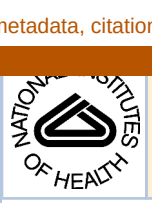

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Regional specificity in the real-time development of phasic dopamine transmission patterns during acquisition of a cue-cocaine association

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Abstract

Drug seeking is significantly regulated by drug-associated cues and associative learning between environmental cues and cocaine reward is mediated by dopamine transmission within the nucleus accumbens (NAc). However, dopamine transmission during early acquisition of a cue-cocaine association has never been assessed because of the technical difficulties associated with resolving cue-evoked and cocaine-evoked dopamine release within the same conditioning trial. Here, we used fast-scan cyclic voltammetry to measure sub-second fluctuations in dopamine concentration within the NAc core and shell during the initial acquisition of a cue-cocaine Pavlovian association. Within the NAc core, cue-evoked dopamine release developed during conditioning. However, within the NAc shell, the predictive cue appeared to cause an unconditioned decrease in dopamine concentration. The pharmacological effects of cocaine also differed between sub-regions, as cocaine increased phasic dopamine release events within the NAc shell but not the core. Thus, real-time measurements not only revealed the initial development of a conditioned neurochemical response but also demonstrated differential phasic dopamine transmission patterns across NAc sub-regions during the acquisition of a cue-cocaine association.

Keywords

in vivo voltammetry; neurotransmission; carbon-fiber microelectrode; drug abuse; addiction; reward

Environmental cues paired with cocaine for just one day increase cocaine seeking behavior nearly one year later (Ciccocioppo *et al.*, 2004). This demonstrates the importance of associations formed during initial drug exposure. However, the neural regulation of early acquisition of cue-cocaine associations is poorly understood. While it is known that dopamine transmission within the nucleus accumbens (NAc) is critical for the acquisition of a Pavlovian association (Kelley, 2004), fluctuations in dopamine concentration during the first day of cue-cocaine conditioning have never been measured. This is primarily because, until recently, it has been a technical impossibility to resolve cue-evoked and cocaine-evoked dopamine release

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within the same conditioning trial. Specifically, the neurochemical consequences of cue presentation and the pharmacological effects of cocaine are separated by just seconds (Stuber *et al.*, 2005a) whereas the temporal resolution of traditional measurement technology (microdialysis) is in the range of minutes (Pan *et al.*, 1991; Watson *et al.*, 2006).

Here, we circumvent past technical limitations by using fast-scan cyclic voltammetry (FSCV) (Wightman, 2006) to measure sub-second fluctuations in dopamine concentration ([DA]) within the NAc core and shell during the initial acquisition of a cue-cocaine association. As cue-cocaine associations are formed via classical conditioning mechanisms (Robinson & Berridge, 2008), we employed a recently described Pavlovian conditioning design in which presentation of a discrete cue was presented with non-contingent intravenous (i.v.) cocaine delivery (Uslaner *et al.*, 2006). This paradigm ensured equal number and timing of cue-cocaine pairings on the first day of Pavlovian conditioning in drug naive rats. Rapid dopamine measurements during this paradigm readily distinguished between the development of conditioned dopamine transmission associated with the predictive cue (Phillips *et al.*, 2003; Stuber *et al.*, 2005b) and increased dopamine transmission resulting from pharmacological effects of cocaine (Cheer *et al.*, 2004; Heien *et al.*, 2005; Aragona *et al.*, 2008; Sombers *et al.*, 2009).

With respect to conditioned dopamine transmission, the current study directly addressed a long-standing controversy regarding phasic dopamine signaling following the presentation of a conditioned stimulus. Prior to real-time neurochemical measurements, development of conditioned dopamine signaling has been monitored with extracellular electrophysiology measures of dopaminergic neurons (Pan *et al.*, 2005). Such studies have suggested that conditioned stimuli cause a phasic increase in firing among the great majority of dopaminergic neurons and it is often assumed that this results in a uniform increase [DA] across sub-regions of the NAc (Schultz, 1998). Conversely, microdialysis studies suggest conditioned stimuli differentially increase [DA] across NAc sub-regions (Ito *et al.*, 2000; Bassareo *et al.*, 2006).

Several methodological issues may explain this discrepancy. For example, electrophysiological identification of a dopaminergic phenotype is unambiguous only in anesthetized subjects (Ungless *et al.*, 2004; Margolis *et al.*, 2006b) and release from dopaminergic neurons depends on firing history (Montague *et al.*, 2004). Further, dopaminergic neurons exist in sub-populations that project to different forebrain locations (Margolis *et al.*, 2006a; Lammel *et al.*, 2008). Measurements of [DA] in forebrain terminal fields avoids such concerns, but microdialysis utilizes probes that sample [DA] over a rather large area and this technique lacks the temporal resolution to examine phasic dopamine signaling (Robinson *et al.*, 2003). Conversely, FSCV employs sensors that sample from discrete microenvironments (Wightman *et al.*, 2007) downstream from specific midbrain dopaminergic sub-populations (Ikemoto, 2007) and measures phasic dopamine communication on a similar time scale to single unit recording (Hyland *et al.*, 2002; Schultz, 2002). Thus, FSCV was used to resolve the controversy regarding conditioned phasic dopamine communication across distinct terminal fields, in this case, the NAc core and shell.

With respect to the pharmacological effects of cocaine, we have recently used FSCV to show that (in addition to slowing dopamine uptake (Giros *et al.*, 1996)), i.v. cocaine administration also evokes a direct increase in phasic dopamine release events within the NAc shell, but not the core (Aragona *et al.*, 2008). However, our previous study only tested a single high dose cocaine infusion (Aragona *et al.*, 2008). Here, we examined if cocaine-evoked release events within the NAc shell continue to occur following multiple cocaine infusions using a lower dose consistent with those that are self-administered. The current study demonstrates regionally specific dopamine transmission patterns during the formation of a cue-cocaine association, with conditioned (cue-evoked) dopamine transmission specific to the NAc core and

unconditioned (cocaine-evoked) dopamine transmission specific to the NAc shell. Thus, our data support recent evidence for anatomically and functionally separate mesolimbic dopamine pathways (Ikemoto, 2007) and suggest that these systems mediate distinct aspects of cue-cocaine associations.

Methods

Animals and surgery

Male Sprague-Dawley rats were purchased with implanted jugular vein catheters (n = 24, ~ 375 g, Charles River Laboratories, Wilmington, MA). Rats were anesthetized with intra-muscular ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (20 mg/kg). Bipolar stimulating electrodes (Plastics One, Roanoke, VA) (placed in the VTA; 5.2 mm posterior, 1.0 mm lateral, 7.5 mm ventral relative to bregma) and Ag/AgCl reference electrodes (placed in contralateral cortex) were secured as described in detail elsewhere (Phillips *et al.*, 2003; Wightman *et al.*, 2007). Guide cannula (Bioanalytical Systems, West Lafayette, IN) were aimed at the NAc core (1.3 mm anterior, 1.3 mm lateral, −2.5 mm ventral) or shell (1.7 mm anterior, 0.8 mm lateral, −2.5 mm ventral; relative to bregma). Following the experiment, an electrolytic lesion was made at the micro-drive setting used during the experiment and verified histologically. Experiments were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

Fast-scan cyclic voltammetry

Following 5 to 7 days recovery from surgery, glass-encased carbon-fiber electrodes were lowered using a locally constructed micro-drive (University of North Carolina at Chapel Hill, Department of Chemistry Instrument Shop) and positioned where both electrically evoked (biphasic pulses, 2 ms/phase, 24 pulses, 60 Hz, 120 μA) and transients were detected (Wightman *et al.*, 2007). Waveform generation and processing, current transduction, and data collection and filtering have been described in detail elsewhere (Wightman *et al.*, 2007). Background subtraction employed the cyclic voltammograms with the lowest current within the 10 s pre-cue/infusion baseline period. Current was converted to [DA] using principal component regression as previously described (Heien *et al.*, 2005). A dopamine transient was defined as a five fold or greater increase in [DA] relative to the root-mean-square noise value taken from the same electrode. Average transient duration is ~ 1 s (Wightman *et al.*, 2007; Aragona *et al.*, 2008) and due to their brief duration, they are routinely described as phasic (Phillips *et al.*, 2003). Events below 30 nM were excluded from the analysis because events below this magnitude were not reliably detected across electrodes. Transient frequency was determined with Mini Analysis (Synaptosoft, Decatur, GA). Average transient frequency in the current study is similar to our recent experiments (Cheer *et al.*, 2007; Aragona *et al.*, 2008) but higher than our earlier work (Robinson *et al.*, 2002; Stuber *et al.*, 2005a) and this is due to improvements in sensitivity and conducting experiments in locations where naturally occurring dopamine transients are detected (Robinson & Wightman, 2007; Wightman *et al.*, 2007). Assessment of acute alteration in mean [DA] during conditioning trials was restricted to a 90 s sampling period because electrode drift prevents reliable analysis for longer times for many electrodes (Heien *et al.*, 2005). Maximal [DA] within the sampling window is referred to as `peak' [DA]. In the case of cocaine-evoked increases in dopamine signaling, peak [DA] is the result of transients superimposed on gradual increases in [DA] that is due to blockade of terminal dopamine transporters (Heien *et al.*, 2005; Cheer *et al.*, 2007; Aragona *et al.*, 2008). `Cue-evoked dopamine release' is obviously also a transient event, but since it appears to be the result of synchronous burst firing of dopaminergic neurons (Aragona *et al.*, 2008; Sombers *et al.*, 2009), it is given this separate designation and the maximal [DA] within 2 s of cue-onset represents its peak. Following the experiment, electrodes were calibrated as previously

described (Wightman *et al.*, 2007). All chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Pavlovian Conditioning (cocaine)

Consistent with established parameters for Pavlovian conditioning utilizing cocaine reward (Uslaner *et al*., 2006), a compound stimulus (cue light and tone) was simultaneously presented with the onset of a non-contingent i.v. infusion of cocaine (0.16 mg/inf over 3s; ~ 0.5 mg/kg/infusion). Cue onset and i.v. infusion onset were simultaneous because cue onset was designed to predict central cocaine effects, not the infusion itself. The duration of the predictive cue was 20 s so that cue presentation overlapped with the previously established time of increased dopamine transmission by cocaine (Aragona *et al*., 2008). The conditioning session consisted of 30 cue-cocaine pairings with an average inter-trial interval of 5 minutes, for a total of 30 cocaine infusions in the conditioning session.

Previous studies using a similar design have shown that animals exhibit approach responses toward cocaine predictive cues (termed sign-tracking or autoshaping behavior) with no attempt to consume the conditioned stimulus (Uslaner *et al*., 2006). Approach behavior was analyzed using offline video-analysis and was defined as the subject bringing its nose to within ~ 1 cm of the cue light (Uslaner *et al*., 2006) and the duration the subject spent sniffing the light was used to calculate cue `investigation'. While novel cue investigation was observed in previous studies (Uslaner *et al.*, 2006), our subjects did not show cue investigation when i.v. saline was given instead of cocaine (n = 3; data not shown). As such, our subjects did not receive environmental or cue habituation described in previous experiments (Uslaner *et al*., 2006).

For Pavlovian conditioning using i.v. cocaine, it is critical for the inter-trial interval (ITI) to be long enough to allow the animal to distinguish between the acute effects of the most recent cocaine infusion and the residual effects of the preceding cocaine infusions (Uslaner *et al*., 2006). Given that accumbal dopamine transmission does not begin to decline until 2 to 3 min following i.v. infusion (Aragona *et al*., 2008) the Pavlovian ITI must exceed this duration. However, the effects of i.v. cocaine infusion are not completely absent for ~ 45 min (Pan *et al*., 1991) and an ITI of this length would render the current study impossible given that stable performance of carbon-fiber electrodes limits experimental duration. The ITI for self-administration of the dose of cocaine used in this present study (0.16 mg/infusion) is ~ 3.3 min (Carelli & Deadwyler, 1996). To minimize stereotypy and because additional cocaine metabolism is desired in this paradigm (Uslaner *et al*., 2006), the ITI was increased to a mean duration of 5 min (4.75, 5.00, and 5.25 min).

Pavlovian Conditioning (sucrose)

Male Sprague-Dawley rats (n = 7) received bilateral intra-oral catheters and voltammetry surgery on the same day. During the experimental day, the behavioral chamber was illuminated by a light on the side of the chamber. After a 2 minute delay, the light was extinguished and a cue was presented. The cue consisted of a tone stimulus (65 dB, 2900 Hz) paired with illumination of a house light at the top of the chamber presented for 6 s. An infusion pump delivered 200 μl of 0.3 M sucrose intra-orally over 6 s immediately following the cue. Intra-oral delivery of sucrose by an infusion pump is slower than sucrose delivery using a solenoid (Roitman *et al*., 2008). Following the infusion, there was another 2 minute delay before the next trial and a total of 30 trials were administered.

Statistics

As described in our previous study (Aragona *et al*., 2008), changes in [DA] and transient probability across conditioning trials were assessed using a linear mixed model, which was chosen based on its ability to properly handle data in which observations are not independent

(such as repeated measures data), correctly model correlated error terms, and incorporate random subject effects. To determine mean changes of [DA], measurements were averaged into 2.5 s time bins for the cue-cocaine conditioning study and 2.0 s for cue-sucrose conditioning. To estimate transient probability, it was determined whether or not a transient occurred within 2.5 s bins across the conditioning trials. A transient was assigned to a specific bin depending on the time of its peak. For both [DA] and transient probability, bins were treated as the dependent variable, with time serving both as a repeated measure and fixed effect variable. Estimation of time bins at which concentration differed from the pre-cue/infusion baseline was achieved by construction of simple slopes and comparison to the reference period (10 s prior to cue/infusion onset for cue-cocaine conditioning and 6 s prior to cue onset for cue-sucrose conditioning) to obtain *t* values. Significance was assigned if values crossed a critical *t* value (±2 for all studies), which corresponded to an α level of 0.05. Baseline dopamine transients within the NAc core and shell as well as cue-evoked dopamine release and dopamine release evoked by a 4 pulse electrical stimulation at 20 Hz 4 were compared using independent samples T-tests. Statistical significance was designated at $\alpha = 0.05$ and all statistical analyses were carried out in SPSS version 14 for Windows (SPSS).

Results

Carbon-fiber microelectrodes (6 × 100 μm) were secured in portions of the NAc core or shell (Fig. 1A) that supported electrically stimulated dopamine release (Phillips *et al*., 2003) as well as dopamine `transients' (Wightman *et al*., 2007). A dopamine transient is defined as a naturally occurring increase in [DA] that is five times greater than the root-mean-square noise (Heien *et al*., 2005) and is indicative of phasic dopamine release (Aragona *et al*., 2008). Prior to cue-cocaine conditioning, a 10 minute recording period showed that frequency of dopamine transients did not significantly differ (independent samples t-test) between the NAc core and shell ($t_3 = 3.16$, $P = 0.119$; 6.2 + 1.2 transients per minute; collapsed across sub-regions).

Cue-cocaine Pavlovian conditioning was then initiated. The first cocaine infusion (0.16 mg; ~ 0.5mg/kg) increased peak dopamine concentration (Δ [DA] = 229 + 48 nM in the NAc shell and 141 + 31 nM in the NAc core; relative to background subtraction) to magnitudes consistent with our previous study using a similar dose (Heien *et al*., 2005). Initial cocaine-evoked increases in dopamine transmission, in both the NAc shell and core, can be seen in mean [DA] data from the first conditioning trial (Fig 2; dashed box). In subsequent trials, background subtraction removes the residual effects of previous cocaine infusions. Therefore, remaining analysis of increased dopamine signaling by cocaine is focused on acute increases by the cocaine infusions that occur within specific conditioning trials.

Changes in [DA] were continuously assessed over 90 s sampling periods (Heien *et al*., 2005) that began 10 s prior to cue/infusion onset and this portion served as the baseline to determine acute changes in [DA] by the predictive cue and cocaine infusions. Since averaging FSCV data washes out dopamine transients that are not time locked to a specific event (Roitman *et al*., 2008), representative concentration traces (Fig 1B, C, D, and E) are required to demonstrate the phasic nature of dopamine transmission detected by FSCV.

We have recently demonstrated that cocaine directly increases phasic dopamine release events within the NAc shell, but not the core (Aragona *et al*., 2008). Consistent with this previous study, cocaine infusions did not acutely increase phasic dopamine signaling within the NAc core in the seconds following i.v. infusion compared to the pre-cue/infusion baseline (Fig 1B and C). However, within the NAc shell, cocaine infusion increased the probability and magnitude of dopamine transients, beginning at ~ 20 s following drug infusion both early and late within the conditioning session (Fig 1 D and E). Thus, acute phasic increases in dopamine transmission were detected even after multiple cocaine infusions (i.e. in the presence of the

elevated levels of [DA] described by microdialysis studies (Pettit & Justice, 1989)). Importantly, the time-point of this increase is identical to that described in our previous study that did not utilize a predictive cue (Aragona *et al*., 2008), indicating that cocaine-evoked release events are a pharmacological effect.

The sub-second temporal resolution of FSCV allows for cue-evoked dopamine transmission to be distinguished from cocaine-evoked increases in [DA] (Stuber *et al*., 2005a). Within the NAc core, cue onset did not alter dopamine transmission early in the conditioning session (Fig 1B). However, following additional cue-cocaine pairings, cue onset evoked a robust increase in [DA] within the NAc core (Fig 1C). These data demonstrate that cue-evoked dopamine release can emerge in one Pavlovian conditioning session using cocaine reward. Conversely, within the NAc shell, cue onset did not increase dopamine transmission either early (Fig 1D) or late (Fig 1E) within the conditioning session. Thus, cue-evoked dopamine release, during this Pavlovian design using cocaine reward, occurs within the NAc core but not the NAc shell.

Three-dimensional representation of mean changes in [DA] across conditioning trials provides a detailed assessment of the development of real-time dopamine transmission patterns during the establishment of this cue-cocaine association (Fig 2A and C). Within the NAc core, cue onset did not increase [DA] early within the session (Fig 2A). However, cue onset evoked higher [DA]s across conditioning trials and cue-evoked dopamine release was most robust toward the end of the conditioning session (Fig 2A). For statistical analysis, change in [DA] was organized into 2.5 s bins (Fig 2 B and D) and data were averaged into the first (1 to 10) middle (11 to 20) and last (21 to 30) blocks of conditioning trials (the 10 s prior to cue/infusion onset served as the baseline). Within the NAc core, a linear mixed model analysis reveals that [DA] was significantly increased during presentation of the predictive cue during the middle and last conditioning blocks (Fig 2B; Trials 11–20, $t_{125.4} = 7.21$, $P < 0.001$ at peak; Trials 21–30, $t_{93.98} = 6.76$, $P < 0.001$ at peak). Importantly, this was due to cue-evoked dopamine release, as cue onset induced an immediate and significant increase in transient probability, including transients greater than 100 nM (Fig. 3A; Trials 11–20, $t_{109.88} = 5.697$, $P < 0.001$ at peak; Trials 21–30, $t_{114.8} = 5.433$, $P < 0.001$ at peak).

Cue-evoked dopamine release within the NAc core reached a maximum concentration following 30 conditioning trials (Supplemental Figure 1A) and this was not due to the detection capabilities of the electrode (Supplemental Figure 1B). Rather, this appeared to be due to development of synchronous population burst firing of dopaminergic neurons, as an independent samples t-test revealed no difference between peak cue-evoked dopamine release (114 + 38 nM) and dopamine release evoked by electrical stimulation of dopaminergic neurons (122 + 14 nM; $t_3 = 0.072$, $P = 0.947$; n = 4, 2 from the NAc shell, 2 from the NAc core, no difference across sub-regions) that mimicked population burst firing (4 pulse stimulation at 20 Hz) (Pan *et al.*, 2005).

After cue-evoked dopamine release was established, presentation of the predictive cue alone (i.e. in the absence of cocaine infusion) also resulted in time-locked dopamine release within the NAc core (Supplemental Figure 1C). This is consistent with previous studies (Phillips *et al.*, 2003)(which measured exclusively within the NAc core) and indicates that cue-evoked dopamine release was driven by conditioned sensory stimulation (Dommett *et al.*, 2005; Pan & Hyland, 2005) and not an interoceptive signal provided by i.v. infusion (Wise *et al.*, 2008). We have previously established that conditioning is indeed necessary for sensory input to evoke dopamine release (Phillips *et al.*, 2003). However, to confirm that this is the same for Pavlovian conditioning, two additional subjects received 30 `un-paired' cue-cocaine trials. These subjects did not show time-locked dopamine release upon cue presentation (Supplemental Figure 2). Finally, there was a significant positive correlation between the mean change in cue-evoked dopamine release and Pavlovian approach behavior (Uslaner *et al.*, 2006) ($R^2_{28} = 0.445$, $P <$

0.001; Supplemental Figure 1D). These data confirm that cue-evoked dopamine release within the core was a conditioned effect and suggest that this signal carried motivational significance (Robinson & Berridge, 2003).

Within the NAc core, linear mixed model analysis did not show a significant increase in [DA] compared to the pre-cue/infusion baseline following multiple cocaine infusions (Fig 2A and B; all *t* values < 2; P > 0.05). This indicates that while multiple cocaine infusions maintain a global elevation in [DA] within the NAc core (Stuber *et al.*, 2005a) they did not acutely increase [DA] within this region. The lack of an acute increase in [DA] following subsequent cocaine infusions is indicative of the failure of cocaine to increase transient probability in the seconds following cocaine infusion compared to the pre-cue/infusion baseline (Fig 3A; all *t* values < 2; P > 0.05) and this is consistent with our previous study showing that cocaine does not directly increase phasic dopamine release events in the NAc core (Aragona *et al.*, 2008).

With respect to both cue- and cocaine-evoked dopamine transmission, dopamine signaling within the NAc shell (Fig 2C) showed nearly the opposite pattern compared to that seen within the NAc core (Fig 2A). Within the NAc shell, [DA] was lowest during cue presentation compared to the pre-cue/infusion baseline (Fig 2C). Linear mixed model analysis shows that cue presentation significantly decreased [DA] within the NAc shell (Fig 2D) and that this decrease was present during the first conditioning block, suggesting that the cue-evoked decrease was unconditioned (*t* value < −2 for at least one comparison during cue period for each trial block, P < 0.05). Transient probability was not different during the cue period (Fig 3B; all *t* values between −2 and 2; P > 0.05), suggesting that the decrease was not due to detectable changes in dopamine transients. However, linear mixed model analysis shows that the probability of high concentration transients (defined as transients >100 nM) was significantly increased in the last conditioning block (Fig 3B; $t_{128} = 3.082$, P = 0.003 at peak). As a result, there was an acute increase in [DA] during the final conditioning block (Fig 2D; $t_{47.29} = 3.654$, P = 0.001 at peak), which is defined as a significant increase compared to the pre-cue/infusion baseline.

We next determined if this cue-evoked decrease in [DA] within the NAc shell generalized to a similar conditioning paradigm using sucrose reward. In a separate group of subjects (n = 7), a predictive cue was presented six seconds prior to non-contingent intra-oral delivery of sucrose that was readily ingested. Consistent with cue-cocaine pairings, cue presentation resulted in a time-locked decrease in mean [DA] within the NAc shell (Fig 4A; $t_{123.65} = -2.438$, P = 0.016 at trough), that was present during the first block of cue-sucrose pairings (Fig 4B; $t_{122.62} = -2.424$, P = 0.017 at trough). Together, these data demonstrate that cue-evoked decreases in [DA] within the NAc shell during early Pavlovian acquisition generalized to both drug and natural reward.

Mean [DA] traces for the NAc core and shell during the last cue-cocaine conditioning block (final 10 pairings) were superimposed to emphasize the differential dopamine transmission patterns between the NAc core and shell (Fig 5). Despite nearly opposite directionality in transmission patterns, peak [DA] following cue-evoked dopamine release within the NAc core (114 + 38 nM) and cocaine-evoked dopamine release within the shell (131 + 18 nM) were similar in magnitude. It is possible that peak [DA] within the NAc shell resulting from cocaine-evoked dopamine release required the preceding cue-evoked decrease in [DA] because this would reduce autoreceptor activation (Sulzer & Pothos, 2000) and thus potentiate subsequent release events. Finally, peak cocaine-evoked [DA] within the NAc shell represents a 3.9 fold increase in the signal to baseline, relative to the lowest [DA] value in the conditioning trial, i.e. during the cue-evoked decrease in dopamine signaling. This is comparable to the 3.7 signal to baseline achieved by cue-evoked dopamine release within the NAc core, relative to [DA] during the pre-cue/infusion baseline. Thus, the cue-evoked decrease in [DA] in the NAc shell

allowed cocaine-evoked dopamine release within the shell (implicated in primary reinforcement by drugs of abuse) to achieve a similar signal to baseline increase as cue-evoked dopamine release within the core (implicated in conditioned reinforcement).

Discussion

Real-time fluctuations in [DA] were measured within the NAc core and shell during the first session of a Pavlovian conditioning paradigm in which a discrete cue was paired with non-contingent i.v. infusions of cocaine. The sub-second measurements provided by FSCV unambiguously distinguished between cue-evoked and cocaine-evoked alterations in dopamine transmission, which allowed novel characterization of the initial development of dopamine transmission patterns during the acquisition of a cue-cocaine association. Within the NAc core, the predictive cue had no effect on dopamine transmission early in the conditioning session. However, cue onset evoked phasic dopamine release toward the end of the same conditioning session. Cue-evoked dopamine release developed as a function of conditioning as it was correlated with Pavlovian approach behavior and was not observed in unpaired subjects. In contrast to the NAc core, cue onset decreased [DA] within in the NAc shell. While initial cocaine infusions elevated [DA] levels in both the NAc core and shell compared to pre-drug levels, subsequent cocaine infusions acutely increased phasic dopamine release events only within the NAc shell. Together, the current data demonstrate dramatic regional specificity in phasic dopamine signaling during the formation of a Pavlovian association utilizing cocaine reward with conditioned dopamine transmission within the NAc core and unconditioned dopamine transmission within the NAc shell.

Conditioned dopamine transmission within the NAc core

In humans, drug-associated cues induce craving and increase drug-seeking behavior (Grant *et al.*, 1996; Childress *et al.*, 1999; Garavan *et al.*, 2000). In laboratory models, drug-associated cues maintain cocaine self-administration (Ito *et al.*, 2004) and cues paired with a single exposure to cocaine can potentiate drug seeking nearly one year later (Ciccocioppo *et al.*, 2004). It is well established that enhanced reward seeking by conditioned stimuli is processed within the NAc core as lesions of this area impair approach behavior toward conditioned stimuli (Parkinson *et al.*, 1999; Di Ciano *et al.*, 2001) and reduce the ability of conditioned stimuli to reinforce and potentiate operant behavior (Hall *et al.*, 2001; de Borchgrave *et al.*, 2002). Microdialysis studies have shown that, in subjects trained to self-administer cocaine, non-contingent presentation of a conditioned stimulus (previously paired with the operant response) increase [DA] selectively within the NAc core (Ito *et al.*, 2000). Further, studies using FSCV (which provides a faster temporal resolution compared to microdialysis (Watson *et al.*, 2006; Wightman, 2006)) have shown that cue-evoked increases in [DA] within the NAc core during cocaine self-administration (Ito *et al.*, 2000) are due to phasic dopamine release at cue onset that results in brief but robust increases in [DA] (Phillips *et al.*, 2003; Stuber *et al.*, 2005a).

In the current study, we show that cue-evoked dopamine release within the NAc core fully developed in just one session of Pavlovian conditioning utilizing cocaine reward. Consistent with our previously published self-administration study (Phillips *et al.*, 2003), cue onset did not increase [DA] in unpaired subjects. This indicates that cue-evoked dopamine release required conditioning and was not merely the result of enhanced dopamine detection following blockade of dopamine transporters (Robinson & Wightman, 2004). Additionally, there was a significant positive correlation between cue-evoked dopamine release within the NAc core and investigation of the conditioned stimulus (Uslaner *et al.*, 2006). This relationship confirms that the cue served as a conditioned stimulus and that cue-evoked dopamine release was a conditioned effect.

Extracellular electrophysiology studies show that presentation of a conditioned stimulus results in synchronous population bursting of dopaminergic neurons located approximately 1 mm off the midline (Pan *et al.*, 2005) (i.e. neurons that primarily project to the NAc core (Ikemoto, 2007)). In the current study, electrical stimulation of dopaminergic neurons at parameters within the physiological range of population bursting (4 pulses at 20 Hz) (Pan *et al.*, 2005) resulted in an equivalent increase in [DA] compared to cue-evoked dopamine release. This suggests that cue-evoked dopamine release was due to the development of synchronous bursting among dopaminergic neurons projecting to the NAc core (Ikemoto, 2007). Consistent with this hypothesis, dopaminergic neuron bursting is mediated by glutamate (Overton & Clark, 1997) and glutamatergic projections (Geisler *et al.*, 2007) are activated by visual/auditory stimulation to increase dopaminergic neuron firing (Dommett *et al.*, 2005; Pan & Hyland, 2005). Further, we have recently demonstrated that cue-evoked dopamine release that predicts intracranial self-stimulation was abolished by blockade of NMDA receptors within the ventral tegmental area (Sombers *et al.*, 2009). Additionally, a recent FSCV study demonstrated that cue-evoked dopamine release within the NAc core fully developed on the third conditioning session using a Pavlovian conditioning paradigm which utilized a natural reward (sucrose pellets) and this occurred on the same day as enhancement of glutamatergic synapses on midbrain dopaminergic neurons (Stuber *et al.*, 2008). It is known that a single cocaine exposure causes a similar alteration in glutamate synapses (Ungless *et al.*, 2001) and thus may be related to the more rapid development of cue-evoked dopamine release with Pavlovian conditioning utilizing cocaine reward.

Unconditioned dopamine transmission within the NAc shell

In contrast to the NAc core, cue onset appeared to cause a brief unconditioned decrease in [DA] within the NAc shell during Pavlovian conditioning utilizing both non-contingent cocaine and sucrose reward. This regional specificity is in contrast to electrophysiology studies suggesting that conditioned stimuli increase firing in the majority of dopaminergic neurons and thus increase [DA] across all striatal regions (Schultz, 2002). However, caution must be taken when attempting to infer terminal dopamine communication solely from neuronal activity for several reasons. First, identification of a dopaminergic phenotype is reliable only in anesthetized preparations (Margolis *et al.*, 2006b). Second, while certain sub-populations of dopaminergic neurons are disproportionately sampled in freely moving electrophysiology studies, recent electrophysiology studies support functionally distinct sub-populations of dopaminergic neurons (Brischoux *et al.*, 2009; Matsumoto & Hikosaka, 2009). Behavioral studies have focused primarily on `conventional' dopaminergic neurons (Lammel *et al.*, 2008) that preferentially project to the NAc core (Hyland *et al.*, 2002; Pan *et al.*, 2005), whereas dopaminergic neurons that project to the NAc shell (Ikemoto, 2007) and have not been examined in freely moving electrophysiology studies. Finally, there is not a one to one correspondence between action potential generation and terminal dopamine release (Montague *et al.*, 2004) and [DA] can be significantly modulated at the terminal level (Cragg, 2006; Britt & McGehee, 2008).

By directly measuring changes in terminal [DA] with FSCV, the current study avoided these concerns and revealed that cue onset decreases [DA] within the NAc shell in this paradigm. This finding supports recent descriptions of functionally distinct dopamine projection pathways between the NAc core and shell (Ikemoto, 2007; Liu *et al.*, 2008). It is only recently that we have demonstrated that it is possible to detect decreases in [DA] using FSCV (Roitman *et al.*, 2008). FSCV is a differential technique in which raw data show phasic increases in [DA] relative to a background subtracted time point (Wightman, 2006). However, averaging across trials can reveal time-locked decreases in [DA] under certain behavioral situations, such as intra-oral infusion of an aversive tastant (Roitman *et al.*, 2008). Future studies are needed to address if this shell-specific decrease is primarily due to decreased dopaminergic neuronal

firing (Ungless *et al.*, 2004) or to differences in terminal modulation among regionally specific afferents (Zahm, 2000).

Consistent with our previous study, which used a single high dose infusion of cocaine (Aragona *et al.*, 2008), the lower dose infusions used in the current study also caused unconditioned increases in phasic dopamine release events within the NAc shell. Sucrose did not significantly increase [DA] compared to baseline values and this is most likely due to the relatively slow intra-oral infusion rate by pump infusion (current study) compared to the rapid solenoid deliver used in our previous study (Roitman *et al.*, 2008), which did result in a significant increase in [DA]. Acute increases in [DA] by cocaine are in addition to the global elevation in [DA] described by microdialysis studies (Pettit & Justice, 1989) and are critical for the timing of drug intake in rats self-administering cocaine (Stuber *et al.*, 2005a). Previous studies from our lab show that cocaine-evoked increases in transient frequency within the NAc shell are due to a true increase in the number of phasic dopamine release events and are not merely the result of enhanced dopamine detection (Aragona *et al.*, 2008; Sombers *et al.*, 2009). The magnitude of increased transient frequency is equal to that of autoreceptor blockade (Aragona *et al.*, 2008), a manipulation known to increase burst firing of dopaminergic neurons (Andersson *et al.*, 1995). Cocaine-evoked dopamine transients within the shell were eliminated by infusion of GABA agonists (Aragona *et al.*, 2008) and lidocaine directly into the ventral tegmental area (VTA) (Sombers *et al.*, 2009) demonstrating that this increase is indeed due to increased firing of dopaminergic neurons. Finally, burst firing is mediated glutamate activation of NMDA receptors (Overton & Clark, 1997), peripheral cocaine administration increases glutamate within the VTA (Kalivas & Duffy, 1995; Wise *et al.*, 2008; You *et al.*, 2008), and blockade of NMDA receptors within the VTA reduces the frequency of dopamine transients within the NAc shell (Sombers *et al.*, 2009). Together these data strongly suggest that the unconditioned increase in phasic dopamine release events within the NAc shell following cocaine infusion is due to a brief increase in burst firing among dopaminergic neurons projecting to the NAc shell.

While conditioning is not required for cocaine-evoked dopamine release (Aragona *et al.*, 2008; Sombers *et al.*, 2009), acute increases in [DA] by cocaine were greatest toward the end of the conditioning session (current study). The most parsimonious explanation for this is that cocaine accumulated within the brain because it was delivered every ~ 5 minutes and it takes longer for cocaine to be fully metabolized following an i.v. infusion (Pan *et al.*, 1991). As such, cocaine concentration increases in the brain with each infusion causing later infusions to be the functional equivalent of higher dose infusions (see figure 3A in (Stuber *et al.*, 2005a)). Due to the differential nature of FSCV, only acute increases in [DA] during each conditioning trial were assessed. Future studies are needed that measure long-term changes in [DA] across conditioning trials (Hermans *et al.*, 2008). While these data suggest that increased cocaine-evoked [DA] across the conditioning session is a pharmacological effect, we cannot exclude the possibility that progressively greater [DA]s were due to sensitization of the mesolimbic system by early cocaine infusions (Robinson & Berridge, 2003) or to the development of an association between interoceptive signals related to drug infusion and central cocaine action resulting in later infusions causing greater dopamine release (Wise *et al.*, 2008).

Regionally specific dopamine function in reward processing

Increased mesolimbic dopamine transmission is often interpreted within the confines of a specific theory regarding its role in behavioral regulation including: learning and memory (Berke, 2003; Kelley, 2004; Wise, 2004), reward prediction (Bayer & Glimcher, 2005; Schultz, 2007), incentive salience (Robinson & Berridge, 2003; Berridge, 2007), general motivation and effort (Horvitz, 2000; Salamone *et al.*, 2003), or hedonic processing (Di Chiara & Bassareo, 2007; Volkow *et al.*, 2007). However, the current data emphasize that dopamine transmission in different brain regions may require different theories to best explain dopamine function. For

instance, cue-evoked dopamine release within the NAc core developed more rapidly with cue-cocaine pairings compared to Pavlovian conditioning utilizing sucrose reward (Day *et al.*, 2007; Stuber *et al.*, 2008). This is consistent with dopamine involvement in associative processes and enhanced learning with drug reinforcement (Berke, 2003; Kelley, 2004; Hyman *et al.*, 2006; Kalivas, 2007). However, cue-evoked dopamine release was correlated with investigation of the conditioned stimulus, suggesting that it also mediates incentive/motivational aspects of learned associations (Uslaner *et al.*, 2006). Thus, phasic dopamine transmission within the NAc core may serve as an incentive signal (Robinson & Berridge, 2003) during early acquisition (current study) as well as maintenance (Phillips *et al.*, 2003; Stuber *et al.*, 2005b) of a learned association. However, in well-trained animals, core dopamine transmission may continue to signal reinforcement (Wise, 2004; Day *et al.*, 2007) while direct behavioral regulation by dopamine may shift to the dorsal striatum in over trained animals (Everitt & Robbins, 2005; Vanderschuren *et al.*, 2005).

The function of cue-evoked alterations in dopamine transmission within the NAc shell is less apparent. Previous microdialysis studies using cocaine self-administration have shown that conditioned stimuli do not increase [DA] within the NAc shell (Ito *et al.*, 2000). Therefore, the brief pause in dopamine transmission described in the current study (that could not be detected with microdialysis) is not inconsistent with previous cocaine studies. However, pharmacological enhancement of dopamine transmission in the shell improves the ability of conditioned stimuli to increase reward seeking (Wyvell & Berridge, 2000). Therefore, dopamine transmission within the shell can enhance the incentive impact of a conditioned stimulus, even if the stimulus itself does not directly increase dopamine release. However, presentation of a non-acute conditioned stimulus (10 min object presentation) that precedes morphine reward has been shown to increase [DA] in the NAc shell but not the core (Bassareo *et al.*, 2006), suggesting that conditioned stimuli may evoke dopamine release in a reward specific manner. Alternatively, differential dopaminergic processing of conditioned information within the NAc may depend on the nature of the information provided by the cue. For instance, the NAc shell (but not the core) is critical for context conditioning (achieved, in part, via interaction with the hippocampus) (Ito *et al.*, 2008), which may explain why discriminative cues that signal the availability to self-administer electrical brain stimulation evoke phasic dopamine release within the NAc shell (Owesson-White *et al.*, 2008). Additionally, the NAc shell may be more important for regulation of drug seeking by discriminative stimuli following drug abstinence (Ghitza *et al.*, 2003), while the NAc core may be important for drug seeking related to conditioned stimuli following abstinence (Hollander & Carelli, 2007). Together, these studies suggest that the function of conditioned dopamine signaling within the NAc shell is context specific.

Conversely, there is convergent evidence across behavioral paradigms indicating that dopamine transmission within the NAc shell is involved in primary reward processing associated with abused drugs. Lesions of the NAc shell attenuate the unconditioned potentiating effects of psychostimulants (Cardinal & Everitt, 2004) and these drugs are self-administered directly into the NAc shell (and olfactory tuberacle) but not the NAc core (Ikemoto, 2003). Psychostimulants preferentially increase [DA] within the NAc shell (Pontieri *et al.*, 1995; Aragona *et al.*, 2008; Frank *et al.*, 2008) and dopamine receptor blockade within the NAc shell prevents the acquisition of drug-induced conditioned place preferences (Fenu *et al.*, 2006). Importantly, there is evidence that activation of D1-like receptors is especially important for mediating the unconditioned aspects of associative learning (Di Chiara & Bassareo, 2007) and cocaine directly increases the probability of high concentration phasic release events within the NAc shell which likely increases the proportion of low affinity D1-like receptors that are activated (Richfield *et al.*, 1989). Thus, the current data are relevant to the hypothesis that rapid and robust increases in [DA] mediate reward associated with abused drugs (Di Chiara & Bassareo, 2007; Volkow *et al.*, 2007).

In conclusion, the current data suggest that dopamine transmission within the NAc core and shell mediate distinct aspects of motivational and associative processes. These regions either regulate different components of a particular theoretical framework describing dopamine function, or perhaps separate theories are required to best describe dopamine transmission in a given brain region. Regardless, it is clear that all theories of dopamine function must account for regional specificity regarding phasic dopamine signaling during the acquisition of a cue-cocaine association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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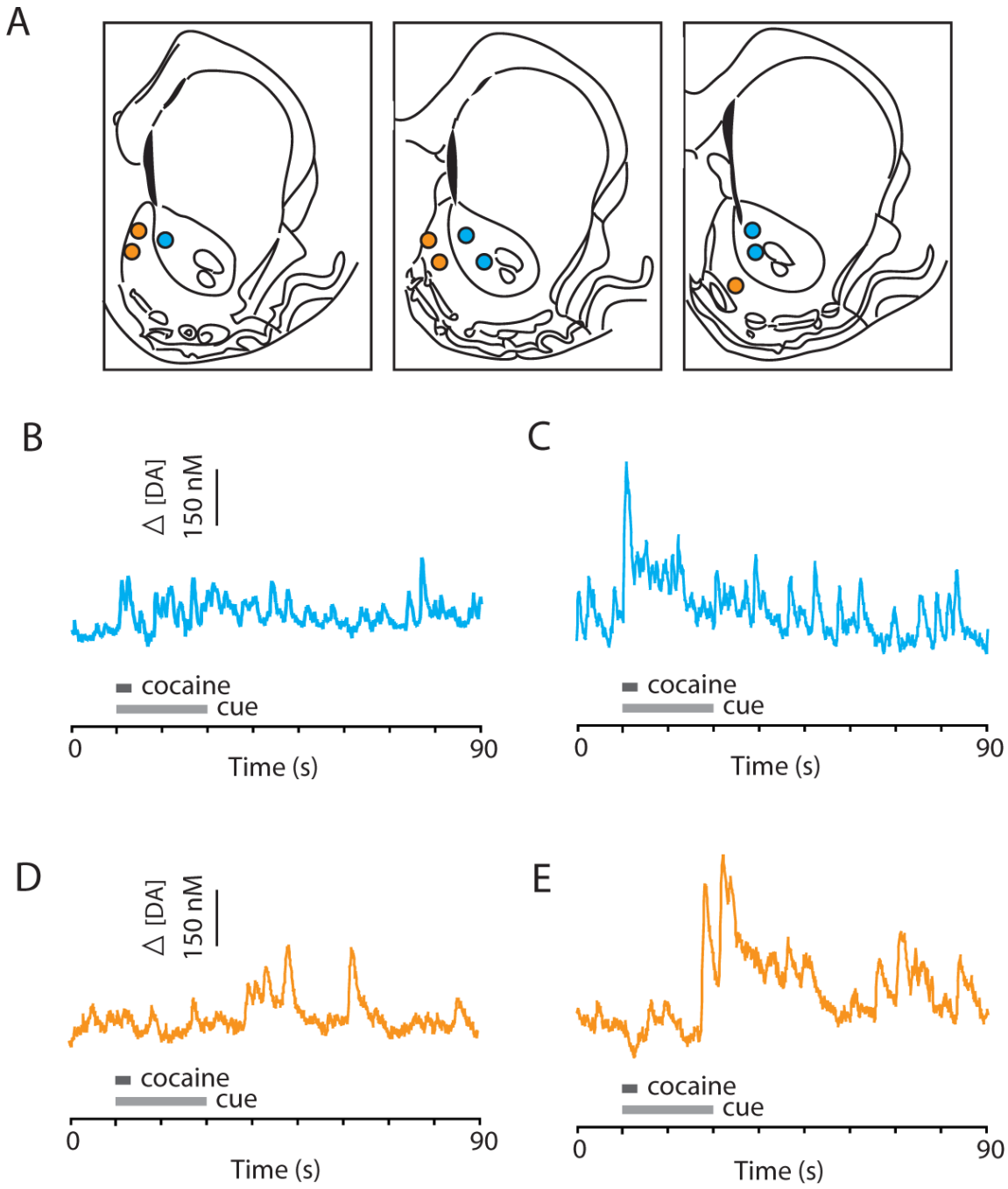

Figure 1.

Representative [DA] traces reveal region-specific phasic dopamine communication during the acquisition of a cue-cocaine association. Probe placements (one placement per rat) and individual [DA] traces early or late within the conditioning session. A) Measurements were made in the NAc core (blue; n = 5) or the NAc shell (orange; n = 5). B) [DA] trace from the NAc core during the first conditioning block (trial 7) C) and during the last conditioning block (trial 25). D) [DA] trace from the NAc shell during the first conditioning block (trial 5) E) and during the last conditioning block (trial 28).

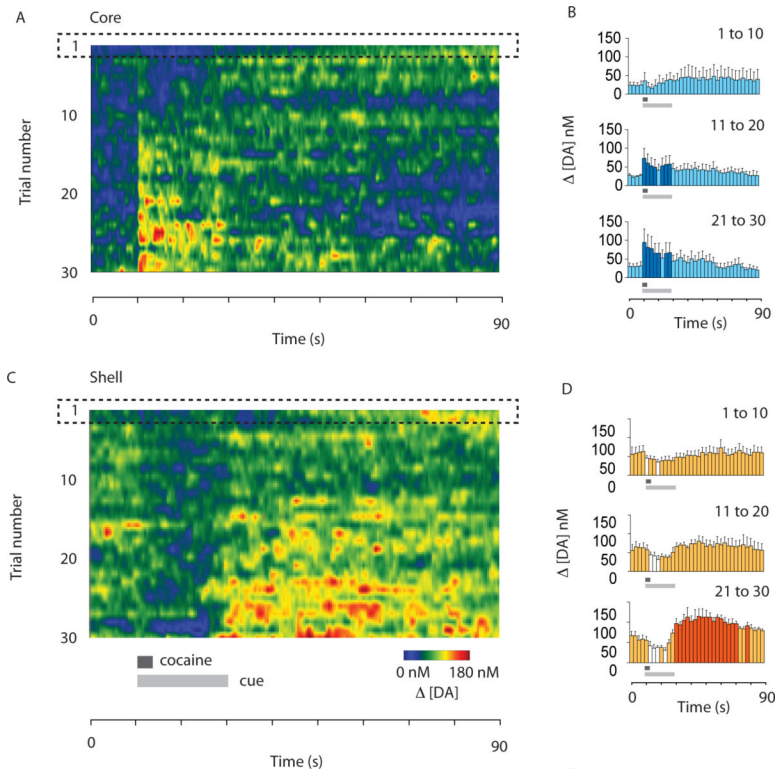

Figure 2.

Real-time dopamine transmission patterns during early acquisition of a cue-cocaine association. A and C) Mean change in [DA] is represented as change in color during the 90 s sampling window (x-axis) for each conditioning trial (y-axis). The i.v. cocaine infusion (3 s) is represented by the dark grey box and the predictive cue (20 s) is represented by the light grey box. B and D) Quantification of [DA] within the NAc core and shell during cue-cocaine association. Trial numbers are indicated on the figure and dopamine concentrations were binned in 2.5 s intervals. A) Within the NAc core (n = 5), cue-evoked dopamine release is increased across conditioning trials. Cocaine-evoked increases in [DA] were present early in the conditioning session but inconsistent thereafter. B) Within the NAc core (blue; n = 5), [DA] was not altered during the first conditioning block (trials 1 to 10) by either cue presentation or cocaine infusions. However, during the second (trials 11 to 20) and third conditioning blocks (trial 21 to 30), [DA] was significantly increased during cue presentation with dark blue bars indicating a significant increase over the the pre-cue/infusion baseline (i.e. the first four bins) ($p < 0.05$). C) Within the NAc shell (n = 5), [DA] is lowest during cue presentation and the cue-evoked attenuation in [DA] was present from the onset of the conditioning session. Cocaine robustly increased [DA] at later time points consistent with its known pharmacokinetics. Cocaine-evoked dopamine release continued following multiple drug infusions and [DA] levels were highest toward the end of the session. D) Within the NAc shell (light orange; n = 5), [DA] was significantly decreased (indicated by white bars) during cue presentation during all conditioning blocks. In the final conditioning block (trials 21 to 30) [DA] was significantly increased (dark orange) over the pre-cue/infusion baseline ($p < 0.05$) (beginning 20 s after drug infusion). B and D) error bars equal standard error from the mean.

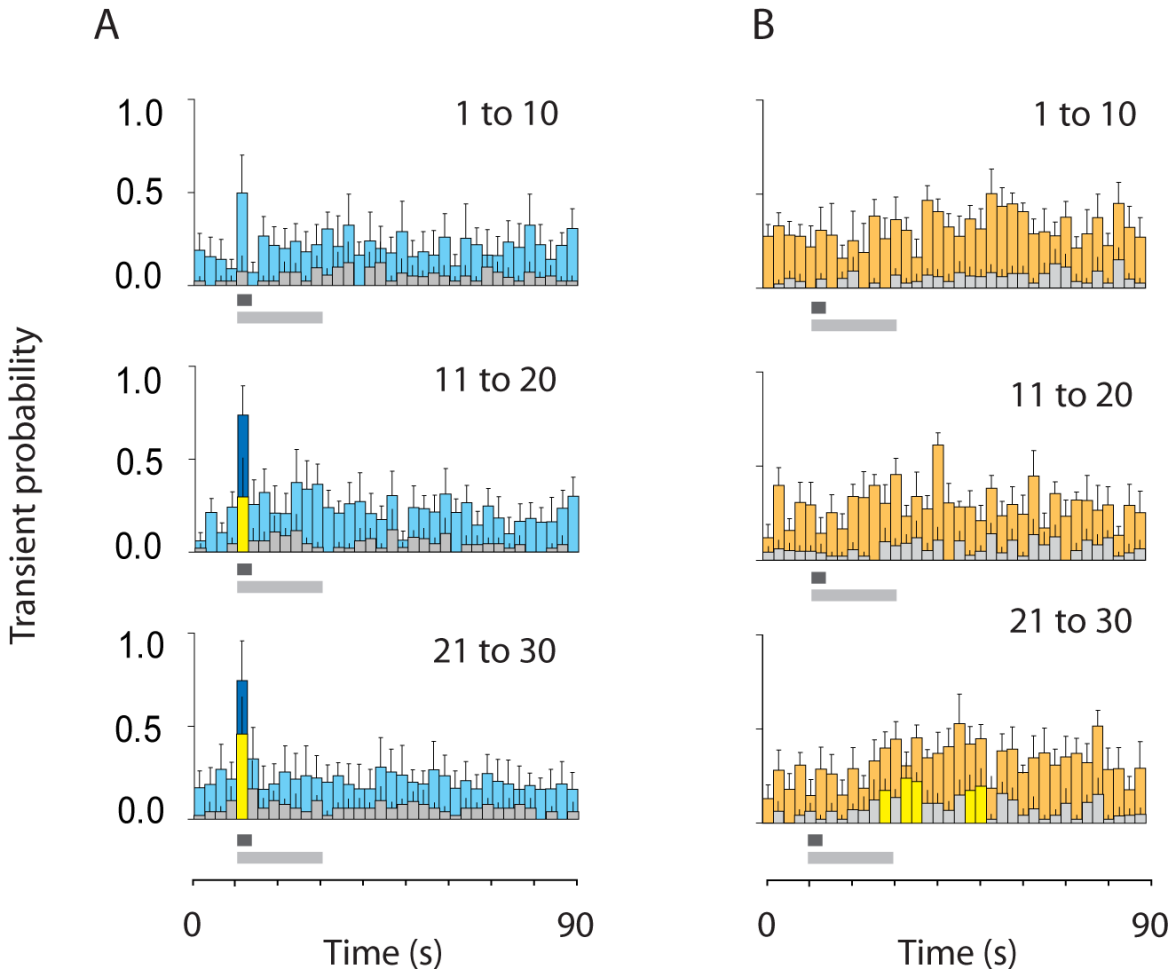

Figure 3.

Transient probability within the NAc core and shell during early acquisition of a cue-cocaine association. A and B) The presence or absence of dopamine transients during 2.5 s bins across the conditioning trials was used to determine transient probability. A) Within the NAc core, the probability of all dopamine transients is coded in light blue and the probability of transients greater than 100 nM is coded in grey. During the first conditioning block (trials 1 to 10) transient probability was not altered by the predictive cue or cocaine infusion. However, during the middle and last conditioning blocks, the probability of all dopamine transients was significantly increased (dark blue) and the probability of transients over 100 nM was also significantly increased (yellow) during the first bin of cue onset compared to the pre-cue/infusion baseline. B) Within the NAc shell, the probability of all dopamine transients is coded as orange and the probability of transients greater than 100 nM is coded as grey. For all conditioning blocks, neither the predictive cue nor cocaine infusion significantly altered the probability of dopamine transients if transients of all concentration were assessed. However, cocaine significantly increased the probability of transients over 100 nM in the final conditioning block (trials 21 to 30) relative to the pre-cue/infusion baseline (indicated by yellow bars). A and B) Significance level was $p < 0.05$; error bars equal standard error from the mean.

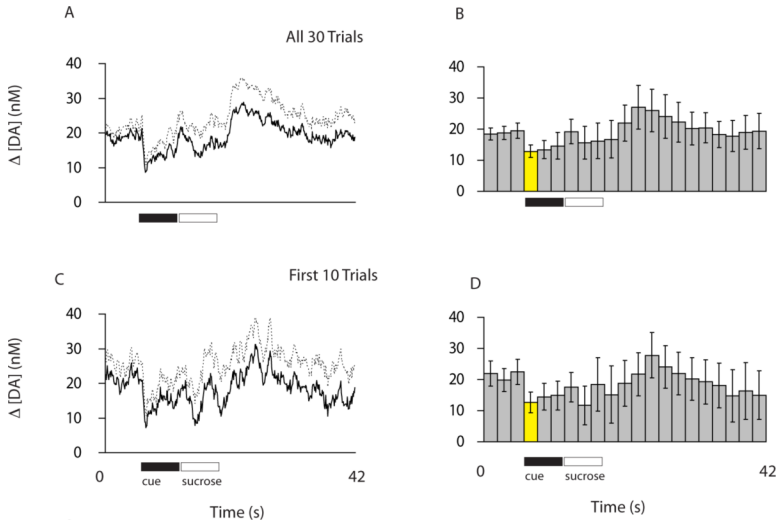

Figure 4.

Discrete cues predictive of sucrose delivery decrease [DA] within the NAc shell during the first session in a Pavlovian conditioning paradigm. A, B, C, and D) Black box indicates onset and duration of the predictive cue and the white box indicates onset and duration of computer controlled intra-oral sucrose delivery by an infusion pump. A) Mean [DA] across all 30 conditioning trials (solid line) and mean plus standard error (dashed line). B) Mean [DA] averaged into 2 s bins for statistical analysis across all 30 trials. C) Mean [DA] across the first 10 conditioning trials (solid line) and mean plus standard error (dashed line). D) Mean [DA] averaged into 2 s bins for statistical analysis across the first 10 trials. B and D) Yellow box indicate significant difference at $p < 0.05$; error bars indicate standard error from the mean.

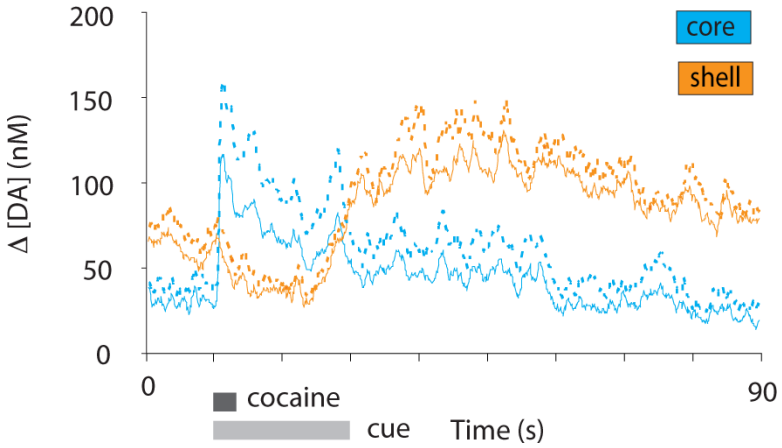

Figure 5.

Sub-region differences in dopamine transmission patterns. and Pavlovian approach behavior. Mean (solid lines) and mean plus standard error (dashed lines) for 100 ms fluctuations in [DA] from the NAc core (blue) and NAc shell (orange). Change in [DA] from the final conditioning block (trials 21 to 30). For each 90 s collection window, the lowest current value during the 10 s pre-cue/infusion baseline period was the point chosen for background subtraction