Mode of sucrose delivery alters reward-related phasic dopamine signals in nucleus accumbens

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Abstract

Nucleus accumbens (NAc) dopamine correlates with rewards and reward-predictive cues. However, the mode of reward delivery may have important implications for how reward itself, as well as associated stimuli and behaviours, are encoded by dopamine. We compared two modes of delivery: sucrose pellets, which require goal-directed action for their collection, and intraoral infusions, which require no action. To assess the role of Pavlovian cues in evoking phasic dopamine, rats were trained to associate distinct cues with subsequent delivery of either a sucrose pellet or an intraoral infusion of sucrose solution directly into the mouth. Fast-scan cyclic voltammetry was used to measure phasic dopamine in NAc while rats experienced both cued and uncued rewards within a single session. Behavioural discrimination between pellet-paired and infusion-paired cues was observed with rats making more anticipatory receptacle port entries during pellet-predictive cues than infusion-predictive cues. Both pellet-predictive and infusion-predictive cues evoked dopamine release, however, concordant with the behavioural difference, greater dopamine was evoked by pellet cues than infusion cues. In addition, in cued trials, delivery of pellets increased dopamine above baseline whereas delivery of infusions did not. In uncued trials, both pellets and

infusions evoked dopamine release. Responses were generally similar across NAc subregions with core and shell dopamine release appearing qualitatively similar, although dopamine events were broader in shell than in core. Thus, phasic dopamine responses to intraoral infusions and infusion-predictive cues demonstrate a potential role for dopamine in encoding both reward prediction and reward evaluation.

Introduction

The mode in which a reward is delivered may affect how it is perceived and encoded by neural circuits. First, different modes of reward delivery may affect the subsequent behaviour required to receive reward. For example, if food rewards – either in pellet or liquid form – are delivered to a receptacle animals need to attend to the delivery and organize approach behaviour before the food can be consumed. In contrast, solutions delivered through an intraoral cannula directly into the oral cavity are immediately available for consumption without requiring effort or movement (Grill and Norgren, 1978). Second, the sensory processes that are engaged by different modes of delivery will differ between foods in a solid or liquid form. Finally, the rate at which a reward is ingested or absorbed may affect its rewarding or reinforcing properties and the neural processes that are engaged (Avena et al., 2008; Ferrario et al., 2008; Furlong et al., 2014; Samaha et al., 2002). All of these factors may influence both how reward delivery and receipt are encoded by the brain and how information about associated stimuli and contexts is processed.

Dopamine signaling in nucleus accumbens (NAc) has been heavily implicated in encoding rewards and reward-related stimuli (Bassareo and Di Chiara, 1997;

Gunaydin et al., 2014; Roitman et al., 2004; Steinberg et al., 2013; Stuber et al., 2005). Particularly important are brief, phasic, increases in concentration as seen in NAc following unpredicted presentation of rewards, such as food pellets, as well as after presentation of initially neutral stimuli (cues) that become reliable predictors of reward (Brown et al., 2011; Day et al., 2007; Flagel et al., 2011). These studies - of dopamine release in terminal regions - have predominantly been conducted in rodents using sugar pellets or solution delivered to a receptacle and therefore require the organization of appetitive behaviors for collection. Another less commonly used mode of reward delivery is intraoral infusions, which via an implanted cannula, allow solutions to be delivered directly to the oral cavity (Grill and Norgren, 1978). This technique is favoured in certain situations as it allows exquisite experimenter control over stimulus exposure and may allow the consummatory phase of ingestive behaviour to be isolated from appetitive behaviours (Hudson and Ritter, 2004; Seeley et al., 1995). A small number of studies have examined phasic dopamine responses to unpredicted intraoral sucrose infusions with most evidence showing, relative to pellets, responses to the infusions themselves are smaller and less phasic in nature (McCutcheon et al., 2012; Roitman et al., 2008). However, to our knowledge, dopamine responses to

pellets and infusions have never been directly compared and, furthermore, responses to cues that predict intraoral sucrose infusions have not been examined (see Cone et al 2016 for responses to sodium infusion-paired cues).

Here, we have directly compared phasic dopamine responses in NAc to sucrose pellets and intraoral infusions in the same rats and, importantly, within the same dopamine recording session. In addition, all rats were trained to associate distinct predictive cues with delivery of each mode of sucrose delivery and dopamine responses to each cue were compared. Finally, due to the proposed functional heterogeneity of striatal subregions (Kelley, 1999) and identification in previous reports of regional differences in responses to rewards and predictive cues (Brown et al., 2011; Cacciapaglia et al., 2012; Wheeler et al., 2011), we compared responses across NAc core and shell.

Materials & Methods

Subjects

Male Sprague-Dawley rats (n=7; Charles River) weighing 325-375 g and aged approximately 10-12 weeks at the start of the experiment were used. Rats were individually housed with lights on from 7:00 to 19:00. Animal care and use was in

accordance with the National Institutes for Health Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

Surgical procedures

Procedures were as described elsewhere (Cone et al., 2016; Fortin et al., 2015). Briefly, under general anaesthesia (100 mg/kg ketamine + 10 mg/kg xylazine; i.p.), rats were implanted with an intraoral cannula in a first surgery and, after initial training (see below), were implanted with apparatus for performing fast-scan cyclic voltammetry recordings in a second surgery. This apparatus consisted of: a guide cannula (Bioanalytical Systems; West Lafayette, IN) directed towards NAc core (mm from Bregma: +1.3 AP, +1.3 ML; n=4) or shell (mm from Bregma: +1.7, +0.9 ML; n=3) and a Ag/AgCl reference electrode in contralateral cortex secured to the skull using stainless steel screws and dental cement. Rats were given meloxicam (1 mg/kg) and enrofloxacin (10 mg/kg) at time of surgery and for two days post-operatively. Rats had at least 1 week of recovery between each surgery and continuation of the experiment.

Behavioural procedures

All rats were food-restricted to 90-95% of free-feeding weight. Behavioural training, testing, and fast-scan cyclic voltammetry experiments took place in the same chambers (20 cm length x 12 cm width x 14 cm height) equipped with: a pellet receptacle; a house light; a white noise generator; two levers and two cue lights flanking the pellet receptacle; and a fluid line attached to a solenoid valve and solution reservoir. The solenoid valve was positioned outside of the behavioural chamber to minimize the likelihood that rats could use the audible click of the valve as a predictor of sucrose delivery. In all sessions the house light was illuminated and the white noise generator was applied (60 dB). Two different reward stimuli, matched for caloric content, were used: 45 mg sucrose pellets (BioServ) and ~329 µL intraoral infusions of 0.4 M sucrose solution. Intraoral infusions (6.5 s duration) were delivered by activating the solenoid valve to allow sucrose solution to flow directly into the rats' mouth at a rate of ~50 µL/s. Rats were given 2-6 pre-testing sessions to become familiar with each type of reward. First, rats were presented with a session in which 30 sucrose pellets were delivered to the receptacle at pseudorandom intervals (mean ITI 45 s; range 30 – 60 s). The number of pellets consumed was recorded and the following day, each rat was placed back into the chamber and received the same number of infusions in a similar temporal pattern

to match pellet consumption from the previous day. This pattern (pellet day, infusion day) was continued until rats consumed all pellets delivered, typically 2-3 repetitions. Once rats reached criteria, they received one day of training in which pellets and infusions were delivered at pseudorandom intervals and in pseudorandom order. Following this session, Pavlovian conditioning sessions began. In these sessions, delivery of each reward was preceded by extension of a lever and illumination of the cue light above the lever (e.g. right lever and cue light → sucrose pellet vs. left lever and light → sucrose infusion; sides counterbalanced across rats). Importantly, interaction with the levers had no programmed consequence. Each reward was delivered 3 s after the cue onset and the cue remained on until 6.5 s after reward delivery, the time at which the infusion stopped. An infrared beam was situated above the pellet receptacle allowing head entries into the receptacle to be recorded. For training sessions, rats were presented with 28-30 trials of each cue-reward pair in a pseudorandom manner. Rats experienced five of these sessions before undergoing surgery for fast-scan cyclic voltammetry. Following recovery from surgery, rats experienced 1-2 post-surgical training sessions before a test session during which phasic dopamine was recorded. For this test session, rats were additionally presented with 14-15 trials in

which each reward was presented in the absence of a cue (probe trials) and 14-15 trials in which a solenoid valve was activated without resulting in infusion to control for the possibility that rats were able to use the solenoid click as a predictor of sucrose delivery (dummy trials).

Fast-scan cyclic voltammetry

On a single test day (behavioural component described above), phasic dopamine was recorded by lowering a carbon fibre microelectrode into NAc using a custom-built micromanipulator (UIC Research Resources Center). Procedures were similar to those described in detail elsewhere (Fortin et al., 2015). Briefly, a triangular voltage waveform (-0.4V → +1.3 V → -0.4 V relative to Ag/AgCl; 400 V/s; 10 Hz) was applied to the electrode using custom-built hardware (University of Washington Electromechanical Engineering). This results in the oxidation and reduction of electroactive species at the electrode surface. After subtracting the non-faradaic background signal, dopamine can be extracted from the data using principal component analysis (PCA). Training sets were derived in each rat by injecting a cocktail of cocaine (10 mg/kg) and raclopride (1 mg/kg) at the end of the recording session to evoke robust dopamine and pH changes. Electrodes were

pre-calibrated in a custom flow cell (Sinkala et al., 2012) to derive a calibration factor. Across all electrodes, the average calibration factor was 45.55 nM/nA.

Histology

Following completion of the experiment, rats were terminally anaesthetised with sodium pentobarbital (50 mg/kg). The position of each recording site was lesioned by lowering a polyimide-insulated stainless steel electrode to the same depth as the carbon fibre and passing current (4 x 4 s, 1 mA; Ugo-Basile Lesion Making Device) before rats were transcardially perfused with phosphate-buffered saline followed by neutral buffered formalin (10%). Fixed brains were sectioned on a cryostat (50 µm) and sections containing lesion marks were identified under light microscopy with assistance from a rat brain atlas (Paxinos and Watson, 1998).

Data analysis and statistical methods

Behavioural data were acquired using Med-PC (Med Associates) and analysed using custom MATLAB scripts. Voltammetry data were acquired and initially analysed using TarHeel CV (Fortin et al., 2015) and then imported into MATLAB for further analysis. All analysis code is available on request. Cue-evoked approach was defined as total time spent in food cup between cue onset and

reward delivery. To analyse this behaviour during the recording session, a paired t-test was used to compare cued pellet trials to cued infusion trials. Dopamine concentration traces were extracted from voltammetry data using principal component analysis (PCA). Trials in which the summed residual (Q) exceeded the threshold indicating satisfactory PCA (Qα) were automatically excluded (Keithley et al., 2009). Data were subsequently analysed by comparing average dopamine concentration across different epochs. For cued trials these epochs were: baseline (1 s before cue onset), cue (1 s following cue onset), and reward (1 s following reward delivery). For uncued trials these epochs were: baseline (3 s before reward delivery) and reward (3 s following reward delivery). Epoch lengths were based on differences in pellet retrieval latency across trials and were designed to capture the large majority (>70%) of latencies. Two-way within-subjects ANOVA with Trial Type and Epoch as factors was used with appropriate *post hoc* tests (Sidak or Dunnett's). To probe regional differences, receiver operator characteristic (ROC) analysis was applied to dopamine concentration traces after they were binned into 500 ms. All trials from dopamine measurements in the core were compared to all trials from measurements in the shell for each trial type. $p < 0.05$ was considered as significance level.

Results

Phasic dopamine signals during uncued trials

To assess whether different patterns of dopamine release were associated with the different modes of sucrose delivery (trial types), we used fast-scan cyclic voltammetry to measure changes in dopamine concentration during behaviour (Fig. 1). During the test session, uncued trials were interleaved with trials in which each reward was preceded by a distinct, predictive cue.

First, we compared trials in which pellets and infusions were delivered without a predictive cue to examine whether, in this situation, each type of reward was encoded differentially by dopamine (Fig. 1*C*). In addition, we included trials in which a dummy solenoid click was used but no infusion was delivered to ensure that any responses to the infusion were not a conditioned response to the potentially audible opening of the valve (importantly, both the real solenoid and dummy solenoid were housed outside a large sound-insulated chamber and should be inaudible to the rat). Two-way within-subjects repeated measures ANOVA revealed a significant main effect of Trial Type ($F(2,12)=4.729$, $p=0.031$) and a trend towards a main effect of Epoch ($F(1,6)=5.882$, $p=0.051$), and a significant

Epoch x Trial type interaction ($F(2,12)=13.135$, $p=0.001$). Further testing revealed that on both pellet trials ($p=0.048$) and infusion trials ($p=0.028$) dopamine was significantly elevated above baseline at time of reward delivery whereas there was no modulation of dopamine levels on the dummy trials ($p=0.175$). In addition, analysing each epoch separately, dopamine was similar during the baseline epoch for all trial types ($F(2,12)=0.624$, $p=0.552$) whereas, during the reward epoch, there was modulation by trial type ($F(2,12)=11.911$, $p=0.001$). *Post hoc* tests revealed that, relative to dummy trials, dopamine was elevated by both pellets ($p=0.014$) and infusions ($p=0.009$) whereas the concentration of dopamine evoked by pellets and infusions did not differ ($p=0.638$). Thus, when rewards were uncued, both pellets and infusions were encoded in the same manner and evoked a dopamine increase of similar magnitude.

Phasic dopamine signals during cued trials

Next, we examined trials in which Pavlovian cues were included to assess whether dopamine encoded predictive cues differentially dependent on the reward that they predicted. In addition, we examined how the presence of a predictive cue altered dopamine signalling at the time of reward delivery. To determine whether rats had learned to discriminate between the cues, we monitored the time spent nose-

poking in the pellet receptacle during presentation of each cue, but before reward delivery, as a measure of cue-evoked approach behaviour. On test day, comparison of this parameter during cued pellet and cued infusion trials revealed that the pellet-predictive cue elicited a greater amount of time spent nose-poking in the pellet receptacle, relative to the infusion-predictive cue (pellet cue, 1.93 ± 0.26 s; infusion cue, 1.54 ± 0.23 s; $t(6)=3.767$, $p=0.009$).

Comparison of phasic dopamine signals during these cued trials showed that mode of reward delivery influenced dopamine encoding of cues and rewards (Fig. 1*D*). Two-way within-subjects repeated measures ANOVA revealed significant main effects of both Trial Type ($F(1,6)=13.238$; $p=0.011$) and Epoch ($F(2,12)=11.625$; $p=0.002$) with a significant Trial Type x Epoch interaction ($F(2,12)=11.285$; $p=0.002$). For cued pellet trials there was a significant modulation of the dopamine concentration across epochs ($F(2,12)=12.370$, $p=0.001$) with *post hoc* tests revealing that, relative to baseline, there was elevated dopamine concentration during both the cue epoch ($p<0.001$) and the reward epoch ($p=0.029$). For cued infusion trials, a significant modulation of dopamine was seen across epochs ($F(2,12)=10.227$, $p=0.003$). However, although dopamine was elevated, relative to baseline, during the cue epoch ($p<0.001$) dopamine was not elevated during

infusion delivery (p=0.174). Direct comparison of dopamine concentration during each epoch in pellet and infusion trials revealed that there was no difference in concentration during baseline epoch (p=0.919) but that, in pellet trials relative to infusion trials, dopamine was elevated in both the cue epoch (p=0.037) and the reward epoch (p=0.015). Taken together, these analyses show that both cues evoked similar patterns of dopamine release, however, the magnitude of this release was greater for rewards that require an additional response (e.g. approach to consume sucrose pellets) vs. passively-received rewards (sucrose infusions). In addition, while delivery of pellet rewards increased dopamine, delivery of infusions did not.

Subtle differences are seen between phasic dopamine release in nucleus accumbens subregions

Finally, we examined whether the pattern of responses differed across NAc subregions as there is a substantial body of work indicating that core and shell may encode different aspects of reward-related tasks (Kelley, 1999). Histological examination of lesion sites confirmed that recordings from four rats were made in NAc core and three rats were made in NAc shell. We binned data from each trial type into 500 ms bins and used receiver-operator characteristic analysis to ask if

dopamine responses in core and shell differed significantly at any time point (Fig. 2). We found that, for each trial type, subtle differences existed between core and shell subregions. In trials involving pellets, greater dopamine release was seen in core than shell in response to the earliest predictor of reward (e.g. cue in cued trials and pellet delivery in uncued trials; red circles on Fig. 2 show time points at which ROC analysis produced $p < 0.05$). In addition, a prolonged reward-evoked elevation of dopamine in shell, relative to core, was seen on cued pellet trials but not on uncued pellet trials. In trials involving infusions, differences between core and shell were only seen during time of infusion when elevated dopamine release was seen in shell, relative to core.

Discussion

We demonstrate that during presentation of uncued rewards, a requirement for goal-directed action had little effect on dopamine encoding of reward as both pellets and infusions evoked a similar increase in dopamine concentration. When predictive cues were present, subtle differences emerged. Although cue-evoked dopamine was qualitatively similar in both pellet and infusion trials, dopamine evoked by pellet cues was of greater magnitude than that evoked by infusion cues. In addition, when rewards were cued, ‘primary’ reward evoked dopamine release

only on cued pellet trials, not on cued infusion trials. These results suggest that dopamine's role in encoding reward prediction and reward evaluation is nuanced and partially influenced by the mode of reward delivery.

Recent work suggests a tight link between high concentration, phasic increases in dopamine and the engagement in proactive behaviours for the consumption of food reward (du Hoffmann and Nicola, 2014; Hamid et al., 2015). Consistent with this framework, phasic dopamine signals emerge during Pavlovian conditioning along with conditioned approach (Aragona et al., 2009; Cone et al., 2016; Day et al., 2007; Stuber et al., 2008). The demonstration here that cues predicting passively-received intraoral infusions evoke dopamine is important because in most studies linking dopamine fluctuations to appetitive Pavlovian learning, approach behaviour of some kind has been required to retrieve the reward. The present study is the first to assess matched rewards (i.e. sugar pellets vs. sugar solution) to compare the magnitude and dynamics of dopamine release in each case. Thus, the finding that cues predicting intraoral delivery evoke dopamine is consistent with a role for dopamine in reward prediction independent of the action the cues instruct. Although work in monkeys recording somatic action potentials in dopamine neurons has often used behavioural paradigms in which rewards were presented

within licking distance (Bromberg-Martin and Hikosaka, 2009; Schultz et al., 1993), this study is the first, to our knowledge, where dopamine release in terminal regions has been recorded during a task that requires no movement to retrieve rewards. We have recently shown that cues that predict intraoral salt differentially evoke NAc dopamine depending on physiological state (sodium appetite; Cone et al., 2016). The present study extends these findings by showing that intraoral sucrose rewards behave similarly and lead to cue-evoked dopamine responses.

Despite this demonstration that a goal-directed action was not required to receive reward on infusion trials, our data do not rule out a contribution of motor generation to dopamine signals. Indeed, goal-directed action could underlie the difference in magnitude that we observed between pellet-predictive and infusion-predictive cues. In our paradigm, although rats did not need to make any motor movement to acquire intraoral infusions, they were also not compelled to stay still and, although making fewer entries into the food receptacle on infusion trials than on pellet trials, performed head entries on many trials nonetheless. This likely reflects either generalization of the cue-reward contingency, incomplete learning, and/or the fact that inappropriate head entries were not punished. Different training conditions may be needed to disentangle these possibilities such as a paradigm where errors

are punished with timeouts or withholding of reward. For example, in a Go-NoGo paradigm when rats are required to suppress all movement and remain still during presentation of a reward-predictive cue, suppression of dopamine in NAc core is observed (Syed et al., 2015). In a different paradigm, under a Go-NoGo schedule in which rats must withhold prepotent responding on a lever to avoid a timeout, differential activity in NAc neurons is observed (Roitman and Loriaux, 2014).

Although we tried to match rewards in terms of their value to the rats, there were differences between rewards that may have affected our results. For example, pellet delivery involves additional audiovisual elements not present during intraoral delivery (e.g. magazine turn, rattling of the pellet down the chute and into the receptacle). For intraoral delivery, we explicitly masked any proximal cues that can be associated with these infusions such as the click of the solenoid valve. Thus, dopamine evoked at time of reward delivery in pellet trials may reflect cue-driven processes as well as goal-directed action needed to retrieve pellets. Another potential difference between trial types that could drive differential dopamine responses is the time course of reward receipt. Although rewards were matched for quantity/caloric value, in the case of infusions, delivery of sucrose spanned several seconds whereas, for pellets, rats received the sucrose at a single point in time

and then, presumably, took a few seconds to chew and swallow the pellet. These differences between modes of reward delivery may influence the ability of rats to learn cue-reward associations that in turn affect how these stimuli and associations are encoded by dopamine and other brain structures.

With respect to dopamine signals at time of reward, it was of interest that in cued trials an increase in dopamine was observed when pellets, but not infusions, were delivered. Interestingly, during uncued trials we saw no difference between the amount of dopamine evoked by pellets or infusions. Thus, a simple explanation involving reduced detection ability of infusions, relative to pellets, is ruled out. One potential explanation that lends support to a dual-encoding hypothesis is that, in the uncued situation, increases in dopamine subserve primarily a 'reward-predictive' role; both pellets and infusions are equally unexpected and so similar signals are seen. In cued trials, classic reward prediction error theory would suggest that no signal should be present at time of reward if the cue is completely predictive (Schultz, 1998). This is indeed what is observed on infusion trials. However, on pellet trials when additional action is required to retrieve reward, a robust increase in dopamine is observed, which may be necessary to invigorate retrieval (Nicola, 2016). An alternative explanation is that there are differences in

the rate of learning for each cue-reward association. As such, it is possible that the cue-reward association develops more rapidly on infusion trials than on pellet trials, potentially because of the tighter temporal coincidence between events (e.g. infusions are always received exactly three seconds after cues whereas pellet receipt may be more delayed especially in early training sessions). Therefore, the predictive power of the infusion cue may be stronger than the pellet cue leading to a reduced dopamine response to cued infusions, relative to cued pellets. Longitudinal recordings from NAc across training sessions will help to disentangle these possibilities.

Phasic dopamine signals in NAc core and shell subregions may reflect different aspects of reward encoding (Brown et al., 2011; Cacciapaglia et al., 2012; Saddoris et al., 2015; Wheeler et al., 2011). In particular, in naïve rats, dopamine in NAc shell responds to delivery of rewarding intraoral stimuli (McCutcheon et al., 2012; Roitman et al., 2008; Wheeler et al., 2011) whereas dopamine in NAc core appears unresponsive (Wheeler et al. 2011). In contrast, in trained rats, responses to food pellets or food-associated cues are seen in NAc core but not in NAc shell (Brown et al., 2011). Thus, it has been proposed that NAc shell is associated with responses to primary reward and that NAc core is more important for signalling

environmental associations consistent with a later role in prediction error learning (Aragona et al., 2009). This framework follows an influential hypothesis involving the spiralling of information from ventromedial to dorsolateral parts of striatum during associative learning (Everitt and Robbins, 2005; Haber et al., 2000; Willuhn et al., 2012). Based on these previous findings we expected to observe clear differences in the current study between core and shell. Specifically, our hypothesis was that responses to cues would only be seen in NAc core and that NAc shell would only respond to 'primary' rewards. Surprisingly, this distinction was not present in our data; instead we found that NAc core and shell responses to each event were, in general, remarkably similar. We did observe differences in the time course of dopamine release events: responses tended to be sharper, more time-locked and shorter in duration in the core relative to shell. The latter pattern could reflect the known differences in dopamine transporter expression between these regions; low levels of expression in the shell allow dopamine responses to be prolonged (Ciliax et al., 1995). Reasons for the discrepancy between the findings here and other studies may reflect the different tasks animals were engaged in, the level of training animals received, or even different rewards under study. One hypothesis is that, in the present study, the (relatively) low level of training and

competing cue-reward associations may have kept NAc shell online and engaged in processing stimuli. In fact, it is not unprecedented to find responses to cues in NAc shell in studies using self-paced operant conditioning and more complex tasks (Cacciapaglia et al., 2012; Saddoris et al., 2015).

To summarize, different modes of reward delivery allow the appetitive and consummatory phases of ingestive behaviour to be studied independently. Here, we used a combination of cued and uncued intraoral infusions and sucrose pellets to assess how dopamine encodes multiple events in the sequence of actions that ultimately produce feeding. Our results demonstrate that dopamine has a broad, nuanced role that does not seem restricted to either phase of behaviour but rather may contribute to goal-directed action, reward prediction, and stimulus evaluation.

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Declaration of Conflicting Interests

The authors declare no conflicts of interest.

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Author Contributions

JM designed experiments, carried out experiments, analysed data and wrote the manuscript. MR designed experiments and wrote the manuscript.

Data Accessibility Statement

All data sets will be deposited on Dryad upon publication. Analysis code is available from the corresponding author on request.

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Figure captions

Figure 1. Dopamine responses to cues and primary rewards are qualitatively similar despite different types of reward delivery. ***A,*** Representative fast-scan cyclic voltammetry data from a single rat showing single trial examples of each trial type. Upper panels show colour plots with time shown on the x-axis, electrode holding potential shown on the y-axis, and background-subtracted current shown in pseudocolour. Vertical dashed white lines show events. Dopamine concentration, extracted using principal component analysis, is shown in lower panels. ***B,*** Averaged colour plots derived for each trial type for the same rat shown in ***A***. Mean dopamine concentration traces ± SEM are shown below. ***C,*** Dopamine response averaged from all rats for cued trials (left) and background-subtracted dopamine concentration during baseline (B), cue presentation (C), and reward delivery (R) epochs (right). Bars show mean and circles show data from individual rats. ***D,*** Average dopamine concentration data from all rats for probe trials in which no cue was presented (left) and background-subtracted dopamine concentration during

baseline (B) and reward delivery (R) epochs. Bars show mean and circles show data from individual rats. ***, p<0.001; *, p<0.05 vs. baseline epoch. ##, p<0.01, #, p<0.05 vs. corresponding epoch during pellet trials in ***C*** and dummy trials in ***D***.

Figure 2. Dopamine responses in nucleus accumbens core and shell show differences in time course but not in their principal features. ***A,*** Dopamine concentration traces evoked by different trial types in NAc core (solid lines, coloured shading) and shell (dashed lines, grey shading). Schematics on left show electrode placements (yellow circles) in coronal sections at +2.0 mm (left) and +1.7 mm (right) from Bregma. ***B,*** Receiver-operator characteristic (ROC) analysis to determine time points at which core and shell responses deviate. ROC values greater than 0.5 indicate elevated dopamine in core vs. shell and ROC values less than 0.5 indicate elevated dopamine in shell vs. core. Circles denote time points at which this difference is statistically significant (p<0.05). Although the shapes of each response are similar in both regions, peaks are sharper in core than in shell leading to elevated dopamine at time of cue in cued pellet trials and at time of pellet delivery in uncued pellet trials. In addition, responses are more prolonged in shell following reward delivery, for example elevated dopamine seen in shell vs.

core after reward delivery in cued pellet trials, cued infusion trials, and uncued infusion trials.