Dopamine supports reward prediction to constrain reward seeking

Melissa Malvaez[1], Andrea Suarez*[1], Nicholas K. Griffin*[1], Sean B Ostlund [2], Kate M. Wassum[1, 3]
*[1]Dept. of Psychology, UCLA, Los Angeles, CA 90095, USA. [2]Department of Anesthesiology. UCI, Irvine, CA [3]Brain Research Institute, UCLA, Los Angeles, CA 92697, USA. *equal contribution.*

Correspondence:
Kate Wassum: kwassum@ucla.edu
Dept. of Psychology, UCLA
1285 Franz Hall
Box 951563
Los Angeles, CA 90095-1563

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ABSTRACT

Reward-predictive cues trigger dopamine release in the nucleus accumbens core (NAc). This signal has long been thought to mediate motivation. However, understanding of dopamine function is complicated by the fact that reward cues not only motivate reward pursuit, but also enable the reward predictions that shape how reward is pursued. Thus, here we used dopamine sensor fiber photometry, cell-type and pathway-specific optogenetic inhibition, Pavlovian cue–reward conditioning, and test of cue-induced reward-pursuit strategy in male and female rats, to ask how cue-evoked phasic dopamine release supports reward pursuit. Cues that predicted imminent reward with high probability triggered a large NAc dopamine response and checking for the expected reward in the delivery location, rather than exploratory reward seeking. Cues that predicted reward with low probability elicited less dopamine and biased towards motivation to seek, rather than check for reward. Cue-evoked dopamine inversely related to and predicted reward seeking. Correspondingly, yet surprisingly, inhibition of cue-evoked NAc dopamine increased reward-seeking motivation and decreased reward checking. Thus, cue-evoked NAc dopamine is positively modulated by high imminent reward prediction to constrain exploratory reward-seeking motivation in favor of a more focal reward-checking strategy. These data advance understanding of dopamine function by indicating dopamine does not simply promote reward-seeking motivation, but rather supports reward predictions to shape adaptive reward pursuit strategy.

SIGNIFICANCE STATEMENT

Cues that signal reward availability can motivate us. To ensure this motivation is adaptive, we use the predictions these cues enable to help us decide how to pursue reward. When reward prediction is low, we'll seek out new reward opportunities. When it is high, we'll exploit the expected reward by checking for it in its usual location. Dopamine is typically thought to promote reward seeking. But here we discovered that transient, cue-evoked nucleus accumbens dopamine fluctuations support reward predictions to shape how reward is pursued, actually constraining reward seeking and promoting reward checking when reward is predicted imminently. These findings help understand the link between motivational and dopamine disruptions in psychiatric conditions such as addictions and depression.

Reward cues motivate our behavior. By signaling reward availability, they can incentivize reward pursuit (Corbit and Balleine, 2016; Robinson and Berridge, 2025). Cues can also influence *how* we pursue reward. By enabling reward predictions, cues influence how motivation is translated into action (Marshall and Ostlund, 2018). When a cue only sporadically signals a desired resource, it is more adaptive to engage in exploratory reward seeking than to check for the unlikely reward. But if reward is more reliably predicted, it is better to go wait for it at its usual location than to waste effort seeking it out (Azrin and Hake, 1969; Lovibond, 1981; Stephens and Krebs, 1986; Timberlake, 1994; Anselme and Güntürkün, 2018; Marshall and Ostlund, 2018). This is particularly true for cues that signal imminent reward. When time is of the essence, exploring new reward opportunities can prevent exploiting an expected reward, and vice versa. So, we tend to seek reward when its presence is unlikely, but check for it when it is probable. For example, with only a few minutes before a meeting, if snacks are offered only sporadically, you might seek one out at the vending machine, but, if they are more reliably predicted, you'll head straight to the conference room to look for a treat. Inability to regulate motivational impulses or exploit available adaptive rewards can lead to unnecessary opportunity costs, loss of control, or maladaptive reward seeking. Apathy can result from insufficient motivation. Thus, reward prediction is critical for adaptive behavior. Impairment in this process can lead to symptoms that characterize psychiatric conditions such as addictions and depression (Levy and Dubois, 2006; Jentsch and Pennington, 2014; Le Heron et al., 2018; Robinson and Berridge, 2025). Yet, little is known of how the brain supports reward prediction to regulate reward-pursuit strategy.

Dopamine may contribute. Reward-predictive cues trigger dopamine release in the nucleus accumbens core (NAc) (Roitman et al., 2004; Day et al., 2007; Day et al., 2010; Wassum et al., 2013; Aitken et al., 2015; Saddoris et al., 2015; Collins et al., 2016; Sun et al., 2018; Kutlu et al., 2021; Garr et al., 2024; Bornhoft et al., 2025). Motivational accounts suggest this phasic signal is a critical substrate of cue-motivated, exploratory, reward-seeking behavior (Beeler et al., 2012; Saunders and Robinson, 2012; Wassum et al., 2013; Syed et al., 2016; Berke, 2018; Robinson and Berridge, 2025). Yet, cue-evoked dopamine can be shaped by elements of reward prediction (Gan et al., 2009; Day et al., 2010; Hamid et al., 2015; Hart et al., 2015; Saddoris et al., 2015; Aitken et al., 2016; Garr et al., 2024). This raises the possibility that cue-evoked NAc dopamine may instead shape how reward is pursued by supporting reward prediction. Thus, here we asked whether cue-evoked dopamine directly promotes reward-seeking motivation, or rather supports reward prediction to shape reward pursuit. The latter makes the intriguing prediction that cue-evoked NAc dopamine could actually constrain reward seeking when the cue strongly signals imminent reward. We combined Pavlovian cue–reward conditioning and a Pavlovian-to-instrumental transfer test of cue-motivated behavior with dopamine measurement and manipulation to arbitrate between these possibilities. We used cues that predicted reward with either low or high probability and short-delay conditioning so that reward-seeking motivation would be constrained by reward prediction.

METHODS

Subjects

Male and female wildtype Long-Evans rats and transgenic Long-Evans rats expressing Cre recombinase under control of the tyrosine hydroxylase promoter (Th-cre+) aged 10 - 12 weeks at the time of surgery served as subjects. Rats were housed in a temperature (68 - 79°F) and humidity (30 - 70%) regulated vivarium. They were

initially housed in same-sex pairs and then, following surgery, housed individually to preserve implants. Rats were provided with water *ad libitum* in the home cage and, during experimental training and testing, were maintained on a food-restricted 12-18-g daily diet (Lab Diet, St. Louis, MO) to maintain approximately 85 - 90% free-feeding body weight. Rats were habituated to handling for 3 - 5 days prior to the onset of each experiment. Separate groups of naïve rats were used for each experiment. Experiments were performed during the dark phase of a 12:12 hr reverse dark:light cycle (lights off at 7 AM). All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the UCLA Institutional Animal Care and Use Committee.

Surgery

Rats were anesthetized with isoflurane (4 - 5% induction, 1 - 2% maintenance) and positioned in a digital stereotaxic frame (Kopf, Tujunga, CA). Subcutaneous Rimadyl (Carprofen; 5 mg/kg; Zoetis, Parsippany, NJ) was given pre-operatively for analgesia and anti-inflammatory purposes. An incision was made along the midline to expose the skull. After performing a small craniotomy, virus was injected using a 28-g infusion needle (PlasticsOne, Roanoke, VA) connected to a 1-mL syringe (Hamilton Company, Reno, NV) by intramedic polyethylene tubing (BD; Franklin Lakes, NJ) and controlled by a syringe pump (Harvard Apparatus, Holliston, MA). Virus was injected at a rate of 0.1 µl/min and the needle was left in place for 10 min post-injection to allow diffusion. Further experiment-specific surgical details are provided below. After surgery, rats were kept on a heating pad maintained at 35 °C for 1 hr and then single-housed in a clean homecage for recovery and monitoring. Rats received chow containing the antibiotic Trimethoprim/Sulfamethoxazole (TMS; Inotiv, West Lafayette, IN) for 7 days following surgery to prevent infection, after which they were returned to standard rodent chow.

Behavioral procedures

Apparatus. Training took place in Med Associates conditioning chambers (East Fairfield, VT) housed within sound- and light-attenuating boxes, described previously (Malvaez et al., 2015; Collins et al., 2019). Each chamber had grid floors and contained a retractable lever that could be inserted to the left of a recessed food-delivery port (magazine) on the front wall. A photobeam entry detector was positioned at the entry to the food port. Each chamber was equipped with a pellet dispenser to deliver 45-mg food pellets (Bio-Serv, Frenchtown, NJ) into a well of the food port. A tone generator was attached to a speaker on the wall opposite the lever and food-delivery port. A clicker was also mounted on this wall. A fan mounted to the outer chamber provided ventilation and external noise reduction. A 3-watt, 24-volt house light mounted on the top of the back wall opposite the food port provided illumination. For optogenetic manipulations, chambers were outfitted with an Intensity Division Fiberoptic Rotary Joint (Doric Lenses, Quebec, QC, Canada) connecting the output fiber optic patch cords to a laser (Changchun New Industries Optoelectronics Technology Co., ChangChun, JiLin, China) positioned outside of the chamber.

Short-delay Pavlovian conditioning with Pavlovian-to-instrumental transfer test

Magazine conditioning. Rats first received 2 sessions (1 session/day) of training to learn where to receive the food-pellet reward (45-mg grain pellet; Bio-Serv). Each session included 30 non-contingent pellet deliveries (60-s intertrial interval, ITI).

Pavlovian conditioning. All rats received 8 sessions of short-delay Pavlovian conditioning (1 session/day) to learn to associate each of 2, 10-s auditory (75 – 78 db) cues (aka conditioned stimuli, CS), click (10 Hz) and tone (1.5kHz), with the delivery of grain pellets. For 10 subjects in the fiber photometry experiment the tone was continuous for 10-s, for all other subjects, the tone was pulsed in 0.25-s on/off intervals (4 Hz). Each 10-s cue co-terminated with the delivery of a single pellet. The high-probability cue (CS_{90}) co-terminated with reward delivery on 90% of the trials. The low-probability cue (CS_{30}) co-terminated with reward delivery on only 30% of the trials. For half the subjects, click served as the CS_{90} and tone as the CS_{30}, with the other half receiving the opposite arrangement. Each session consisted of 20 click and 20 tone presentations. Cues were delivered pseudo-randomly with a variable 60 - 160-s ITI (mean = 110 s).

Instrumental conditioning. Rats next received 8 sessions, minimum, of instrumental conditioning (1 session/day), in which lever-press actions were reinforced with delivery of a single grain pellet. Each session terminated after 30 outcomes had been earned or 40 min had elapsed. Lever presses were continuously reinforced during the first session and then the reinforcement schedule was shifted to random interval (RI) in which a variable interval must elapse following a reinforcer for another press to be reinforced. The variable interval started at an average

of 15-s (RI-15s) for one session, then escalated to 30 s (RI-30s) for one session, and finally averaged 60 s (RI-60s) for the remaining sessions.

Pavlovian retraining. Following instrumental training, rats received 4 additional sessions of Pavlovian conditioning, as described above.

Pavlovian-to-instrumental transfer test. Rats next received a Pavlovian-to-instrumental transfer (PIT) test. During the PIT test, the lever was continuously present, but pressing was not reinforced. After 10 min of lever-pressing extinction, each 10-s cue was presented separately 10 times, separated by a fixed 110-s ITI. Cues were presented in pseudorandom order with no more than 2 of the same cue type occurring in succession. The cue type that started the session was counterbalanced across subjects. No rewards were delivered following cue presentation.

Data collection and analysis

Entries into the food-delivery port and/or lever presses were recorded continuously for each session and processed with Microsoft Excel (Microsoft, Redmond, WA). For Pavlovian conditioning, conditional food-port approach responses were assessed by comparing entries into the food-delivery port during the 10-s cue to the 10-s baseline periods immediately prior to cue onset (precue). Data were averaged across trials and then averaged across the last 2 conditioning sessions. For instrumental conditioning, press rates (presses/min) were averaged across the last 2 training sessions. For PIT tests, we evaluated entries into the food-delivery port and lever presses for a 15-s cue window combining the 10-s cue and 5-s post-cue period. This allowed us to capture behavior during the cue itself and the post-cue period, when reward was delivered during training. See Supplemental Figures 1-1 and 2-1 for a breakdown of food-port entries and lever presses in 2-s time bins before, during, and after cue presentation during PIT. Lever presses during the 15-s cue periods were compared to that during the 15-s precue baseline. As shown previously (Halbout et al., 2019; Halbout et al., 2022), we found that many food-port entries occurred immediately (within 2 s) following a lever press (Supplemental Figure 1-2). Therefore, to isolate Pavlovian conditional food-port checks from these entries that occurred as part of an instrumental press-enter sequence, we excluded food-port entries that directly followed a lever press within 2 s. All food-port entries and press-linked food-port entries are shown in Supplemental Figure 1-2. Food-port entries during the 15-s cue periods were compared to that during the 15-s precue baseline. To account for baseline behavior rates and evaluate the cue-induced change in behavior, we also computed the change (Δ) in lever presses and unlinked food-port entries by subtracting those during the 15-s cue period from baseline. Data were averaged across trials for each cue type. Trial x trial results are presented in Supplemental Figure 1-3.

Fiber photometry recordings of NAc dopamine release during Pavlovian-to-instrumental transfer

Subjects. Naïve, male and female Long Evans rats (Final *N* = 14, 7 male) were used in this experiment to record dopamine release in the NAc during PIT. 2 subjects were removed from the experiment. 1 due to illness and 1 because it did not acquire Pavlovian conditional behavior. 3 subjects with tissue damage that prevented placement validation were excluded from the analysis.

Behavioral training. Rats were food restricted and received magazine conditioning, followed by Pavlovian and instrumental training as above. Following surgery (see below) and recovery, rats were again food restricted and received 4 additional Pavlovian and 4 additional instrumental training sessions. Rats were tethered for the retraining sessions, but no light was delivered.

Surgery. Rats were infused unilaterally (Right hemisphere *N* = 13, Left: *N* = 1) with AAV encoding the GPCR-activation-based dopamine sensor $GRAB_{DA2m}$ (pAAV9-hsyn-GRAB_DA4.4, Addgene). Virus (0.5 µl) was infused at 0.1 µl/min into the NAc (AP: +1.3; ML: ±1.3; DV: -7.2, from bregma). 5 min later, viral injectors were dorsally repositioned in the NAc for a second viral infusion (0.5 µl; DV: -6.4). Optical fibers (200-µm diameter, 0.37 NA, Neurophotometrics Ltd, San Diego, CA) were implanted (DV: -6.8). Rats were allowed to recover for 5 days before retraining commenced.

Fiber photometry recordings during Pavlovian-to-instrumental transfer. Fiber photometry was used to image $GRAB_{DA2m}$ fluorescent changes in NAc neurons during PIT using a commercial fiber-photometry system (Neurophotometrics Ltd.). 470-nm excitation light was adjusted to approximately 80 - 100 µW at the tip of the patch cord (fiber core diameter: 400 µm; Doric Lenses). Fluorescence emission was passed through a 535-nm bandpass filter and focused onto the complementary metal-oxide semiconductor (CMOS) camera sensor through a tube lens. Samples were collected at 20 Hz using a custom Bonsai (Lopes et al., 2015) workflow. Time stamps of task events were collected simultaneously through an additional synchronized camera aimed at the

Med Associates interface, which sent light pulses coincident with task events. Signals were saved using Bonsai software and exported to MATLAB (MathWorks, Natick, MA) for analysis.

Fiber photometry analysis. Data were pre-processed using a custom-written pipeline in MATLAB (MathWorks). The 415-nm and 470-nm signals were fit using an exponential curve. Change in fluorescence (ΔF/F) at each time point was calculated by subtracting the fitted 415-nm signal from the 470-nm signal and normalizing to the fitted 415-nm data [(470-fitted 415)/fitted 415)]. Non-normalized 470-nm signal is provided in Supplemental Figure 1-4. The ΔF/F data was Z-scored to the average of the whole session [(ΔF/F - mean ΔF/F)/std(ΔF/F)]. Z-scored traces were then aligned to behavioral event timestamps. Dopamine fluctuations were quantified using the peak value within a specified window. To quantify cue-evoked dopamine, we used the 2 s period immediately following cue onset compared to the 2-s precue baseline periods. To quantify dopamine related to entry and lever press behaviors during the cue, we used the 4-s period prior to entries or lever presses compared to the baseline 4-s precue periods. To quantify dips in dopamine detected following cue offset, when the predicted reward was omitted at test, we calculated the minimum $GRAB_{DA}$ Z-score in the 5-s postcue period compared to 5-s period before cue offset. Individual trial data were excluded if the patch cord became detached or if artifactual signal due to excessive motion or patch cord twisting was detected (n = 6 CS30 trials from 4 subjects; n = 7 CS90 trials from 6 subjects). Quantifications and signal aligned to events were averaged across trials within a session and compared between groups.

Optogenetic inhibition of VTA_{DA}→NAc terminals at cue onset during Pavlovian-to-instrumental transfer

Subjects. Naïve, male and female Th-cre+ (hemizygous) Long Evans rats and wildtype (Th-cre-) littermates aged approximately 10 - 12 weeks at the time of surgery served as subjects in this experiment to assess the necessity of activity in ventral tegmental area dopamine projections to the NAc (VTA_{DA}→NAc) for cue-motivated behavior (Control, Final *N* = 12, 6 male, 9 Th-cre+/cre-dependent tdTomato, 3 Th-cre-/cre-dependent ArchT; ArchT, *N* = 14 Th-cre+, 9 male). Subjects with misplaced optic fibers (ArchT, *N* =1), lack of terminal expression (*N* = 1), or broken optic fibers (ArchT, *N* = 4; Control, *N* = 1) were excluded from the dataset.

Surgery. Th-cre+ rats were randomly assigned to a viral group and received bilateral infusions (0.2 µl) of AAV encoding a cre-inducible inhibitory opsin archaerhodopsin T (AAV5-CAG-FLEX-ArchT-tdTomato, Addgene), or a tdTomato fluorescent protein control (tdTomato; AAV5-CAG-FLEX-tdTomato, University of North Carolina Vector Core, Chapel Hill, NC). Th-cre- rats all received bilateral infusions (0.2 µl) of AAV encoding an mCherry fluorescent protein control (AAV8-hSyn-mCherry) into the ventral tegmental area (VTA; AP: -5.3; ML: ±0.7; DV: -8.3 mm from bregma). Optical fibers (200-µm core, 0.39 NA, Thorlabs, Newton, NJ) held in ceramic ferrules (Kientec Systems, Stuart, FL) were implanted bilaterally in the NAc (AP: +1.3; ML: ±1.4; DV: -6.8, from bregma). Rats were allowed to recover for 3 - 4 weeks prior to the start of food restriction and training.

Behavioral training. Rats received magazine, Pavlovian, and instrumental conditioning as above. They were attached to the optical tether (200 µm, 0.22 NA, Doric Lenses) throughout Pavlovian and instrumental conditioning, but no light was delivered. One cohort (*N* = 6, 3 control, 3 ArchT) received 12 rather than 8 Pavlovian conditioning sessions.

Optogenetic inhibition of VTA_{DA}→NAc projections during the Pavlovian-to-instrumental transfer test. PIT testing occurred 7 - 9 weeks post-surgery to allow sufficient ArchT expression in VTA_{DA}→NAc terminals at the time of manipulation. Optogenetic inhibition was used to attenuate the activity of ArchT-expressing VTA_{DA} axons and terminals in the NAc at cue onset. Green light (532 nm; 10 mW) was delivered to the NAc via a laser (Changchun New Industries Optoelectronics Technology Co.) connected through a ceramic mating sleeve (Thorlabs) to the ferrule implanted on the rat. During the PIT test, 2.5 s of continuous light was delivered beginning 0.5 s before each cue onset. Light effects were estimated to be restricted to the NAc core based on predicted irradiance values (https://web.stanford.edu/group/dlab/cgi-bin/graph/chart.php). Individual trials in which the patch cord became detached, and therefore light was not delivered, were removed from the analysis (Controls: n = 1 CS90 trial in 1 subject; ArchT: n = 3 CS30 trials across 3 subjects; n = 5 CS90 trials across 4 subjects). Behavioral data did not differ between the Th-cre+ and TH-cre- control types (PIT lever presses: 3-way RM-ANOVA, Control type: $F_{1,20}$ = 1.52, *P* = 0.23; Control type x CS presence: $F_{1,20}$ = 2.11, *P* = 0.16; Control type x CS type: $F_{1,20}$ = 0.13, *P* = 0.72; Control type x CS presence x CS type: $F_{1,20}$ = 0.67, *P* = 0.42; PIT food-port entries: 3-way RM-ANOVA, Control type: $F_{1,20}$ = 0.01, *P* = 0.94; Control type x CS presence: $F_{1,20}$ = 0.02, *P* = 0.89; Control type x CS type: $F_{1,20}$ = 0.22, *P* = 0.64; Control type x CS presence x CS type: $F_{1,20}$ = 1.80, *P* = 0.19). The controls were, therefore, collapsed into a single group.

Histology

Following behavioral experiments, rats were deeply anesthetized with pentobarbital (Vortech Pharmaceuticals, Dearborn, MI) and transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Brains were removed and post-fixed in 4% PFA overnight, placed into 30% sucrose solution, then sectioned into 30-µm slices using a cryostat (CM1950, Leica Biosystems, Nusslock, Germany) and stored in cryoprotectant. Slices were rinsed in a DAPI (Thermofisher Scientific, Waltham, MA) solution for 4 min (5 mg/mL stock, 1:10000), washed 3 times in PBS for 15 min, mounted on slides and coverslipped with ProLong Gold mounting medium (Thermofisher Scientific). All images were acquired using a fluorescence microscope (BZ-X710; Keyence, Osaka, Japan) with 4X, 10X, and 20X objectives (CFI Plan Apo), CCD camera, and BZ-X Analyze software (Keyence), to confirm viral expressions and optic fiber placement.

Immunofluorescence was used to confirm expression of $GRAB_{DA}$ in the NAc. Floating coronal sections were washed 3 times in 1x PBS for 30 min and then blocked for 1 – 1.5 hr at room temperature in a solution of 3% normal goat serum (Thermofisher Scientific) and 0.3% Triton X-100 (Thermofisher Scientific) dissolved in PBS. Sections were then washed 3 times in PBS for 15 min and incubated in blocking solution containing chicken anti-GFP polyclonal antibody (1:1000; Abcam, Cambridge, MA) with gentle agitation at 4°C for 18 – 22 hr. Sections were next rinsed 3 times in PBS for 30 min and incubated with goat anti-chicken IgY, Alexa Fluor 488 conjugate (1:500; Abcam) in blocking solution at room temperature for 2 hr. Sections were washed a final 2 times in PBS for 10 min.

tdTomato fluorescence with a Th costain was used to confirm expression of ArchT-tdTomato in VTA_{DA} neurons. Floating coronal sections were washed 3 times in 1x PBS for 30 min and then blocked for 2 hr at room temperature in a solution of 3% normal donkey serum (Thermo Fisher Scientific) and 0.2% Triton X-100 dissolved in PBS. Sections were then washed 3 times in PBS for 15 min and incubated in blocking solution containing rabbit anti-TH antibody (1:1000; EMD Millipore, Burlington, MA) with gentle agitation at 4°C for 44 - 48 hr. Sections were next rinsed 3 times in PBS for 30 min and incubated with goat anti-rabbit IgG, Alexa Fluor 488 conjugate (1:500; Thermofisher Scientific) in blocking solution at room temperature for 2 hr. Sections were washed a final 2 times in PBS for 10 min. Immunofluorescence was also used to confirm expression of ArchT-tdTomato in axons and terminals in the NAc. Floating coronal sections were washed 2 times in 1x PBS for 10 min and then blocked for 2 hr at room temperature in a solution of 10% normal goat serum and 0.5% Triton X-100 dissolved in PBS. Sections were then washed 3 times in PBS for 15 min and incubated in blocking solution containing rabbit anti DsRed polyclonal antibody (1:1000; EMD Millipore, Burlington, MA) with gentle agitation at 4°C for 18 - 22 hr. Sections were next rinsed 3 times in blocking solution for 30 min and incubated with goat anti-rabbit IgG, Alexa Fluor 594 conjugate (1:500; Thermofisher Scientific) in blocking solution at room temperature for 2 hr. Sections were washed a final 2 times in PBS for 10 min.

Statistical analysis

Datasets were checked for normality using the Kolmogorov-Smirnov test and then analyzed by two-tailed, paired and unpaired Student's *t* tests, one-, two-, or three-way repeated-measures analysis of variance (ANOVA; GraphPad Prism, GraphPad, San Diego, CA; SPSS, IBM, Chicago, IL). *Post hoc* tests were corrected for multiple comparisons using the Bonferroni method and used to clarify main and interaction effects. To assess the relationship between cue-evoked dopamine and task and behavioral variables we used multivariate general linear model (Covariance predictor: peak cue-evoked dopamine; Dependent variables: test trial, cue-evoked change in lever presses, and cue-evoked change in food-port entries), Pearson and partial correlation (controlling for subject), and linear mixed-model analysis (Repeated factors: CS, trial; Covariance predictor: peak cue-evoked dopamine; Random factor: subject; Dependent variable: cue-evoked change in lever presses or food-port entries) (GraphPad Prism, GraphPad, San Diego, CA; SPSS, IBM, Chicago, IL). Alpha levels were set at $P < 0.05$.

Sex as a biological variable

Male and female rats were used in approximately equal numbers for each cohort of each experiment. Individual data points are visually disaggregated by sex. We did not detect any significant effect of sex or interaction between sex and variables of interest (lowest $P = 0.09$).

Rigor and reproducibility

Group sizes were estimated *a priori* based on prior work using male Long Evans rats in this behavioral task (Malvaez et al., 2015; Lichtenberg and Wassum, 2016; Lichtenberg et al., 2017; Sias et al., 2021; Sias et al., 2024) and to ensure counterbalancing of cue-reward probability pairings. Investigators were not blinded to viral

group because they were required to administer virus. All behaviors were scored using automated software (MedPC). Each experiment included 2 replication cohorts and cohorts were balanced by viral group, cue-reward probability pairings, sex etc. prior to the start of the experiment.

Data and code availability

All data that support the findings of this study will be available as source data provided with this publication and from the corresponding author upon request. Custom-written MATLAB code is accessible via Dryad repository (https://zenodo.org/records/4926719)(Sias et al., 2021) and available from the corresponding author upon request.

RESULTS

Reward prediction shapes reward-pursuit strategy.

We first monitored NAc dopamine release during cue-motivated behavior (Figure 1a-c). Male and female rats were food restricted and received Pavlovian conditioning. We used short-delay conditioning to encourage imminent reward prediction and competing reward-seeking and reward-checking strategies at test (Ostlund and Marshall, 2021). To evaluate whether reward prediction influences cue-evoked dopamine, we used two distinct, 10-s auditory cues (conditioned stimuli, CS) each of which predicted food-pellet reward with different probabilities. The high-probability cue (CS_{90}) co-terminated with reward delivery on 90% of the trials. The low-probability cue (CS_{30}) co-terminated with reward on only 30% of the trials. Rats learned these differential reward-probability predictions. By the end of training, they entered the food-delivery port more during the high-probability cue than the low-probability cue (Figure 1d). To provide rats a reward-seeking action, we next gave them instrumental conditioning to lever press to earn the food-pellet reward. All rats acquired the instrumental behavior (final average press rate = 17.27, s.e.m. = 2.39). To ask how the cues affected reward-seeking v. -checking behavioral strategy, we gave rats a Pavlovian-to-instrumental transfer test (Holmes et al., 2010; Corbit and Balleine, 2016; Ostlund and Marshall, 2021). At test, each cue was presented in pseudorandom order to assess its influence over lever pressing and checks of the food-delivery port. No rewards were delivered, such that cued reward predictions decreased across the test. Motivation to seek reward is constrained by reward prediction (Konorski, 1967; Ostlund and Marshall, 2021). Whereas cues that predict reward with low probability motivate instrumental, reward-seeking behaviors, this motivational impulse is suppressed in favor of checking for an expected reward during highly predictive cues (Marshall et al., 2020). Indeed, at test, the low-probability cue increased lever pressing (Figure 1f-g). Such reward-seeking motivation was not triggered in response to the high-probability cue. Instead, this cue preferentially elicited a reward-checking strategy. To isolate conditional food-port checks from those that occurred as part of an instrumental press-enter sequence, we excluded food-port entries directly linked to lever presses (see Supplemental Figure 1-2). The high-probability cue caused more of these conditional reward-checking responses than the low-probability cue (Figure 1h-i). The cue-induced change in seeking and checking were not correlated (Partial correlation, controlling for subject, $r_{255} = -0.08$, $P = 0.19$), suggesting that the ability of the cue to trigger reward seeking did not depend on the extent to which it triggered reward checking and vice versa. There were modest changes in elicited behavioral strategy, most notably an increase in reward seeking to the high-probability cue, as reward prediction weakened across test trials (Supplemental Figure 1-3a-b). Thus, reward prediction shapes reward pursuit. Cues that signal imminent

reward with high probability preferentially elicit a strategy of checking for the expected reward. Conversely, cues that signal reward with low probability bias towards exploratory, instrumental reward seeking.

Cue-evoked NAc dopamine is positively modulated by reward prediction and inversely relates to reward-seeking motivation.

We exploited this task to ask the extent to which cue-evoked NAc dopamine release is shaped by reward-seeking motivation or reward prediction. We used fiber photometry to record fluorescent activity of the G-protein-coupled-receptor-activation-based dopamine sensor ($GRAB_{DA}$) in the NAc core during the Pavlovian-to-instrumental transfer test (Fig. 1a-c,e). If dopamine signals reward-seeking motivation, it should be higher in response to the low-probability cue than the high-probability cue, because this cue triggered more reward seeking. If, however, dopamine is shaped by reward prediction, it should be higher to the high-probability cue than the low-probability cue. The onset of both cues triggered robust, transient dopamine release (Figure 1j-k, see Supplemental Figure 1-2 for non-subtracted $GRAB_{DA}$ fluorescence response). We also detected a dopamine dip at the offset of both cues, when the predicted reward would have been delivered, but was omitted (Figure 1j,l). The cue-evoked dopamine response was larger for the high-probability cue than the low-probability cue (Figure 1j-k). Thus, cue-evoked dopamine is shaped by reward prediction. If true, then the cue-evoked dopamine response should decline as the reward prediction weakened across trials of the unrewarded test. Indeed, this was the case (Figure 1m; Supplemental Figure 1-3c). Thus, cues that predict imminent reward with high probability elicit a strong NAc core dopamine response and this is associated with checking for the expected reward in the delivery location, rather than seeking for it. Cues that predict reward with low probability elicit less NAc core dopamine and this is associated with preferential motivation to seek reward, rather than check for it.

We next further probed the relationship between cue-evoked NAc dopamine and reward prediction and asked how this signal relates to cue-motivated behavior. We first used a multivariate general linear model including data from each trial for each subject to evaluate the extent to which cue-evoked dopamine relates to declining reward prediction resulting from extinction across test (defined by test trial; Figure 1m) and cue-induced changes in instrumental reward-seeking (lever presses; Figure 1n) and -checking (food-port entries; Figure 1o) behavior. Cue-evoked dopamine significantly related to this multivariate combination ($F_{3,254}$ = 15.79, P < 0.001, η^2 = 0.16). Follow-up univariate analysis indicated that cue-evoked dopamine significantly inversely related to test trial ($F_{3,129}$ = 40.05, P < 0.001, η^2 = 0.14; β = -0.67, SE = 0.11, t_{256}= -6.33, P < 0.001, 95% CI -0.88 - -0.46). Indeed, there was a significant negative correlation between cue-evoked dopamine and trial (Figure 1m), indicating that as reward prediction weakened, so too did the cue-evoked dopamine. Thus, cue-evoked dopamine tracks trial-dependent changes in reward prediction. Cue-evoked dopamine also significantly inversely related to cue-induced reward seeking ($F_{1,129}$ = 10.88, P = 0.001, η^2 = 0.04; β = -0.37, SE = 0.08, t_{256}= -3.30, P = 0.001, 95% CI -0.44 - -0.11), but did not significantly relate to reward-checking behavior ($F_{1,129}$ 2.54, P = 0.11, η^2 = 0.01; β = 0.14, SE = 0.09, t_{256}= 1.59, P = 0.11, 95% CI -0.03 - 0.32). There was a negative correlation between cue-evoked dopamine and cue-evoked reward seeking (Figure 1n) that held true both between- and within-subjects (Supplemental Figure 1-5). Subjects for which the cues elicited a larger dopamine response showed less cue-evoked reward-seeking motivation. On trials in which the cue elicited a larger dopamine response it also tended

to trigger less reward seeking. Thus, larger cue-evoked dopamine is associated with stronger reward predictions and less reward seeking. We next asked whether cue-evoked dopamine could predict cue-induced behavior. Using a linear mixed-model analysis, we were able to reliably inversely predict reward seeking from the cue-evoked dopamine response (Figure 1p; Supplemental Figure 1-3d), indicating larger cue-evoked NAc dopamine predicts less reward seeking. Cue-evoked reward checking could be positively predicted with marginal significance from cue-evoked dopamine (Figure 1q; Supplemental Figure 1-3d). Thus, cue-evoked NAc dopamine is positively modulated by reward prediction and inversely relates to reward-seeking motivation.

Lastly, we asked whether NAc dopamine relates to the execution of behavioral strategies. Consistent with prior evidence (Wassum et al., 2013), we found that dopamine is transiently released in the NAc during cue-induced reward seeking (Figure 1r-s). This was similar for both the high and low-probability cue. We also found transient dopamine release prior to reward-port checks (Figure 1t-u, see Supplemental Figure 1-6 for dopamine release around all food-port entries). Dopamine release was larger prior to checks during the high-probability cue than those during the low-probability cue. Thus, higher reward prediction causes greater dopamine release prior to reward-checking behavior.

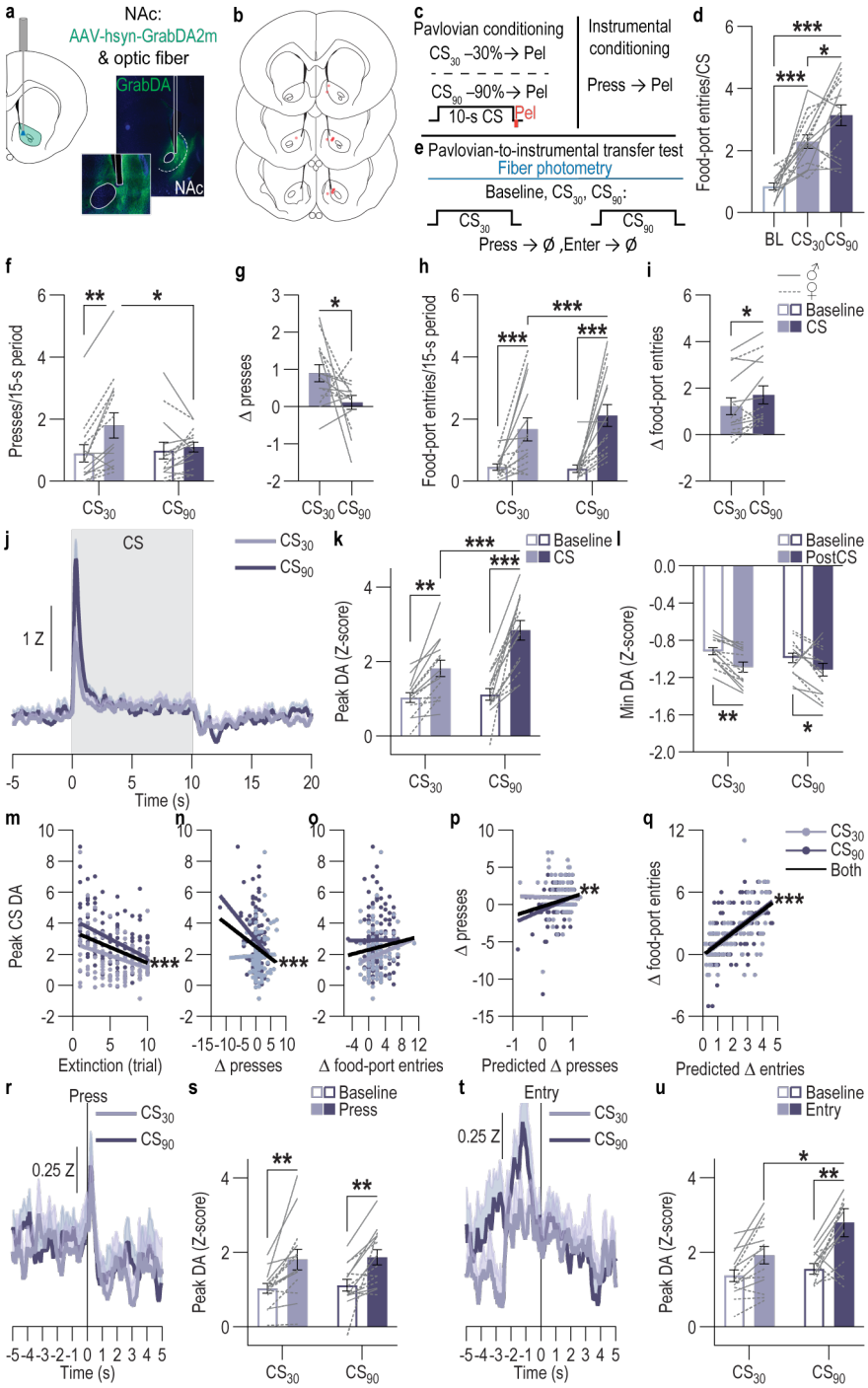

Figure 1. Cue-evoked NAc dopamine is positively modulated by reward prediction and inversely relates to reward-seeking motivation. ***A,*** Left, fiber photometry approach for imaging $GRAB_{DA}$ fluorescence changes in NAc core neurons. Right, Representative immunofluorescence image of NAc $GRAB_{DA2m}$ expression and fiber placement. ***B,*** Fiber placement map. ***C,*** Pavlovian and instrumental conditioning schematic. CS, 10-s conditioned stimulus (Click or Tone) coterminating with food pellet (Pel) reward on 30% (CS_{30}) or 90% (CS_{90}) of the trials. Press, lever press earns food pellet (Pel) on a random-interval reinforcement schedule. ***D,*** Food-port entries per 10-s baseline (BL) and CS period averaged across the last 2 days of Pavlovian conditioning. 1-way repeated measures (RM)-ANOVA, $F_{2,26}$ = 24.18, P < 0.0001. ***E,*** Pavlovian-to-instrumental transfer test schematic. Ø, no rewards were delivered at test. ***F,*** Lever presses for the 15-s CS (10-s CS + 5-s postCS) and preceding 15-s baseline periods. 2-way RM-ANOVA, CS presence x CS type: $F_{1,13}$ = 4.83, P = 0.047; CS presence: $F_{1,13}$ = 21.48, P = 0.0005; CS type: $F_{1,13}$ = 2.50, P = 0.14. ***G,*** CS-induced change (Δ) in lever presses from baseline. 2-tailed t-test, t_{13} = 2.20, P = 0.047, 95% Confidence interval (CI) -1.56 to -0.01. ***H,*** Food-port entries, excluding those directly linked to lever presses, for the 15-s CS and baseline periods. 2-way RM-ANOVA, CS presence x CS type: $F_{1,13}$ = 7.69, P = 0.02; CS presence: $F_{1,13}$ = 16.40, P = 0.001; CS type: $F_{1,13}$ =

2.56, $P = 0.13$. ***I,*** CS-induced change in food-port entries. 2-tailed t-test, $t_{13} = 2.77$, $P = 0.02$, 95% CI 0.11 to 0.88. ***J,*** CS-evoked $GRAB_{DA}$ fluorescence changes (Z-score). ***K,*** Peak CS-evoked $GRAB_{DA}$ (DA) Z-score compared to preCS baseline. 2-way RM-ANOVA, CS presence x CS type: $F_{1,13} = 13.88$, $P = 0.003$; CS presence: $F_{1,13} = 49.91$, $P < 0.0001$; CS type: $F_{1,13} = 23.25$, $P = 0.0003$. ***L,*** Minimum (min) post-CS $GRAB_{DA}$ Z-score relative to baseline before CS offset. 2-way RM-ANOVA, CS offset: $F_{1,13} = 20.20$, $P = 0.0006$; CS type: $F_{1,13} = 1.08$, $P = 0.32$; CS offset x CS type: $F_{1,13} = 0.56$, $P = 0.47$. ***M-O,*** Relationship between peak CS-evoked $GRAB_{DA}$ Z-score and test trial (m; 2-tailed Partial correlation, controlling for subject, $r_{261} = -0.37$, $P < 0.001$.), CS-evoked change in lever presses (n; 2-tailed Partial correlation, $r_{255} = -0.20$, $P < 0.001$), or food-port entries (o; 2-tailed Partial correlation, $r_{255} = -0.12$, $P = 0.06$). ***P,*** Correlation between actual CS-induced change in presses and that predicted by CS-evoked dopamine using a linear mixed model (Repeated factors: CS, trial; Covariance predictor: peak CS-evoked dopamine; Random factor: subject; $\beta = -0.21$, standard error (SE) = 0.07, $t_{136.60} = -3.06$, $P = 0.003$, 95% CI -0.35 - -0.08; Pearson $r_{258} = 0.20$, $P = 0.001$). ***Q,*** Correlation between actual CS-induced change in food-port entries and that predicted by CS-evoked dopamine using a linear mixed model (Repeated factors: CS, trial; Covariance predictor: peak CS-evoked dopamine; Random factor: subject; $\beta = 0.15$, SE = 0.08, $t_{139.73} = 1.89$, $P = 0.06$, 95% CI -0.007 - 0.30; Pearson $r_{258} = 0.59$, $P < 0.0001$). ***R,*** $GRAB_{DA}$ fluorescence changes (Z-score) around lever presses during the CSs. ***S,*** Peak $GRAB_{DA}$ Z-score before press during CS. 2-way RM-ANOVA, Press v. baseline: $F_{1,13} = 24.22$, $P = 0.0003$; CS type: $F_{1,13} = 0.14$, $P = 0.71$; Press x CS type: $F_{1,13} = 0.007$, $P = 0.94$. ***T,*** $GRAB_{DA}$ fluorescence changes (Z-score) around food-port entries, excluding those directly linked to lever presses, during the CSs. ***U,*** Peak $GRAB_{DA}$ Z-score before food-port entry. 2-way RM-ANOVA, Entry v. baseline: $F_{1,13} = 14.48$, $P = 0.002$; CS type: $F_{1,13} = 7.49$, $P = 0.02$; Entry x CS type: $F_{1,13} = 1.99$, $P = 0.18$. $N = 14$ rats (7 male). Data presented as mean ± s.e.m. Males = solid lines, Females = dashed lines. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Bonferroni corrected.

Cue-evoked NAc core dopamine constrains reward seeking and promotes reward checking.

Having found that cue-evoked NAc dopamine is positively modulated by reward prediction, we next asked whether this signal mediates the influence of such prediction on behavior. Motivational accounts propose that cue-evoked dopamine promotes reward-seeking motivation (Wyvell and Berridge, 2000; Berridge, 2007; Wassum et al., 2011; Saunders and Robinson, 2012; Peciña and Berridge, 2013; Wassum et al., 2013; du Hoffmann and Nicola, 2014; Ostlund et al., 2014; Halbout et al., 2019; Jovanoski et al., 2023). Yet, we found cue-evoked dopamine release is positively modulated by reward prediction and negatively relates to reward seeking. Thus, large cue-evoked NAc core dopamine may support high imminent reward prediction to constrain exploratory reward seeking in favor of checking for the strongly predicted reward. This leads to the intriguing, perhaps surprising, prediction that inhibition of cue-evoked NAc dopamine release will increase reward seeking and decrease reward checking.

We tested this using optogenetic inhibition of cue-evoked NAc dopamine release during a Pavlovian-to-instrumental transfer test (Figure 2a-c). We cre-dependently expressed the inhibitory opsin archaerhodopsin T (ArchT) bilaterally in ventral tegmental area dopamine (VTA_{DA}) neurons of male and female tyrosine hydroxylase (Th)-cre rats (Figure 2a-b) and implanted optical fibers bilaterally over NAc core (Figure 2b). For controls, we either cre-dependently expressed tdTomato bilaterally in VTA_{DA} neurons of Th-cre rats or infused the cre-dependent ArchT AAV in wildtype (Th-cre-) rats and, in both cases, implanted optical fibers bilaterally over NAc core. Rats were food restricted and received Pavlovian short-delay conditioning, as above, during which two distinct, 10-s auditory cues each predicted a food-pellet reward with either high (90%) or low (30%) probability. Rats learned these differential reward-probability predictions (Supplemental Figure 2-1a). Rats then received instrumental conditioning to lever press to earn food-pellet rewards (Supplemental Figure 2-1b). They were then given a PIT test during which the lever was available and each cue was presented in pseudorandom order to assess its influence over lever pressing and checks of the food-delivery port. No rewards were delivered at test. We optically inhibited (532 nm, 10 mW, 2.5 s) VTA_{DA} axons and terminals in the NAc coincident with each cue during this test. We restricted optical inhibition to cue onset to inhibit only the phasic cue-induced dopamine elevation. Reward prediction influenced behavioral strategy in controls. Only the low-probability cue increased instrumental reward seeking (Figure 2d-e). The high-probability cue increased reward checking more than the

low-probability cue (Figure 2f-g). Optical inhibition of cue-evoked VTA_{DA}→NAc activity elevated the reward-seeking motivational response to both cues (Figure 2d-e) and decreased cue-evoked reward checking during the high-probability cue (Figure 2f-g; see Supplemental Figure 2-2 for data on all entries and press-linked entries). These two effects were not correlated (two-tailed, between-subject Pearson r, Control, CS_{30}: $r_{12} = 0.27$, $P = 0.39$; CS_{90}: $r_{12} = -0.10$, $P = 0.76$; Both CSs: CS_{30}: $r_{24} = -0.01$, $P = 0.95$; ArchT, CS_{30}: $r_{14} = 0.22$, $P = 0.44$; CS_{90}: $r_{14} = -0.18$, $P = 0.55$; Both CSs: CS_{30}: $r_{28} = -0.03$, $P = 0.88$), indicating the increase in reward seeking was not a secondary consequence of the decrease in reward checking or vice versa. Despite inhibition occurring only at cue onset, these effects persisted throughout the duration of the cue period (Supplemental Figure 2-3). Thus, cue-evoked, transient, NAc core dopamine mediates the influence of reward prediction over behavioral strategy, which, in this case, opposes exploratory reward-seeking motivation and promotes checking for a highly predicted reward.

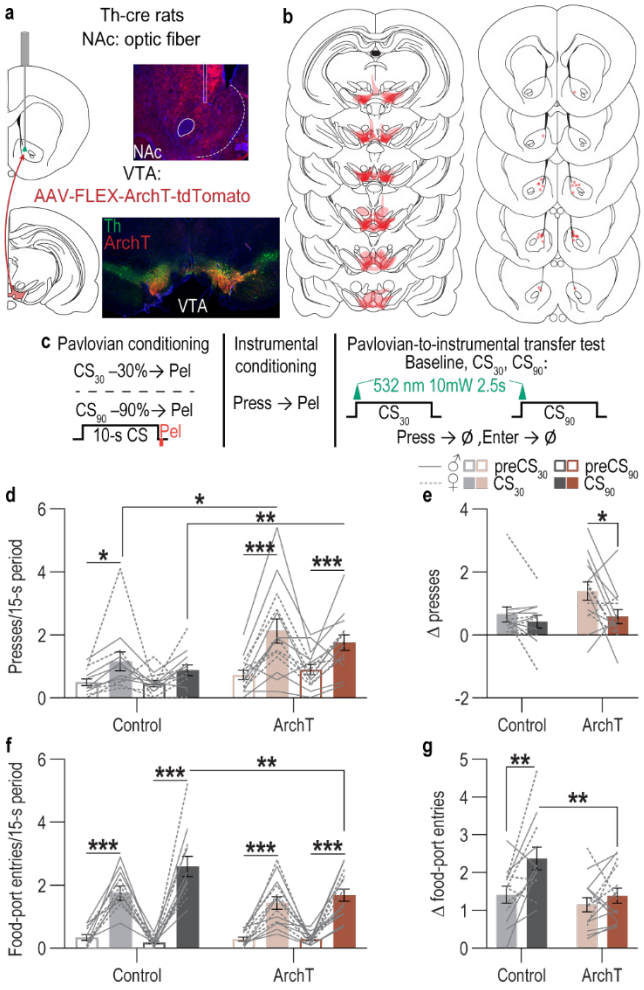

Figure 2. Cue-evoked NAc core dopamine constrains reward seeking and promotes reward checking. ***A,*** Left, optogenetic approach for bilateral inhibition of VTA_{DA}→NAc projections. Right top, representative image of fiber placement in the vicinity of immunofluorescent ArchT-tdTomato-expressing VTA_{DA} axons and terminals in NAc. Right bottom, representative immunofluorescent image of ArchT-tdTomato expression in VTA_{DA} (Th+) neurons. ***B,*** VTA ArchT expression and NAc fiber placement maps. ***C,*** Task training and test schematic. CS, 10-s conditioned stimulus (Click or Tone) coterminating with food pellet (Pel) reward on 30% (CS_{30}) or 90% (CS_{90}) of the trials. Press, lever press earns food-pellet (Pel) reward on a random-interval reinforcement schedule. Ø, no rewards were delivered. ***D,*** Lever presses for the 15-s CS (10-s CS + 5-s postCS) and preceding baseline periods. 3-way RM-ANOVA, Virus x CS presence: $F_{1,24} = 4.48$, $P = 0.04$; Virus: $F_{1,24} = 6.75$, $P = 0.02$; CS presence: $F_{1,24} = 36.92$, $P < 0.0001$; CS presence x CS type: $F_{1,24} = 3.44$, $P = 0.07$; CS type: $F_{1,24} = 1.07$, $P = 0.31$; Virus x CS type: $F_{1,24} = 0.06$, $P = 0.81$; Virus x CS presence x CS type: $F_{1,24} = 0.57$, $P = 0.46$. ***E,*** CS-induced change (Δ) in lever presses from baseline. 2-way RM-ANOVA, CS type: $F_{1,24} = 6.33$, $P = 0.02$; Virus: $F_{1,24} = 2.58$, $P = 0.12$; Virus x CS type: $F_{1,24} = 1.97$, $P = 0.17$. ***F,*** Food-port entries, excluding those directly linked to lever presses, for the 15-s CS and preceding baseline periods. 3-way RM-ANOVA, Virus x CS presence: $F_{1,24} = 5.68$, $P = 0.03$; CS presence x CS type: $F_{1,24} = 9.90$, $P = 0.004$; Virus x CS presence x CS type: $F_{1,24} = 3.72$, $P = 0.07$; CS presence: $F_{1,24} = 137.90$, $P < 0.0001$; CS type: $F_{1,24} = 5.86$, $P = 0.02$; Virus: $F_{1,24} = 3.82$, $P = 0.06$; Virus x CS type: $F_{1,24} = 1.22$, $P = 0.28$. ***G,*** CS-induced change in food-port entries. 2-way RM-ANOVA, Virus: $F_{1,24} = 5.66$, $P = 0.03$; CS type: $F_{1,24} = 10.18$, $P = 0.004$; Virus x CS type: $F_{1,24} = 3.65$, $P = 0.07$. Control, $N = 12$ rats (6 male; 9 Th-cre+, 3 TH-cre-) ArchT, $N = 14$ Th-cre+ rats (8 male). Data presented as mean ± s.e.m. Males = solid lines, Females = dashed lines. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, Bonferroni corrected.

DISCUSSION

Here we investigated whether cue-evoked dopamine directly promotes reward-seeking motivation or instead supports reward prediction to shape reward-pursuit strategy. We found that cues that predict imminent reward with high probability trigger a large dopamine response and checking for the expected reward in the delivery location, rather than exploratory reward seeking. Cues that predict reward with low probability elicit less dopamine and bias towards motivation to seek, rather than check for reward. Moreover, cue-evoked dopamine

inversely relates to and predicts reward seeking. Correspondingly, we discovered that NAc dopamine release to short-delay cues opposes exploratory reward-seeking behavior and promotes checking for a highly predicted reward. Thus, cue-evoked NAc dopamine can support high imminent reward prediction to constrain exploratory reward-seeking motivation in favor of a reward-checking strategy.

Reward prediction shapes how reward is pursued. We found that cues that signaled imminent reward with high probability tended to elicit a strategy of checking for the expected reward in its delivery location rather than seeking for it. Conversely, low-probability cues preferentially triggered instrumental reward seeking. These seeking v. checking effects were not correlated, indicating they were not simply due to response competition. Rather they reflect distinct exploratory, global, v. focal reward pursuit strategies (Timberlake, 1994). This occurred in a novel situation in which behavioral strategy was selected on-the-fly. The test was the first opportunity to seek reward during the cues and these actions were never reinforced. Thus, reward predictions rather than reinforcement regulated reward seeking. These results are consistent with evidence that cue-evoked predictions of the probability (Marshall et al., 2020), timing (Lovibond, 1981; Crombag et al., 2008; Marshall and Ostlund, 2018), and value (Marshall et al., 2023) of reward regulate reward-pursuit strategy. That strong reward prediction constrains exploratory reward-seeking motivation in favor of checking for the expected reward is particularly the case for cues that signal imminent, non-contingent reward (Konorski, 1967; Ostlund and Marshall, 2021), as we used here. Indeed, long-duration cues that set the occasion for sporadic, infrequent reward tend to vigorously motivate reward seeking (Crombag et al., 2008; Wassum et al., 2013; Collins et al., 2016; Halbout et al., 2019). Moreover, diminishing reward prediction, either by extinction (Delamater, 1996) or reward devaluation (Marshall et al., 2023), promotes reward seeking. Thus, while reward-predictive cues acquire motivational properties, this motivation is suppressed by strong, imminent reward prediction to allow a more adaptive, focal checking strategy.

NAc core dopamine is positively modulated by cue-evoked reward prediction. Consistent with prior reports (Roitman et al., 2004; Day et al., 2007; Day et al., 2010; Flagel et al., 2011; Wassum et al., 2013; Aitken et al., 2015; Saddoris et al., 2015; Collins et al., 2016; Sun et al., 2018; Kutlu et al., 2021; Garr et al., 2024; Bornhoft et al., 2025), we found that reward-predictive cues elicit phasic dopamine release in the NAc core. Cues that signaled reward with high probability elicited a larger NAc dopamine response than low-probability cues and cue-evoked dopamine tracked trial-dependent changes in reward prediction. This accords with prior evidence of reward probability encoding in both VTA dopamine neuron firing (Fiorillo et al., 2003; Tobler et al., 2005; Eshel et al., 2016) and NAc dopamine release (Hart et al., 2015; Garr et al., 2024). It also aligns with findings that cue-evoked dopamine is modulated by the value of the predicted reward (Gan et al., 2009; Day et al., 2010; Aitken et al., 2016; Syed et al., 2016) and that dopamine signals transitions in reward availability state (Kalmbach et al., 2022). An important future question is the extent to which dopamine is modulated by other features of the predicted reward. Whereas probability and general value shape NAc cue-evoked dopamine, specific features of the predicted reward may not (Halbout et al., 2019; Taira et al., 2024), at least in NAc core (Sias et al., 2024). Whether dopamine's modulation by reward prediction is associated with its signaling of prediction error (Schultz

et al., 1997; Steinberg et al., 2013; Hart et al., 2014) and/or salience (Kutlu et al., 2021; Kutlu et al., 2022), is also ripe for investigation.

Cue-evoked NAc dopamine inversely predicts and constrains reward seeking. Cue-evoked dopamine inversely related to and predicted the ability of the cue to promote reward seeking. Larger cue-evoked dopamine was associated with stronger reward predictions and less reward seeking. Correspondingly, inhibition of cue-evoked NAc dopamine increased reward seeking. It also decreased reward checking. Therefore, the dopamine triggered by short-delay cues constrains exploratory reward-seeking motivation and promotes the reward-checking strategy. Despite only brief inhibition at cue onset, the behavioral effects persisted during and immediately after the cue. Thus, the cue-evoked dopamine transients can support a transition in the reward-prediction state that controls subsequent behavioral strategy, in this case constraining reward seeking.

Three pieces of evidence argue against the alternative explanation that cue-evoked NAc dopamine mediates conditional reward-checking behavior to secondarily affect reward seeking via removal of response competition. First, cue-evoked dopamine more strongly inversely related to reward seeking than positively related to reward checking. Second, inhibition of cue-evoked dopamine did not fully suppress reward checking, consistent with considerable evidence that dopamine is not required for conditional reward-checking behaviors (Lex and Hauber, 2008; Flagel et al., 2011; Saunders and Robinson, 2012; Halbout et al., 2019). Third, the influence of dopamine manipulations on reward-seeking and -checking behaviors were not correlated, indicating the effect of dopamine inhibition to increase reward seeking was not secondary to a reduction in reward checking. Rather, the results indicate that cue-evoked NAc dopamine supports reward prediction to constrain reward seeking and promote reward checking.

These results update motivational accounts of dopamine function. Such theories presume cue-evoked reward-seeking motivation is directly related to incentive value (Dayan and Balleine, 2002; McClure et al., 2003) and posit that cue-evoked dopamine reflects such value to incentivize reward seeking (Beeler et al., 2012; Saunders and Robinson, 2012; Wassum et al., 2013; Syed et al., 2016; Berke, 2018; Robinson and Berridge, 2025). Indeed, this is the case for long-duration cues that signal delayed or temporally uncertain reward (Wassum et al., 2013; Ostlund et al., 2014; Aitken et al., 2015; Collins et al., 2016). But when reward is strongly and imminently predicted, the seeking motivational impulse should be suppressed to allow one to retrieve the expected reward (Konorski, 1967; Ostlund and Marshall, 2021). As we show, strong, imminent reward prediction biases away from exploratory, global, reward seeking to a more focal reward pursuit strategy at the expected reward location (Timberlake, 1994). Here we show that cue-evoked NAc core dopamine does not promote motivation for exploratory reward-seeking behavior, but rather supports reward prediction, which can flexibly constrain reward seeking and promote more focal reward checking. This seemingly contrasts with findings that cues elicit a larger NAc dopamine response when they promote incentive motivation (Flagel et al., 2011) or the need to instrumentally seek reward (Syed et al., 2016), than when they encourage waiting at the reward-delivery location. Unlike the present design, in these studies the behavioral strategy was reinforced. Reinforcement may cache the value of action to the predictive cue, such that the cue-evoked dopamine not only supports reward prediction, but also the reinforced motivational value of reward seeking (Hamid et al., 2015; Syed et al., 2016).

Thus, the information dopamine conveys and how it influences reward pursuit may differ if reward-pursuit strategies have been reinforced v. are being determined on-the-fly. Our results also seemingly contrast with those indicating that tonic inactivation of NAc dopamine input (Halbout et al., 2019) or dopamine receptors (Lex and Hauber, 2008) attenuates reward seeking in response to long-duration cues that loosely predict temporally uncertain reward. This could result from differences in the function of tonic v. phasic dopamine release (Niv et al., 2007; Schultz, 2007). It could also indicate that dopamine function depends on the duration, strength, and nature of reward prediction. These variables may shape the downstream neuronal circuitry engaged by the cue. Whereas, strong, imminent reward prediction may engage circuitry to constrain global, exploratory reward seeking and promote focal reward checking, cues that signal less, delayed, uncertain, and/or infrequent reward may engage distinct circuitry to promote reward seeking. Cue-evoked dopamine may accentuate whichever reward-pursuit circuit is engaged by the cue. These intriguing possibilities warrant further investigation.

Dopamine, including that elicited by cues (Maes et al., 2020), has long been known to function as a teaching signal to support learning (Waelti et al., 2001; Schultz, 2002; Lerner et al., 2021). These results add to a growing body of evidence that dopamine does not simply function retrospectively in learning (Schultz, 2002; Glimcher, 2011; Kahnt and Schoenbaum, 2025), but also prospectively to regulate behavior (Howe and Dombeck, 2016; Schultz, 2016; Berke, 2018; Kaźmierczak and Nicola, 2022). They add that this prospective function is not simply to promote reward-seeking motivation, but rather NAc core dopamine can support reward prediction to flexibly regulate reward-pursuit strategy, constraining exploratory, reward seeking when reward is predicted imminently. Motivational attenuation and inability to adaptively regulate motivational impulses are symptoms of psychiatric conditions, including depression and addictions, conditions that are also marked by dopamine disruption. Thus, these data also aid our understanding and treatment of motivational symptoms of psychiatric disease.

AUTHOR CONTRIBUTIONS

KMW, SBO, AS, and MM conceptualized and designed the experiments. MM & KW analyzed and interpreted the data, and wrote the paper. AS executed the fiber photometry experiments and did the initial analysis. NKG and MM conducted the optogenetic experiments and did the initial analysis. SBO provided guidance on experimental execution, analysis, and interpretation and contributed to manuscript preparation.

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COMPETING FINANCIAL INTERESTS

The authors have no biomedical financial interests or potential conflicts of interest to declare.

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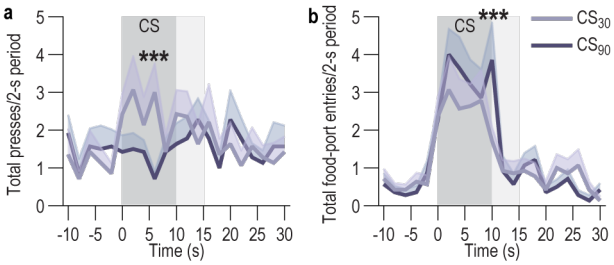
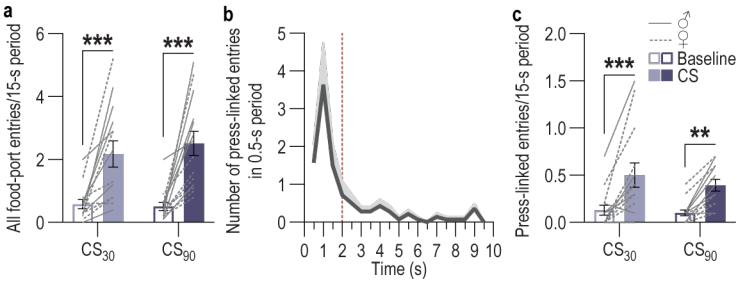
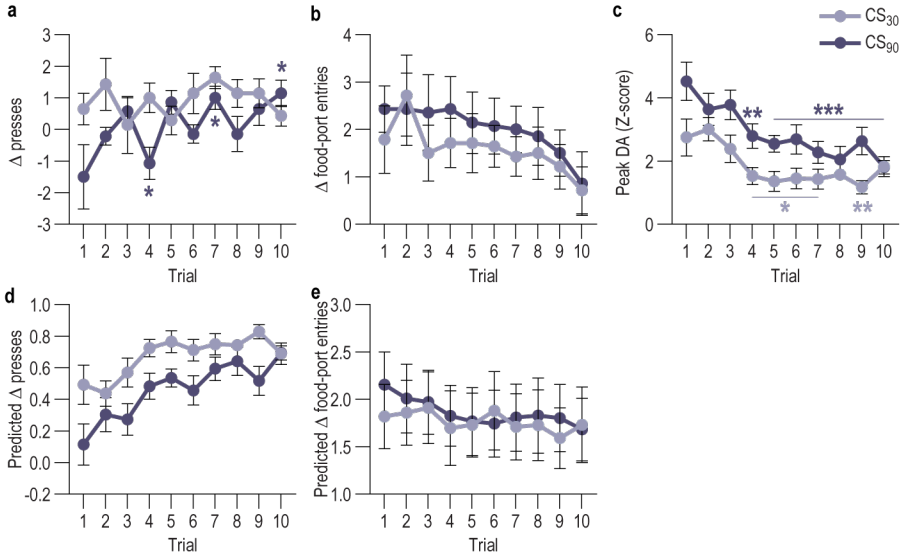
SUPPLEMENTAL FIGURES



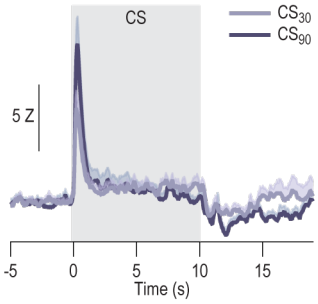
Supplemental Figure 1-1. Cues differentially promote reward seeking v. checking based on reward prediction throughout the cue during Pavlovian-to-instrumental transfer. *A-B,* Lever presses *(A)* and food-port entries, excluding those directly linked to lever presses, *(B)* in 2-s bins before, during, and after cue (conditioned stimulus, CS) presentation. Gray, 10-s CS; Light gray, 5-s post-CS period. 2-way RM-ANOVA, Presses, Time x CS type: $F_{20,260} = 2.07$, $P = 0.005$; Time: $F_{20,260} = 1.69$, $P = 0.03$; CS type: $F_{1,13} = 1.18$, $P = 0.30$; Food-port entries, 2-way RM-ANOVA, Time x CS type: $F_{20,260} = 2.12$, $P = 0.004$; Time: $F_{20,260} = 10.98$, $P < 0.0001$; CS type: $F_{1,13} = 1.41$, $P = 0.26$. Data presented as mean ± s.e.m. ***$P < 0.001$, CS_{30} v. CS90, Bonferroni corrected. Cues that signal reward with low probability biased behavior towards exploratory, instrumental reward seeking. This reward-seeking strategy was not triggered in response to the high-probability cue. Instead, this cue preferentially elicited a reward-checking strategy (entries into the food-delivery port). Because cue-evoked changes in reward-seeking and reward-checking behavior persisted into the 5-s post-cue period, when reward was delivered during training, we used a 15-s CS (10-s CS + 5-s postCS) to quantify CS-evoked behavioral changes during PIT. $N = 14$ rats (7 male).



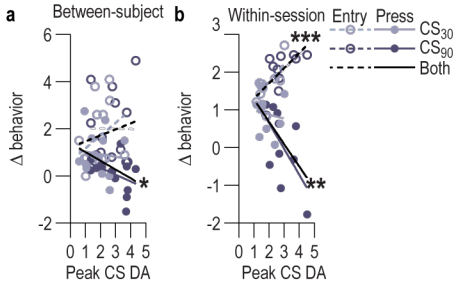
Supplemental Figure 1-2. All and press-linked cue-evoked entries into the food-delivery port during Pavlovian-to-instrumental transfer. *A,* All food-port entries for the 15-s CS (10-s CS + 5-s postCS) and preceding baseline period. 2-way RM-ANOVA, CS presence x CS type: $F_{1,13}$ = 3.17, P = 0.10; CS presence: $F_{1,13}$ = 24.56, P = 0.0003; CS type: $F_{1,13}$ = 0.60, P = 0.45. Both cues cause conditional entries into the food-delivery port. ***B,*** Distribution of the time of food-port entries occurring directly after a lever press. Many entries into the food-delivery port occurred immediately (within 2-s) directly following a lever press, indicating they are part of an instrumental press-check sequence (Halbout et al., 2019; Halbout et al., 2022). Therefore, in the main figures, to isolate conditional food-port checks from those that occurred as part of an instrumental press-enter sequence, we excluded food-port entries that directly followed a lever press within 2 s. ***C,*** Press-linked food-port entries (entry occurring directly after and within 2-s of a press) for the 15-s CS and preceding baseline periods. 2-way RM-ANOVA, CS presence: $F_{1,13}$ = 34.73, P < 0.0001; CS type: $F_{1,13}$ = 0.67, P = 0.43; CS presence x CS type: $F_{1,13}$ = 0.62, P = 0.44. N = 14 rats (7 male). Data presented as mean ± s.e.m. Males = solid lines, Females = dashed lines. **P < 0.01, ***P < 0.001, Bonferroni corrected.



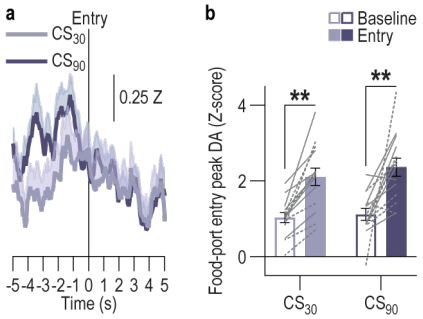
Supplemental Figure 1-3. Reward-seeking and -checking behaviors across trials during Pavlovian-to-instrumental transfer test and seeking and checking behaviors predicted by cue-evoked dopamine. *A-B,* Change (Δ) in lever presses *(A)* and food-port entries, excluding those directly linked to lever presses *(B)*, during the cue (conditioned stimulus, CS) from baseline across trials within test. 2-way RM-ANOVA, Presses, Trial: $F_{9,117}$ = 2.20, P = 0.03; CS type: $F_{1,13}$ = 4.83, P = 0.046; Trial x CS type: $F_{9,117}$ = 1.69, P = 0.10; Entries, Trial: $F_{9,117}$ = 1.76, P = 0.08; CS type: $F_{1,13}$ = 3.17, P = 0.09; Trial x CS type: $F_{9,117}$ = 0.22, P = 0.99. The high-probability cue initially suppressed instrumental reward seeking, but as the reward prediction weakened with repeated presentation of this cue without reward, came to elicit a reward-seeking response similar to the low-probability cue. ***C,*** Peak CS-evoked $GRAB_{DA}$ Z-score compared to baseline across trials within test. Mixed-effects analysis, Trial: $F_{9,117}$ = 10.22, P < 0.0001; CS type: $F_{1,13}$ = 26.25, P = 0.0002; Trial x CS type: $F_{9,101}$ = 1.37, P = 0.21. Dopamine responses were larger to the high-probability cue than the low-probability cue. Cue-evoked dopamine declined as the reward prediction weakened across test trials. ***D,*** Cue-induced change in lever presses predicted by cue-evoked dopamine. We used a linear mixed-model analysis to determine the extent to which cue-evoked instrumental reward seeking could be predicted from the cue-evoked dopamine response. To capture both between-subject variability and with-subject variability occurring across the test we used each trial for each subject. The dependent variable was change in lever presses, CS and trial were repeated factors, peak CS-evoked dopamine was the predictor, and we included subject as a random factor to control for incidental variability due to expression density and fiber placement. β = -0.21, standard error (SE) = 0.07, $t_{136.60}$ = -3.06, P = 0.003, 95% CI -0.35 - -0.08. ***E,*** Cue-induced change in food-port entries predicted by cue-evoked dopamine. We used a similar linear mixed-model analysis to determine the extent to which cue-evoked conditional reward-checking behavior could be predicted from the cue-evoked dopamine response. The dependent variable was change in food-port entries (excluding those directly linked to lever presses), CS and trial were repeated factors, peak CS-evoked dopamine was the predictor, and subject was a random factor. β= 0.15, SE = 0.08, $t_{139.73}$ = 1.89, P = 0.06, 95% CI -0.007 - 0.30. We were able to reliably, inversely predict cue-induced changes in reward-seeking behavior from the cue-evoked dopamine response. Thus, between-subjects and across trials, larger cue-evoked NAc core dopamine predicts a lower reward-seeking response. Data presented as mean ± s.e.m. *P < 0.05, **P < 0.01, Bonferroni corrected, relative to the first trial.



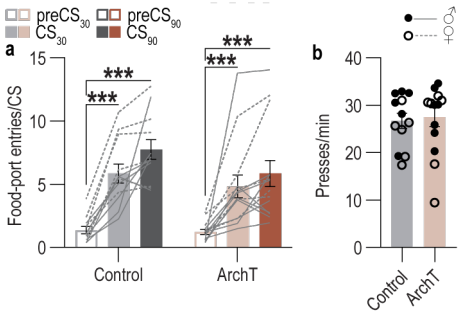
Supplemental Figure 1-4. Cue-evoked NAc dopamine without 415 nm signal subtraction. $GRAB_{DA}$ fluorescence changes (drift-corrected 470 nm without subtraction of 415 nm signal) in response to CS presentation during test. Data presented as mean ± s.e.m. As shown in the main data, the onset of both cues triggered robust, transient dopamine release that was larger for the high-probability cue than the low-probability cue.



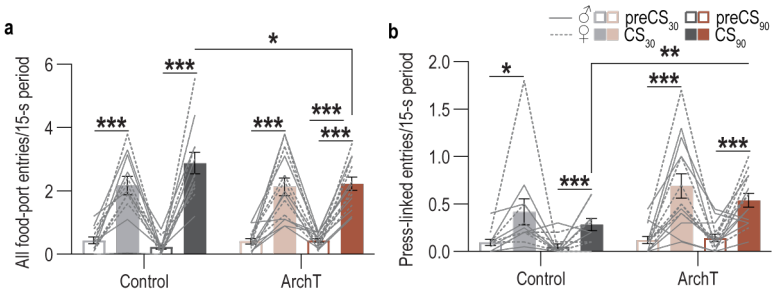
Supplemental Figure 1-5. Relationship between cue-evoked dopamine and reward-seeking and reward-checking behavior. *A,* Between-subject (averaged across trial for each subject) correlation between peak CS-evoked $GRAB_{DA}$ Z-score and change in lever presses and food-port entries, excluding those directly linked to lever presses. 2-tailed Pearson correlation, Presses, $r_{28} = -0.42$, $P = 0.03$, Entries, $r_{28} = 0.19$, $P = 0.34$. Between subjects there is a negative relationship between cue-evoked dopamine and cue-evoked instrumental reward seeking. Subjects for which the cues elicited a larger dopamine response, on average, showed a lower reward-seeking response. ***B,*** Within-session (averaged across subjects for each trial) correlation between peak CS-evoked $GRAB_{DA}$ Z-score and change in lever presses and food-port entries, excluding those directly linked to lever presses, for the 15-s CS period. 2-tailed Pearson correlation, Presses, $r_{20} = -0.61$, $P = 0.004$, Entries, $r_{20} = 0.69$, $P = 0.0009$. Across trials, cue-evoked dopamine positively relates to cue-induced reward checking and negatively relates to cue-induced reward seeking. On trials in which the cue elicited a larger dopamine response it also tended to trigger a larger reward-checking and smaller reward-seeking response. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.



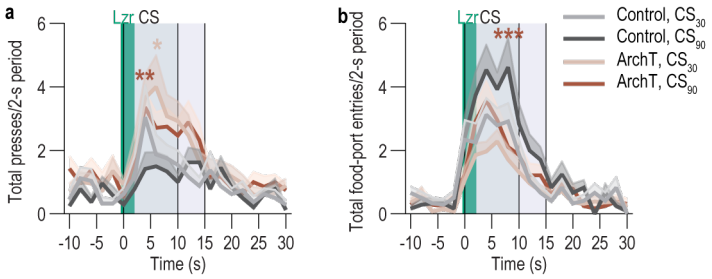
Supplemental Figure 1-6. NAc dopamine around all food-port entries. *A,* $GRAB_{DA}$ fluorescence changes (Z-score) around all food-port entries during CS at test. ***B,*** Peak $GRAB_{DA}$ Z-score within 4 s before food-port entry. 2-way RM-ANOVA, Entry v. baseline: $F_{1,13} = 48.26$, $P < 0.0001$; CS type: $F_{1,13} = 1.09$, $P = 0.31$; Entry x CS type: $F_{1,13} = 0.19$, $P = 0.67$. $N = 14$ rats (7 male). There is a significant elevation in dopamine prior to all entries into the food-delivery port (whether linked or not to presses) during both cues. Data presented as mean ± s.e.m. Males = solid lines, Females = dashed lines. **$P < 0.01$, Bonferroni corrected.



Supplemental Figure 2-1. Subjects in the VTA_{DA}→NAc inhibition experiment learned the Pavlovian reward-probability predictions and acquire instrumental behavior. *A,* Food-port entries per 10-s pre-CS baseline and CS period averaged across the last 2 days of Pavlovian training. 2-way RM-ANOVA, CS period: $F_{2,48} = 53.48$, $P < 0.0001$; Virus: $F_{1,24} = 1.48$, $P = 0.24$; Virus x CS period: $F_{2,48} = 1.28$, $P = 0.29$. Both groups of rats acquired a conditional goal-approach response and learned the differential reward-probability predictions. By the end of training, they showed stronger conditional checks of the food-delivery port to the high-probability cue than the low-probability cue. ***B,*** Lever presses averaged across the last 2 days of instrumental training. 2-tailed t-test, $t_{24} = 0.30$, $P = 0.77$, 95% CI -4.57 to 6.12. Rats in both groups similarly acquired the instrumental behavior. Control, $N = 12$ rats (6 male; 9 Th-cre+, 3 TH-cre-) ArchT, $N = 14$ Th-cre+ rats (8 male). Data presented as mean ± s.e.m. Males = close circles/solid lines, Females = open circles/dashed lines. *$P < 0.05$, ***$P < 0.001$, Bonferroni corrected.



Supplemental Figure 2-2. Effect of VTA_{DA}→NAc inhibition on all and press-linked cue-evoked entries into the food-delivery port during Pavlovian-to-instrumental transfer. *A,* All food-port entries for the 15-s CS (10-s CS + 5-s postCS) and preceding baseline period. 3-way RM-ANOVA, Virus x CS presence x CS type: $F_{1,24}$ = 2.76, P = 0.11; CS presence x CS type: $F_{1,24}$ = 3.70, P = 0.07; CS presence: $F_{1,24}$ = 166.40, P < 0.0001; Virus: $F_{1,24}$ = 0.54, P = 0.47; CS type: $F_{1,24}$ = 1.52, P = 0.23; Virus x CS presence: $F_{1,24}$ = 2.04, P = 0.17; Virus x CS type: $F_{1,24}$ = 0.54, P = 0.47. Inhibition of cue-evoked NAc dopamine release tended to reduce overall entries into the food-delivery port, similar to the effect seen only cue-evoked entries that were not directly linked to lever pressing. ***B,*** Press-linked food-port entries (entry occurring directly after and within 2-s of a press) for the 15-s CS and preceding baseline periods. 3-way RM-ANOVA, Virus x CS presence: $F_{1,24}$ = 3.80, P = 0.06; Virus: $F_{1,24}$ = 5.98, P = 0.02; CS presence: $F_{1,24}$ = 51.09, P < 0.0001; CS presence x CS type: $F_{1,24}$ = 1.61, P = 0.22; CS type: $F_{1,24}$ = 2.31, P = 0.14; Virus x CS type: $F_{1,24}$ = 0.06, P = 0.81; Virus x CS presence x CS type: $F_{1,24}$ = 0.17, P = 0.69. Inhibition of cue-evoked NAc dopamine release tended to increase entries into the food-delivery port that are directly linked to lever presses, similar to the effect seen lever pressing itself. Control, N = 12 rats (6 male; 9 Th-cre+, 3 TH-cre-) ArchT, N = 14 Th-cre+ rats (8 male). Data presented as mean ± s.e.m. Males = solid lines, Females = dashed lines. *P < 0.05, **P < 0.01, ***P < 0.001, Bonferroni corrected.



Supplemental Figure 2-3. The transient cue-evoked NAc core dopamine response influences behavior throughout the cue. *A-B,* Lever presses *(A)* and food-port entries, excluding those directly linked to lever presses, *(B)* in 2-s bins before, during, and after cue (conditioned stimulus, CS) presentation. Gray, 10-s CS; Light gray, 5-s post-CS period; Green, 2.5-s laser (lzr) period. 3-way RM-ANOVA, Presses, Virus x Time: $F_{20,480} = 1.64$, $P = 0.04$; Time x CS type: $F_{20,480} = 1.75$, $P = 0.02$; Virus: $F_{1,24} = 5.27$, $P = 0.03$; Time: $F_{20,480} = 12.09$, $P < 0.0001$; CS type: $F_{1,24} = 0.47$, $P = 0.50$; Virus x CS type: $F_{1,24} = 0.16$, $P = 0.69$; Virus x Time x CS type: $F_{20,480} = 0.79$, $P = 0.72$; Entries, Virus x Time: $F_{20,480} = 2.15$, $P = 0.003$; Time x CS type: $F_{20,480} = 3.14$, $P < 0.0001$; Virus: $F_{1,24} = 5.47$, $P = 0.03$; Time: $F_{20,480} = 35.76$, $P < 0.0001$; CS type: $F_{1,24} = 14.50$, $P = 0.0009$; Virus x CS type: $F_{1,24} = 0.28$, $P = 0.60$; Virus x Time x CS type: $F_{20,480} = 0.70$, $P = 084$. Although inhibition of VTA_{DA}→NAc projections was restricted to the first 2 s of the cue, this was sufficient to cause a sustained effect to elevate reward seeking (lever presses) and decrease reward checking (food-port entries) throughout the duration of the cue and post-cue period. Control, $N = 12$ rats (6 male; 9 Th-cre+, 3 TH-cre-) ArchT, $N = 14$ Th-cre+ rats (8 male). Data presented as mean ± s.e.m. Males = solid lines, Females = dashed lines. *$P < 0.05$, **$P < 0.01$ ArchT relative to control, Bonferroni corrected.