Neural encoding of cocaine-seeking behavior is coincident with phasic dopamine release in the accumbens core and shell

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Abstract

Mesolimbic dopamine neurons projecting from the ventral tegmental area to the nucleus accumbens (NAc) are part of a complex circuit mediating cocaine-directed behaviors. However, the precise role of rapid (subsecond) dopamine release within the primary subregions of the NAc (the core and shell) and its relationship to NAc cell firing during this behavior remain unknown. Here, using fast-scan cyclic voltammetry we report rapid dopamine signaling in both the core and shell; however, significant differences were observed in the timing of dopamine release events within seconds of the cocaine-reinforced response during self-administration sessions. Importantly, simultaneous voltammetric and electrophysiological recordings from the same electrode reveal that, at certain sites within both subregions, neurons exhibiting patterned activation were observed at locations where rapid dopamine release was present; the greater the strength of the neural signal the larger the dopamine release event. In addition, it was at those locations that electrically-evoked stimulated release was greatest. No changes in dopamine were observed where nonphasic neurons were recorded. Thus, although differences are evident in dopamine release dynamics relative to cocaine-reinforced responding within the core and shell, dopamine release is heterogeneous within each structure and varies as a function of precise neuronal targets during cocaine-seeking behavior.

Introduction

Electrophysiological recordings in behaving animals show that distinct populations of nucleus accumbens (NAc) neurons encode the important features of goal-directed behaviors. During cocaine self-administration, we have shown that a subset of NAc neurons exhibit changes (increases and/or decreases) in firing rate within seconds of lever pressing for intravenous drug. Some NAc neurons discharge seconds before the cocaine-reinforced response and appear to encode ‘anticipation’ of impending drug reward. Other NAc cells exhibit changes in firing rate within seconds following response completion and are activated by stimuli paired with drug infusion. Similar types of neuronal firing patterns have been observed during behavioral responding for natural (e.g. food/water/sucrose) reward (Wheeler & Carelli, 2008) and intracranial self-stimulation (Cheer et al., 2007).

The NAc consists of two primary subregions (the core and shell), which differ in their anatomical connections and functional properties. Each subregion receives afferent projections from a variety of cortical and subcortical structures including the basolateral amygdala, prefrontal cortex and hippocampus (Groenewegen et al., 1991; Zahm & Brog, 1992; Brog et al., 1993; Wright et al., 1996). In addition, the core and shell receive dense dopaminergic input from the ventral tegmental area (VTA) (Zahm & Brog, 1992), which is believed to modulate cortical and subcortical activation of NAc neurons (Mogenson et al., 1980; Pennartz et al., 1994; Nicola et al., 2000). However, the inability of VTA electrophysiology studies to selectively record from dopamine neurons that project exclusively to the core or shell makes it impossible to determine whether dopamine is differentially released across NAc subregions during behavior. In terminal regions, rapid dopamine signaling (transients) occurs with the same temporal and spatial resolution as NAc cell firing relative to goal-directed behaviors for cocaine (Phillips et al., 2003b), natural rewards (Roitman et al., 2004) and intracranial self-stimulation (Cheer et al., 2007). Importantly, we recently demonstrated that rapid dopamine transients in the NAc arise from burst firing in the VTA (Sombers et al., 2009). Voltammetric measurements of dopamine can reveal if dopamine release is similar across the core and shell of the NAc during cocaine-seeking, and if dopamine release is positioned to differentially modulate NAc cell firing within discrete locations.
We completed two studies to address these issues. Experiment 1 utilized fast-scan cyclic voltammetry alone to examine if differences are observed in rapid dopamine release dynamics in the core vs. shell during cocaine self-administration. In Experiment 2, we examined the relationship of rapid dopamine signaling to NAc cell firing during the same task using simultaneous voltammetric and electrophysiological recordings from the same electrode. We found that dopamine is released within seconds of the cocaine-reinforced response in both the core and shell; however, differences were observed in the temporal properties of rapid dopamine signaling across subregions relative to the response. In addition, at certain sites within both subregions, neurons exhibiting patterned activation were observed at locations where behavior-related rapid dopamine release was present; no changes in dopamine concentration were observed where nonphasic neurons were recorded. These findings reveal that dopamine release within each subregion is heterogeneous and varies as a function of ongoing drug-seeking behavior and precise neuronal targets in the NAc.

Materials and methods

Subjects

Male Sprague–Dawley rats, ~90–120 days old (275–350 g) were used as subjects (n = 31 total; eight for Experiment 1, 23 for Experiment 2). All animals were surgically prepared for self-administration via implantation of a catheter into the jugular vein under ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg) anesthesia using established procedures (Carelli, 2000). All surgical procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee and in concordance with the NIH Guide for the Care and Use of Animals.

Cocaine self-administration

At 1 week after catheter implantation, animals were trained to self-administer cocaine during daily 2 h sessions conducted in a 43 × 43 × 53 cm Plexiglas chamber (Med. Associates, Inc., St Albans, VT, USA). The beginning of the session was signaled by the onset of a cue light positioned above a lever and lever extension (the lever remained extended throughout the session). Each lever press (fixed ratio 1 schedule) initiated intravenous cocaine delivery (0.33 mg/kg-infusion, over 6 s) controlled by a syringe pump (Model PHM-100, Med. Associates, Inc.), termination of the cue light and simultaneous onset of a 20 s tone (67 dB, 1 kHz) and houselight (25 W) stimulus; lever presses during stimulus presentation had no programmed consequences. Subsequent trials were initiated by the animals and typically occurred with intertrial intervals of ~5–6 min. Training was complete when stable responding was established (i.e. < 10% variability in press number during a consecutive 2–3 day period; total training period of 8–12 days).

Electrochemistry/ electrophysiology surgery

Once cocaine self-administration was stable rats were anesthetized with xylazine hydrochloride (10 mg/kg, i.p.) and ketamine hydrochloride (100 mg/kg, i.p.) and placed in a stereotaxic frame. Surgery for electrochemical recordings followed previously described procedures (Phillips et al., 2003a). A microdialysis guide cannula (Locking Intracerebral Guide and Styel, Bioanalytical Systems, West Lafayette, IL, USA) was implanted above the NAc shell (1.7 mm anterior, 0.8 mm lateral, coordinates relative to bregma) or NAc core (2.2 mm anterior, 1.5 mm lateral). Shell placements were within rostral portions of the NAc shell, which has greater dopamine concentration (Heidbreder & Feldon, 1998) and negligible noradrenergic input compared with caudal portions (Delfs et al., 1998; Baldo et al., 2003). A bipolar stimulating electrode (tip diameter 0.2 mm, tips spaced 1 mm apart, Plastics One, Roanoke, VA, USA) was lowered to the substantia nigra/VTA (5.2 mm posterior, 1 mm lateral and 7.8 mm dorso-ventral) (Paxinos & Watson, 1986). An Ag/AgCl reference electrode was placed in the contralateral hemisphere, ~3.6 mm posterior to bregma. The implanted items were permanently affixed to the skull with cranioplastic cement.

Fast-scan cyclic voltammetry

Cylindrical carbon-fiber microelectrodes (50–100 μm of exposed fiber) were prepared as described previously (Cahill et al., 1996) with T650 fibers (6 μm diameter, Amoco Corporation, Greenville, SC, USA) inserted into a glass pipette (A-M Systems, Carlsborg, WA, USA). The carbon-fiber electrode was held at ~0.4 V vs. Ag/AgCl and periodically a cyclic voltammogram was acquired (100 ms intervals for chemical measurements and 200 ms intervals for combined recordings). The applied potential was scanned to +1.3 V and back in a triangular fashion at 400 V/s in 8.5 ms (Heien et al., 2003). Stimulation timing, voltage application and data collection were achieved with an interface board (National Instruments, Austin, TX, USA) in a Pentium IV computer running custom-designed LABVIEW (National Instruments) software.

On the experimental day, the reference electrode was connected to ground and a fresh carbon-fiber microelectrode was lowered into the NAc with a micromanipulator based on a previous design (Rebec et al., 1993; Garris et al., 1997). The micromanipulator was modified (Robinson & Wightman, 2007) to fit tightly into the locking guide cannula; one rotation of the device moved the electrode 300 μm. Wires from the reference and stimulating electrodes and a head-stage amplifier connected to the carbon-fiber electrode were routed to a swivel (Med. Associates, Inc.), which allowed free movement of the animal, and then to custom-built amplifiers (Chemistry Department Electronics Facility, University of North Carolina Chapel Hill, Chapel Hill, NC, USA) and into a computer interface. The carbon-fiber electrode was lowered in 75 μm increments until it was in a site where electrical stimulation (125 μA biphasic pulses, 2 ms per phase, 60 Hz, 24 pulses) evoked dopamine release, establishing that the electrode was near functional dopaminergic terminals. Once the behavioral session started, data were collected in 60 s files.

Fast-scan cyclic voltammetry is a differential technique that measures changes in analyte concentration. To remove background and charging currents, 10 cyclic voltammograms were recorded before an event (VTA stimulation or behavioral response), averaged and subtracted from all of the cyclic voltammograms collected in the data file. The voltammograms were then plotted with the abscissa as acquisition time of the cyclic voltammogram, the ordinate as the applied potential and the current in false color (Michael et al., 1998).

Combined electrophysiology/ electrochemistry

The technique for combined electrophysiological and electrochemical recordings has been described previously (Cheer et al., 2005). The apparatus was identical to that described above except that a solid-state relay in the head-stage alternated between a current amplifier for voltammetric scans and a voltage follower for unit recording. Units
were recorded for 180 ms between voltammetric scans that were collected at 5 Hz. Each scan occupied 20 ms (8.5 ms for the scan and the remainder to allow the amplifiers to settle). Both signals were referenced to the Ag/AgCl electrode. In these experiments, recordings were made at sites where single units were isolated. Electrical stimulations were made to determine whether dopamine release could be evoked. Following collection of data at one site, the electrode was lowered ~300 μm until another unit was found. This approach often resulted in two sessions per day separated by 5–10 min and in some instances partial sessions were recorded (i.e. ~9–15 trials/sessions vs. full sessions of ~20–25 trials/session).

Extracellular cell firing recorded from carbon-fiber electrodes (impedance at the tip: ~500 kΩ at 1 kHz sine wave in saline) was amplified (×1000) and bandpass-filtered (300 Hz–3 kHz). The units were digitized with commercially available software (Neurosurgery WorkStation, Plexon Inc., Dallas, TX, USA). The waveforms were discriminated using principal component analysis in Offline Sorter (Plexon Inc.). One to two neurons could typically be discriminated at each location. Custom-written software (LABVIEW, National Instruments) was used to timestamp each behavioral event to the electrochemical record allowing analysis of the relationships among behavior, dopamine release events and NAc cell firing.

**Data analysis**

**Dopamine signal identification and analysis**

Principal component regression was used to extract the dopamine component from the voltammetric data (Heien et al., 2004). For each experiment a calibration set of cyclic voltammograms of dopamine and pH changes was obtained in vivo during different stimulations employing 6, 12 or 24 stimulus pulses as well as two different frequencies (30 or 60 Hz). Principal component regression analysis yielded dopamine and pH contributions as well as a residual from the measured data. Data were retained only if a low residual was found (Heien et al., 2005). The scaling factor to relate current concentration was obtained by calibration of the carbon-fiber electrodes after the in-vivo experiment (Logman et al., 2000).

Following extraction, the concentration of dopamine ([DA]) was averaged into 1 s bins across a 20 s time window (10 s before and 10 s following the cocaine-reinforced lever-press response). All data sets were tested for normality using the D’Agostino & Pearson omnibus normality test, prior to ANOVA or t-test analysis. One-way repeated-measures ANOVAs were performed on [DA] across the 20 s window, followed by Dunnett’s test comparing [DA] within each 1 s bin with baseline (defined as dopamine concentration within 10–9 s before the response). A t-test analysis was used to compare stimulated [DA] at phasic vs. nonphasic cell firing locations across the core and shell. Signal-to-baseline (S:B) ratios for dopamine were determined by dividing the mean [DA] during the period of peak changes (1 s of peak increases within 0.6 s before to 2 s following the response, i.e. the signal) by the mean [DA] during a baseline period.

**Classification of nucleus accumbens cell firing**

Neuronal cell firing was classified into one of four types of discharges within previously defined epochs relative to the reinforced response (Carelli, 2000). Briefly, neurons were classified as type pre-response (PR) if significant increases in cell firing were observed within a 1 s window of the 2.5 s pre-response epoch. Type reinforcement-excitation (RFe) or reinforcement-inhibition (RFi) neurons exhibited significant increases or decreases, respectively, in firing that occurred within a 1 s window within the 2.5 s epoch immediately after response completion. Nonphasic (NP) cells showed no change in cell firing relative to baseline to the reinforced response for cocaine. The 1 s ‘sliding’ window of peak/trough changes in cell firing within the 2.5 s epochs was used as there is variability in the precise timing of neural changes across cells relative to the response, as described previously (Carelli, 2000). The classification of each cell type into type PR, RFe, RFi or NP cells was determined with z-score statistics for each neuron (P < 0.05). Z-scores were calculated as (x + b)/s, where x is the mean firing rate during the 1 s period of peak or trough firing relative to the response, s is the mean firing rate during the baseline period and b is the SD of the baseline period. Baseline consists of firing during the −10 to −7.5 s preceding the reinforced response for each cell. S:B ratios for phasic cell firing were determined by dividing the mean firing rate during the 1 s period of peak or trough firing relative to the response (i.e. the signal) by the mean firing rate during the baseline period.

**Verification of carbon-fiber microelectrode placement**

Following experiments rats were anesthetized with a lethal dose of sodium urethane (2 g/kg, i.p.). A tungsten electrode was inserted into the location where recordings had been made because the carbon-fiber electrode causes little damage to brain tissue (Peters et al., 2004). To create an electrolytic lesion a constant current of 500 μA was applied twice for 5 s through the tungsten electrode. Rats were then transcardially perfused with 300 mL of saline followed by 300 mL of a 10% formalin solution. Brains were dissected out, cryoprotected and coronally sectioned on a cryostat. Brain sections were mounted on slides, stained with thionin, coverslipped and viewed with bright-field microscopy.

**Results**

**Differential dopamine release dynamics in the core vs. shell relative to cocaine-reinforced responding during self-administration**

For all rats (n = 31) in Experiments 1 and 2, stable self-administration behavior for intravenous cocaine was observed on recording days with mean interinfusion intervals of 327.5 ± 22.3 s.

In Experiment 1 (n = 8 rats), the precise temporal characteristics of rapid dopamine release events relative to the reinforced response for intravenous cocaine during self-administration was compared across the NAc core and shell using recordings every 100 ms. The carbon-fiber electrode was inserted into the desired region of the NAc via a micromanipulator and its position was adjusted until high concentrations of dopamine were observed during electrical stimulation. This procedure was used to ensure that the electrode was located among dopamine terminals (Wightman et al., 2007). Figure 1A shows a color representation of a set of background-subtracted cyclic voltammograms and the corresponding [DA] changes in the NAc core time-locked to a single operant response. Consistent with our previous reports (Phillips et al., 2003b; Stuber et al., 2005a,b), dopaminergic signals at the lever press for intravenous cocaine in the core had two distinct components, i.e. an initial increase in dopamine within seconds before the response followed by a larger more sustained increase following response completion. As in previous studies, the pre-response increase began ~1 s before the response and reached a maximum of 80 nM. This was followed by a larger post-response increase in dopamine that peaked within 1 s following the operant response (maximum [DA] = 200 nM) and declined thereafter. Increases in [DA] were also observed before and following the response in the shell (representative example in Fig. 1B). However,
these included a larger pre-response increase and a post-response that was less synchronized to the operant response than observed in the core. Specifically, the increase in dopamine signal in the shell began ~6 s preceding the response with a peak maximum concentration of ~165 nM, exhibited a delayed post-response that peaked ~4 s after the press (250 nM) and remained elevated for ~9 s following response completion. Thus, dopamine signaling relative to the reinforced response for cocaine occurred in the shell but appeared to be of longer duration and was not so tightly time-locked to the response as that observed in the core.

Similar changes in dopamine signaling in the core and shell were maintained across all animals and recording sites, as shown in Fig. 2. As previously reported (Phillips et al., 2003b), the pre-response component of dopamine release in the core was variable in its synchronization to the lever press and was therefore diminished in the average trace in Fig. 2A. As in previous reports (Phillips et al., 2003b; Stuber et al., 2005b), rats typically move around the chamber to varying degrees during this time period, then approach and press the lever. A larger increase in [DA] began immediately following the operant response and peaked at 2–3 s following response completion.

Fig. 1. Representative dopamine release dynamics in the core and shell relative to a lever-press response for intravenous cocaine. Two-dimensional color representation of cyclic voltammetric data collected for 20 s around single self-administration trials in the core (A) and shell (B). The ordinate is the applied voltage ($E_{\text{app}}$) and the abscissa is time (in s). Changes in current at the carbon-fiber electrode are indicated in color; dopamine has features at 0.6 V on the positive-going scan and ~0.2 V on the negative-going scan. Differential [DA] concentrations determined via principal component analysis are plotted above color plots.

Fig. 2. Average voltammetric data measured in the core and shell relative to cocaine-reinforced responding across all animals. Mean (solid line) ± SEM (dashed line) changes in dopamine relative to the reinforced response (R) across all animals in the core (A) ($n = 4$) and shell (B) ($n = 4$). Averages were determined for each animal and then averaged together; only full sessions were included. The dashed vertical bar shows the time of R and the horizontal line indicates the drug-infusion period. *Significant increases in dopamine relative to baseline concentrations ($P = 0.05$).
second increase in dopamine (maximum of 157 nM) Dunnett’s multiple comparison test revealed that dopamine concentration was significantly increased relative to the lever-press response for intravenous cocaine (F_{19,173} = 3.034, \( P < 0.001 \)) that occurred from 1 to 4 s following response completion compared with baseline (Dunnett’s multiple comparison test, \( P < 0.05 \)). Although dopamine levels in the shell also increased relative to lever-press responding for intravenous cocaine, the profile of those changes differed from that observed in the core across all shell recording sites (Fig. 2B). Specifically, a pronounced and relatively prolonged increase in [DA] began \(~8 \text{ s}~\) preceding the response followed by a slight decline at response completion (mean pre-response maximum increase was 27.2 \(\pm 7.9 \text{ nM}~\)). This was followed by a larger increase in [DA] that began \(~2 \text{ s}~\) following the response and peaked at 52.1 \(\pm 4.9 \text{ nM}~\) \(~6 \text{ s}~\) after the operant response. This post-response increase in [DA] remained elevated for \(~9 \text{ s}~\). A one-way ANOVA revealed significant fluctuations in [DA] in the shell relative to the lever-press response (F_{19,171} = 4.819, \( P < 0.001 \)). Dunnett’s multiple comparison test revealed that dopamine concentrations were significantly elevated \((P < 0.05)~\) elevated relative to baseline 2–3 s prior to the response and during 1–8 s following response completion.

Simultaneous measurement of subsecond dopamine release and adjacent accumbens cell firing during cocaine self-administration

It has been well documented that a subset of NAc neurons (\(~30\%)~\) exhibit patterned discharges (i.e. increases and/or decreases in cell firing) relative to the lever-press response for intravenous cocaine (Carelli, 2000). An important issue addressed in the present study was to determine whether the locations at which phasic changes in dopamine release occur are the same locations at which NAc neurons exhibit patterned discharges. To this end, rapid dopamine release and NAc cell firing were simultaneously measured in another set of animals (\(n = 23\)) at discrete locations within the NAc core and shell during cocaine self-administration. In these experiments, the carbon-fiber electrode was inserted into the desired region of the NAc and its position was adjusted until extracellular activity of a single neuron was detected. At each of these locations electrical stimulation of the VTA was applied before the session to ensure that the electrode was in the midst of dopamine terminals. In these experiments, fast-scan cyclic voltammetry had 200 ms resolution to allow sufficient time for single unit collection.

A total of 75 NAc neurons (34 cells in the core; 41 neurons in the shell) were recorded in combination with voltammetric measurements of phasic dopamine release. Across all neurons, the average baseline firing rate was 2.5 \(\pm 0.3 \text{ Hz}~\). Of the 75 NAc cells, 29 neurons (39%) exhibited one of three types of patterned discharges within seconds of the reinforced response for cocaine similar to that previously described (Carelli, 2000). Figure 3 shows an example of a single NAc neuron recorded in the shell that exhibited patterned cell firing and phasic dopamine release relative to the cocaine-reinforced response across a single self-administration session. As seen in the color plot averaged across all self-administration trials (Fig. 3, top), the cyclic voltammetric data show an increase in [DA] that reached a maximum concentration of 130 nM immediately before the response, with a second increase in dopamine (maximum of 157 nM) \(~4 \text{ s}~\) following the lever-press response. The [DA] increase relative to the lever press was confirmed with principal component regression [Fig. 3, bottom, blue trace superimposed on the peri-event histogram (PEH)]. Also illustrated in the bottom portion of Fig. 3 are a PEH and corresponding raster display that show the simultaneously recorded activity of a single NAc neuron. In this case, the neuron showed an increase in cell firing that began within 1 s following response completion and maintained a sustained increase in firing (maximum of 11.7 Hz) during the 10 s post-response period.

Of the 29 phasically active cells recorded in both the core and shell, three types of neuronal firing patterns were observed relative to the reinforced response for intravenous cocaine, as illustrated by the population PEHs in Fig. 4. Average (and SEM) changes in [DA] corresponding to locations at which each type of patterned discharge was observed are illustrated by the blue traces superimposed above the PEHs (Fig. 4A–C). A subset of NAc neurons (\(n = 14\)) displayed increases in cell firing (peak increase of 2.23 \(\pm 0.07 \text{ Hz}~\) within seconds preceding the reinforced response with an abrupt decline in cell firing at response completion, termed type PR cells, shown in Fig. 4A. Significant increases in dopamine were observed at these locations (F_{13,247} = 2.9, \( P < 0.0001 \), i.e. a slight but nonsignificant
remarkably, at only two locations at which NP cells were recorded relative to the cocaine-reinforced response (termed NP cells).

Of the 75 NAc neurons, 46 cells (61%) showed no change in cell firing. However, due to the variability in dopamine release across NAc sites, only five of the nine type RFi cells were significantly correlated with decreases in S : B dopamine levels and NAc phasic cell firing, linear regression analyses were completed that correlated S : B ratios for peak [DA]s vs. S : B ratios for peak changes in NAc cell firing across cell types. For neurons that displayed increases in firing rate relative to the response (types PR and RFe), a significant positive linear regression was observed between S : B dopamine concentration and S : B cell firing (F_{1,15} = 6.326, P = 0.05, r^2 = 0.30, Fig. 5A). Conversely, a significant negative correlation was observed for neurons displaying inhibitions in cell firing relative to the response (type RFi neurons), i.e. increases in S : B dopamine levels were significantly correlated with decreases in S : B cell firing of type RFi cells (F_{1,5} = 36.41, P = 0.01, r^2 = 0.88, Fig. 5B).

To examine whether differences exist in the relationship between dopamine release and NAc phasic cell firing, linear regression analyses were completed that correlated S : B ratios for peak [DA]s vs. S : B ratios for peak changes in NAc cell firing across cell types. For neurons that displayed increases in firing rate relative to the response (types PR and RFe), a significant positive linear regression was observed between S : B dopamine concentration and S : B cell firing (F_{1,15} = 6.326, P = 0.05, r^2 = 0.30, Fig. 5A). Conversely, a significant negative correlation was observed for neurons displaying inhibitions in cell firing relative to the response (type RFi neurons), i.e. increases in S : B dopamine levels were significantly correlated with decreases in S : B cell firing of type RFi cells (F_{1,5} = 36.41, P = 0.01, r^2 = 0.88, Fig. 5B).
which only phasic (type PR, RFe and RFi) cells were recorded, significant increases in dopamine were observed in both the core ($F_{13,247} = 2.6, \ P < 0.0001$, Fig. 6C) and shell ($F_{10,190} = 31.7, \ P < 0.0001$; Fig. 6D). There were only four locations (two in the core and two in the shell) at which phasic cells were recorded and no significant changes in dopamine were observed (data not shown).

As noted above, to ensure that cell recordings were made at dopamine-rich regions of the NAc, prior to the start of each session electrical stimulation was applied to the VTA and stimulated dopamine release was measured. Interestingly, the average concentration of stimulated dopamine was approximately double at phasic locations compared with NP locations. For the core, stimulated dopamine release in phasic locations was $1270 \pm 320 \text{ nM}$ vs. $590 \pm 130 \text{ nM}$ in NP locations ($t_{29} = 2.10, \ P < 0.01$). Likewise, for the shell, stimulated

| Table 1. Distribution of NAc neurons across cell types and subregions |
|-----------------------------|-----------------------------|-----------------------------|
| Cell type | NAc core (cells/dopamine sites) | NAc shell (cells/dopamine sites) | Totals (cells/dopamine sites) |
| PR | 9/8 | 5/4 | 14/12 |
| RFe | 2/2 | 4/4 | 6/6 |
| RFi | 5/4 | 4/3 | 9/7 |
| NP | 18/2 | 28/0 | 46/2 |

NAc, nucleus accumbens; NP, nonphasic; PR, pre-response; RFe, reinforcement-excitation; RFi, reinforcement-inhibition.

Fig. 5. Linear regression analyses correlating S : B ratios for peak [DA]s vs. S : B ratios for peak changes in NAc cell firing across cell types. (A) For excitatory neurons (types PR and RFe) a significant positive linear regression was observed. (B) For inhibitory neurons (type RFi) a significant negative linear regression was evident.

Fig. 6. Dopamine changes around the lever press for cocaine sorted by NAc subregion and degree of phasic activity. Dopamine concentrations at locations where NP neurons were recorded in the core (A) or shell (B) reveal no significant increases in dopamine release events. In contrast, when dopamine concentration changes were plotted at sites in which only phasic (type PR, RFe and RFi) cells were recorded, significant increases in dopamine were observed in both the core (C) and shell (D). Mean [DA] is indicated by solid lines and SEM is indicated by dashed lines. Significant increases in dopamine ($^*P = 0.05, ^{**}P = 0.01$). R, reinforced response.
dopamine release at phasic locations was 650 ± 260 nM vs. 280 ± 40 nM at NP locations (t_{11} = 2.29, P < 0.05). Furthermore, examination of [DA] changes following electrical stimulation prior to each experiment revealed differences in dopamine reuptake between core and shell locations. Specifically, calculation of the dopamine half-life (when dopamine concentration is half of total stimulated release) revealed significant differences between the core (1315 ± 85 ms) and shell (1546 ± 75 ms) (t_{13} = 2.06, P < 0.05). Importantly, the differences in stimulated dopamine release peak concentrations and reuptake values across the core and shell could not be attributed to dissimilarities in electrode sensitivity. Specifically, carbon-fiber electrodes post-calibrated for [DA] in vitro in a flow injection system showed similar calibration factors regardless of whether measurements were made at nonphasic (7.55 ± 0.56 nA/μM) or phasic (7.78 ± 0.65 nA/μM) locations. Furthermore, there were a number of instances (n = 10) in which both nonphasic and phasic cells were recorded from the same electrode track.

In addition to stimulated dopamine release, we also measured the frequency of naturally occurring phasic dopamine release events, i.e. dopamine ‘transients’ prior to the start of each behavioral session (Wightman et al., 2007; Aragona et al., 2008). Interestingly, significantly more dopamine transients per minute were observed in the core (2.4 ± 0.19) than in the shell (1.5 ± 0.15; t_{63} = 4.053, P < 0.001). However, there were no significant differences in the frequency of transients observed in nonphasic vs. phasic cell locations in either the core (2.0 ± 0.24 vs. 2.7 ± 0.26; t_{11} = 2.008, P > 0.05) or shell (1.3 ± 0.19 vs. 1.6 ± 0.18; t_{28} = 0.93, P > 0.05).

Figure 7 shows the distribution of electrode placements within the core and shell of the NAc.

**Discussion**

Here, we determined the dynamics of rapid (subsecond) dopamine signaling during cocaine self-administration within the NAc core and shell, and its relationship to NAc cell firing during the same task. In Experiment 1, rapid dopamine signaling was observed within seconds of the reinforced response in both the core and shell, although significant differences were evident in the temporal properties of dopamine release between regions, i.e. dopamine responses in the core were closely time-locked to the reinforced response, whereas dopamine events in the shell were of longer duration and less synchronized to the lever press. In Experiment 2, electrophysiological recordings revealed that a subset of NAc neurons displayed increases and/or decreases in cell firing within seconds of the lever press for cocaine in both the core and shell, similar to previous reports (Carelli, 2000). However, within both subregions, phasic dopamine release was not uniform but occurred primarily at locations where NAc neurons exhibited patterned activation; in addition, the greater the strength of the neural signal the larger the dopamine release event. Furthermore, it was at those locations that electrically-evoked stimulated release was greatest. Together, these data demonstrate that: (i) dopamine release is evident in both the core and shell relative to the cocaine-reinforced response, although there are significant differences in the temporal properties of release dynamics across regions, and (ii) within both regions dopamine release is heterogeneous and anatomically positioned to modulate the activation of specific NAc neurons that encode cocaine-seeking behaviors. The implications of these findings are considered below.

**Rapid dopamine signaling during cocaine self-administration: core vs. shell**

In well-trained animals ~55–80% of dopamine neurons discharge in synchrony during reward-based tasks (Schultz et al., 1997; Schultz, 2007). However, those studies did not consider VTA projection targets and as such may have provided information on only a subpopulation of VTA neurons. By directly measuring dopamine within the core and shell (as opposed to extrapolating release from VTA extracellular recordings) we revealed that, although dopamine release is observed in both subregions, significant differences exist in the temporal properties of dopamine release across terminal regions. These differences may be related to a more potent dopamine uptake in the core than the shell, thus enabling dopamine to diffuse further from its release site in the shell (Jones et al., 1996). These temporal distinctions may contribute to the different functional roles of the core and shell in reward-seeking behaviors (Ito et al., 2000; Di Chiara, 2002; Cadoni & Di Chiara, 2007; Di Ciano et al., 2008).

Rapid dopamine release in the NAc core is consistent with previous studies showing an increase in [DA] within seconds before and immediately following the response for intravenous cocaine (Phillips et al., 2003b; Stuber et al., 2005a,b). We previously showed that electrical stimulation of the VTA and consequent dopamine release in the core evoke cocaine self-administration, indicating that phasic dopamine transients preceding the response may function to promote reward-seeking (Phillips et al., 2003b). In addition, rapid dopamine release in the core within seconds following the cocaine-reinforced response appears to be related to external cues (i.e. tone/houselight) paired with drug infusion during self-administration (Phillips et al., 2003b). The finding that these chemical signals are significantly attenuated during extinction (Stuber et al., 2005b) supports the notion that the changes in dopamine at the time of the lever press are not related to a direct pharmacological action of cocaine but instead reflect learned associations between response completion, cue onset and cocaine reward.

Our voltammetric recordings also revealed increases in dopamine release in the shell prior to and following the response for cocaine. However, shell increases in dopamine were of higher concentration, less synchronized to the response and of longer duration than in the
core. As noted above, a cause of this variation may be related to the existence of more potent dopamine uptake in the core than the shell allowing dopamine to diffuse further from its release site in the shell (Jones et al., 1996). In addition, subregion differences in release dynamics may be related to differences in afferent projections to the core and shell. For example, the medial shell of the NAc (where measurements were made in this study) receive dopamine inputs from more medial portions of the VTA, compared with the core where projections originate in the lateral VTA (Ikemoto, 2007). Moreover, intertwined within this dopamine projection system are highly complex neuroanatomical ‘loops’ involving distinct cortical and subcortical structures (Haber et al., 2000; Everitt et al., 2008), as well as direct connections between the core and shell (van Dongen et al., 2005). Indeed, it has been postulated that the shell may modulate activity in the core through these circuit connections (Haber et al., 2000; Luscher & Bellone, 2008). Consistent with different anatomical inputs, our voltammetric measurements reveal that dopamine neurotransmission clearly operates on different timescales within the core and shell during the same goal-directed behavior.

**Relationship between rapid dopamine release and accumbens activity**

To examine the relationship between rapid dopamine release and NAc cell firing, Experiment 2 employed a combined electrochemical and electrophysiological technique (Cheer et al., 2005). This powerful method allowed for the simultaneous recording of NAc cell firing and dopamine release from the same electrode during behavior. This study revealed that dopamine changes that occur within seconds of lever pressing for intravenous cocaine are accompanied by changes in unit activity of some NAc neurons. We previously established several patterns of cell firing during cocaine self-administration using multiple electrode arrays (Carelli, 2000; Hollander et al., 2002) and also demonstrated equal distribution of cell types across the core and shell (Carelli & Wodolowski, 2006). These distinct firing patterns were maintained when collecting with a single carbon-fiber electrode. Remarkably, dynamic increases in the extracellular concentration of dopamine were found at the majority of the sites where cells responded to the lever press with a phasic change in activity. At those same locations significantly larger increases in stimulated dopamine release were measured, indicating stronger dopaminergic influences compared with sites at which nonphasic neurons were recorded.

Anatomical studies show that, in the nigrostriatal system, dopamine neurons have axons that arborize, resulting in terminals that are clustered in multiple locations (Prensa & Parent, 2001). Moreover, dopaminergic clusters make synaptic contact with the necks of the dendritic spines on MSNs in the striatum (Surmeier et al., 2007) and a similar architecture exists in the NAc (Shen et al., 2008). MSNs in the NAc have cell bodies with a radius of ~10 μm and their dendritic fields are approximately oval and extend > 250 μm (van Dongen et al., 2008). DAT is located ubiquitously on dopaminergic terminals and thereby provides a index of the density and distribution of dopamine terminals (Nirenberg et al., 1997). Mathematical models suggest that an electrode should be within five times the radius of the cell body for a measurable extracellular signal (Prochazka, 1984). Furthermore, from the uptake rate of dopamine by the DAT, we can estimate the distance that it can diffuse from the release sites to the electrode. Dopamine has a half life of ~0.05 s in the NAc and therefore its concentration will halve in ~5 μm (Garris et al., 1994). In the NAc shell, with fewer uptake sites (Jones et al., 1996), dopamine can diffuse twice as far before its concentration is halved (i.e. ~10 μm). Thus, to detect released dopamine in our experiments, its release site must be in very close proximity to the detecting electrode. Similarly, the release sites must be a similar distance from receptor sites on the dendrites. To measure both dopamine release and NAc cell firing, the electrode must also be positioned close to the cell body.

Here, 39% of NAc neurons displayed phasic activity; all but four cells also had simultaneous dopamine fluctuations around the lever press for cocaine. Of the cells that did not show phasic activity (61%), dopamine fluctuations were observed at only two locations. These sites supported stimulated dopamine release although it was smaller than that found at phasic locations. Stimulated release measured with carbon-fiber electrodes has been shown to depend on the specific terminal region in the NAc (Wightman et al., 2007), with release amounts differing by ~two-fold in most locations. Thus, even at the length scale of our electrode (~100 μm), the NAc appears heterogeneous within each subregion. This is consistent with anatomical studies showing neurochemical heterogeneity in the NAc, perhaps corresponding to a ‘patch-matrix’ organization (Gerfen et al., 1987; Voorn et al., 2004). Alternatively, it may be that the dopaminergic nerve terminals at nonphasic cells simply did not receive signals to cause release during lever pressing, perhaps due to pre-synaptic modulation of terminals (Howland et al., 2002; Britt & McGhee, 2008). Although this heterogeneity in dopamine release is evident in both the core and shell, we have shown that transient frequency increases significantly in the shell compared with the core following cocaine administration (Aragona et al., 2008). However, during cocaine self-administration we observed spontaneous transients (i.e. dopamine release that occurred independent of ongoing behavior) equally at both phasic and nonphasic cell locations. Thus, dopamine can be released at nonphasic cell locations but is not apparent relative to goal-directed behaviors. Taken together these data suggest a complex and highly modifiable role for dopamine in modulating information processing in the NAc by both pharmacological agents and during cocaine-seeking behavior.

Another level of this complexity is represented by findings indicating that dopamine release and NAc cell firing that occur within seconds of the response represent an associative aspect of the task and not a direct pharmacological action of cocaine. For example, we have shown that both dopamine release and phasic accumbens neuronal responses are not evident within seconds following experimenter-delivered cocaine delivery but are selectively activated by the tone/houselight stimulus paired with cocaine infusion during self-administration (Carelli & Deadwyler, 1996; Carelli, 2000; Phillips et al., 2003b). This same activation is absent in animals without a history of cue/drug pairings (Phillips et al., 2003b; Hollander & Carelli, 2007). In addition, we have shown that NAc cell firing and dopamine release are also influenced by instrumental contingencies (i.e. lever press requirement) implicit in the behavioral task as post-response activity can be extinguished and reinstated (Carelli & Ijames, 2000; Stuber et al., 2005b). These findings support the view that, although cocaine clearly has direct pharmacological actions that occur over a longer time period (e.g. Peoples & West, 1996; Stuber et al., 2005b; Aragona et al., 2008), those that occur within seconds of the response (the focus of the current study) represent associative factors operating within the task.

**Functional organization of the accumbens**

It has been postulated that the NAc is comprised of ‘neuronal ensembles’ or groups of cells with similar functional properties that are activated by synchronous excitation of their inputs (Fennartz et al., 1994). Within this framework, dopamine functions as a neuromodulator, gating the activation of NAc neurons by glutamatergic inputs from the BLA, hippocampus and PFC, and influences NAc output (Mogenson et al.,...
Dopamine signals modulate NAc activity via complex interactions between D1- and D2-like dopamine receptors (Hu & White, 1997; Ikemoto et al., 1997). For example, NAc neurons periodically exhibit “up states” during which action potentials may occur (O’Donnell, 2003), which dopamine can facilitate while simultaneously reducing the firing rate (Goto & O’Donnell, 2001). In addition, dopamine is a modulator of striatal GABAergic interneuron coupling, indirectly affecting cell firing patterns (Cummings et al., 2008). This action increases the S : B ratio of phasically active neurons resulting in stronger effenter signals. Indeed, inactivation of VTA neurons decreases NAc phasic excitations for reward predictive cues (Yun et al., 2004). Thus, it may be the case that the neurons that exhibit pre- vs. post-response activity relative to the reinforced response may represent different sets of NAc neurons defined by differential distribution of D1 vs. D2 receptors and projection targets (Gertler et al., 2008). As such, the ability of dopamine to drive NAc neurons that encode distinct aspects of cocaine-reinforced responding (PR, RFe and RFi) may be related to dopamine receptor distribution on those cells but is probably also a function of the ability of dopamine to modulate distinct afferent inputs that in turn drive select populations of NAc neurons.

We previously reported similar coincident changes in rapid dopamine release and phasic NAc cell firing during intracranial self-stimulation that were reversed via iontophoretic application of a dopamine D1 receptor antagonist (Cheer et al., 2007). Although iontophoretic drug application was not attempted here due to cocaine-induced stereotypy, significant linear regressions were obtained between the strength of the neural code for phasically active cells and dopamine release events. These findings support the view that rapid dopamine signaling may indeed be modulating the activity of NAc neurons during behavior. Interestingly, ongoing studies in our laboratory indicate similar relationships between rapid dopamine release and NAc cell firing during food-reinforced behaviors. Thus, our findings reveal that the NAc (both the core and shell) is a functionally heterogeneous structure and that rapid dopamine release is anatomically situated, and released at unique periods, to play a critical role in the activation of NAc neurons that encode reward-directed behaviors.

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Abbreviations
[DA], concentration of dopamine; NAc, nucleus accumbens; NP, nonphasic; PEH, peri-event histogram; PR, pre-response; RFe, reinforcement-excitation; RFi, reinforcement-inhibition; S : B, signal-to-baseline; VTA, ventral tegmental area.

References


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