RAPID DOPAMINE SIGNALING IN THE NUCLEUS ACCUMBENS SHELL, BUT NOT CORE, ENCODES REWARD MAGNITUDE-BASED DECISION MAKING

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ABSTRACT

DEIRDRE A. SACKETT: Rapid dopamine signaling in the nucleus accumbens shell, but not core, encodes reward magnitude-based decision making
(Under the direction of Regina M. Carelli)

Effective decision making requires organisms to predict reward values and bias behavior toward the best available option. The mesolimbic dopamine system, including the nucleus accumbens (NAc) core and shell, is involved in this process. While studies support a differential role of the core and shell in subjective versus outcome based decision making, no studies have examined dopamine release to cues signaling the availability of different reward magnitudes. Here, electrochemical methods were used in rats to measure shell versus core dopamine release during a magnitude decision making task in which discrete cues signaled the availability of different reward sizes. Dopamine release in the shell (not core), preferentially tracks cues that predict the large preferred reward. Further, unique dopamine release dynamics are observed in the shell, but not core, upon lever press. These findings indicate a differential role of the core and shell in subjective, versus outcome-based, aspects of value-based decision-making.

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INTRODUCTION

Effective decision making depends on an organism's ability to predict the outcome of its choices, and bias behavior toward the option of greatest value. Value-based decision making recruits the mesolimbic system, including the nucleus accumbens (NAc) and its dopaminergic input (Day et al., 2007, Fields et al., 2007, Clark et al., 2012). Dopamine neurons increase activity to cues that predict rewards, and track choice behaviors related to a range of decision making including effort, delay, risk, and delay discounting (Schultz, 1997, Roesch et al., 2007, Day et al., 2011). Rapid DA release in the NAc reflects this pattern of cellular activity (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Indeed, increases in transient dopamine release have been measured during cues predicting food, liquid, cocaine and intracranial self-stimulation (Phillips et al., 2003, Roitman et al., 2004, Day et al., 2007, Owesson-White et al., 2008). Further, pharmacological disruption or lesions of mesolimbic circuitry, including the NAc, results in maladaptive decision making, such that animals cannot update behavior to reflect changes in reward value (Cardinal et al., 2001, St Onge and Floresco, 2009, Ghods-Sharifi and Floresco, 2010).

The NAc contains two primary subregions, the core and shell, which differ in their afferent and efferent connections (Heimer et al., 1991, Zahm and Brog, 1992, Jongen-Relo et al., 1994, Ikemoto, 2007). For example, the core projects primarily to motor-related structures such as the globus pallidus and substantia nigra, while the shell projects largely to limbic regions (e.g.,, lateral hypothalamus, ventral part of the bed nucleus of the stria terminalis and ventral

tegmental area) (Zahm and Brog, 1992, Zahm and Heimer, 1993, Corbit et al., 2001). This anatomic distinction appears to reflect different functional properties of the NAc subregions during cue-reward learning and motivated behavior. The NAc core has been implicated in goal directed behavior and reward prediction (Carelli, 2004, Saddoris et al., 2013). Conversely, the NAc shell appears to maintain the valence and novelty of rewards (Kelley, 2004, Zorrilla and Koob, 2013, Castro et al., 2015, Saddoris et al., 2015a).

Recent studies also suggest that the core and shell subregions uniquely encode different aspects of value-based decision making. Lesion and pharmacological inactivation studies have linked the NAc core to subjective-based decision making (Cardinal et al., 2001, Cardinal and Cheung, 2005, Cardinal and Howes, 2005, Pothuizen et al., 2005, Hauber and Sommer, 2009, Ghods-Sharifi and Floresco, 2010). In support, rapid dopamine release in the NAc core, but not shell, encodes subjective preference during delay, risk, effort, and delay discounting tasks (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Conversely, dopamine activity in the NAc shell appears to encode reward outcome, such as objectively larger reward magnitudes (Beyene et al., 2010, Stopper and Floresco, 2011). Indeed, cues predicting large (compared to smaller) reward magnitude elicit greater dopamine cell firing (Tobler et al., 2005, Roesch et al., 2007), which is reflected in distinct dopamine release dynamics to rewards of different magnitudes (Beyene et al., 2010, Wanat et al., 2010). While recent optogenetic studies support the view that the NAc is causally linked to subjective as opposed to outcome-based aspects of value-based decision-making, the contribution of the core versus shell in these processes was not specifically examined (Saddoris et al., 2015b).

Although numerous studies clearly support a differential role of the NAc core and shell in subjective versus outcome based decision making (Day et al., 2010, Sugam et al., 2012, Saddoris

et al., 2015b), no studies have specifically examined dopamine release dynamics in these subregions to cues signaling the availability of different reward magnitudes. Here, we used fast-scan cyclic voltammetry (FSCV) in freely behaving rats to examine how rapid dopamine signaling in the NAc shell versus core encodes information about cues signaling different reward magnitudes. Specifically, during a reward magnitude based decision-making task, rats learned that distinct cues signaled the availability of either a small (one sugar pellet) or large (two sugar pellet) reward, and then were given an option to choose their preferred option. We demonstrate that phasic dopamine in the NAc shell, but not core, tracks the large, objectively preferred reward magnitude option compared to the small reward, and changes in dopamine release dynamics are observed in the shell (not core) upon lever press.

METHODS

Animals

Male Sprague-Dawley rats (n = 15) weighing between 275-330 g were used. Rats were singly housed under reverse light:dark cycle and experiments were conducted during the dark cycle. Animals were maintained at no less than 85% of pre-experimental body weights by food restriction, except during the post-operative recovery period when food was given ad libitum (Harlan Lab Chow). Water was available *ad libitum* throughout the duration of the experiment. Animal procedures were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, and were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee (IACUC).

Apparatus

Behavioral testing was conducted in 43x43x53 cm Plexiglas chambers housed in sound-blocking boxes (Med Associates, St. Albans, VT) described in detail previously (Saddoris et al., 2011). Briefly, one side of each chamber is equipped with two retractable levers (Coulbourn Instruments, Allentown, PA) 17 cm apart, with a stimulus light 6 cm above each lever. Sucrose pellets (45 mg) were delivered to a food receptacle, which was located equidistantly between the levers. A house light (100 mA) was mounted on the opposite side of the chamber.

Behavioral Procedures

The timeline for training on the task and the final task design are summarized in Figure 1 A & B. Rats were initially trained to press both levers for a single sucrose pellet on a fixed ratio 1 schedule of reinforcement. Here, cue lights above both levers were illuminated and both levers were extended into the chamber. A maximum of 100 reinforcers (50 per lever) were available and rats were trained to criterion of 50 responses on each lever over 3 sessions.

Next, rats were trained on a task that involved three types of contingencies (30 trials each) intermixed within 90 total trials per session. At this stage, only a single sucrose pellet was available for each lever press throughout the session. The first two trial types were classified as 'Forced-Choice' trials. For one trial type, a single cue light was illuminated for 5 s over one lever, followed by extension of both levers. Responses on the cue light illuminated lever (within 15 s) were immediately reinforced with 1 sucrose pellet. During the other forced-choice trial type, the cue light over the other lever was illuminated for 5 s, followed by extension of both levers. Responses within 15 s on the cue-associated lever were reinforced as above. For both forced-choice trials, responses on the unsignaled lever were counted as 'errors', and resulted in termination of the houselight for the remainder of the trial period, and no reward delivery. During the third trial type, termed 'Free Choice' trials, both cue lights were illuminated for 5 s, after which both levers were extended, and responses on either lever within 15 s were reinforced with 1 sucrose pellet. Following a press on either lever, both levers were retracted and a sucrose pellet was immediately delivered into the food receptacle. In order to move on to the next phase of training, rats needed to maintain at least 3 days of stable accuracy (80% correct responses).

After reaching accuracy criteria, the reward contingency on one of the levers was altered to reflect the reward magnitude decision-making task. Here, the task remained identical except

the reward contingency on one of the levers was changed to 2 sucrose pellets, and responses on the other lever remained at 1 sucrose pellet (Figure 1B). These assignments were counterbalanced across animals and remained constant for each rat throughout training. Animals were trained on the reward magnitude task until accuracy was stable (80% correct responses) and a clear discrimination between the levers observed. Preference discrimination was classified as at least 60% responding on one lever during free choice trials. Following acquisition of stable responding and magnitude preference, all rats were prepared for electrochemical recording in either the NAc core or shell. After recovery, animals underwent additional training sessions until behavior reached pre-surgery baseline levels (at least 3 sessions).

Surgery

Rats were surgically prepared for voltammetric recording using established procedures (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Animals were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg), and placed in a stereotaxic frame. A guide cannula (Bioanalytical Systems, West Lafayette, IN) was positioned above the NAc core (1.3 mm anterior, 1.3 mm lateral from bregma) or shell (1.3 mm anterior, 0.8 mm lateral from bregma). Another guide cannula (for the Ag/AgCl reference electrode) was placed contralateral to the NAc cannula. A bipolar stimulating electrode (Plastics 1 Inc., Roanoke, VA) was placed dorsally to the ventral tegmental area (VTA; 5.2 mm posterior, 1.0 mm lateral from bregma and 7 mm ventral from dural surface) and ipsilateral to the NAc cannula. Correct placement of the stimulating electrode in the VTA was determined by applying a range of stimulation parameters (12-24 biphasic pulses, 20-60 Hz) and observing tail movement. The stimulating electrode was lowered in increments of 0.1 mm until slight to no tail movement was observed at 60 Hz, 24 pulses. Stainless steel screws and dental cement were then used to secure all items. Animals were

given an anti-inflammatory medication (meloxicam) for two days post-surgery and were allowed access to food and water *ad libitum.*

Fast Scan Cyclic Voltammetry (FSCV)

One week following surgery, animals were food restricted and retrained on the magnitude decision-making task until they reached pre-surgery performance (maximum of 5 sessions). Changes in dopamine concentration during the task were assessed using fast-scan cyclic voltammetry as previously described (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). On the test day, a carbon-fiber microelectrode was lowered into the NAc core or shell with a locally constructed microdrive (Chemistry Department Electronic Facility, University of North Carolina, Chapel Hill), after placing an Ag/AgCl reference electrode in the contralateral hemisphere. The carbon-fiber microelectrode was held at -0.4 V vs. Ag/AgCl reference electrode. Periodically a cyclic voltammogram was acquired (100 ms intervals) by applying a triangular waveform that drove the potential to 1.3 V and back to -0.4 V. Changes in current at the oxidation potentiation for dopamine were compared to electrically-stimulated dopamine release at the same location. Chemometric analysis was used to identify dopamine concentrations using HDCV software (UNC Chemistry Electronics, Chapel Hill, NC) and aligned to behavioral events (Trans IV, MED Associates). In a subset of rats (n=3), after recording a full session of 90 trials, the electrode was lowered another ~300 μm until another release site was found, at which point another recording was taken for another session of 90 trials.

Histology

After completion of the experiments, rats were deeply anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). To mark the placement of the electrode tip, a tungsten electrode housed in a micromanipulator was lowered to the recording site and a small electrolytic lesion was made. Brains were then extracted, sliced on a freezing cryostat, and placed onto slides. The location of electrode tips were assessed by visual examination of successive coronal sections in comparison to visual landmarks and the anatomical organization of the NAc core and shell, as represented in a stereotaxic atlas (Paxinos and Watson).

Data Analysis

Rats were only included in the analyses if they demonstrated at least 80% accuracy on forced choice trials and at least 60% preference on free choice trials. For behavioral analysis of forced choice trials, paired t-tests were used to compare accuracy (percent rewarded trials) and percent errors during high and low reward trial types. For free choice trials, a paired t-test was used to compare reward magnitude preferences.

Analysis of FSCV recordings was similar to previous reports (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Briefly, each subject received electrical stimulation of VTA afferents (frequency: 12-60 Hz, pulses: 1-20) to generate a training set of dopamine release at the recording location in the NAc. To analyze recorded FSCV data, each subject's training set collected from the site of recording was used to chemometrically convert recorded current during the session into dopamine concentrations (Rodeberg et al., 2015). Concentrations were then aligned to behavioral events to assess dopamine release dynamics relative to task stimuli.

Changes in dopamine concentration from baseline in response to cue presentation were evaluated separately for each trial type (forced choice high magnitude, forced choice low

magnitude, and free choice) using a one-way repeated measures ANOVA with Dunnett's correction for multiple comparisons. This analysis compared the baseline mean dopamine concentration (5 s prior to cue onset) to each data point (100 msec bin) obtained within 2 s following the cue presentation. To assess the differential effects of the three cue types on dopamine release, peak dopamine concentrations within 2 s following cue presentation were analyzed using a one-way ANOVA with Fisher's LSD correction. Because free choice trials allowed rats to choose a large versus small sucrose reward, there were unequal numbers of responses to high versus low magnitude options. Thus, because of differences in variance, it would be inappropriate to compare dopamine release for the average of 26 high magnitude choices compared to 4 low magnitude choice. Therefore, we combined all dopamine concentrations for the 30 free choice trials, regardless of what option the rat eventually chose. To compare amounts of dopamine release in the shell versus core, we examined the cumulative dopamine release in each region by summating the concentration of dopamine in each bin during 0.5 to 4 s following cue onset for the forced and free choice trials to provide an estimate area under the curve, and completed a 2-way ANOVA on these data. During lever press, changes in NAc dopamine concentration from baseline were evaluated separately for each trial type (forced choice high magnitude, forced choice low magnitude, and free choice) using a one-way repeated measure ANOVA with Dunnett's correction for multiple comparisons. This analysis compared baseline mean dopamine concentration (1s prior to lever press) to each data point (100 msec bin) obtained within 1 s following lever press.

All analysis were considered significant at $\alpha=0.05$. Statistical and graphical analysis were performed using GraphPad Prism 6.0 for Windows (GraphPad Software, La Jolla, CA) and Neuroexplorer for Windows version 4.034 (Plexon Inc., Dallas, TX).

RESULTS

Behavior

Rats rapidly learned the magnitude decision-making task and discriminated between the cue types. Specifically, rats completed significantly more correct responses during high forced trials ($t_{14} = 4.06$, $p < 0.01$; Figure 1C) and made significantly more errors ($t_{14} = 3.769$, $p < 0.01$; Figure 1D) during low forced choice trials. On free choice trials, rats exhibited a significant preference for the high magnitude option over the low magnitude option (Figure 1E, $t_{14} = 14.34$, $p < 0.001$).

Differential Dopamine Release Patterns in the NAc Shell to Cues Signaling High Versus Low Reward Options

Reward-predictive cues evoked rapid dopamine release in the NAc shell, consistent with previous reports (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). However, forced choice high magnitude trials induced higher concentrations of dopamine release than forced choice low magnitude trials. This finding is illustrated for a representative trial in a single animal in Figure 2. Here, color representation of a set of background-subtracted cyclic voltammograms and the corresponding dopamine concentration trace are averaged across all forced high and forced low trials of a single session. During the forced high magnitude trial (left), the onset of the cue (indicated by dashed line at time 0) resulted in an increase in rapid dopamine release that reached a maximum of ~ 170 nM. While a similar increase in NAc

dopamine release was observed during forced low trials, it was of lower concentration (~ 100 nM).

Figure 3 (left) shows the average dopamine response measured across 8 locations (7 rats) in the NAc shell during all trial types. In the NAc shell, cues predictive of different reward magnitudes evoked significant increases in rapid dopamine release during high forced ($F_{21,147}$ = 4.643, $p < 0.0001$), low forced ($F_{21,147}$ = 1.88, $p < 0.05$) and free choice ($F_{21,147}$ = 4.282, $p < 0.0001$) trials. However, the amplitude of *peak* cue-evoked dopamine release varied depending on the trial type ($F_{2,14}$ = 5.656, $P < 0.05$; Figure 3, right). Post hoc analysis revealed that forced choice high magnitude cues evoked significantly larger peak dopamine concentration than forced choice low magnitude cues (Fisher's LSD, $p = 0.0054$).

As described previously (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b), cue-evoked dopamine may signal the value of the best available option, regardless of what is eventually chosen. Thus, we investigated dopamine signaling on free choice trials, where the best option available and the chosen outcome are not always identical (Day et al., 2010, Sugam et al., 2012). Because free choice trials required rats to allocate choice responses based on preference, there were unequal numbers of responses to high versus low magnitude options (Figure 1D). Due to these differences in variance, we combined all dopamine concentrations for free choice trials, regardless of what option the rat eventually chose. As shown in Figure 3 (right), in the NAc shell, peak dopamine concentration during free choice cues was similar to cues predicting the forced high magnitude (Fisher's LSD, $p = 0.329$), but not the forced low magnitude option (Fisher's LSD, $p = 0.0393$).

Dopamine Release in the NAc Core does not Differentiate Cues Signaling High Versus Low Reward Options

In the NAc core, reward-predictive cues also evoked significant increases in rapid dopamine release during both forced and free choice trials (Figure 4 left, high forced: $F(21, 126) = 3.381$, $p < 0.0001$, low forced: $F(21, 126) = 2.924$, $p = 0.0001$, free choice: $F(21, 126) = 4.892$, $p < 0.0001$). However, there was no significant difference in peak dopamine concentration between trial types in the core ($F(2,12) = 2.597$, $P = 0.1155$; Figure 4 right).

Differential Dopamine Release Dynamics In The NAc Shell Versus Core To Cues And At Lever Press

Figure 5 shows dopamine release dynamics across the entire session and all trial types in the NAc core (left) versus the shell (right). It is immediately apparent that while dopamine was released to cues in both regions, it was higher in concentration in the shell than core. To quantify this finding, we examined the cumulative dopamine release in each region by summating the concentration of dopamine in each bin during 0.5 to 4 s following cue onset for the forced and free choice trials to provide an estimate area under the curve. A two way ANOVA revealed a main effect of region ($F_{1,13} = 5.124$, $p < 0.05$), a main effect of trial type ($F_{2, 26} = 14.40$, $p < 0.05$) and no significant interaction ($F_{2,26} = 2.371$, $p > 0.05$). These findings confirm that while all cue types significantly increased dopamine release in both regions, release was significantly higher in the NAc shell, compared to core.

Five seconds following cue presentation, both levers were extended into the chamber and rats could press a single lever indicating either one reward outcome or no reward (forced choice trials) or choice of high versus low reward options (free choice trials). In the NAc shell, a dip in dopamine release occurred upon lever press compared to 1 s before press (Figure 5B). This

decrease was significant in forced choice high magnitude ($F_{10, 70} = 2.245$, $p < 0.05$) and free choice trials ($F_{10, 70} = 2.018$, $p < 0.05$), but not forced choice low magnitude trials ($F_{10, 70} = 1.860$, $p > 0.05$). There was no change in rapid dopamine release in the NAc core upon lever press across any trial type (Figure 5A; high forced: $F_{(10,40)} = 1.359$, $p = 0.2344$; low forced: $F_{(10,40)} = 0.6222$, $p = 0.7858$; choice: $F_{(10,40)} = 0.8809$, $p = 0.5583$).

DISCUSSION

The present findings reveal that while cue-evoked dopamine release was observed within both the NAc core and shell, it was only in the shell that dopamine differentially scaled to cues that signaled the large reward. During forced choice trials, when rats choose between the large or small reward or nothing, dopamine release was higher for the large reward. During free choice trials, when rats were presented with both cues simultaneously, dopamine release in the NAc shell tracked the best available option, regardless of the animals' eventual choice. Five seconds following cue presentation, rats were given the opportunity to press the lever underneath the corresponding cue light to receive the high or low magnitude reward. In the NAc shell, but not core, a brief dip in rapid dopamine release was observed upon lever press. These findings highlight differential dopamine release dynamics across the NAc core and shell related to discrete aspects of the magnitude based decision making task. The results and implications of these findings are considered in detail below.

Rapid Dopamine Signaling In The NAc Shell Differentiates Preferred Magnitude

During forced choice trials, distinct cue lights indicated the availability of either a low or high magnitude reward (1 versus 2 sugar pellets) or nothing. Peak dopamine release in the NAc shell, but not core, was greater for the cue associated with the high magnitude option than for the low magnitude option. This finding indicates that dopamine signaling in the shell encodes the availability of an objectively favorable outcome. These findings are consistent with previous

studies implicating the shell in encoding reward magnitude (Beyene et al., 2010, Stopper and Floresco, 2011). Indeed, prior electrochemistry studies revealed that dopamine release in the NAc shell scales to cues predicting different magnitudes of intracranial self-stimulation (Beyene et al., 2010). The current study demonstrates that this pattern of dopamine activity also tracks cues predicting different magnitudes of sucrose reward.

During free choice trials in the present study, rats were given the option to choose either the large or small magnitude reward via simultaneous presentation of both cue lights. Peak dopamine release during the free choice trials was significantly greater than during the low forced magnitude trials, but was similar in concentration to dopamine release during high forced choice trials. This finding reveals that upon simultaneous cue presentation, dopamine release in the NAc shell signaled the objectively better option, regardless of the rats' eventual choice. Previous work reveals that dopamine release in the NAc core encodes the best available option regardless of the action chosen during delay, risk, and effort based decision making tasks (Day et al., 2010, Sugam et al., 2012). The current findings reveal a similar dynamic in the NAc shell during outcome-based decision making, and provide further evidence that these NAc subregions encode different aspects of value-based decision making.

The current findings complement previous literature associating the NAc shell with reward hedonics and valence (Pecina and Berridge, 2000, Kelley, 2004, Pecina and Berridge, 2005, Zorrilla and Koob, 2013, Castro et al., 2015, Saddoris et al., 2015a). For example, glutamate antagonists microinfused into the shell enhanced appetitive behavior in rats (Maldonado-Irizarry et al., 1995, Kelley and Swanson, 1997), whereas NAc shell inactivation disrupts the ability of rats to judge reward magnitude options (Stopper and Floresco, 2011) or availability of cued rewards (Ambroggi et al., 2011). Novel, uncued delivery of food-related

stimuli evoked increases in NAc shell dopamine levels (Bassareo and Di Chiara, 1997, 1999, Roitman et al., 2008). Further, the shell (not core) processes outcome-selective information about predictive cues, and updates information based on current reward value relative to the animal's motivational state (Floresco et al., 2008, Corbit and Balleine, 2011, Saddoris et al., 2015a). The present study adds to this body of literature by revealing how rapid dopamine release tracks reward magnitude information, and encodes the best objective outcome.

Rapid Dopamine Signaling In The NAc Core Responds To Cues Signaling Reward Availability, But Does Not Differentiate Preferred Magnitude

Elements of value-based decision making may be separable into related but distinct components (Saddoris et al., 2015b). That is, assessment of reward value appears to involve outcome-based features of the association (e.g., reward magnitude), as well as subjective components that may be more variable across individuals (e.g., willingness to engage in risky behaviors). In the present study, a significant increase in rapid dopamine release occurred in the NAc core during all reward-predictive cues. However, dopamine release in the core did not significantly encode the availability of discrete reward magnitudes (1 versus 2 pellets). In contrast, prior studies show that rapid dopamine release in the core does differentiate subjective-based decision making (delay, effort, risk delay discounting). These findings support the view that discrete features of value-based decision making are functionally related to the different NAc subregions.

Our findings imply that while dopamine release in the NAc core may not differentially encode information related to different reward magnitudes, it still does respond to learned cues that indicate reward availability. Indeed, previous studies implicate the NAc core in goal-directed behavior, cue response, and reward prediction (Carelli, 2004, Ambroggi et al., 2008, Saddoris et

al., 2013). Likewise, increases in phasic dopamine release have been previously observed in the NAc core during presentation of cues that reliably predicted sucrose reward (Roitman et al., 2004). It is interesting to note that the core sends dense projections to the shell, but the shell only sends sparse projections to the core (van Dongen et al., 2005). Therefore, the NAc core may provide information about the learned cue–outcome associations to the shell, which tracks expected outcome value. Then, the shell may integrate this parallel information to update cue-encoding to reflect the updated outcome value. This integration of information may underlie the proposed role of the shell in gating behavior (Ambroggi et al., 2011).

Differential Dopamine Signaling In NAc Shell Versus Core To Lever Press

Five seconds following cue presentation, rats could press a lever underneath the corresponding cue light to receive either a high or low magnitude reward. In the NAc shell, a significant, yet brief decrease in dopamine release occurred upon lever press during all trial types (forced and free choice trials). Notably, the dip in dopamine appears to briefly extend down to baseline dopamine activity (i.e., the differential dopamine concentration before the cue). This effect was not observed in the core.

It is well known that in well-trained animals that have learned cue-reward associations, mesolimbic dopamine cell firing shifts from reward consumption to cue presentation (Schultz et al., 1997, Schultz, 2002, Day et al., 2007). Our present findings, that show large increases in dopamine to the cues, not reward, are consistent with these and our prior findings (Day et al., 2007). While we did not expect to see an increase in dopamine release during lever press and subsequent reward consumption, the dip in NAc shell dopamine observed in the present study was unprecedented. One logical conclusion of this finding is that this decrease in dopamine

concentration at lever press might be indicative of a reward prediction error, in which dopamine decreases upon the omission of an expected reward (Schultz et al., 1997, Schultz, 2002). However, there are flaws to this theory with respect to interpretation of the current findings. First, recent data show that the NAc core, not shell, differentially encodes reward prediction error (Sugam et al., 2012, Saddoris et al., 2015a). Second, rats are well-trained on this task in the present study, and reliably received rewards immediately (within 500 ms) upon lever press. As such, rats do not experience an unexpected omission of reward. Therefore, it is unlikely that this decrease in dopamine reflects reward prediction error.

One potential cause of this brief decrease in shell dopamine signaling could be the termination of cue lights upon lever press. While rats are well trained and should expect the cues to turn off, dopamine signaling may encode this disappearance of the predictive cues even in well-trained rats. That is, dopamine release in the shell may reflect the termination of the cues predicting outcome value. This would support both current and prior assertions that the shell updates information about outcome value (Beyene et al., 2010, Saddoris et al., 2015a). The decrease in shell dopamine could also be indicative of a behavioral shift from food seeking to consummatory behavior. Indeed, neurons in the NAc shell, but not core, were inhibited upon lever press for a reward (Ambroggi et al., 2011). This brief alteration to dopamine activity could be attributed to components of the shell's unique "feeding" circuit, such as the ventral pallidum and lateral hypothalamus, exerting inhibitory effects on the ventral tegmental area (Stratford and Wirtshafter, 2012).

Concluding Remarks

The current findings contribute to a growing body of evidence demonstrating that the NAc core and shell differentially encode unique aspects of value-based decision making (Day et al., 2010, Sugam et al., 2012, Saddoris et al., 2015b). Taken together with previous findings, these results reveal a distinct role of dopamine release in the NAc shell during encoding outcome-based decision making. However, it is unknown if dopamine release in the NAc shell is *causally* linked to outcome-based decision making. A prior study investigated the causal role of dopamine signaling during both magnitude and delay-based decision making tasks using optogenetics (Saddoris et al., 2015b). Stimulation of the NAc during cues shifted behavior to the non-preferred option during the delay, but not magnitude, task. These findings indicate a possible causal role between dopamine signaling and subjective (delay), but not outcome (magnitude)-based, decision making. Importantly, however, this study did not differentiate between the core and shell subregions of the NAc (i.e., optogenetic stimulation targeted both subregions equally). As such, ongoing studies in the Carelli lab are examining a specific causal link between outcome-based decision making and dopamine release in the NAc shell.

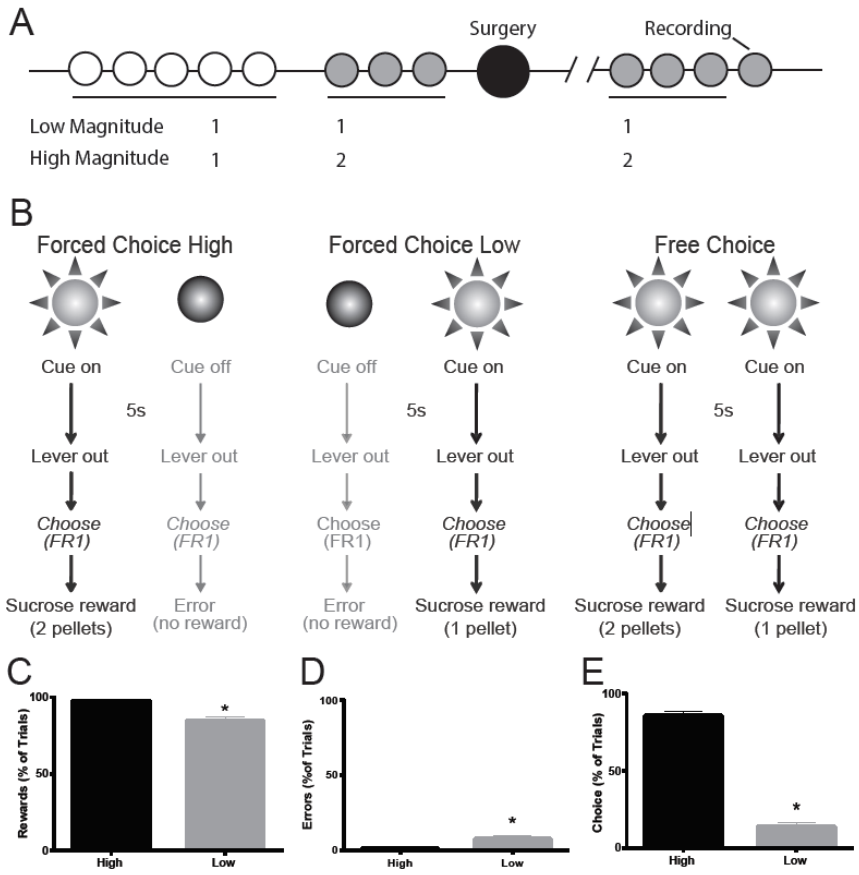

Figure 1. Magnitude-based decision-making task and behavior. A. Schematic diagram of behavioral training timeline. Animals received ~8 total training sessions before surgical preparation for FSCV (each circle = 1 session). Reward magnitudes were equal during early training (white circles), and differentiated into small versus large magnitude later (gray circles). Number of sucrose pellets/lever press indicated below timeline. Following surgery, at least 3 additional training sessions were completed then dopamine [DA] was measured during the task. B. Behavioral task. Left: During forced choice high magnitude (30 trials), one cue light was illuminated for 5 s followed by extension of both levers. Presses on the correct lever resulted in 2 sucrose pellets. Presses on the non-signaled lever were counted as errors, resulting in termination of the houselight for the remainder of the trial period, and no reward delivery. Middle: On forced choice low magnitude trials (30 trials), the other cue light was illuminated for 5 s followed by extension of both levers. If the correct lever was chosen, 1 sucrose pellet was delivered. Presses on the non-signaled lever were counted as errors and unrewarded. Right: During free choice trials (30 trials, right), both cue lights illuminated for 5 s followed by extension of both levers. Responses were rewarded based on the contingency of the lever chosen. C. Percentage of correct reinforced responses during high and low magnitude forced choice trials. Animals made significantly more correct responses during forced choice high magnitude trials ($p < 0.01$). D. Rats made significantly more errors on forced choice low magnitude trials than forced choice high magnitude trials ($p < 0.01$). E. Responses during free choice trials indicate that rats significantly preferred the high (compared to low) magnitude option ($p < 0.001$).

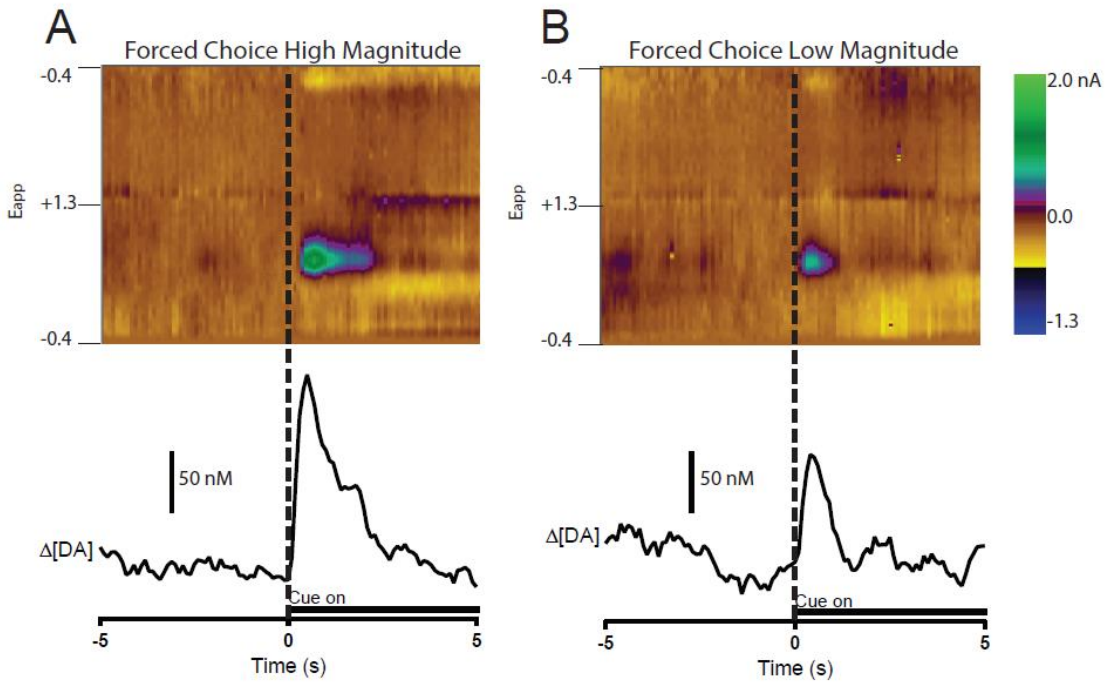

Figure 2. Example of differential dopamine release dynamics to the cue signaling high and low forced choice trials. Two-dimensional color representations of cyclic voltammetric data collected for 10 s around single forced high (A) and forced low (B) magnitude trial in the NAc shell for a single representative animal. The ordinate is the applied voltage (Eapp) and the abscissa is time (s). Differential dopamine [DA] determined via principal component analysis are plotted below the color plots.

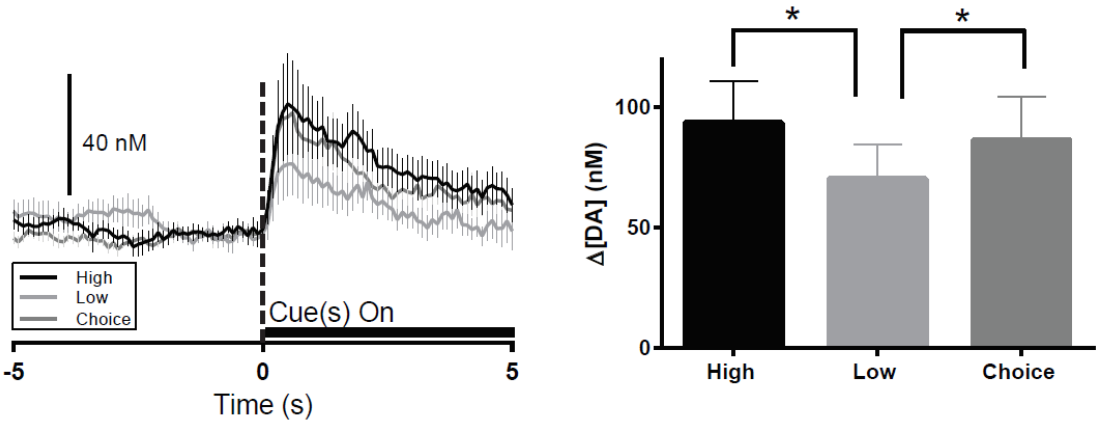

Figure 3. Dopamine release in the NAc shell across all rats encodes the value of the best available magnitude option. A. Change in dopamine [DA] concentration in the NAc shell on forced and free choice trials, aligned to cue onset (dashed line, time 0). [DA] increased significantly from baseline during the cue. B. Peak [DA] on high magnitude forced choice trials was significantly larger than low magnitude forced choice trials. On free choice trials, cue-evoked dopamine reflected the more preferred high magnitude option. All data are mean ± SEM. *$p < 0.05$.

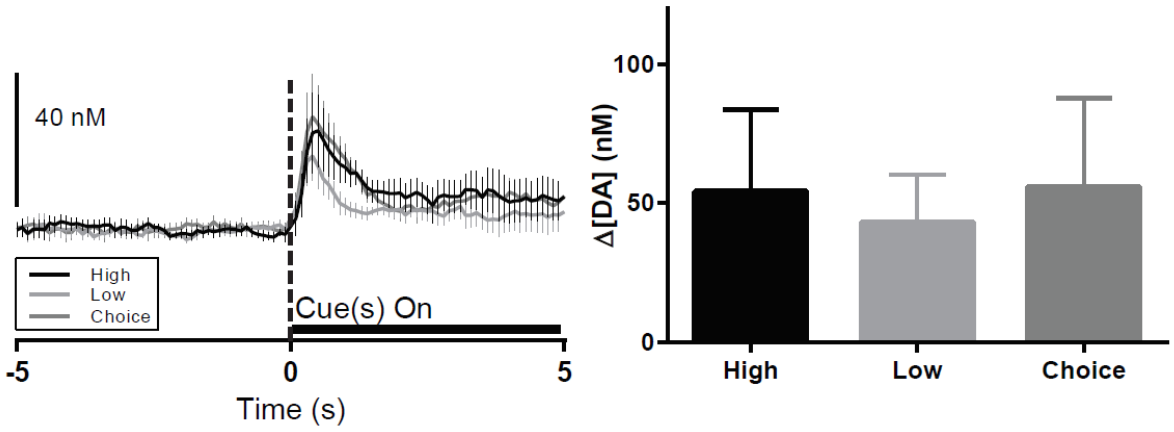

Figure 4. In the NAc core, cues elicit increases in dopamine [DA] but do not reflect differences in reward magnitude. A. Change in [DA] on forced and free choice trials, aligned to cue onset (dashed line, time 0). B. Peak cue-evoked dopamine signal on forced and free choice trials. Across all trial types, cue-evoked [DA] did not differentiate between the value of available options. All data are mean ± SEM. $p > 0.05$.

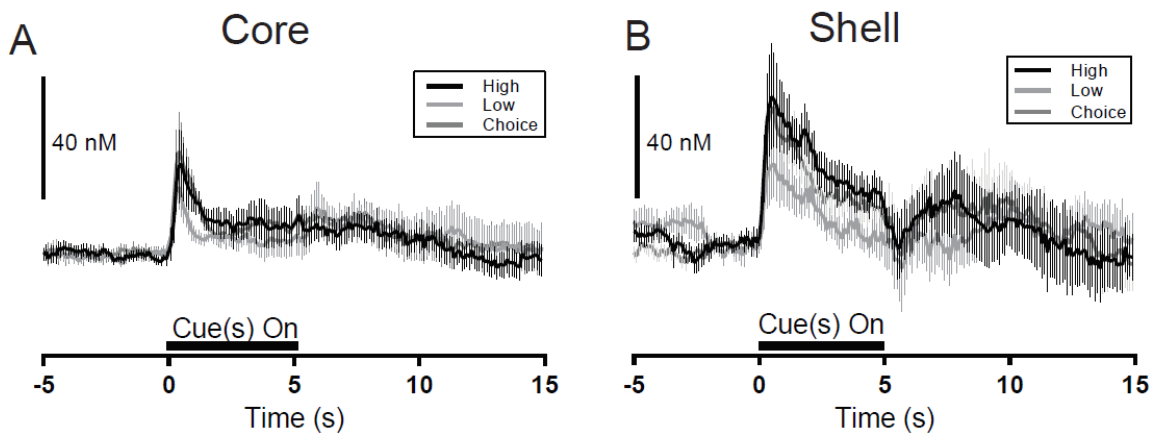

Figure 5. Average dopamine release for all animals across the entire session and all trial types in the NAc core (A) and shell (B). While cue-evoked dopamine release was observed in both regions, it was significantly higher in the NAc shell than the core. Further, a significant dip in dopamine release occurred in the NAc shell, but not NAc core, upon lever press.

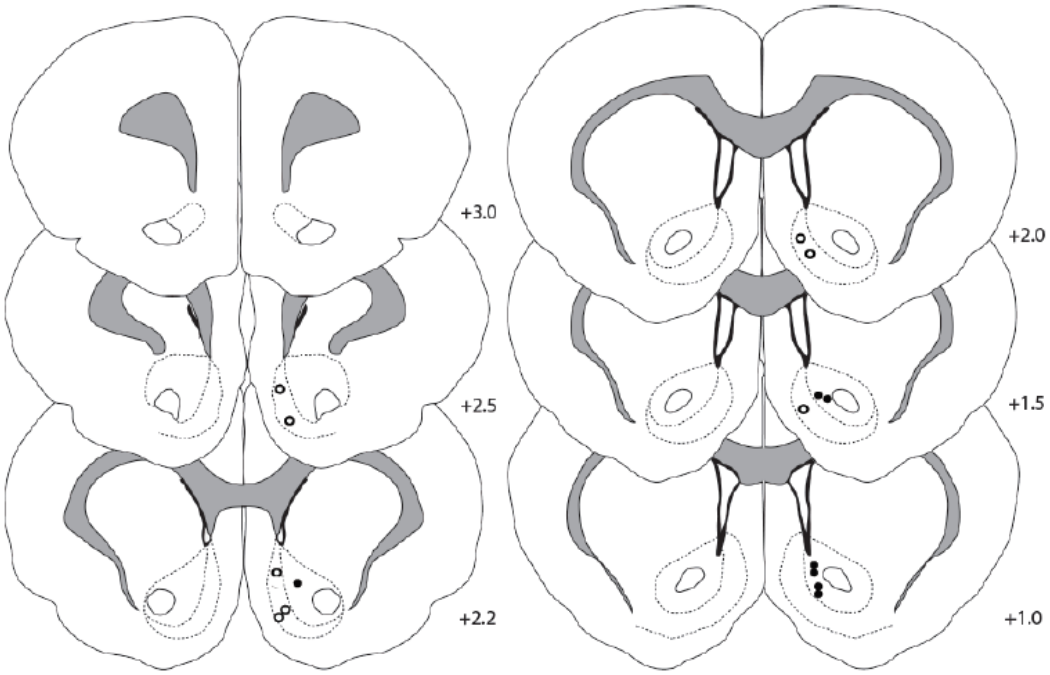

Figure 6. Histologically confirmed location of carbon-fiber microelectrodes in the NAc shell (*n* = 8, white circles) or core (*n* = 7, black circles). Units indicate coronal placement anterior to bregma.

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