Neuron, Vol. 46, 661–669, May 19, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.neuron.2005.04.036

Extinction of Cocaine Self-Administration Reveals Functionally and Temporally Distinct Dopaminergic Signals in the Nucleus Accumbens

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Summary

While Pavlovian and operant conditioning influence drug-seeking behavior, the role of rapid dopamine signaling in modulating these processes is unknown. During self-administration of cocaine, two dopaminergic signals, measured with 100 ms resolution, occurred immediately before and after the lever press (termed pre- and postresponse dopamine transients). Extinction of self-administration revealed that these two signals were functionally distinct. Preresponse transients, which could reflect the motivation to obtain the drug, did not decline during extinction. Remarkably, postresponse dopamine transients attenuated as extinction progressed, suggesting that they encode the learned association between environmental cues and cocaine. A third type of dopamine transient, not time locked to overt stimuli, decreased in frequency during extinction and correlated with calculated cocaine concentrations. These results show that dopamine release transients involved in different aspects of cocaine self-administration are highly plastic—differentially governed by motivation, learned associations linked with environmental stimuli, and the pharmacological actions of cocaine.

Introduction

Dopaminergic neurons originating in the ventral tegmental area and projecting to the amygdala, prefrontal cortex, and nucleus accumbens (NAc) are hypothesized to relate information about a primary reinforcer, such as cocaine, with goal-directed behavior and/or environmental stimuli (McClure et al., 2003; Schultz, 2004). This idea is supported by electrophysiological studies in behaving animals, which have demonstrated that dopaminergic neurons are initially activated by primary reinforcers but adapt over repeated pairings to respond to conditioned stimuli that are associated with the reinforcer (Schultz, 1998). Furthermore, the degree of phasic activity of dopamine neurons is a function of the probability in which a conditioned stimulus predicts a subsequent reward (Fiorillo et al., 2003), suggesting that dopaminergic transmission can adapt to changing degrees of uncertainty of which a cue can predict a reward.

Virtually all drugs of abuse cause the release of dopamine in the NAc (Di Chiara and Imperato, 1988; Wise, 2004), which can then become associated with neutral environmental stimuli. Because of this, dopaminergic transmission within the NAc may promote the formation of an unnatural relationship between drugs of abuse and environmental cues (O'Brien et al., 1993; Everitt et al., 1999; Hyman and Malenka, 2001; Wise, 2004). However, because cocaine has a potent pharmacological effect to inhibit monoamine uptake (Jones et al., 1995; Wu et al., 2001), it has been difficult to separate increases in dopamine due to either the primary pharmacological or secondary conditioned effects of the drug. Microdialysis studies in self-administrating animals have shown that NAc dopamine decreases over minutes leading up to a lever press for cocaine and then increases slowly after the drug infusion (Wise et al., 1995). When dopamine changes are examined with greater temporal resolution, electrochemical studies have revealed phasic changes in dopamine (lasting only a few seconds), which are time locked to the operant response for the drug (Phillips et al., 2003b; Stuber et al., 2005). Consistent with this, subsequent exposure to drug-associated cues led to an increase of dopamine within the NAc (Ito et al., 2000; Phillips et al., 2003b) and can lead to the relapse of drug taking, thus playing an important role in the manifestation and maintenance of addiction (Weiss et al., 2001; Crombag et al., 2002).

While reward-related learning utilizes dopaminergic transmission to associate environmental stimuli with a primary reinforcer (Schultz, 1998; Ito et al., 2000), the precise role that dopaminergic signaling plays during drug self-administration remains unknown. To address this, cyclic voltammetry with 100 ms temporal resolution was used to monitor rapid dopamine release during regular cocaine self-administration (maintenance), extinction, and reinstatement. The superb temporal resolution of this technique revealed that dopaminergic signals play three temporally and functionally distinct roles that are differentially influenced by extinction.

Results

Cocaine Self-Administration during Extinction/Reinstatement

Intravenously catheterized rats were initially trained to self-administer cocaine (0.33 mg/infusion; delivered over 6 s) in 2 hr daily sessions and were surgically prepared for monitoring rapid dopamine signaling in the NAc core after 2–3 weeks of training. Following recovery from surgery and 3–5 days of retraining, rats underwent a within-session extinction/reinstatement experiment conducted in three phases. In phase one (termed maintenance), rats completed two to four lever press responses in quick succession followed by five lever presses with mean interinfusion intervals of 439 ± 33 s. During maintenance, each lever press resulted in an intravenous cocaine infusion (0.33 mg, 6 s) paired with drug-associated cues (20 s). Lever pressing behavior remained stable throughout maintenance, with no

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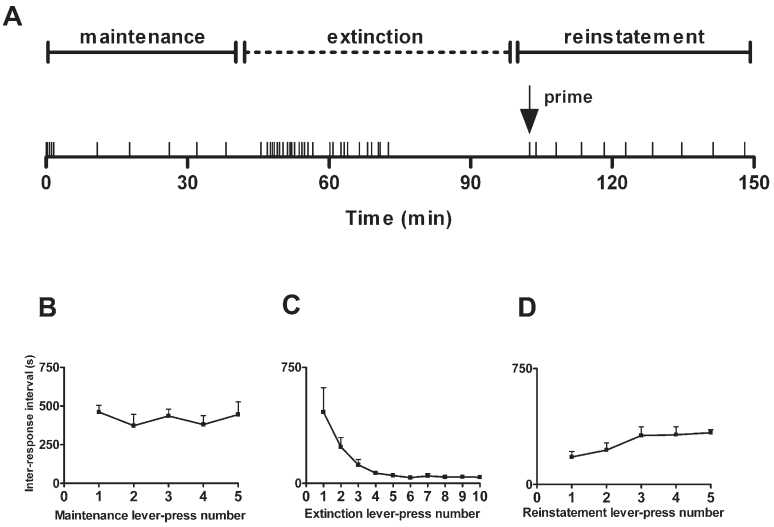

Figure 1. Lever Press Behavior during Maintenance, Extinction, and Reinstatement of Cocaine Self-Administration

(A) Representative behavioral response record from one animal throughout the three experimental phases. Each vertical tick mark represents a lever press response. The animal was reinstated to self-administer cocaine after extinction by issuing a noncontingent "priming" infusion of cocaine paired with a 20 s presentation of the drug-associated cues (indicated by the arrow).

(B–D) Interresponse intervals for lever presses in maintenance, extinction, and reinstatement.

significant difference in the interresponse interval over the trials [F(4, 39) = 0.48; p = 0.74; Figures 1A and 1B]. In phase two (extinction), saline was substituted for cocaine. Each lever press resulted in saline infusion (6 s) paired with the drug-associated cues (20 s). This led to a dramatic increase in the rate of responding measured over the first ten trials of extinction [F(9, 79) = 6.2; $p \leq 0.0001$; Figures 1A and 1C] and the eventual cessation of responding after 20.3 ± 3.0 trials. Following 30 min of no responding, phase three (reinstatement) was initiated. Specifically, self-administration behavior was reestablished by a noncontingent intravenous infusion of cocaine (0.33 mg, 6 s) paired with the same drug-associated stimuli. During the reinstatement phase, rats were allowed to respond five times, in which each response resulted in cocaine paired with the drug-associated cues, as in maintenance. Response rates decreased and eventually stabilized over the five reinstatement trials [F(4, 39) = 3.1; $p < 0.05$; Figure 1D].

Phasic Dopamine Signals Are Associated with the Operant Response during Maintenance

Phasic dopamine time locked to a single operant response during maintenance is shown in Figure 2A. As reported previously (Phillips et al., 2003b; Stuber et al., 2005), dopaminergic signals at the lever press in maintenance have two distinct components: an initial increase in dopamine in the time interval 10 s before the lever press response (termed preresponse) followed by a larger increase within 10 s after the response (termed postresponse). The mean preresponse dopaminergic signal was 52.6 ± 6.2 nM and remained stable in amplitude from trial to trial during the maintenance phase [F(4, 39) = 0.57; p = 0.73; Figure 2B]. Similarly, the mean postresponse dopaminergic signal was 74.4 ± 5.8 nM and also did not significantly change in amplitude throughout the maintenance phase [F(4, 39) = 0.31; p = 0.75; Figure 2C].

Extinction of Cocaine Self-Administration Reveals a Functional Dissociation between Preresponse versus Postresponse Dopamine Signaling

We have previously shown that preresponse dopamine transients promote cocaine seeking, while postresponse dopamine signaling relates to the association of lever press responding and the cues that predict cocaine delivery (Phillips et al., 2003b). Therefore, we hypothesized that the preresponse phasic dopamine signal would remain during extinction, while the postresponse dopamine signal would attenuate as the learned association between the reinforcer and the drug-associated cues decreased across extinction trials. For analysis purposes, extinction was broken up into early (first ten) and late (last five) lever presses. Figure 3A shows a single trial in early extinction. Even though each lever press resulted in a saline infusion paired with the drug-associated cues, both the pre- and postresponses were associated with the lever press. A repeated measures ANOVA revealed that preresponse DA transients remained remarkably stable in amplitude throughout early extinction and were not significantly different relative to the maintenance phase [F(10, 87) = 0.83; p = 0.60; Figure 3B]. In contrast, the amplitude of postresponse dopamine transients was maintained during early extinction (Figures 3A and 3C) but significantly declined as extinction proceeded [repeated measures ANOVA; F(10, 87) = 2.44; $p \leq 0.05$; Figure 3C]. Post hoc comparisons revealed that the postresponse dopamine signal on trial 10 was significantly less than the average dopamine signal during the maintenance phase ($p \leq 0.05$).

As the extinction phase proceeded, a clear dissociation between pre- and postresponse dopamine transients became apparent. Figure 3D shows a single lever press response (from the same animal shown in Figure 3A) during late extinction. A dopamine preresponse is evident; however, there is no significant dopamine postresponse. A repeated measures ANOVA revealed that the amplitude of the preresponse dopamine transients during the last five trials of extinction did not significantly decrease relative to maintenance [F(5, 47) = 1.55; p = 0.20; Figure 3E]. In contrast, the amplitude of the postresponse signal was significantly attenuated during the last five trials of extinction relative to postresponses during maintenance [F(5, 47) = 14.89; $p \leq 0.0001$; Figure 3F].

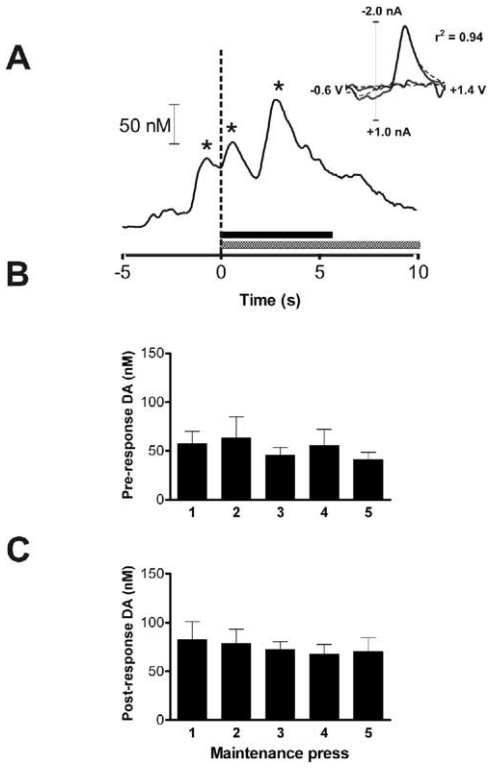

Figure 2. Phasic Dopamine in the NAc Time Locked to the Operant Response during the Maintenance Phase

(A) Pre- and postresponse dopamine associated with the operant response for a single trial. The lever press response occurs at t = 0, the black bar indicates the duration of the 0.33 mg cocaine infusion, and the shaded bar indicates the presentation of the drug-associated cues (lasts for 20 s after the lever press response). Asterisks indicate signals that are dopamine. Inset shows the cyclic voltammogram from the maximum dopamine change (solid line) compared to the voltammogram for electrically evoked dopamine release from the same animal. Linear regression analysis revealed a high correlation ($r^2 = 0.94$) between the voltammograms, indicative that the measured signal was dopamine.

(B and C) Mean (±SEM) preresponse (B) or postresponse (C) dopamine concentrations during maintenance.

Linear regression analysis across all animals (n = 8) and all trials of the session revealed no significant correlation with the amplitude of postresponse time-locked transients and calculated cocaine concentrations [$r^2 = 0.01$; $F(1, 136) = 1.4$; $p = 0.24$; slope not significantly different from zero]. The brain cocaine concentration was calculated using the method of Pan et al. (1991). Similarly, changes in the amplitude of preresponse dopamine transients were also not correlated with calculated brain cocaine concentrations [$r^2 = 0.02$; $F(1, 132) = 3.1$; $p = 0.08$; slope not significantly different from zero].

Postresponse Dopamine Transients Become Reassociated with the Operant Response during Reinstatement

After 30 min without lever press responding, cocaine self-administration was reestablished by noncontingent intravenous infusion of 0.33 mg cocaine paired with the drug-associated cues (reinstatement). Figure 4A shows that dopamine was again associated with the operant response during reinstatement. A repeated measures ANOVA revealed no significant change in the amplitude of the preresponse dopamine transients during reinstatement relative to maintenance or extinction [$F(6, 55) = 0.68$; $p = 0.66$; Figure 4B], further demonstrating that the preresponse is variable, but persistent. In contrast, the postresponse dopamine transients, while attenuated during extinction, reemerged by the second trial of the reinstatement phase. A repeated measures ANOVA revealed a significant main effect of trial number on the postresponse dopamine amplitude during reinstatement [$F(7, 63) = 4.61$; $p \leq 0.0005$; Figure 4C]. Post hoc tests showed that the postresponse dopamine transients were significantly lower during the "priming" infusion and during the first lever press of reinstatement relative to the average dopamine concentration during the maintenance phase.

"Spontaneous" Dopamine Transients Correlate with Calculated Brain Cocaine Concentrations

While dopamine transients are consistently associated with each operant response during maintenance and reinstatement (Figures 2 and 4), we have also identified another aspect of dopamine signaling: spontaneous dopamine transients, which are not associated to any overt behavior (e.g., lever press) or environmental stimuli. These transients occur randomly and increase in frequency upon application of cocaine (Stuber et al., 2005). Examples of spontaneous dopamine transients occurring during each of the three experimental phases are shown in Figures 5A–5C. Across all rats (n = 8), the frequency of spontaneous dopamine transients was 5.6 ± 1.7 min^{-1}, 1.8 ± 0.4 min^{-1}, 3.2 ± 0.8 min^{-1} in maintenance, extinction, and reinstatement, respectively [$F(2, 23) = 5.6$; $p \leq 0.05$]. To determine whether falling cocaine levels during extinction correlated with the attenuation of the postresponse (see above) or spontaneous dopamine transients, brain cocaine levels were computed using the model of Pan et al. (1991). This model revealed that calculated cocaine levels fluctuated little during maintenance and reinstatement and fell exponentially during extinction (results from one animal are shown in Figure 5D; dopamine transient frequency across the three phases is shown for the same animal in Figure 5E).

During extinction, calculated cocaine levels fall (Figure 6A). At the same time, the frequency of spontaneous dopamine transients also decline (Figure 6B). Indeed, linear regression analysis (Figure 6C) revealed a highly significant correlation between the rate of spontaneous dopamine transients and calculated brain cocaine levels during extinction across all animals ($r^2 = 0.93$; $p \leq 0.0001$).

The Decrease in Spontaneous Dopamine Transient Frequency Precedes the Increase in Lever Pressing and Activity during Extinction

Analysis of locomotor activity during extinction, which in this study corresponded with the rate of lever pressing, was conducted to determine where in time the decrease in spontaneous dopamine transient frequency

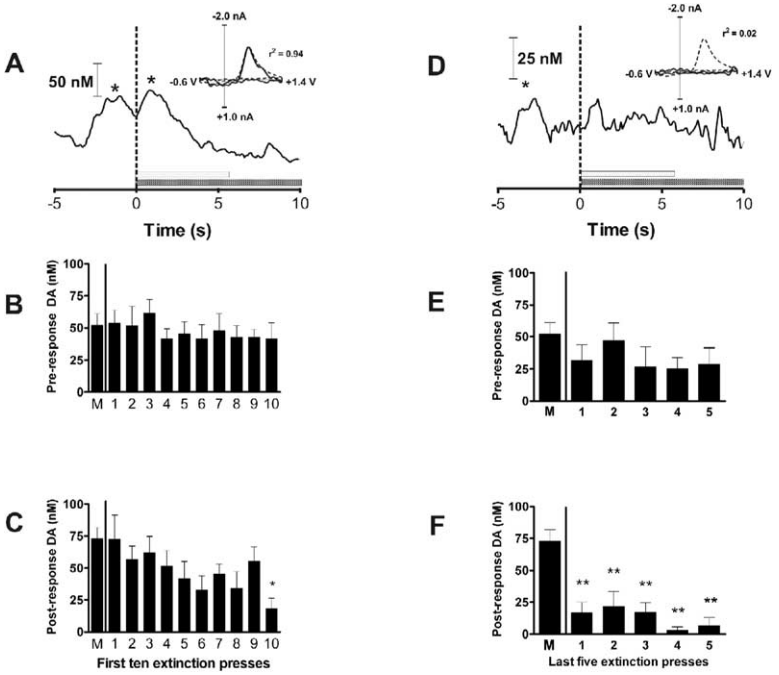

Figure 3. Phasic Dopamine in the NAc within ±10 s of the Operant Response during Early and Late Extinction of Cocaine Self-Administration

Early extinction: (A) Pre- and postresponse dopamine time locked to the operant response for a single trial in early extinction. The lever press response occurs at t = 0, the clear bar indicates the duration of the saline infusion, and the shaded bar indicates the presentation of the drug-associated cues (lasts for 20 s after the lever press response). Asterisks indicate signals that are dopamine. Inset shows the cyclic voltammogram at the maximal dopamine response (solid line) compared to the voltammogram for electrically evoked dopamine release from the same animal. Linear regression analysis revealed a high correlation ($r^2 = 0.94$) between the voltammograms, indicative that the measured signal was dopamine. (B and C) Mean (±SEM) preresponse (B) or postresponse (C) dopamine concentrations during the first ten trials of extinction compared to maintenance ("M"). Late extinction: (D) Lack of postresponse dopamine time locked to the operant response for a single trial in late extinction from the same animals shown in (A). Asterisks denote that the cyclic voltammograms for the preresponse were ≥ 0.75. Inset voltammograms, taken less than 1 s after the lever press response, show little similarity to that of electrically evoked dopamine release, indicative that the measured signal is not dopamine. (E and F) Mean (±SEM) preresponse (E) or postresponse (F) dopamine concentrations during the last five trials of extinction compared to maintenance ("M"). *$p \leq 0.05$, **$p \leq 0.01$ compared to maintenance.

occurs relative to the increase in behavioral responding. While individual spontaneous dopamine transients do not appear to be time locked to specific behavioral or environmental stimuli, the decrease in overall transient frequency occurs before locomotor activity increases. Figure 6B shows that spontaneous dopamine transient frequency is maximal at 4 min into extinction, while behavioral activity is maximal at 8 min (Figure 6D).

Discussion

The present data demonstrate that phasic dopamine signals in the NAc play three distinct roles as revealed by their variation during the maintenance, extinction, and reinstatement of cocaine self-administration. With respect to the lever press response, two distinct signals are observed: preresponse and postresponse dopamine transients. The preresponse transients are variable in amplitude but persist over the course of all three phases of the experiment. In contrast, postresponse dopamine transients were stable during the maintenance phase, decreased in amplitude during extinction, and were reassociated with the lever press during reinstatement. A third mode of signaling, an increased frequency of spontaneous dopamine transients, was observed when calculated brain cocaine concentrations were high. Their frequency declined during extinction and was restored during reinstatement. These spontaneous transients correlated well with calculated brain cocaine levels, while the pre- and postresponse dopamine transients did not. Thus, these dopamine transients, revealed by fast-scan cyclic voltammetry, reflect both conditioned and pharmacological actions.

Phasic Dopamine Time Locked to the Operant Response during Maintenance, Extinction, and Reinstatement

Pre- and postresponse dopamine transients serve distinct functions within the NAc (Phillips et al., 2003b). Electrically evoked dopamine transients during a cocaine self-administration session induce the animal to press the lever within a similar time period in which the preresponse dopamine transients occur, suggesting that transients before the lever press promote the goal-directed behavior or seeking of the drug (Phillips et al., 2003b). Consistent with this idea, preresponse transients remain intact and linked with lever pressing throughout the extinction phase.

While the preresponse transients may promote cocaine seeking, the postresponse dopamine transients may encode for learned associations between the drug-associated stimuli and cocaine. Previous studies (Ito et al., 2000; Phillips et al., 2003b) have demonstrated that NAc dopamine increases upon noncontingent presentation of drug-associated cues and that inactivation of either the ventral tegmental area or the NAc core (Di Ciano and Everitt, 2004) can reduce responding for cocaine-associated stimuli. Consistent with this, postresponses occur during the initial phase of extinction (Figure 3C) even though cocaine is withheld. As extinction progressed, the postresponse amplitudes decreased but at a different rate than the increase in lever pressing behavior. This shows that the postresponse transients do not linearly encode for the behavior but instead reflect a conditioned response (i.e., related to operant and/or classical conditioning aspects of the self-administration task).

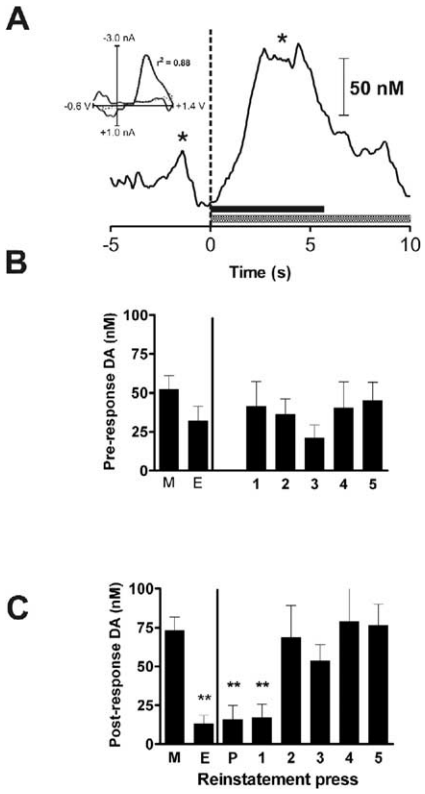

Figure 4. Phasic Dopamine in the NAc within ±10 s of the Operant Response during Reinstatement of Cocaine Self-Administration

(A) Pre- and postresponse dopamine time locked to the operant response for a single trial during reinstatement. The lever press response occurs at t = 0, the black bar indicates the duration of the 0.33 mg cocaine infusion, and the shaded bar indicates the presentation of the drug-associated cues (lasts for 20 s after the lever press response). Asterisks indicate signals that are dopamine. Inset shows the cyclic voltammogram from the trial (solid line) compared to the voltammogram for electrically evoked dopamine release from the same animal. Linear regression analysis revealed a high correlation ($r^2 = 0.88$) between the voltammograms, indicative that the measured signal was dopamine.

(B and C) Mean (±SEM) preresponse (B) or postresponse (C) dopamine concentrations during the five trials of reinstatement, the average concentration during late extinction ("E"), or the reinstating prime ("P"), compared to maintenance ("M"). **$p \leq 0.01$ compared to maintenance.

It is important to note that a clear dissociation exists between pharmacological levels of cocaine and the occurrence of postresponse dopamine transients. First, modeled cocaine concentrations did not correlate with the amplitude of these events. Second, examination of Figure 4C shows that no postresponse transients were observed during late extinction, following the cocaine prime, and relative to the first lever press during reinstatement. However, calculated cocaine levels are dramatically different at these time points (see Figure 5D). Finally, during early extinction (by trial 4) calculated cocaine concentrations were similar to levels modeled during the first lever press in reinstatement, yet postresponse dopamine transients were still observed at this point in extinction (Figure 3C), as opposed to their absence during trial 1 of reinstatement (Figure 4C). Collectively, these findings support the view that the postresponse dopamine transients reflect conditioned as opposed to pharmacological actions of cocaine.

The data here are also consistent with the hypothesized role of dopaminergic signaling as a prediction error signal (Schultz et al., 1997; McClure et al., 2003; Redish, 2004). Phasic activation of dopaminergic neurons is determined by how well a stimulus predicts the reward (Fiorillo et al., 2003). Here, as extinction progressed, both the probability that the drug-associated cues predict cocaine delivery and the postresponse dopamine transient amplitude decreased. Importantly, the dynamic decrease in postresponse dopamine signaling during extinction reported here is exactly the response predicted by temporal difference reinforcement learning models for learning-associated signals (Schultz et al., 1997; Redish, 2004). Likewise, the dissociation between the drug-associated cues and cocaine delivery may explain why no significant increase in dopamine is observed relative to the priming infusion of cocaine paired with drug-associated cues following extinction. At this time, the probability that the cues predict cocaine delivery is the lowest. While the present findings demonstrate that time-locked DA responses are highly plastic over short time periods, future studies are necessary to determine whether these signals are modulated with other more long-duration drug-seeking paradigms that mirror human drug withdrawal and relapse.

Spontaneous Dopamine Transients Are Induced by the Pharmacological Effects of Cocaine

While two different types of dopamine signals occurred that are associated with the operant response for cocaine, spontaneous dopamine transients occurred randomly in time, but their frequency correlated with calculated cocaine levels. Because they occur in both drug- and self-administration-naive animals (Stuber et al., 2005) as well as animals with a history of cocaine self-administration, they arise from a pharmacological effect of the drug. Similarly, both noncontingent (Schwarz et al., 2004) and self-administered (Wise et al., 1995) cocaine led to an increase in tonic dopamine levels, as measured by microdialysis, and are linearly related to cocaine concentrations (Nicolaysen et al., 1988). Indeed, increases in tonic dopamine occurring over minutes may simply be due to a summation of all spontaneous dopamine transients. Furthermore, spontaneous dopamine transients may be able to diffuse greater distances and interact with a greater population of dopamine receptors in the presence of cocaine. As such, the significance of spontaneous events may be to activate a larger neuronal network in the presence of cocaine compared to nondrug conditions, in which spontaneous dopamine transients are observed much less frequently (Roitman et al., 2004).

Although spontaneous dopamine transients do not appear to be time locked to any overt behavior or environmental stimuli, they may play an important role by encoding for the hedonic aspects of drug-taking. Consistent with this, locomotor activity, which in this study was associated with the rate of lever pressing during extinction is maximal following a decline in spontaneous dopamine transient frequency (Figure 6D). This is

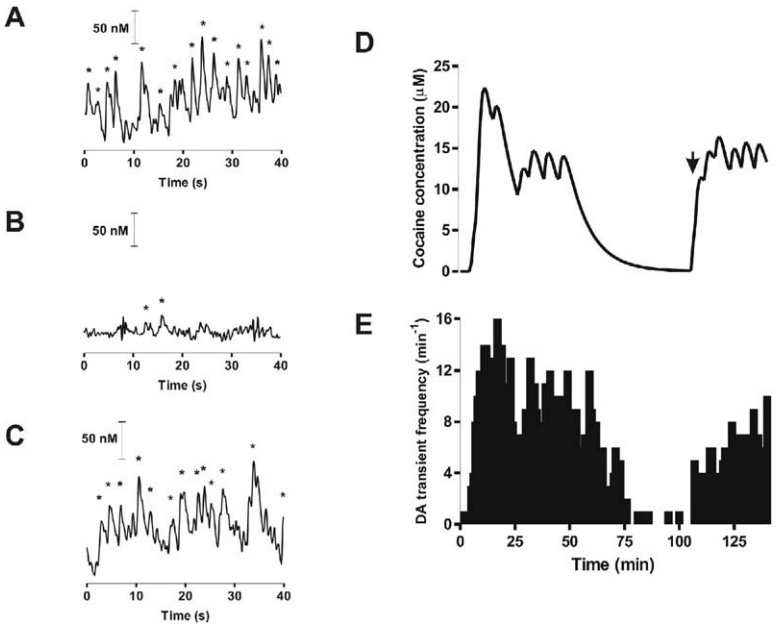

Figure 5. Brain Cocaine Concentrations and Frequency of "Spontaneous" Dopamine Transients Decrease during Extinction

(A–C) Traces showing spontaneous dopamine transients during maintenance (A), late extinction (B), or reinstatement (C) from the same animal. Asterisks denote voltammograms at that time which have an $r^2 \geq 0.75$ compared to stimulated dopamine release.

(D) Calculated brain cocaine concentrations during maintenance, extinction, and reinstatement of cocaine self-administration. The animal was reinstated to self-administer cocaine by administering a 0.33 mg priming infusion of cocaine paired with a 20 s exposure to the drug-associated cues (denoted by the arrow).

(E) Frequency of spontaneous dopamine transients during maintenance, extinction, and reinstatement from the same animal used for the calculated brain cocaine concentrations (D).

also consistent with the "threshold hypothesis" proposed by Wise et al. (1995), in which falling tonic dopamine concentrations were thought to trigger successive operant responses for the drug.

The Role of Dopamine Signaling within NAc Microcircuits

In this study, we describe dopamine signals that occur during three different phases of cocaine self-administration. While each of these are distinctly modulated by the dissociation of cocaine from the drug-associated stimuli during extinction, little is known about the function dopamine transients play within the NAc to influence subsequent cellular activity. NAc neurons show distinct patterned discharges time locked to the operant response for cocaine, natural rewards, and the stimuli that are associated with these (Carelli, 2004). Furthermore, within-session extinction experiments conducted during cocaine self-administration (Carelli and Ijames, 2000) show that NAc neurons exhibiting postresponse patterned discharges are attenuated during extinction and again become phasically active during reinstatement in much the same way that phasic postresponse dopamine signaling is seen in the NAc. Furthermore, NAc neurons exhibiting preresponse patterned discharges are not altered during extinction similar to preresponse dopamine transients reported here. Whether phasic dopamine signaling is directly affecting cells within the NAc remains unknown, but understanding this will further elucidate the neurobiologi-

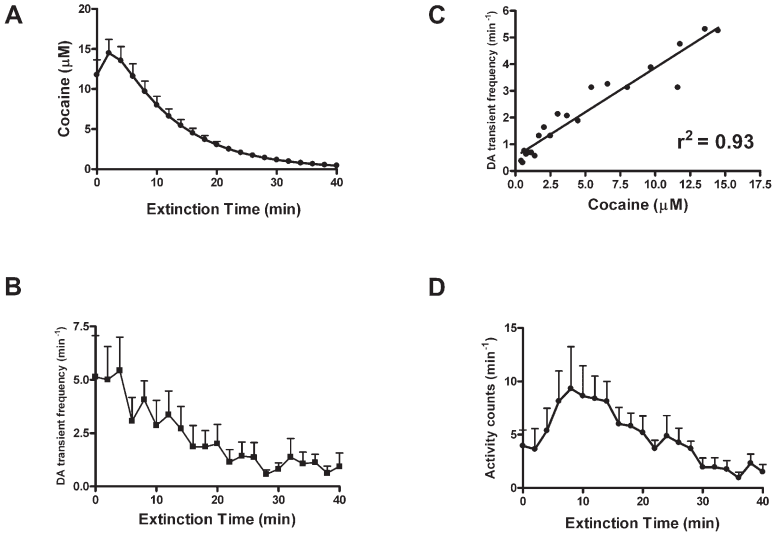

Figure 6. Declining Cocaine Concentrations and Dopamine Transient Frequency Precede the Increase in Behavioral Activity

(A) Average (mean ± SEM) cocaine concentrations across all animals during the extinction phase.

(B) Average (mean ± SEM) spontaneous dopamine transient frequency across all animals during the extinction phase.

(C) Linear regression analysis showing the correlation of spontaneous dopamine transient frequency with brain cocaine concentrations (from [A] and [B]) over the extinction phase.

(D) Average (mean ± SEM) locomotor activity across all animals over the extinction phase. Time 0 is the beginning of the extinction phase immediately after the last maintenance response.

cal function of dopamine within the NAc, and the role it plays in reinforcement and drug addiction.

Experimental Procedures

Cocaine Self-Administration

Male Sprague-Dawley (Harlan, Raleigh, NC) rats (280–300 g; n = 8) were housed individually and kept on a 12:12 light cycle (lights on at 07:00). Rats had ad libitum access to food, while water intake was restricted to 30 ml/day to maintain body weight. Rats were anesthetized with ketamine hydrochloride (100 mg/kg i.m.) and xylazine hydrochloride (20 mg/kg i.m.) and implanted with a chronic indwelling SILASTIC catheter into their right jugular vein as described previously (Carelli and Deadwyler, 1996). A syringe pump was connected to a liquid swivel system in sound-attenuated experimental chambers, which enabled intravenous infusion of cocaine. An additional nine animals were removed from the study; four were removed due to faulty i.v. catheters, four had no detectable electrically evoked DA release at the beginning or end of the experiment, and one would not initiate lever pressing.

Following a 1 week recovery period, rats were trained daily to self-administer i.v. cocaine in 2 hr sessions. At the beginning of each self-administration session, rats were placed in the operant chambers (Med Associates, St. Albans, VT), connected to the syringe pump, and a cue light located 6.5 cm above the lever was illuminated to signal drug availability. Each lever depression resulted in a 6 s cocaine infusion (FR-1 schedule; 0.33 mg/infusion dissolved in sterile heperanized saline) via a computer-controlled syringe pump. Cocaine infusions were paired with the immediate onset of a tone/house light stimulus (65 dB, 2900 Hz) lasting for 20 s. During the 20 s postresponse interval, responding on the lever had no programmed consequences. Stable self-administration behavior was typically achieved by 10 to 15 training sessions. Once stable self-administration behavior was achieved, rats were surgically prepared for voltammetric recording. Behavioral events during self-administration were logged on a PC with Med-PC software (Med Associates, St. Albans, VT). A separate PC for voltammetry data acquisition was synchronized with Med-PC via a series of TTL pulses.

Voltammetric Recording

Implantation and voltammetric recording procedures were carried out as previously described (Phillips et al., 2003a). Rats were anesthetized with ketamine hydrochloride (100 mg/kg i.m.) and xylazine hydrochloride (20 mg/kg i.m.) and placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IL) was positioned above the NAc core (+1.3 mm AP, +1.3 mm ML, with its tip −2.5 mm DV; all coordinates relative to bregma). An Ag/AgCl reference electrode was placed contralateral to the guide cannula. All items were secured to the skull with machine screws and cranioplastic cement. A detachable micromanipulator containing a carbon fiber electrode (75–100 μm length cylinders, T-650; Amoco, Greenville, SC) was inserted into the guide cannula, and the electrode was lowered into the dorsal NAc core. A bipolar stimulating electrode was placed directly above the ventral tegmental area (−5.2 mm AP, +1.0 mm ML, and −7.7 to −8.8 mm DV). It was lowered in 0.1 mm increments until electrically evoked (60 biphasic pulses, 60 Hz, 120 μA, 2 ms/phase) dopamine release was detected at the carbon fiber electrode. The stimulating electrode was then secured in place with cranioplastic cement, and the carbon fiber electrode was removed and replaced with a stylet. Following voltammetric surgery, all rats were allowed to recover to their presurgery body weight.

Extinction/Reinstatement Procedure

Following recovery from surgery, rats were retrained for an additional 2–5 days to reestablish their self-administration behavior. On the day of the experiment, a new carbon fiber electrode was lowered into the NAc core. The carbon fiber and Ag/AgCl electrodes were connected to a head-mounted voltammetric amplifier attached to a commutator (Med Associates, St. Albans, VT) located at the top of the test chamber. Voltammetric recordings were made every 100 ms by applying a triangular waveform (−0.6 V to +1.4 V, 400 V/s), stored to a PC using software written in LabVIEW (National Instruments, Austin, TX). Dopamine release was optimized within the NAc core by adjusting the vertical position of the working electrode (0.1 mm increments). All recording sites had a signal-to-noise ratio of electrically evoked (24 biphasic pulses, 60 Hz, 120 μA, 2 ms/phase) dopamine release of at least 30. The electrode was then locked in place, and the voltammetric waveform was applied for an additional 45–60 min (Phillips et al., 2003a).

Following equilibration of the voltammetric electrode (~1 hr), the experiment was begun by turning on white noise and illuminating the cue light above the lever, signaling drug availability. Rats typically responded two to four times in quick succession, then three distinct experimental phases followed: maintenance, extinction, and reinstatement. The maintenance phase consisted of five lever press responses in which each response was reinforced with a 0.33 mg infusion of cocaine (delivered over 6 s), paired with a 20 s presentation of the drug-associated cues (as described above). Following maintenance, the extinction phase of the experiment began, and heparinized saline was substituted for cocaine. During extinction, each lever press response resulted in a 6 s infusion of saline paired with a 20 s presentation of the drug-associated cues. This caused a dramatic increase in the rate of responding (see Figure 1) and eventually the termination of lever presses. The extinction phase of the experiment was determined to be complete following 30 min without a lever press. The reinstatement phase was initiated by noncontingently priming each animal with an infusion of cocaine (0.33 mg/6 s) paired with the drug-associated cues (20 s). In seven of eight rats, a single priming infusion was sufficient to reinstate responding on the lever (the remaining rat was reinstated with three priming infusions). Following the priming infusion, rats were allowed to respond five times during reinstatement in which each response resulted in an infusion of cocaine (0.33 mg/6 s) paired with a 20 s presentation of the drug-associated cues.

After the experiment, the VTA was again electrically stimulated to ensure that viable dopamine release could still be detected at the carbon fiber electrode. The carbon fiber electrode was then removed, cleaned, and calibrated with 1 μM dopamine in a flow injection analysis system (Phillips et al., 2003a).

Signal Identification and Correction

Chemical identification for dopamine was obtained from the background-subtracted cyclic voltammograms (Heien et al., 2003; Phillips and Wightman, 2003). Typical noise levels were equivalent to 6 nM dopamine. Only signals larger than this were evaluated for the presence of dopamine. Collected data files were evaluated with a program written in LabVIEW. To examine the cyclic voltammograms within ±10 s of the lever press, a local minimum in the current (0.5 s duration) at the potential where dopamine is oxidized was found during the 10 s preceding each lever press. The cyclic voltammograms in this interval were subtracted from the remainder of this set. To evaluate each cyclic voltammogram in the 20 s interval around the lever press for the presence of dopamine, it was compared to a template cyclic voltammogram obtained from stimulated dopamine release, and a correlation coefficient for the similarity of their shape was obtained. In vitro data analysis revealed that a cutoff of $r^2 \geq 0.75$ distinguished dopamine from interferences (Heien et al., 2003) such as ascorbic acid, DOPAC, ionic changes in the extracellular space (e.g., changes in local pH [Venton et al., 2003]), and noise. Typically cyclic voltammograms near the lever press had a correlation coefficient of $r^2 \geq 0.80$. Current recorded at the potential where dopamine is oxidized was pH corrected as described previously (Stuber et al., 2005). Amplitude of dopamine transients time locked to the lever press was calculated as described in detail previously (Stuber et al., 2005). Briefly, for postresponse dopamine transients, the first transient that occurred immediately after the lever press response was used for data analysis (Stuber et al., 2005).

Identification of Spontaneous Dopamine Transients

Spontaneous dopamine transients were identified using an algorithm written in LabVIEW. The average of five voltammograms was subtracted from a voltammogram recorded 1 s later, and this pro-

cedure was followed sequentially for each cyclic voltammogram in the file. Template voltammograms obtained from stimulated dopamine release were statistically compared to the resulting subtracted voltammograms using linear regression analysis. Spontaneous dopamine signals were identified if their voltammograms had an $r^2 \geq 0.75$ compared to stimulated dopamine release (Heien et al., 2003). Spontaneous dopamine transients were considered to be separate events if they were separated by at least 0.5 s by voltammograms in which dopamine was not detected.

Modeling Brain Cocaine Concentrations

Brain cocaine concentrations throughout maintenance, extinction, and reinstatement were computed using the model for rats chronically treated with i.v. cocaine developed by Pan et al. (1991). Briefly, the brain cocaine concentration, C_{brain}, is expressed by the equation $C_{brain} = A * (e^{-\alpha t} - e^{-\beta t})$, where A = 9.65 (a multiplicative factor included the injected cocaine dose), $\alpha = 0.097$, $\beta = 0.642$, and t is the time in min since the cocaine infusion. The time course of each cocaine infusion was calculated independent of the others. The actual brain cocaine concentration was obtained by summing the cocaine concentrations that each infusion contributed in 30 s intervals using a macro written in Microsoft Excel.

Locomotor Activity Analysis

Locomotor activity was quantified offline by analysis of videotapes from the extinction/reinstatement sessions. The operant chamber was divided into four equal quadrants, and an activity count of one was made every time the animal's head completely entered a different quadrant. Locomotor activity was analyzed in 2 min bins, consistent with the length of voltammetric data files.

Histology

Following completion of experiments, rats were deeply anesthetized (ketamine, 150 mg/kg; xylazine, 20 mg/kg), and a stainless steel electrode (50 μm tip radius), housed in the same micromanipulator used during the experiment, was lowered to the recording site, and an electrolytic lesion was made (50 μA, 10 s). Rats were then transcardially perfused with physiological saline, followed by 4% paraformaldehyde. Brains were stored in 4% paraformaldehyde for at least 3 days and frozen, and 50 μm sections were made using a cryostat. Brain sections were mounted on slides, stained with thionin, and coverslipped. All recording sites were verified to be located in the core region of the NAc based on the atlas of Paxinos and Watson (1997).

Statistical Analysis

All behavioral and voltammetric data were analyzed in GraphPad Prism and InStat (GraphPad Software, San Diego, CA). One-way repeated measures ANOVAs were performed on data from each of the three experimental phases. Upon conformation of a main effect, Dunnett's test for multiple comparisons post hoc analysis was performed for all voltammetric data that were compared to the average concentration during the maintenance phase. For all other post hoc analyses, Tukey's test for multiple comparisons was used. All results were considered statistically significant if $p \leq 0.05$.

Acknowledgments

The authors would like to thank M.L.A.V. Heien, P.E.M. Phillips, and the UNC Department of Chemistry Electronics Facility. This work was supported by grants from the National Institute on Drug Abuse: DA10900 (R.M.W.), DA017318 (R.M.C. and R.M.W.), and DA015923 (G.D.S.).

Received: December 22, 2004
Revised: March 11, 2005
Accepted: April 10, 2005
Published: May 18, 2005

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