbens (NAS) is critically implicated in cocaine-directed behavior. Cocaine, administered either contingently (Pettit and Justice 1989) or non-contingently (Di Chiara and Imperato 1988), elevates extracellular dopamine in NAS. The extent of cocaine-evoked dopamine is greater during self-administration than during yoked administration (Hemby et al. 1997; Lecca et al. 2007) and is augmented (sensitized) in animals that previously underwent cocaine self-administration (Zapata et al. 2003). While permanent dopamine depletion of NAS extinguishes cocaine self-administration (Roberts et al. 1977, 1980), low and moderate doses of dopamine antagonists administered systemically (Bergman et al. 1990; de Wit and Wise 1977; Koob et al. 1987) or directly into NAS (Caine et al. 1995; Maldonado et al. 1993; McGregor and Roberts 1993; Suto et al. 2009) increase cocaine intake. The neuroleptic-induced increase in cocaine intake is similar to the effects of lowering drug dose (Yokel and Wise 1976) and thus suggests that dopamine receptor-mediated neurotransmission in NAS underlies the rewarding property of cocaine (Wise 2004).

In addition to dopaminergic afferents from the ventral tegmental area (Swanson 1982), NAS receives glutamatergic inputs from various brain areas, including the prefrontal cortex, amygdala, hippocampus, and thalamus (Christie et al. 1987; Gimenez-Amaya et al. 1995; Meredith et al. 1993). In NAS, glutamate and dopamine afferents converge on medium spiny neurons (Sesack and Pickel 1990) and thus are in a position to interactively regulate striatal outputs (Sesack et al. 2003). Cocaine elevates extracellular glutamate in NAS of animals previously trained to self-administer cocaine but not in naïve rats (Madayag et al. 2007; McFarland et al. 2003; Miguens et al. 2008; but see Reid and Berger 1996; Smith et al. 1995). In a reinstatement paradigm, the ability of cocaine to promote lever-pressing is linked to glutamate elevation in NAS (McFarland et al. 2003). Pharmacological activation of α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors in NAS reinstates extinguished lever-pressing (Cornish et al. 1999; Suto et al. 2004), while blockade of AMPA receptors decreases the priming effects of cocaine (Cornish and Kalivas 2000; Park et al. 2002) and cocaine-associated cues (Backstrom and Hyttia 2007). By contrast, blockade of *N*-methyl-D-aspartate (NMDA) receptors in NAS failed to affect lever-pressing for cocaine (Di Ciano and Everitt 2001; Suto et al. 2009) and cocaine-associated cues (Cornish and Kalivas 2000; Di Ciano and Everitt 2001; but see Backstrom and Hyttia 2007). Therefore, AMPA, rather than NMDA, receptor-mediated neurotransmission in NAS appears to be necessary for cocaine-directed behavior.

Based on its neuroanatomical differences, NAS is sub-divided into “core” and “shell” subregions (Alheid and Heimer 1988). Lever-pressing during cocaine self-administration, which is typically maintained by contingent co-administration of cocaine and cocaine-associated cues, is associated with greater dopamine elevation in shell than in core (Lecca et al. 2007). In the absence of cocaine, non-contingent presentation of cocaine-associated cues reinstates extinguished lever-pressing (Kruzich et al. 2001) and elevates dopamine in core but not shell (Ito et al. 2000). However, contingent presentation of cocaine-associated cues promotes lever-pressing but fails to affect dopamine in either core or shell (Bradberry et al. 2000; Ito et al. 2000; Neisewander et al. 1996). Therefore, further study is necessary to elucidate the association between cocaine-directed behavior and dopamine fluctuation in core and shell. Lever-pressing during cocaine self-administration (Miguens et al. 2008) and

cocaine-primed reinstatement (McFarland et al. 2003; also see Miguens et al. 2008) is associated with glutamate elevation in core, although little is known about the relationship between lever-pressing and glutamate fluctuations in core versus shell during cocaine self-administration and yoked cocaine administration. Similarly, little is known about glutamate fluctuations in core and shell associated with lever-pressing for cocaine-associated cues in the absence of cocaine. Thus, we sought to identify extracellular fluctuations of dopamine and glutamate in core versus shell associated with lever-pressing during cocaine self-administration, extinction, and yoked administration of cocaine.

Materials and methods

The present experiment comprised two phases: self-administration training (10 days) and microdialysis testing (1 day). One hundred seventeen male Long-Evans rats (Charles-River, Raleigh, NC), weighing 350–400 g at the time of the surgery, were used. All experimental procedures were approved by the local Animal Care and Use Committee. Throughout the entire experiment, each rat was housed, trained and tested in a dedicated operant conditioning chamber (Med Associates, Georgia, VT) equipped with two levers (one removable “active” lever and one stationary “inactive” lever) and a liquid swivel system. Upon arrival, rats were randomly assigned to one of ten experimental groups defined by site of microdialysis (core or shell) and experimental condition. There were five experimental conditions defined by different combinations of training (cocaine or saline self-administration) and testing (cocaine self-administration, extinction, yoked cocaine, or yoked saline administration). The ten groups are summarized in Table 1. Each rat was surgically implanted with bilateral guide cannulae (CMA, North Chelmsford, MA) aimed at core or shell and an intravenous catheter (Braintree Science, Braintree, MA) as previously described (Suto et al. 2009). The catheters were flushed daily with 0.05 ml gentamicin (4.0 mg/ml in sterile saline) and 0.05 ml heparin solution (30 U). Six rats did not complete the experiment because of non-patent or leaking catheters.

Rats in six of the ten experimental groups were designated as “cocaine-trained” rats and underwent daily self-administration training for cocaine. The training began 3 days after the surgery and lasted for 10 days. At the beginning of each training session, the active lever was inserted into the operant conditioning chamber, and rats received a priming infusion of cocaine (1.0 mg/kg, i.v.). Rats were then allowed to self-administer cocaine (1.0 mg/kg/infusion, i.v.) under a fixed ratio 1 (FR1) schedule of reinforcement for 4 h. Presses on the active lever delivered cocaine infusions (0.13 ml) and cocaine-associated cues, including cue-light and other stimuli associated with infusion, such as sound of pump motor and peripheral sensation of infusion. Presses on the inactive lever were without scheduled consequence. During a 20-s “time-out” period immediately following a reinforced lever press, the cue-light was illuminated, and active lever presses were ineffective. Rats were required to demonstrate two consecutive days of stable responding (less than 10% day-to-day variability) with a minimum of 30 infusions per session. Five rats failed to achieve these criteria and were excluded. Rats in the remaining four experimental groups were designated as “saline-trained” rats and served as cocaine-naïve controls. These rats were subjected to saline, instead of cocaine, self-administration during the training. Each earned saline infusion was paired with saline-associated cues, including cue-light and other stimuli associated with the infusion.

Upon completion of the last training session, microdialysis probes with 1.0 mm active membrane (CMA/12-14/01) were bilaterally inserted into core or shell and perfused with artificial cerebrospinal fluid at a rate of 2.0 µl/min. On the following day, rats underwent microdialysis testing. In all cases, the testing included two phases: 1-h “baseline” period followed by a 4-h period under an experimental condition. The rats in each cocaine-trained

group were tested under one of three conditions: cocaine self-administration or extinction or yoked cocaine administration. The rats in each saline-trained group were tested under one of two conditions: yoked cocaine administration or yoked saline administration. For the testing under cocaine self-administration, rats were allowed to self-administer cocaine as in the training sessions. For the testing under extinction, a response on the active lever delivered cocaine-associated cues coupled with a saline infusion rather than a cocaine infusion. During yoked cocaine or saline administration, each yoked infusion was delivered with cocaine- or saline-associated cues (depending on the training history of rats) when a cocaine-trained “executive” rat made a reinforcing active lever press for a cocaine infusion. Total numbers of active lever presses per session were recorded and used for statistical analyses. Microdialysis samples were collected every 20 min in plastic vials located outside of each operant conditioning chamber. The samples were analyzed for their dopamine and glutamate contents with high-performance liquid chromatography (Wise et al. 2008). The volumes of samples used for dopamine and glutamate analyses were 20 and 10 μ l, respectively.

After completion of all experimental procedures, rats were anesthetized with sodium pentobarbital. Brains were removed and fixed in 10% formalin. Coronal sections (40 μ m) were mounted onto slides and stained with cresyl violet for verification of probe placements. Figure 1 depicts representative probe placements. Only data obtained from animals with both probes placed within core or shell were retained for statistical analyses. Fifteen animals were excluded from statistical analysis because one or both probes were found to be outside of the targeted area.

The two groups (one core and one shell) that were trained with saline and tested with yoked saline served as the “control” for all other groups to determine significant changes in lever-pressing as well as extracellular fluctuations of dopamine and glutamate. Numbers of active lever presses per 20 min during testing were analyzed using two-way between and one-way within analyses of variance (ANOVA) with site (two levels) and experimental condition (five levels) as the between factors and time (12 levels) as the within factor. Extracellular levels of dopamine and glutamate were expressed as a percentage of baseline values: the mean concentration of the three samples preceding the actual testing was defined as 100%. Extracellular levels of dopamine and glutamate were analyzed with two-way between and one-way within ANOVA with site (two levels) and experimental condition (five levels) as the between factors and time (12 levels) as the within factor. Because earlier reports indicated that basal levels of extracellular glutamate in core are significantly reduced in rats that previously underwent cocaine self-administration (e.g., McFarland et al. 2003), additional analyses were conducted to identify the effects of cocaine self-administration training on basal concentrations of glutamate in core and shell during the testing. For this, the mean concentrations of the three baseline (cocaine-free) samples from the four groups tested with yoked cocaine were analyzed with two-way ANOVA with training (two levels: cocaine or saline self-administration) and site (two levels: core or shell) as the between-group factors. Basal concentrations of dopamine were determined in similar analyses. For all cases, Fisher’s protected least significant difference test was used for planned between-group comparisons, following appropriate ANOVAs. All analyses were conducted with Statistica (1999 version) statistical software.

Results

Lever press

The patterns of active lever-pressing differed according to experimental conditions and time (condition [$F_{4,81}=83.85$, $p<0.001$], time [$F_{11,891}=28.35$, $p<0.001$], and condition $\times$ time [$F_{44,891}=9.60$, $p<0.001$]; Fig. 2a). Because the site of microdialysis did not significantly affect lever-pressing, the data from the core and shell groups were combined per

experimental condition for further analyses and graphic representation. Cocaine-trained rats lever-pressed significantly more during the first 40 min of extinction (Fig. 2a (2): $p<0.05$ – 0.001) compared to cocaine self-administration (Fig. 2a (1)). During yoked cocaine administration, cocaine-trained rats failed to maintain lever-pressing after the first 20 min (Fig. 2a (3)), and saline-trained rats did not lever-press (Fig. 2a (4)). Cocaine-trained rats lever-pressed significantly less during yoked administration than during cocaine self-administration ($p<0.001$: Fig. 2a (1) versus (3)). In fact, 19 out of 22 cocaine-trained rats made less than ten lever presses during the 4-h testing under yoked cocaine administration (the average number $\pm$ SEM of total active lever-pressing was 5.59 ± 1.01).

Dopamine

Extracellular fluctuations of NAS dopamine differed according to experimental conditions, brain sites, and time (condition [$F_{4,81}=151.95$, $p<0.001$], site [$F_{1,81}=14.71$, $p<0.001$], time [$F_{11,891}=10.44$, $p<0.001$], condition $\times$ site [$F_{4,81}=3.22$, $p<0.05$], condition $\times$ time [$F_{44,891}=3.22$, $p<0.001$], site $\times$ time [$F_{44,891}=3.85$, $p<0.001$]: Fig. 2b). Compared to the control groups (i.e., core or shell group trained with saline and tested with yoked saline), dopamine in either core or shell was significantly elevated during cocaine self-administration (Fig. 2b (1): $p<0.001$) as well as during yoked cocaine administration in cocaine-trained group (Fig. 2b (3): $p<0.001$) and in saline-trained group (Fig. 2b (4): $p<0.01$ – 0.001). While no significant fluctuation of NAS dopamine was observed during extinction in previous microdialysis studies (Neisewander et al. 1996; Bradberry et al. 2000), small but significant elevations of dopamine were detected during the beginning of extinction (Fig. 2b (2)): dopamine was significantly elevated in core during the first 60 min ($p<0.01$ – 0.05) and in shell during the first 40 min ($p<0.05$). Because of differences in experimental conditions, including analytical methods for dopamine and schedules of reinforcement for extinction, the discrepancies between the previous negative results and the current positive results cannot readily be explained. However, in the absence of cocaine, small but significant elevations of NAS dopamine also accompany lever-pressing promoted by contextual (i.e., non-contingent) presentations of cocaine-associated cues (Weiss et al. 2000).

Consistent with earlier reports (Lecca et al. 2007), the overall elevation of dopamine (the averages of “%change from baseline” across the 240-min testing) was significantly greater in shell than in core during cocaine self-administration ($p<0.001$) as well as during yoked cocaine administration in cocaine-trained ($p<0.01$) and saline-trained ($p<0.01$) groups. By contrast, the overall levels of dopamine elevation in core versus shell were not significantly different during extinction. Consistent with earlier reports (Hemby et al. 1997; Lecca et al. 2007), the extent of dopamine elevation in either core or shell was significantly greater ($p<0.05$) during cocaine self-administration than during yoked cocaine administration in cocaine-trained groups (Fig. 2b (1) versus (3)). Also, consistent with earlier reports (Zapata et al. 2003), the extent of dopamine elevation during yoked administration in either core or shell ($p<0.01$ – 0.05) was significantly greater (sensitized) in cocaine-trained group than in saline-trained group (Fig. 2b (3) versus (4)). There were no significant effects of training, site, or training $\times$ site interaction on basal levels of extracellular dopamine. The mean concentrations ($\pm$ SEM) of basal dopamine in core and shell of cocaine-trained rats were $1.85 (\pm 0.27)$ and $1.58 (\pm 0.28)$ nM, respectively. The mean concentrations ($\pm$ SEM) of basal dopamine in core and shell of saline-trained rats were $1.67 (\pm 0.36)$ and $1.81 (\pm 0.40)$ nM, respectively.

Glutamate

Extracellular fluctuations of NAS glutamate differed according to experimental conditions and time (condition [$F_{4,81}=11.57$, $p<0.001$], time [$F_{11,891}=3.56$, $p<0.001$], condition $\times$ time

[$F_{44,891}=3.71$, $p<0.001$] but not site (Fig. 2c). The overall levels of glutamate in NAS (the averages of “%change from baseline” in core and shell across the 240-min testing) were significantly elevated during cocaine self-administration (Fig. 2c (1): $p<0.05$) and during extinction (Fig. 2c (2): $p<0.05$). During extinction, glutamate was significantly elevated during the first 120 min of extinction (Fig. 2c (2): $p<0.01-0.05$). During yoked cocaine administration, the overall levels of glutamate were significantly depressed below baseline in cocaine-trained rats (Fig. 2c (3): $p<0.01$) but were not affected in saline-trained rats (Fig. 2c (4)). There was a significant effect of training history ($F_{1,1}=20.54$, $p<0.001$) but no significant effect of site or training $\times$ site interaction on basal glutamate. The origin of elevated levels of glutamate in the current dialysates remains to be determined. However, the current microdialysis technique was presumably sensitive enough to detect synaptic fluctuations of glutamate because glutamate elevation measured by a similar microdialysis procedure in the ventral tegmental area is tetrodotoxin-sensitive (You et al. 2007). Basal levels of extracellular glutamate in either core or shell were significantly lower in rats trained to self-administer cocaine compared to rats trained to self-administer saline ($p<0.01$). The mean concentrations ($\pm$ SEM) of basal glutamate in core and shell of cocaine-trained rats were $2.76 (\pm 0.54)$ and $3.05 (\pm 0.61)$ μ M, respectively. The mean concentrations ($\pm$ SEM) of basal glutamate in core and shell of saline-trained rats were $5.57 (\pm 0.35)$ and $5.48 (\pm 0.65)$ μ M, respectively. Basal levels of extracellular glutamate in NAS as measured by microdialysis are sensitive to blockade of cystine–glutamate antiporter and voltage-dependent Ca^{2+} channels (Baker et al. 2002) and thus likely reflect glutamate in both extrasynaptic and synaptic spaces.

Discussion

The current study compared extracellular fluctuations of dopamine and glutamate in core and shell associated with lever-pressing during cocaine self-administration, extinction, and yoked cocaine administration. Dopamine in core and shell were each elevated during cocaine self-administration and yoked cocaine administration. The extent of cocaine-evoked dopamine was greater in shell than in core, greater in rats trained to self-administer cocaine than in rats trained to self-administer saline, and greater during self-administration than during yoked administration. Because a small but significant elevation of dopamine was observed in both core and shell during the first 40–60 min of extinction, it is clear that cocaine-associated cues come to elevate NAS dopamine. Basal concentrations of extracellular glutamate, but not dopamine, in both core and shell were reduced in cocaine-trained rats. During both cocaine self-administration and extinction, however, glutamate in core and shell were elevated. During yoked cocaine administration, glutamate in core and shell were depressed in cocaine-trained but not saline-trained rats. Since the direction of glutamate fluctuation was not dependent on cocaine, NAS glutamate appears to be under the control of cocaine-associated cues rather than cocaine itself. The distinctive patterns of dopamine and glutamate fluctuation in NAS presumably underlie the different patterns of lever-pressing associated with cocaine self-administration, extinction, and yoked cocaine administration.

One possible concern for non-specialists might be the possibility that dialysates collected from core and shell might not be specific to core and shell. Microdialysis is known to collect analytes from more than a 0.25-mm radius from the probe (Borland et al. 2005), and this determination was made with a lower flow rate (0.54 μ l/min) than was used in the present study (2.0 μ l/min). Because of slower clearance of analyte inside the membrane, this would thus underestimate the reach of microdialysis in the present study. However, the concentration gradient in the tissue surrounding the dialysis probe (caused by the zero concentration of analytes *within* the probe) falls off with the square of the distance from the probe surface. Thus, while dialysis with a probe in the shell will always collect analytes

from the adjacent core, the contribution of distal regions is necessarily much weaker—geometrically weaker—than the contribution from tissue close to the probe.

Cocaine-trained rats failed to maintain lever-pressing throughout the current yoked cocaine administration despite the fact that dopamine levels were elevated by the yoked injections in both core and shell and for the duration of each session. While initial yoked injections effectively “primed” the lever-pressing response, their ability to do so was extinguished as sessions continued. That the effectiveness of the yoked injections in instigating or maintaining lever-pressing in cocaine-trained animals was lost—extinguished—when the contingency between lever-pressing and the injections was randomized by the yoked procedure appears to confirm the suggestion that the role of dopamine in motivating responding (“wanting”) is dependent upon the role of dopamine in reinforcing responding (Wise 2004). When the response contingency that is required for training animals to self-administer cocaine is broken, the self-administration habit and the ability of cocaine to “prime” the habitual response extinguishes.

In the current study, basal extracellular levels of NAS glutamate were lower in rats trained to self-administer cocaine than in rats trained to self-administer saline. This reduction in basal glutamate is consistent with earlier reports (McFarland et al. 2003; Miguens et al. 2008) and is likely due to decreased function of cystine–glutamate exchange (Baker et al. 2003) which is thought to underlie the ability of cocaine and cocaine-associated cues to elevate glutamate in NAS and to promote cocaine-directed behavior (Kalivas 2009). Consistent with this view, NAS glutamate was elevated during cocaine self-administration in cocaine-trained rats but was not affected during yoked cocaine administration in saline-trained rats in the current study. However, in rats trained to self-administer cocaine, the current yoked cocaine administration (i.e., multiple non-contingent injections of cocaine with cocaine-associated cues) depressed NAS glutamate below baseline, while a single non-contingent injection of cocaine is sufficient to elevate glutamate in core and to promote lever-pressing (McFarland et al. 2003; Madayag et al. 2007; Miguens et al. 2008). Thus, single and multiple cocaine injections produce fundamentally different conditions. For example, despite reduced levels of basal glutamate, cocaine-induced elevation in NAS glutamate is observed only in rats previously given single injections (Baker et al. 2003; Pierce et al. 1996), but not yoked administration (McFarland et al. 2003), of cocaine. It might also be noted that lever-pressing during the current yoked administration was without any scheduled consequence and was not maintained. By contrast, in the absence of cocaine, contingent presentation of cocaine-associated cues during the current extinction was sufficient to elevate NAS glutamate and to promote lever-pressing. Similarly, in the absence of cocaine, cocaine-associated cues sufficiently elevate NAS glutamate and promote locomotor activity under a Pavlovian conditioning paradigm (Hotsenpiller et al. 2001). Moreover, both reduced levels of basal glutamate (Bell et al. 2000; Hotsenpiller et al. 2001) and cocaine-induced glutamate elevation (Bell et al. 2000) in NAS are observed in a cocaine-associated, but not cocaine-unassociated, environment. Taken together, these findings suggest that cocaine-associated cues, rather than cocaine itself, control glutamate fluctuations in NAS and promote cocaine-directed behavior.

Extending an earlier report on glutamate fluctuations in core (Miguens et al. 2008), in the current study, extracellular glutamate in core and shell of cocaine-trained rats was elevated during both cocaine self-administration and extinction when lever-pressing was actively engaged but was depressed below baseline during yoked cocaine administration when lever-pressing was minimal and apparently suppressed. Thus, elevation and depression of NAS glutamate appear to predict, respectively, activation and suppression of cocaine-directed behavior in rats that previously underwent operant conditioning for cocaine. Paralleling the current results, the ability of cocaine to promote lever-pressing is associated with glutamate

elevation in core (McFarland et al. 2003; Miguens et al. 2008) and is attenuated by glutamate, but not dopamine, antagonists in NAS (Cornish and Kalivas 2000; McFarland and Kalivas 2001), while both dopamine and glutamate *agonists* in NAS reinstate extinguished lever-pressing for cocaine (Cornish et al. 1999; Cornish and Kalivas 2000). The ability of intra-NAS injection of dopamine to reinstate lever-pressing is also attenuated by local glutamate antagonists (Cornish and Kalivas 2000).

Although the direction and extent of glutamate fluctuations in the two subregions of NAS were similar under the same experimental procedure in the current study, there appear to be functional differences between core and shell glutamate for cocaine-directed behavior. For example, similar to the effects of excitotoxic lesion (Ito et al. 2004) and γ -amino butyric acid-induced inactivation (Fuchs et al. 2004; McFarland and Kalivas 2001) of core, but not shell, on cocaine-directed behavior, AMPA antagonists in core, but not shell, reduce lever-pressing for cocaine (Di Ciano and Everitt 2001; Suto et al. 2009) and cocaine-associated cues (Backstrom and Hyttia 2007; Cornish et al. 1999; Cornish and Kalivas 2000). In contrast, NMDA agents in neither core or shell affect lever-pressing for cocaine (Di Ciano and Everitt 2001; Suto et al. 2009) and cocaine-associated cues (Cornish et al. 1999; Di Ciano and Everitt 2001; but see Backstrom and Hyttia 2007). Thus, AMPA-receptor-mediated glutamatergic neurotransmission in core, rather than shell, appears to mediate cocaine-directed behavior.

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Fig. 1.

Cannulae and probe placements. Rats received bilateral guide cannulae and microdialysis probes aimed at either shell or core. The shell cannulae were inserted from the opposite hemisphere through the midline and angled at 160° toward the midline to avoid ventricles and to minimize the diffusion of dopamine and glutamate from the core to the probe. In order to avoid collision of the bilateral cannulae into the shell, two different coordinates were used: (1) +1.2 mm anterior, ± 1.2 mm lateral to bregma, and -8.4 mm from skull; (2) +1.7 mm anterior, ± 1.0 mm lateral to bregma, and -8.0 mm from skull. The two coordinates for the shell were counterbalanced between the left and right hemispheres. For consistency, two different coordinates were also utilized and counterbalanced between the left and right hemispheres of rats for core: (1) +1.2 mm anterior, ± 1.6 mm lateral to bregma, and -7.8 mm from skull; (2) +1.7 mm anterior, ± 1.6 mm lateral to bregma, and -7.4 mm from skull. The core cannulae were angled at 10° toward the midline. All cannulae were positioned at 1.0 mm above the tip of probes. **a** Bars indicate the representative locations and extent of the active portion (1.0 mm) of microdialysis membrane in core (*solid bars*) and shell (*hatched bars*). In order to illustrate the actual extents of microdialysis, the width of the probes are fit to scale. Line drawings were adapted from Paxinos and Watson (1998). The *numbers* indicate the distance (mm) posterior to the bregma. **b** Photomicrograph of representative tracks of shell placements. The brain section, while not clean, was selected to show the tracks of bilateral “cross-hemisphere” placements to shell in a section. The two cross-hemisphere placements were with different anterior/posterior coordinates. The foreground track (marked by two black arrows) belongs to a “posterior” placement, while the background track (marked by two white arrows) belongs to an “anterior” placement. For this section, the “posterior” placement to shell entered from the left hemisphere, crossed the midline, and terminated in the right hemisphere, while the “anterior” placement entered

from the right hemisphere, crossed the midline, and terminated in the left hemisphere. **c** Higher magnification of the bilateral shell placements depicted in **b** showing relatively healthy tissue adjacent to the active probe membranes. The foreground track (*marked by two black arrows*) belongs to the “posterior” placement, while the background track (*marked by two white arrows*) belongs to the “anterior” placement

Fig. 2.

Lever-pressing as well as extracellular fluctuations in dopamine and glutamate in core and shell during cocaine self-administration, extinction and yoked cocaine administration. **a** The mean ($\pm$ SEM) number of lever presses (per 20 min) during cocaine self-administration (**a** (1)), extinction (**a** (2)), as well as during yoked cocaine administration in cocaine-trained (**a** (3)) and saline-trained (**a** (4)) rats (*solid bars*). These numbers are plotted against the mean ($\pm$ SEM) number of lever presses during yoked saline administration in saline-trained rats (*open bars*). **b** The mean ($\pm$ SEM) percent change from baseline in extracellular dopamine in core (*solid circle*) and shell (*solid triangle*) during cocaine self-administration (**b** (1)), extinction (**b** (2)), as well as during yoked cocaine administration in cocaine-trained (**b** (3)) and saline-trained (**b** (4)) rats. These changes are plotted against the mean ($\pm$ SEM) percent change in dopamine in core (*open circle*) and shell (*open triangle*) during yoked saline administration in saline-trained rats. **c** The mean ($\pm$ SEM) percent change from baseline in extracellular glutamate in core (*solid circle*) and shell (*solid triangle*) during cocaine self-administration (**c** (1)), extinction (**c** (2)), as well as during yoked cocaine administration in cocaine-trained (**c** (3)) and saline-trained (**c** (4)) rats. These changes are plotted against the mean ($\pm$ SEM) percent change in glutamate in core (*open circle*) and shell (*open triangle*) during yoked saline administration in saline-trained rats. $N=7-12$ /group

Table 1

Summary of ten experimental groups

Site (2 levels)	Experimental condition (5 levels)		N
	Training	Testing	
Core	Cocaine	Cocaine self-administration	9
Core	Cocaine	Extinction	9
Core	Cocaine	Yoked cocaine	12
Core	Saline	Yoked cocaine	10
Core	Saline	Yoked saline	8
Shell	Cocaine	Cocaine self-administration	9
Shell	Cocaine	Extinction	9
Shell	Cocaine	Yoked cocaine	10
Shell	Saline	Yoked cocaine	8
Shell	Saline	Yoked saline	7

The ten groups were defined by *site* (two levels: core or shell) of microdialysis *testing* and experimental *condition* (five levels). The five experimental conditions were defined by different combinations of *training* (cocaine or saline self-administration) and *testing* (cocaine self-administration, extinction, yoked cocaine, or yoked saline). The numbers (*N*) indicate final numbers of rats in each experimental group retained for statistical analyses