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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 (VTA) 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 sub-regions 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 (FSCV) 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 sub-regions, 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.

Keywords

in vivo voltammetry; neurotransmission; carbon-fiber microelectrode; addiction; behavior; reward

Introduction

Electrophysiological recordings in behaving animals show that distinct populations of 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 (ICSS) (Cheer *et al.*, 2007).

The NAc consists of two primary sub-regions, the core and the shell, which differ in their anatomical connections and functional properties. Each sub-region receives afferent projections from a variety of cortical and subcortical structures including the basolateral amygdala, the prefrontal cortex, and the hippocampus (Groenewegen *et al.*, 1991; Zahm & Brog, 1992; Brog *et al.*, 1993; Wright *et al.*, 1996). Additionally, the core and shell receive dense dopaminergic input from the VTA (Zahm & Brog, 1992) that 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 make it impossible to determine whether dopamine is differentially released across NAc subregions during behavior. In terminal regions, rapid dopamine signaling (transients) occur 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 ICSS (Cheer *et al.*, 2007). Importantly, we recently demonstrated that rapid dopamine transients in the NAc arise from burst firing in the VTA (Somers *et al.*, 2009). Now, 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.

Here, we completed two studies to address these issues. Experiment 1 utilized FSCV alone to examine if differences are observed in rapid dopamine release dynamics in the core versus 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 sub-regions relative to the response. Additionally, at certain sites within both sub-regions, 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 sub-region 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, approximately 90–120 days old (275–350 g) were used as subjects ($n = 31$ total; 8 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 UNC Institutional Animal Care and Use Committee (IACUC) and in concordance with the NIH Guide for the Care and Use of Animals.

Cocaine self-administration

One 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). 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, FR1 schedule) initiated intravenous cocaine delivery (0.33 mg/infusion, over 6 s) controlled by a syringe pump (Model PHM-100, Med Associates, Inc., St. Albans, VT), 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 inter-trial intervals of approximately 5–6 minutes. 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 (10mg/kg, i.p.) and ketamine hydrochloride (100mg/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 Stylet, Bioanalytical Systems, West Lafayette, IL), was implanted above the NAc shell (1.7 mm anterior, 0.8 lateral, coordinates relative to bregma) or NAc core (2.2 mm anterior, 1.5 lateral). Shell placements are within rostral portions of the NAc shell which has greater dopamine concentration (Heidbreder & Feldon, 1998) and negligible noradrenergic input compared to 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) was lowered to the substantia nigra/VTa, 5.2 mm posterior, 1 mm lateral and 7.8 mm dorsoventral (Paxinos & Watson, 1986). An Ag/AgCl reference electrode was placed in the contralateral hemisphere, approximately 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) inserted into a glass pipette (A-M Systems, Carlsborg, WA). The carbon-fiber electrode was held at – 0.4 V versus Ag/AgCl, and periodically a cyclic voltammogram was acquired (100 ms intervals for chemical measurements and 200 ms 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 was achieved with an interface board (National Instruments, Austin, Texas) 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; Garriss *et al.*, 1997). The micromanipulator was modified (Robinson, 2007) to fit tightly into the locking guide cannula; one rotation of the device moves 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, St Albans, VT) which allowed free movement of the animal, and then to custom-built amplifiers (Chemistry Department Electronics Facility, UNC Chapel Hill, Chapel Hill, NC) 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 was collected in 60 s files.

FSCV is a differential technique and measures changes in analyte concentration. To remove background and charging currents, ten cyclic voltammograms were recorded before an event (VTA stimulation or behavioral response), averaged and subtracted from all 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 headstage 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 minutes and in some instances partial sessions were recorded (i.e., ~9–15 trial/sessions versus full sessions of ~20–25 trials/session).

Extracellular cell firing recorded from carbon-fiber electrodes (impedance at the tip: ~500 k Ω at 1 KHz sine wave in saline) were amplified (x1000) and bandpass-filtered (300 Hz to 3 KHz). The units were digitized with commercially available software (Neurosurgery WorkStation, Plexon Inc, Dallas, TX). The waveforms were discriminated using principal component analysis in Offline Sorter (Plexon Inc, Dallas, TX). One to two neurons could typically be discriminated at each location. Custom-written software (LabVIEW, National Instruments, Austin, TX) was used to timestamp each behavioral event to the electrochemical record allowing analysis of 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 2 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 was retained only if a low residual was found (Heien *et al.*, 2005). The scaling factor to relate current to concentration was obtained by calibration of the carbon-fiber electrodes after the *in vivo* experiment (Logman *et al.*, 2000).

Following extraction, [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 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 to baseline (defined as dopamine concentration within 10 to 9 s before the response). T-test analysis

was used to compare stimulated [DA] at phasic versus 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 NAc 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 were used since there is variability in the precise timing of neural changes across cells relative to the response, described previously (Carelli, 2000). Classification of each cell type into type PR, type RFe, type RFi or type 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, *b* is the mean firing rate during the baseline period, and *s* is the standard deviation of the baseline period. Baseline consists of firing during the −10 to −7.5 s preceding the reinforced response for each cell. Signal-to-baseline (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^{−1}, 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 at 40 μm 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 versus 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 inter-infusion 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 during electrical stimulation were observed. This procedure was used to ensure 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 prior reports

(Phillips *et al.*, 2003b; Stuber *et al.*, 2005a; Stuber *et al.*, 2005b), dopaminergic signals at the lever press for intravenous cocaine in the core had two distinct components; an initial increase in dopamine within seconds before the response followed by a larger more sustained increase following response completion. As in prior studies, the pre-response increase began approximately 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] was 200 nM) and declined thereafter. Increases in [DA] were also observed before and following the response in the shell (representative example in Figure 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 approximately 6 seconds preceding the response with a peak maximum concentration of 165 nM, exhibited a delayed post-response that peaked approximately 4 s after the press (250 nM) and remained elevated for approximately 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 Figure 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 therefore was diminished in the average trace in Figure 2A. As in prior 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 (maximum mean [DA] was 30.8 ± 8.9 nM). A one-way analysis of variance (ANOVA) of mean [DA] in the core revealed significant increases in dopamine relative to the lever press response for intravenous cocaine ($F_{19,171} = 3.034$, $p < 0.001$) that occurred from 1 to 4 s following response completion compared to 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 (Figure 2B). Specifically, a pronounced and relatively prolonged increase in [DA] began approximately 8 s preceding the response followed by a slight decline at response completion (mean pre-response maximum increase was 27.2 ± 7.9 nM). This was followed by a larger increase in [DA] that began approximately 2 s following the response and peaked at 52.1 ± 4.9 nM approximately 6 s after the operant response. This post-response increase in [DA] remained elevated for approximately 9 s. A one-way analysis of variance (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 ($p < 0.05$) elevated relative to baseline 2 to 3 s prior to the response and during 1 to 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 (approximately 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 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 was 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 the electrode was in the midst of dopamine terminals. In these experiments, FSCV 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 rates was 2.5 ± 0.3 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 (Figure 3, top), the cyclic voltammetric data shows 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) approximately 4 s following the lever press response. The [DA] increase relative to the lever press was confirmed with principal component regression (Figure 3 bottom, blue trace superimposed on the peri-event histogram, PEH). Also illustrated in the bottom portion of Figure 3 is a PEH and corresponding raster display that show the activity of a single NAc neuron simultaneously recorded. 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 Figure 4. Average (and SEM) changes in [DA] corresponding to locations at which each type of patterned discharge was observed are illustrated in the blue traces superimposed above the PEHs (Figure 4A, B & C). A subset of NAc neurons ($n = 14$) displayed increases in cell firing (peak increase of 2.23 ± 0.07 Hz) within seconds preceding the reinforced response with an abrupt decline in cell firing at response completion, termed type pre-response (PR cells), shown in Figure 4A. At these locations, significant increases in dopamine were observed ($F_{13, 247} = 2.9$, $p < 0.0001$). That is, a slight but non-significant increase in dopamine was observed approximately 8 s before the lever press followed by a more pronounced and significant increase in [DA] (maximum increase of 48.0 ± 1.5 nM) immediately following response completion. Other NAc neurons ($n = 6$) exhibited significant increases in cell firing (peak increase is 2.51 ± 0.17 Hz) immediately following response completion (termed reinforcement-excitation or RFe cells; Figure 4B). At those sites, an overall significant increase in dopamine was observed ($F_{5, 95} = 2.4$, $p < 0.001$). Dopamine levels began to increase approximately 4 s prior to the response, declined slightly at response completion, and reached a peak maximum increase of 56.3 ± 0.4 nM following the response. However, due to the variability in dopamine release across all sites, post hoc tests did not reveal significant increases at specific time points relative to the response. A third set of NAc neurons ($n = 9$) displayed decreases in cell firing within seconds before and/or after the cocaine-reinforced lever press response (termed reinforcement-inhibition or RFi cells; Figure 4C). Note that since inhibitions occurred at varying times relative to the response across individual cells, only 5 of the 9 type RFi cells are shown in the average PEH. For those locations, significant increases in [DA]s were observed ($F_{8, 152} = 4.0$, $p < 0.0001$). Dopamine levels significantly increased 1 s prior to the response and remained significantly elevated 9 s after response completion. Finally, of the 75 NAc neurons, 46 cells (61%) showed no change in cell firing relative to the cocaine-

reinforced response, termed nonphasic or NP cells. Remarkably, at only two locations at which NP cells were recorded did dopamine significantly change (both in the core) in response to the lever press, however no significant overall change in rapid dopamine release was observed when data was averaged (Figure 4D). The distribution of cell types across the core and shell and their relationship to phasic dopamine release are shown in Table 1.

To further examine the relationship between rapid dopamine release and NAc phasic cell firing, linear regression analyses were completed that correlated signal-to-baseline (S:B) ratios for peak [DA]s versus 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 ($F_{1,15} = 6.326$, $p \leq 0.05$, $r^2 = 0.30$, Figure 5A). Conversely, a significant negative correlation was observed for neurons displaying inhibitions in cell firing relative to the response (type RFi neurons). That is, increases in S:B dopamine levels were significantly correlated with decreases in S:B cell firing of type RFi cells ($F_{1,5} = 36.41$, $p \leq 0.01$, $r^2 = 0.88$, Figure 5B).

To examine whether differences exist in the relationship between dopamine release and NAc cell firing across the core and shell, measurements were separated according to NP and phasic cells, and the mean [DA] was plotted relative to the lever press (Figure 6). Examination of [DA]s at locations where only NP neurons were recorded revealed no significant increases in [DA] in the core (Figure 6A) or shell (Figure 6B); in fact a significant decrease in dopamine release was observed immediately following the response at shell locations. In contrast, when [DA] changes were plotted at sites in which only phasic (type PR, RFe and RFi) cells were recorded, significant increases in dopamine was observed in both the core ($F_{13, 247} = 2.6$, $p < 0.0001$, Figure 6C) and shell ($F_{10, 190} = 31.7$, $p < 0.0001$; Figure 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 to NP locations. For the core, stimulated dopamine release in phasic locations was 1270 ± 320 nM vs NP locations; 590 ± 130 nM ($t_{29} = 2.10$, $p < 0.01$). Likewise, for the shell stimulated dopamine release at phasic locations was 650 ± 260 nM vs 280 ± 40 nM at NP locations ($t_{31} = 2.29$, $p < 0.05$). 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 dopamine half-life (when DA concentration is half of total stimulated release) revealed significant differences between the core (1315 ± 85 ms) and shell (1546 ± 75 ms) ($t_{73} = 2.06$, $p < 0.05$). 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/ μ M) or phasic (7.78 ± 0.65 nA/ μ M) locations. Furthermore, there were a number of instances ($n = 10$) in which both nonphasic and phasic cells were recorded from the same electrode within a single 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 shell (1.5 ± 0.15 ; $t_{61} = 4.053$, $p < 0.001$). However, there were no significant differences in the frequency of transients observed in nonphasic versus phasic cell locations in either the core (2.0 ± 0.24 versus 2.7 ± 0.26 ; $t_{31} = 2.008$, $p > 0.05$) or shell (1.3 ± 0.19 versus 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. That is, dopamine responses in the core were closely time-locked to the reinforced response while 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 prior reports (Carelli, 2000). However, within both subregions, phasic dopamine release was not uniform but occurred primarily at locations where NAc neurons exhibited patterned activation; additionally, 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: 1) that 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 2) 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 versus Shell

In well trained animals approximately 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 sub-regions, 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 and, 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 prior studies showing an increase in [DA]s within seconds before and immediately following the response for intravenous cocaine (Phillips *et al.*, 2003b; Stuber *et al.*, 2005a; Stuber *et al.*, 2005b). We previously showed that electrical stimulation of the VTA and consequent dopamine release in the core evokes cocaine self-administration, indicating that phasic dopamine transients preceding the response may function to promote reward-seeking (Phillips *et al.*, 2003b). Additionally, 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). Additionally, sub-region 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 here) receive dopamine inputs from more medial portions of the VTA, compared to 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 electrochemistry and electrophysiology 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 & Wondolowski, 2006). These distinct firing patterns were maintained when collecting with a single carbon fiber-electrode. Remarkably, at the majority of the sites where cells responded to the lever press with a phasic change in activity, dynamic increases in the extracellular concentration of dopamine were found. At those same locations significantly larger increases in stimulated dopamine release were measured indicating stronger dopaminergic influences compared to 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 it can diffuse from the

release sites to the electrode. Dopamine has a half life of ~0.05 s in the NAc, 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 DA 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 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 ~2-fold in most locations. Thus, even at the length scale of our electrode (~100 μm) the NAc appears heterogeneous within each sub-region. 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 presynaptic modulation of terminals (Howland *et al.*, 2002; Britt & McGehee, 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 to 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 to this complexity are findings indicating that dopamine release and NAc cell firing that occur within seconds of the response represent associative aspect of the task, 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 since 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 paper) 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 (Pennartz *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.*, 1980; Pennartz *et al.*, 1994; Nicola

et al., 2000; Carelli & Wightman, 2004). Our findings that dopamine release in the NAc occurs primarily at locations at which patterned activation of NAc neurons are recorded, even in the presence of cocaine, supports this view. The correlation of dopamine release and cell firing across both the core and shell indicates that dopamine is equally positioned within both sub-regions to influence the activation of specific sets of NAc neurons.

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 firing rate (Goto & O'Donnell, 2001). Additionally, dopamine is a modulator of striatal GABAergic interneuron coupling, indirectly affecting cell firing patterns (Cummings *et al.*, 2008). This action increases the signal-to-baseline of phasically active neurons resulting in stronger efferent signals. Indeed, inactivation of VTA neurons decreases NAc phasic excitations for reward predictive cues (Yun *et al.*, 2004). Thus, it may be the case the neurons that exhibit pre versus post response activity relative the reinforced response may represent different sets of NAc neurons defined by differential distribution of D1 versus 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, RFi) may be related to dopamine receptor distribution on those cells, but likely also is 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 ICSS 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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Figure 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 applied voltage (E_{app}) is the ordinate and the abscissa is time ($t(s)$ = time in seconds here and in subsequent figures). 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.

Figure 2. Average voltammetric data measured in the core and shell relative to cocaine-reinforced responding across all animals

Mean (solid line) $\pm$ 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, then averaged together; only full sessions were included. The dashed bar shows the time of R and the horizontal line indicates the drug-infusion period. Asterisks indicate significant increases in dopamine relative to baseline concentrations ($p \leq 0.05$).

Figure 3. A representative example of combined electrochemical and electrophysiological recordings in the NAc shell during a cocaine self-administration session

Upper panel: average of voltammetric data recorded during nine lever-press responses shown as a color representation of the data as in Figure 1. The dopamine signal rises before the lever press, declines slightly after the response, and then rises again. Middle: raster display of single-unit activity on the same trials. Bottom: PEH of the data in the middle panel; the cell was classified as type RFe. The dopamine concentration determined from the color plot by principal component analysis is shown as a blue trace superimposed on the PEH.

Figure 4. Dopamine changes around the lever press for cocaine sorted by type of neural activity of adjacent neurons

PEHs show population firing of neurons relative to lever press responding for intravenous cocaine (indicated by dashed lines at R). Increases in mean [DA] (solid blue lines) $\pm$ SEM (dashed blue lines) are superimposed above each PEH; averages were determined for each session, then averaged together here and in Figure 6. They were recorded at the same locations as the unit activity. A. Type PR ($n = 14$ cells), B. Type RFe ($n = 6$ cells), C. Type RFi ($n = 5$ of 9 cells), D. Type NP ($n=46$ cells). ANOVAs revealed significant overall increases in dopamine concentration at locations at which types PR, RFe and RFi cells were recorded; asterisks indicate significant increases relative to baseline ($p \leq 0.05$). No significant changes in [DA] were measured at locations where type NP cells were recorded. ns = non significant.

Figure 5.

Linear regression analyses correlating signal-to-baseline (S:B) ratios for peak [DA]s versus 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.

Figure 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 was observed in both the core (C) and shell (D). Mean [DA] indicated by solid lines; SEM indicated by dashed lines. Asterisks indicate significant increases in dopamine * $p \leq 0.05$, ** $p \leq 0.01$. R is reinforced response.

Figure 7. Histological reconstruction of electrode tip locations

Coronal sections of the rat brain depicting electrode tip placements (stars) in the core and shell of the NAc. Sections are from the stereotaxic atlas of (Paxinos & Watson, 1986).

Table 1

Distribution of NAc neurons (# cells) across types and sub-region and number of corresponding locations at which phasic dopamine was detected (# dopamine sites)

Cell Type	Core (# cells/# dopamine sites)	Shell (# cells/# dopamine sites)	Total (# 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