Behavioral/Cognitive

Dopamine Invigorates Reward Seeking by Promoting Cue-Evoked Excitation in the Nucleus Accumbens

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Approach to reward is a fundamental adaptive behavior, disruption of which is a core symptom of addiction and depression. Nucleus accumbens (NAc) dopamine is required for reward-predictive cues to activate vigorous reward seeking, but the underlying neural mechanism is unknown. Reward-predictive cues elicit both dopamine release in the NAc and excitations and inhibitions in NAc neurons. However, a direct link has not been established between dopamine receptor activation, NAc cue-evoked neuronal activity, and reward-seeking behavior. Here, we use a novel microelectrode array that enables simultaneous recording of neuronal firing and local dopamine receptor antagonist injection. We demonstrate that, in the NAc of rats performing a discriminative stimulus task for sucrose reward, blockade of either D1 or D2 receptors selectively attenuates excitation, but not inhibition, evoked by reward-predictive cues. Furthermore, we establish that this dopamine-dependent signal is necessary for reward-seeking behavior. These results demonstrate a neural mechanism by which NAc dopamine invigorates environmentally cued reward-seeking behavior.

Key words: **cue-excited neurons; discriminative stimulus; dopamine; nucleus accumbens; reward seeking**

Introduction

The dopamine projection from the ventral tegmental area (VTA) to the NAc is an essential component of the neural circuit that promotes reward-seeking behavior (Nicola, 2007). If NAc dopamine function is reduced experimentally, animals are less likely to exert effort to obtain reward (Salamone and Correa, 2012) and often fail to respond to reward-predictive cues (Di Ciano et al., 2001; Yun et al., 2004; Nicola, 2007, 2010; Saunders and Robinson, 2012). These deficits are due to impairment of a specific component of reward seeking: the latency to initiate approach behavior is increased, whereas the speed of approach, the ability to find the goal and perform the necessary operant behavior required to earn reward, and the ability to consume reward are unaffected (Nicola, 2010). Dopamine must promote approach by influencing the activity of NAc neurons, but the nature of this influence remains unclear. Large proportions of NAc neurons are excited or inhibited by reward-predictive cues (Nicola et al., 2004a; Roitman et al., 2005; Ambroggi et al., 2008, 2011; McGinty et al., 2013), and the excitations begin before onset of cued approach behavior and predict the latency to initiate locomotion (McGinty et al., 2013). Therefore, this activity has the characteristics required of a dopamine-dependent signal that promotes cued approach, but whether it does so is unknown.

Neurons in two structures that send glutamatergic afferents to the NAc, the BLA and dorsal medial PFC (Brog et al., 1993), are excited by reward-predictive cues (Schoenbaum et al., 1998; Ambroggi et al., 2008), and reversible inactivation of either of these structures (Ambroggi et al., 2008; Ishikawa et al., 2008) or of the VTA (Yun et al., 2004) reduces the magnitude of cue-evoked excitations in the NAc. These observations suggest that NAc cue-evoked excitations are driven by glutamatergic inputs, but without NAc dopamine, even these strong excitatory inputs are insufficient to drive cue-evoked firing increases. However, this conclusion is tenuous. Many NAc neurons are inhibited by cues (Nicola et al., 2004a; Ambroggi et al., 2011) and it is unknown whether excitations or inhibitions are more important for activating approach behavior. Additionally, VTA inactivation could reduce discriminative stimulus (DS)-evoked excitations by several dopamine-independent mechanisms: reduced cue encoding in the BLA and PFC, which receive projections from the VTA (Swanson, 1982); reduced firing of GABAergic VTA neurons that project to the NAc (Van Bockstaele and Pickel, 1995); or reduced release of glutamate from dopaminergic neurons (Stuber et al., 2010). Finally, because VTA inactivation reduces not only NAc DS-evoked firing, but also DS-evoked approach behavior (Yun et al., 2004), DS excitation could be secondary to rather than a necessary condition for goal-directed movement.

To directly test the role of NAc dopamine in cue-evoked firing, we devised a novel probe for use in behaving rodents: a circular electrode array surrounding a central injection cannula, which allows for simultaneous recording of unit firing activity

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and infusion of dopamine receptor antagonists into the extracellular space surrounding the recorded neurons (du Hoffmann et al., 2011). This arrangement allows us to establish links between dopamine receptor activation, NAc neuronal firing, and reward-seeking behavior: if blockade of NAc dopamine receptors inhibits both cue-evoked signals and initiation of approach, this would provide strong evidence that the neuronal response depends on endogenous dopamine and that this signal is required for approach behavior.

Materials and Methods

Animals. Fifteen male Long–Evan rats (275–300 g on arrival) were obtained from Charles River and singly housed. One week after their arrival, rats were handled for several minutes daily for 3 d to habituate them to the experimenter. After habituation, the rats were placed on a restricted diet of 13 g of rat chow per day. *Ad libitum* food was provided for 7 d following surgery, after which animals were placed back on the restricted diet. Animal procedures were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Albert Einstein College of Medicine.

Operant chambers. All behavioral experiments and behavioral training took place in custom-made Plexiglas chambers (40 cm square, 60 cm high). These were located inside metal cabinets that served as Faraday cages; cabinets were lined with acoustic foam and white noise was played continuously through a dedicated speaker to minimize audibility of external noise inside the chamber. Operant chambers were equipped with a reward receptacle on one wall with retractable levers on either side of it. A photobeam across the front of the receptacle was used to measure receptacle entry and exit times. The temporal resolution of the behavioral control system (Med Associates) was 1 ms.

DS task. Animals were trained on the DS task following procedures similar to those used previously (Nicola et al., 2004a,b; Ambroggi et al., 2008, 2011; Nicola, 2010; McGinty et al., 2013). Two cues were presented one at a time, either a reward-predictive DS or a neutral stimulus (NS). The auditory cues consisted of a siren tone (which cycled in frequency from 4 to 8 kHz over 400 ms) and an intermittent tone (6 kHz tone on for 40 ms, off for 50 ms); assignment of a particular tone to the DS or NS was randomized across rats. Intertrial intervals (ITIs) were selected at random from a truncated exponential distribution with a mean of 30 s and maximum of 150 s. The NS was always presented for 10 s; lever presses during the NS were recorded but had no programmed consequence. "Active" and "inactive" levers were randomly assigned to left and right levers for each rat at the beginning of training and did not vary subsequently. A lever response on the active lever during the DS terminated the cue, and the first subsequent receptacle entry caused delivery of 10% sucrose reward into a well located in the receptacle. DS presentations during which the animal did not respond were terminated after 10 s. Responses during the ITI (between cue presentations) and responses on the inactive lever were recorded but did not result in reward delivery. Animals were trained on the DS task until they responded to >80% of DSs and <20% NSs in 2 h training sessions.

Cannulated microelectrode arrays. After initial training, rats were implanted with cannulated microarrays consisting of eight tungsten microwire electrodes surrounding a central microinjection guide cannula. These were constructed and mounted in custom-made microdrives as previously described (du Hoffmann et al., 2011). A complete clockwise turn of the drive screw moved the electrodes and cannula as a unit ventrally 300 μm (without rotation of the probes), enabling us to record from several unique populations of neurons in the same animal.

To implant the cannulated arrays, rats were prepared for surgery and placed in a stereotaxic instrument as described previously (du Hoffmann et al., 2011; McGinty et al., 2013). Anesthesia was induced and maintained with isoflurane (0.5–3%). Animals received antibiotic (Baytril) immediately before surgery and 24 h post surgery. Cannulated arrays were implanted bilaterally into the dorsal NAc core (1.4 mm anterior and 1.5 mm lateral from bregma, and 6.5 mm ventral from the skull). Electrodes and microdrives were secured to the skull with bone screws and dental acrylic, and wire obturators were inserted into the guide cannulae so that the ends of the obturators were flush with the ends of the guide cannulae. After surgery, the scalp was treated with Neo-Predef to prevent infection and the animals were allowed 1 week of recovery before proceeding with experiments. For postsurgery analgesia, animals were given 10 mg/kg of the nonsteroidal anti-inflammatory drug ketoprofen.

Drugs. SCH23390 and raclopride were purchased from Sigma. On test days, drugs were freshly prepared by dissolving them in 0.9% sterile saline. Drugs were administered at doses of 1.1 μg SCH233390 in 0.55 μl saline per side and 6.4 μg raclopride in 0.8 μl saline per side. SCH233390 and raclopride were infused over 12 and 17.5 min, respectively. In pilot experiments, we found that bilateral infusions of raclopride lasting 12 min had significant but transient effects on DS response ratio. Thus, to prolong the effect we increased the duration of raclopride infusion such that the temporal profile of its pharmacological effects was similar to that of SCH23390. Only one bilateral or unilateral injection was made per recording session (one session per day). All animals received at least a single bilateral injection of one antagonist, and one (or several) unilateral antagonist injections. During some unilateral antagonist experiments, we concurrently infused saline as a vehicle control contralateral to the hemisphere that received antagonist.

Microinjection and recording procedure. The apparatus for simultaneous microinjection and recording has been described previously (du Hoffmann et al., 2011). The recording cable leading from the head stage terminated in a 24-channel electrical commutator with a central bore hole (Moog), which passed the signals to the electrophysiological recording system. Two syringes were mounted in a single syringe pump located outside the chamber; fluid lines from the syringes led to a dual-channel fluid swivel (Instech Laboratories) mounted above the commutator. Fluid lines descended from the swivel through the commutator's bore hole, ran along the recording cable, and terminated at two 33 gauge microinjectors.

Before the recording session, the microinjectors were backfilled with drug solution and then inserted into the animal's guide cannulae. The microinjector tips extended 0.5 mm beyond the guide cannulae so that the tip of the microinjector was below the electrode tips and ~670 μm from the center of each electrode. Before backfilling with drug, the fluid lines and microinjectors were filled with mineral oil, and the level of the oil-aqueous interface was marked to facilitate *post hoc* confirmation that the drug was injected. Finally, the head stage was connected to the animal and the fluid lines were firmly secured to the recording cable to keep the microinjectors in place for the duration of the experiment. Animals prepared in this way were allowed to perform the DS task for a baseline period of at least 45 min, during which neural activity was recorded; then, the syringe pump was turned on remotely to infuse the drugs into the brain. Injection did not require handling the animal or opening the chamber door, and the behavioral session continued uninterrupted throughout baseline, infusion, and postinfusion periods.

Neural voltage signals were recorded with a head-stage amplifier (unity gain), amplified 10,000 times, and digitized using commercial hardware and software (Plexon). We recorded from 379 neurons in 38 recording/injection sessions in 15 rats. Of the 38 sessions, 7 were discarded due to poor behavior during the preinjection baseline period or because no neurons could be reliably isolated. Thus, our neural analysis focused on 31 recording/injection sessions in which we recorded from 322 well isolated neurons in 12 rats. After each recording/injection session, the microdrive carrying the electrode arrays was advanced ~150 μm (one half turn of the microdrive screw) to move the electrodes ventrally to record from a new population of neurons. If few (or no) neurons were observed, the array was advanced every other day until neurons were detected.

Analysis. Data were divided into preinjection, postinjection, and recovery time periods, which were defined, respectively, as the 45 min before infusion of the antagonists, the 40 min beginning with the end of the injection, and the last 33 min (2000 s) of each session (which lasted, in total, 2–3 h). The postinjection period corresponds to the time during which the drugs have their greatest behavioral effects when injected bilaterally (Fig. 1*C*).

Isolation of single units was performed off-line with Offline Sorter (Plexon) using principal component analysis. Only units with well de-

fined waveforms (>100 μV) that were clearly distinct from noise levels (<20–50 μV) were included in subsequent analyses. Interspike interval distributions and cross-correlograms were used to ensure that single units were well isolated from one another and from background noise (Neural Explorer software; Nex-Tech). Time stamps of verified spikes were analyzed with custom routines in the R software environment. Peristimulus time histograms constructed around the DS and NS, in 50 ms time bins, were used to quantify and detect cue-evoked excitations in Figures 2*A*, 3, 4, 5*A*, 6*A*, 7*A*, 8*A*, and 10*A–C*. To determine whether a neuron exhibited a significant DS-evoked excitation, the Poisson probability distribution function was calculated for the 10 s baseline period before each cue. A neuron was considered DS excited if it exhibited average spike counts above the upper 99% confidence interval of the distribution of baseline firing rates in one or more 50 ms bins between 50 and 200 ms after cue onset. For neurons with significant DS-evoked excitations in the preinjection baseline period, the average firing rate in 50 ms bins time locked to DS and NS onset was obtained for each period in each session, and the average and median (Figs. 2*C–E*, 5*A*, 6*A*, 7*A*, 8*A*, 10*B*,*C*) firing rates across neurons were compared. Because neurons with statistically detectable NS excitation were almost invariably also excited by the DS [not shown, but reported previously (Ambroggi et al., 2011)], we analyzed NS responses for all neurons with a significant DS response. Unless otherwise indicated, all statistical comparisons used within-neuron Wilcoxon rank sum tests.

For Figure 4, we determined whether the effects of bilateral antagonist injection on the latency to reach the lever were correlated with the effects of the antagonists on the magnitude of DS-evoked excitation on a trial-by-trial basis. First, we calculated the average firing rate from 100 to 400 ms after DS onset in every trial for all recorded neurons that exhibited significant DS excitation before bilateral infusion of the antagonists. Next, for each neuron we calculated the Spearman's rank correlation coefficient comparing the trial-by-trial magnitude of DS-evoked excitation and the latency of the rat to reach the lever on corresponding trials. These correlations were plotted in histograms in Figure 4*B*,*D*. All DS trials were included in this analysis; if the animal did not press the lever a latency of 10 s (the maximum length of cue presentation) was assigned to that trial. We computed these correlation coefficients for the preinjection period as defined above; we extended the postinjection period by 1000 s to obtain a broader sampling of latencies on trials in which the animals responded after bilateral infusion. To assess significance of the individual correlations, we used a two-tailed asymptotic *t*-approximation because an exact *p* value cannot be computed when ties are present in the rank data. Then we used paired Wilcoxon tests to compare the medians of the distributions of correlation coefficients before and after antagonist infusion.

Because NAc neurons have low baseline firing rates with lower bounds of the confidence interval very often spanning zero, inhibitions are far more difficult to detect and quantify than excitations. Thus, in addition to the procedure described above, which was used to detect excitation, we also used receiver operating characteristic (ROC) analysis, a more sensitive method, to quantify the likelihood that the firing rate in successive 50 ms time bins after cue onset was different from the firing rate in the 10 s precue baseline. This analysis was performed separately for preinjection and postinjection periods. For each bin, we computed the area under the ROC curve (AUC); AUC values of 0.5 indicate no difference from precue firing, whereas values closer to 0 or 1 indicate greater likelihood that the neuron is inhibited or excited, respectively. To portray in an unbiased fashion the postcue neural activity across the entire population of recorded neurons, firing rates and AUC values were calculated for 50 ms bins; to smooth the data, the bins were advanced by 10 ms for successive AUC computations. The smoothed AUC values were then plotted as heat maps with 10 ms resolution (with each value representing the AUC in the next 50 ms) in Figures 5*B*, 6*B*, 7*B*, 8*B*, and 10*D*,*E*.

Next, we quantified whether AUC values, calculated in nonoverlapping 50 ms bins, reflected a significant difference in firing. For each bin, we first generated 10,000 bootstrapped AUC values from random shuffles of the precue baseline firing rate and firing rate in the corresponding postcue bin. We then determined the two-tailed probability that the actual AUC value was drawn from the distribution of bootstrapped values; if the probability was <0.05, we considered the firing in the bin to be significantly different from precue baseline. Finally, we counted the number of neurons with firing rates in each bin that was significantly greater than or less than the precue baseline firing, and plotted these values as fractions of the total population (Figs. 5*C*, 6*C*, 7*C*, 8*C*, 9*B*,*D*, 10*F*,*G*).

To compare the proportions of neurons excited or inhibited in the preinjection and postinjection periods we used a data reduction approach. First, we calculated the fraction of 50 ms bins between 0 and 1 s after cue onset in which each neuron exhibited significant excitation or inhibition. Next, we compared these fractions in the preinjection and postinjection periods with a paired Wilcoxon test. Neurons that did not exhibit significant modulation in any bin in both preinjection and postinjection periods were excluded from this analysis and were not included in the plots showing the median fraction of significant bins (dot and whisker plots on the right side of each part in Figs. 5*C*, 6*C*, 7*C*, 8*C*, 10*F*,*G*). This procedure eliminated the influence of the large population of neurons with no difference in activity between the post-DS window and pre-DS baseline; this population is of little interest, yet it contributes a large number of null values that bias the median number of significant bins toward 0 and obscure both decreases and increases in the fraction of significant bins after infusion.

Similar analyses were performed for consumption-related firing occurring after entry into the reward receptacle. Animals tended to remain in the receptacle for >5 s; therefore, to capture these relatively long time intervals, we show the results using 200 ms bins (Fig. 9). The time window for comparing proportions of neurons that were excited in the preinjection and postinjection periods was from 0 to 1.5 s, whereas it was from 0 to 5 s for inhibitions; a shorter analysis window was used for excitations because they tended to be more transient. ROC analyses were performed on the Albert Einstein College of Medicine High Performance Computing Cluster using the pROC package for R.

To compare "baseline" firing rates occurring outside of task events, we compared the average firing rate in 10 s bins before each DS preinjection and postinjection of the antagonists. This procedure is functionally equivalent to random sampling of baseline firing rates because DSs are presented with nearly an equal probability at any time during a behavioral session. Neurons were classified as exhibiting significant DS-evoked excitation (before drug infusion) or not, and then baseline firing rates in the preinjection and postinjection periods were compared within these groups with a paired Wilcoxon test (Fig. 10*H*,*I*). We also performed a linear fit for DS-excited neurons and compared the slope of this line to the unity line (slope of 1).

If multiple comparisons were performed on subsets of data that came from the same subject (Figs. 2*C–E*, 5*A*,*C*, 6*A*,*C*, 7*A*,*C*, 8*A*,*C*, 9*B*,*D*, 10*B*,*C*,*F*,*G*), *p* values were Bonferroni corrected; i.e., the *p* value was multiplied by the number of comparisons being made. Corrected *p* values were considered significant if $p < 0.05$. All corrections were made with a factor of 3 except for Figure 2*C–E*, in which the factor was 2.

Video tracking. In a subset of experiments, the rat's position was measured using an overhead camera (30 frames/s) and computerized tracking system (Cineplex; Plexon). The system tracked the *x* and *y* positions of two differently colored LEDs attached to the recording head stage. As previously described (McGinty et al., 2013), we calculated a centroid that describes the center point between LED positions for each video frame. Missing data points up to 10 successive frames were filled in with linear interpolation; in the rare instances in which >10 frames were missing, the data were discarded. For each video frame, we calculated the SD of distances between the centroid's position in that frame and in a time window ±200 ms. These SD measurements constitute the locomotor index (LI) for that frame of the video. Log-transformed LIs were bimodally distributed, with a lower peak representing epochs of little or no movement and an upper peak representing locomotion (Drai et al., 2000). We then fit two Gaussian functions to the distribution of LIs, and determined the movement threshold as the point where these functions overlapped the least.

Movements were defined as at least eight consecutive frames with LIs above the locomotor threshold. To determine the time of movement onset, we restricted the analysis to DS trials in which the animal was still

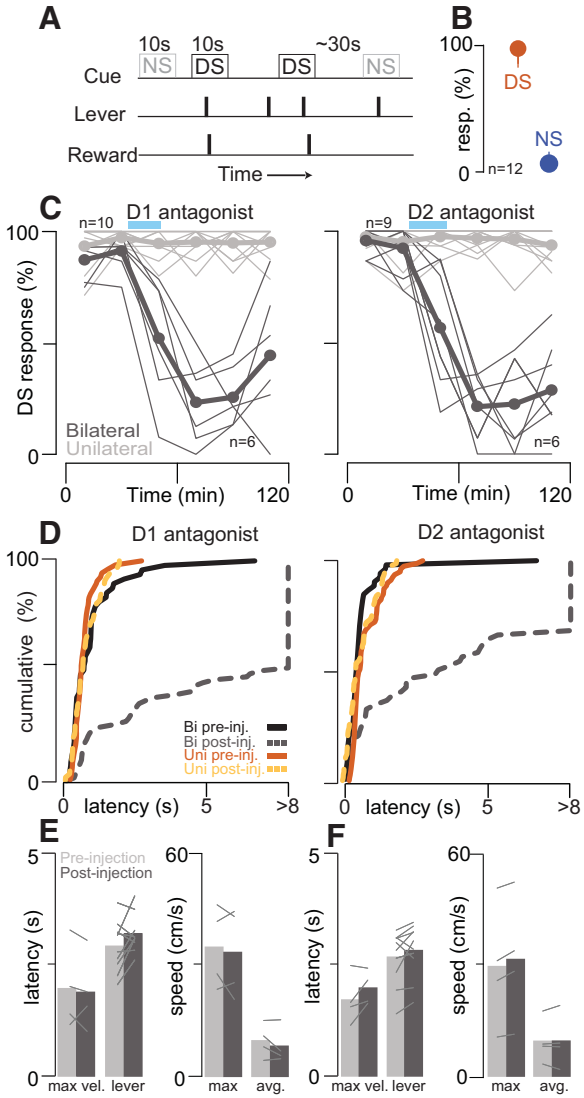

Figure 1. Effects of dopamine receptor antagonists on DS-cued approach behavior. ***A***, Schematic of the DS task. ***B***, Median (dot) and middle quartiles (vertical lines) of DS (orange) and NS (blue) response ratios in the preinjection period for all behavioral sessions that contributed to neural analyses ($p < 0.001$, Wilcoxon). ***C***, Cross-session average DS response ratios before, during, and after unilateral (thick, light gray line) and bilateral infusions (thick, dark gray line) of the D1 antagonist (SCH23390, left) and the D2 antagonist (raclopride, right). Overlaid thin lines show individual session response ratios. Blue lines indicate drug infusion. ***D***, Cumulative movement-onset latencies during DS trials in which rats were not moving at cue onset, before (solid lines) and after (dashed lines) D1 antagonist (left graph) and D2 antagonist (right graph) injection. Bilateral injections increased the latencies (solid black and dashed gray lines, corrected $p < 0.001$, Wilcoxon) whereas unilateral infusions had no effect (solid orange and dashed light orange lines, $p > 0.1$). $N = 51–87$ latencies per trace, which came from three (bilateral D2 antagonist) or four (all other injections) sessions. ***E***, ***F***, Left graphs show latency to reach maximum speed after DS onset (max vel., for experimental sessions with video tracking) and latency to reach the lever (lever, for all sessions) before (light gray) and after (dark gray) unilateral D1 antagonist (***E***) or D2 antagonist (***F***) injection. Right graphs show maximum (max) and mean speed (avg.) attained during DS movement after DS onset. Individual lines superimposed on all bars are the single session data (pre and post drug infusion) that compose the averages. There was no significant effect of either the D1 or D2 antagonist on any of these movement-related variables [$p > 0.1$, within-session paired Wilcoxon, $n = 4$ sessions for all comparisons except bars labeled lever where $n = 10$ (***E***) and $n = 9$ (***F***)].

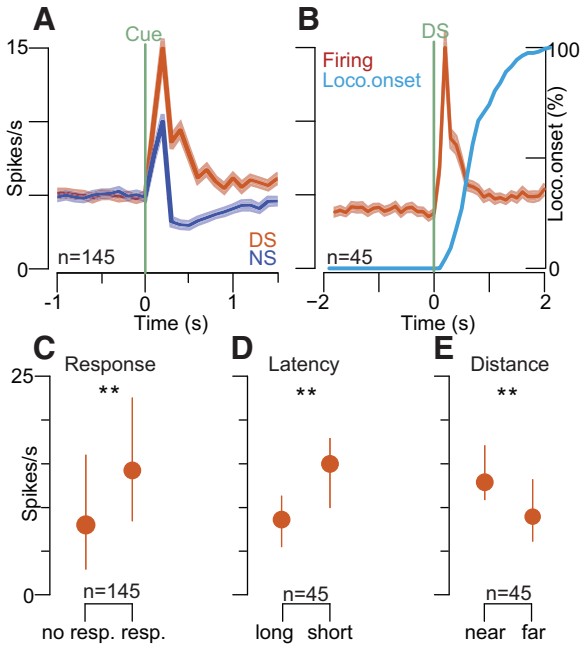

Figure 2. DS-evoked excitations predict subsequent reward-seeking behavior and encode proximity to the lever. ***A***, Average preinjection peri-event time histograms aligned to the onset of the DS (orange trace) or NS (blue trace) for 145 neurons with significant excitatory responses to DS presentation. Bin width = 50 ms. The light clouds around traces in this and subsequent figures indicate ±SEM. DSs elicit greater excitations than NSs (for firing 100–250 ms after cue onset: $p < 0.001$, Wilcoxon). ***B***, Peri-event time histogram (orange line) shows the average firing rate, aligned to DS onset, of the 45 neurons that exhibited significant excitatory responses to DS presentation during the subset of experiments for which video tracking data were available. The blue line represents the cumulative distribution of latencies to movement onset after DS presentation for trials in which animals were still at cue onset. DS-evoked excitations typically preceded the initiation of cued approach behavior. ***C***, DS-evoked excitation was greater on trials in which the animal responded to the DS with a lever press (resp.) than when they fail to make such a response (no resp.); $^{**}p < 0.01$, Wilcoxon. ***D***, DS-evoked excitation was greater on trials in which the latency to reach maximum velocity after DS onset was short than when it was long. Latencies were measured in all DS trials in which the animal made a lever response. Latencies in each session were divided into quartiles, and the firing was compared in trials from the shortest and longest latency quartiles; $^{**}p < 0.01$, Wilcoxon. ***E***, DS-evoked excitation was greater when rats were near the reward-associated lever compared with when they were far. The distribution of distances from the lever at cue onset was bimodal with a distant peak typically >12.5 cm and a proximate peak <12.5 cm (i.e., rats tended to be either near the lever or across the chamber from the lever). Therefore, "near" and "far" trials were those in which the distance from the lever at cue onset was <12.5 cm and >12.5 cm, respectively; $^{**}p < 0.01$, Wilcoxon.

at cue onset and then calculated the latency between cue onset and the first frame in which the LI exceeded the movement threshold (Figs. 1*D–F*, 2*B*, *D*). If no discernible movement was measured on a trial, the latency on that trial was defined as >10 s (the length of cue presentation, Fig. 1*D*). Similar results were obtained when such trials were omitted from the analysis (data not shown). The DS-cued movement latency distributions were then pooled across rats and the medians were compared with a Wilcoxon test. To quantify latency to maximum speed and mean speed of DS-cued lever-directed movements, we used all trials that ended with a lever press even if the rat was moving at DS onset (Fig. 1*E*, *F*).

Histology. Animals were deeply anesthetized with Euthasol and perfused intracardially with saline and 4% formalin. Direct current (15 μA) was passed through each of the electrodes in the arrays for ~30 s to generate lesions. Brains were removed and stored in formalin until they were processed. Before slicing with a cryostat, brains were cryoprotected by immersion in 30% sucrose for several days. Sections (50 μm) were stained for Nissl substance to visualize cannula and electrode tracks and lesions (Fig. 11).

Results

We presented rats with two auditory stimuli at variable intervals averaging 30 s: a reward-predictive DS and an NS (Fig. 1*A*; Nicola

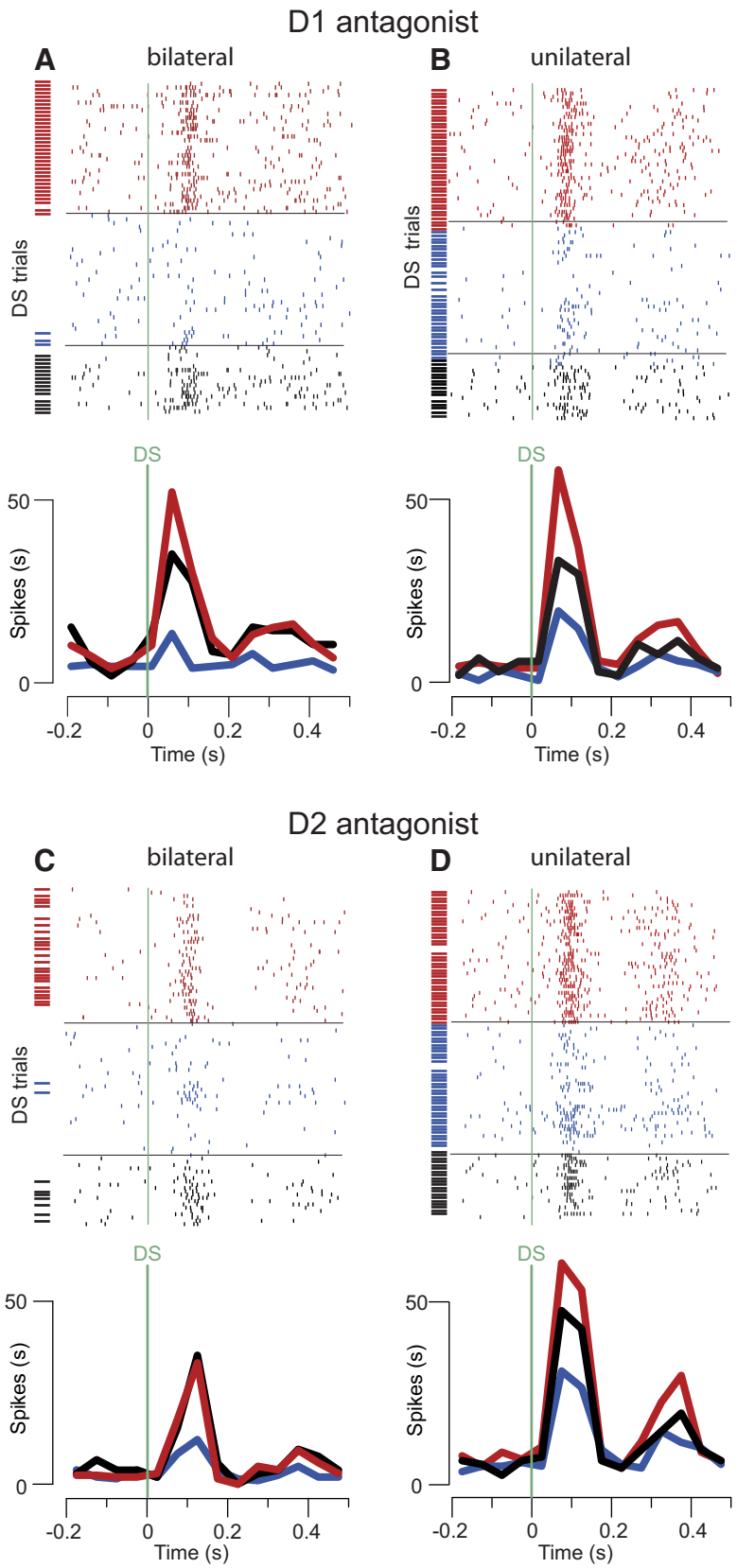

Figure 3. Example neurons show that D1 and D2 antagonists reduce DS-evoked excitation. Rasters and corresponding histograms show the firing of four different DS-excited neurons aligned to DS onset. Data are from the last 40 trials immediately preceding the start of the injection (red), the first 40 trials immediately after the end of the injection (blue), and the last 20 trials of the behavioral session (black). These intervals roughly correspond to the pre and post injection and recovery periods used in Figures 5 and 6. Horizontal lines to the left of the rasters indicate whether a lever-press response occurred on that trial. Neurons shown in ***A*** and ***B*** were recorded during bilateral and unilateral SCH23390 infusion, respectively. Neurons in ***C*** and ***D*** were recorded during bilateral and unilateral raclopride infusions, respectively.

et al., 2004a,b; Ambroggi et al., 2008, 2011; McGinty et al., 2013). A lever press during the DS terminated the cue, and a droplet of sucrose was delivered upon entry into the reward receptacle; if animals did not respond within 10 s, the cue was terminated without reward delivery and the intertrial interval commenced. Responses during this interval and during the NS had no programmed consequence. NSs were always 10 s. Trained animals, which responded to most DSs but few NSs (Fig. 1*B*), were implanted with cannulated arrays targeted to the NAc core. During experiments, animals first performed the task for a 45 min preinjection period during which NAc neural activity was recorded. Next, the D1 receptor antagonist SCH23390 or the D2/3 antagonist raclopride was infused bilaterally or unilaterally into the NAc; animals remained in the chamber with task contingencies in effect throughout the infusion and for at least 75 min afterward.

Consistent with previous studies (Yun et al., 2004; Nicola, 2010), bilateral infusions of either antagonist into the NAc core significantly reduced the proportion of DSs to which the animal responded (Fig. 1*C*, dark gray traces) and increased the latency to initiate locomotion as measured by video tracking in a subset of sessions (Fig. 1*D*, gray dashed traces). In contrast, unilateral infusions of the same doses had no effect on DS response ratio (Fig. 1*C*, light gray traces), latency to initiate movement after DS onset (Fig. 1*D*, dashed light orange traces), and latency to reach the lever or movement speed during lever approaches (Fig. 1*E*,*F*). These behavioral data demonstrate that NAc dopamine in a single hemisphere is sufficient to maintain behavior even though blockade of D1 or D2/3 receptors in both hemispheres severely impairs responding. This dissociation offers a critical experimental advantage, as it allows us to test the effects of dopamine antagonists on neural activity when behavior is impaired (bilateral injection) and when it is not (unilateral injection), thereby ruling out the potential confound that any observed changes in neural activity after antagonist infusion are secondary to changes in behavior.

We recorded from 322 NAc neurons in 31 recording/injection sessions in 12 rats. Approximately 45% of the recorded neurons were significantly excited by DS presentation. These excitations exhibited properties similar to those reported previously (Yun et al., 2004; Nicola et al., 2004a; Ambroggi et al., 2011; McGinty et al., 2013; Morrison and Nicola, 2014):

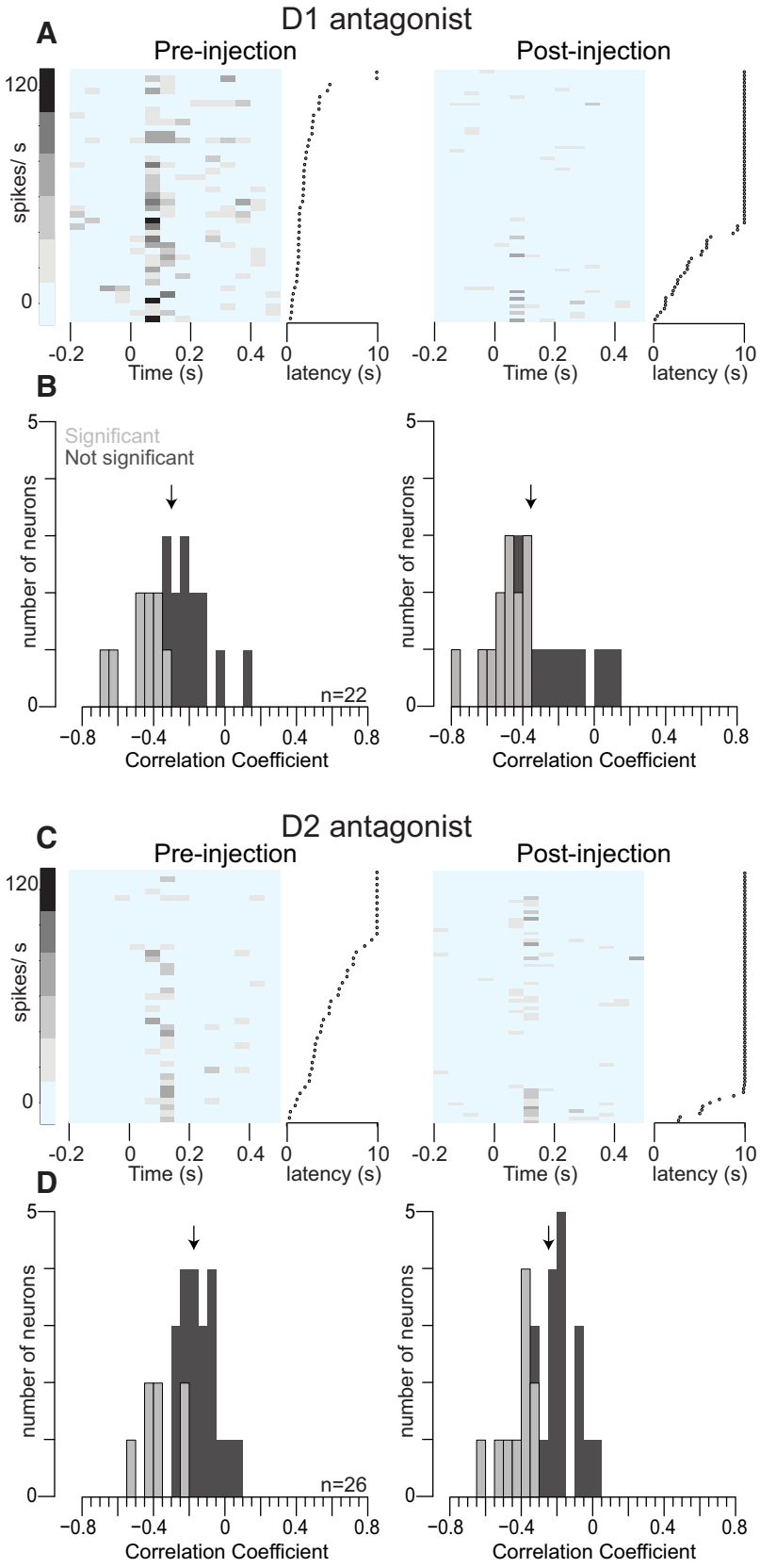

Figure 4. The effects of bilateral dopamine antagonist injection on cue-evoked excitation predict the behavioral effects on a trial-by-trial basis. ***A***, ***C***, Trial-by-trial analysis of neuronal encoding of the rat's latency to reach the lever for the same neurons shown in Figure 3*A* and *C*. Data from the pre and post injection periods are shown on the left and right, respectively; ***A*** shows the effects of SCH23390 and ***C*** shows the effects of raclopride (both injected bilaterally). Within the blue-shaded graphs, each row indicates, in grayscale, the neural firing in 50 ms time bins aligned to DS onset on individual trials. Trials are sorted by latency of the rat to reach the lever, which is indicated by the dots in the graph to the right of each firing plot; latencies equal to 10 s indicate that the rat did not respond to the DS. Overall, the antagonists caused larger increases in latency on those trials in which they caused greater reduction of DS-evoked firing. ***B***, ***D***, Cross-neuron distribution of Spearman rank correlation coefficients relating firing (100–400 ms after DS onset) on each trial to the animal's latency to reach the lever. Trials without lever presses were assigned a latency of 10 s (see Experimental Procedures). Only bilateral injection experiments were used for this analysis, and only neurons exhibiting significant DS-evoked excitation in the preinjection period were included. Graphs on the left and right show the coefficients in the preinjection period and after bilateral antagonist injection, respectively. ***B*** shows results for SCH23390 injection; ***D*** shows results for raclopride injection. Light bars represent neurons with significant correlations ($p < 0.05$); dark bars represent those with nonsignificant correlations. Arrows show the median coefficient, which was significantly <0 in each case (Wilcoxon, $p < 0.05$). In ***D*** the median correlation coefficient was significantly more negative after D2 antagonist injection than preinjection (Wilcoxon, $p < 0.05$).

they were larger than those evoked by NSs (Fig. 2*A*); they began at short latency after cue onset (~120 ms) and occurred before initiation of lever-directed movement (Fig. 2*B*); and their magnitude was correlated with the probability of a behavioral response, movement initiation latency, and proximity to the lever (McGinty et al., 2013; Fig. 2*C–E*).

Bilateral infusion of either the D1or D2/D3 antagonist caused a sharp reduction in the magnitude of DS-evoked excitation. As shown in two example neurons (Fig. 3*A*, *C*), this effect was most pronounced in the minutes immediately after the infusion, corresponding to the maximal reduction in cue-evoked approach behavior caused by the injections (Fig. 3*A*, *C*, blue rasters and histograms). When the behavioral effect recovered, the firing response recovered as well (Fig. 3*A*, *C*, black rasters and histograms). This pattern of results was consistent across cue-excited neurons (Figs. 5*A*, 6*A*, Bilateral histograms and whisker plots). Supporting the hypothesis that these excitations set the vigor of lever approach movement, the magnitude of the cue-evoked excitation during the preinjection period predicted the animal's latency to reach the lever (Fig. 4*A*, *C*, left). After bilateral D1 or D2 antagonist injection, these latencies were markedly shifted to higher values, often so high that there was no response at all within the 10 s cue presentation (Fig. 4*A*, *C*, left and right latency distributions). Strikingly, even though cue-evoked firing was reduced by the antagonists, it continued to predict the vigor of the behavioral response during the postinjection and re-

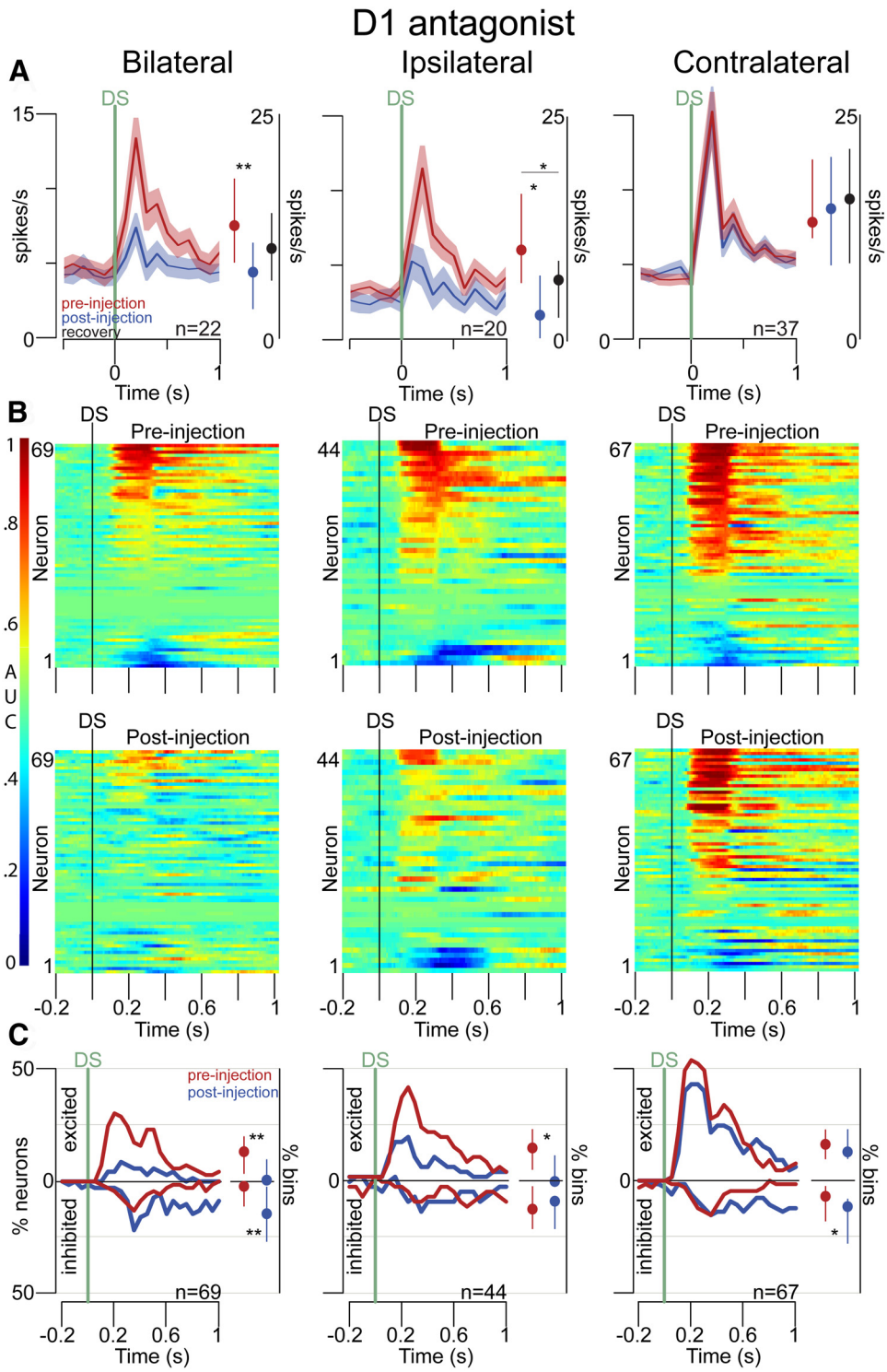

Figure 5. D1 receptor activation is required for DS-evoked excitation. ***A***, Peri-event time histograms aligned to DS onset for neurons with significant DS-evoked excitation in the preinjection period. Traces and clouds indicate the average ±SEM firing rate before (red) and after (blue) infusion of SCH23390 bilaterally (left graph), ipsilateral to the recorded neurons (middle graph), or contralateral to them (right graph). Right side of each graph shows the cross-neuron median (dot) and middle quartiles (vertical lines) of firing between 100 and 250 ms after cue onset for neurons with significant excitation; red, blue, and black dots represent pre and post injection and recovery data, respectively. DS-evoked excitations were reduced by bilateral and ipsilateral injections, but not by contralateral injections. $^{**}p < 0.01$; $^{*}p < 0.05$, Wilcoxon. ***B***, ROC analysis reveals that only DS-evoked excitations, but not inhibitions, are reduced by SCH23390 injection. For each neuron, a ROC curve was generated for individual 10 ms bins aligned to DS onset. The ROC curve compared the firing rate in the bin with that in the 10 s pre-DS baseline. Every recorded neuron was used; data are divided into that obtained in bilateral injection sessions (left graphs), in the hemisphere ipsilateral to the injection in unilateral injection sessions (middle graphs), and in the hemisphere contralateral to injection (right graphs). Within the graphs, each row shows the AUC for an individual neuron's DS-aligned firing; the AUC values are represented by color and smoothed by averaging across a sliding 50 ms window (see Materials and Methods). The neurons are sorted by the magnitude of DS-evoked excitation 200 ms after cue onset in the preinjection period, and the same neuron is shown in a given row in the preinjection (top row) and postinjection (bottom row) graphs. AUC values of 0.5 indicate that firing is not different from baseline, whereas values closer to 1 indicate excitation (warmer colors) and values closer to 0 indicate inhibition (cooler colors). The ROC plots reveal that the reduction in DS-evoked excitation after bilateral and ipsilateral D1 antagonist injection is consistent across neurons, that there were some emergent inhibitions occurring at long post-DS latency after bilateral (but not ipsilateral or contralateral) (*Figure legend continues.*)

covery periods (Fig. 4*A*,*C*, right raster plots). This observation indicates that the behavioral and neural effects of the drug were correlated on a trial-by-trial basis: the greater the reduction in firing caused by a dopamine antagonist, the greater the latency to reach the lever and the lower the probability that the animal reached the lever at all.

To assess the consistency of this trial-by-trial correlation, we computed, for each cue-excited neuron, the Spearman rank correlation between the magnitude of the excitation and the latency to press the lever. We assigned a latency of 10 s to trials in which there was no response; latency in these trials was therefore tied at the highest rank. (Similar results were obtained if trials without a DS-cued lever response were omitted from the analysis; data not shown.) When we compared the correlation coefficients in the preinjection period with those in the combined postinjection/recovery period, we found that almost all of the coefficients were negative in both periods. Moreover, the antagonists either had no significant effect on the median coefficient or shifted the distribution toward even more negative values (Fig. 4*B*,*D*). Therefore, not only does the population of cue-excited neurons reliably predict the behavioral response latency, but the increase in response latency caused by an antagonist on a given trial is robustly predicted by the antagonist's effects on cue-evoked excitation on that trial. These results provide strong evidence for a causal role for endogenous dopamine in setting the vigor of the reward-seeking response to the cue: dopamine increases the cue-evoked excitation of NAc neurons, which in turn causes a short-latency approach to the lever.

An alternative interpretation of these results is that reduced cue-evoked excitation is a consequence of reduced behavioral responding— perhaps because the excitation merely tracks (or anticipates) the behavioral response but is not causal to it. If this were the case, then application of the antagonists in such a way that they do not influence behavior should not result in reduced cue-evoked excitation. However, as demonstrated in two example neurons (Fig. 3*B*,*D*), unilateral injection of either D1or D2/D3 antagonist markedly reduced the magnitude of cue-evoked excitation even though unilateral injections did not alter behavioral performance. Similar results were obtained when averaging across cue-evoked excitations recorded in the injected NAc (Figs. 5*A*, 6*A*, Ipsilateral histograms); in addition, the average data show that cue-evoked excitations in neurons recorded in the NAc contralateral to the injection were unaffected (Figs. 5*A*, 6*A*, Contralateral histograms). To rule out the possibility that the reduction in cue-evoked excitation ipsilateral to the injections was due to small differences in behavioral response probability, we repeated the analysis after excluding all trials in which the animal made no lever press response; similar results were obtained (data not shown; $p < 0.05$ for both D1 and D2 antagonists, Wilcoxon). These results indicate that the antagonist-induced reduction in cue-evoked excitation is unlikely to be a consequence of impaired behavioral performance.

Although the temporal properties of cue-evoked excitation were quite similar across neurons, inhibitions after cue onset were more diverse, typically exhibiting later onset and less stereotyped time courses than excitations (Figs. 5*B*, 6*B*). Analyses of inhibitions (and, to an extent, excitations) that focus on a single time window may therefore miss a significant portion of the signal. Furthermore, standard statistical detection methods cannot consistently identify decreases from very low basal firing rates, including that of many NAc neurons. To circumvent these issues, we took a more inclusive approach in which we quantified, for 50 ms postcue time bins in every recorded neuron, the ROC AUC representing the difference between firing in the bin and the precue baseline. Heat maps of AUC values in time bins aligned to DS onset (Figs. 5*B*, 6*B*) demonstrate that reduction in DS-evoked excitation after bilateral and ipsilateral (but not contralateral) injections of D1 and D2 antagonists was pronounced in almost every cue-excited neuron and occurred across the entire time course of the excitation. In contrast, inhibitions after DS onset were not reduced. To quantify these effects, we determined whether each AUC value indicated a significant difference from baseline by computing a bootstrapped p value representing the likelihood that the AUC was sampled from the distribution of AUCs generated from randomly shuffled baseline and postcue bin firing rates (see Materials and Methods). As shown by plots of the proportion of neurons exhibiting significant ($p < 0.05$) excitation or inhibition in each bin aligned to DS onset (Figs. 5*C*, 6*C*, left plots in each column), the fraction of excitations, but not inhibitions, was reduced by bilateral and ipsilateral injections of the antagonists. This interpretation was confirmed statistically by comparing proportions of significantly excited and inhibited bins across the entire 1 s post-DS window (Figs. 5*C*, 6*C*, dot plots). Thus, excitations after DS onset were reduced by D1 and D2 antagonist injection, but inhibitions were not.

Indeed, the number of neurons showing significant inhibition was increased after some types of injection (Figs. 5*B*,*C*, 6*B*,*C*). These emergent inhibitions are unlikely to have contributed to the behavioral effects of bilateral antagonist infusions because they were not consistent (e.g., they occurred after bilateral and contralateral, but not ipsilateral D1 antagonist injection and after ipsilateral, but not bilateral D2 antagonist injection) and therefore they do not explain the behavioral effects of the antagonists. Furthermore, these late inhibitions were most prominent ~600 ms after DS onset, a time at which, in the control condition, ~50% of goal-directed approach behaviors had already been initiated (Fig. 2*B*). Consequently, it is unlikely that emergent inhibitions contributed to the antagonist-induced increase in approach initiation latency or reduction in response probability. Intriguingly, the great majority of emergent inhibitions occurred in DS-excited neurons, usually toward the end of the excitation (bilateral D1 antagonist: 14/17 neurons, 82%; ipsilateral D2 antagonist: 11/16 neurons, 69%; Figs. 5*B*,*C*, 6*B*,*C*), consistent with the possibility that they were unmasked by the antagonist-induced reduction of the excitatory response and supporting the hypothesis that the firing of DS-excited neurons is causal to initiation of approach behavior.

NS presentations, which rarely elicited lever-press responses (Fig. 1*B*), evoked small but consistent excitation in the same neurons that were excited by the DS (Fig. 2*A*). Surprisingly, NS-evoked excitations were not reduced by the D1 antagonist, either in magnitude (Fig. 7*A*) or in number of excited neurons (Fig.

←

(*Figure legend continued.*) injection, and that DS-evoked excitations persist contralateral to the injection. ***C***, Summary of the ROC analysis, identifying the fraction of all recorded neurons showing significant excitation and inhibition in 50 ms bins aligned to DS onset, before and after bilateral (left), ipsilateral (middle), and contralateral (right) infusions. Left side of each graph shows fraction of recorded neurons that was excited in the indicated time bin (lines above 0) and fraction that was inhibited (lines below 0); red and blue lines indicate pre and post injection periods. Right side of each graph shows the cross-neuron median (dot) and middle quartiles (vertical lines) of the fraction of 50 ms bins between 0 and 1 s after cue onset with significant excitation (points above 0) and inhibition (points below 0); red and blue dots represent pre and post injection data, respectively. Neurons with no significant bins before and after injection were excluded from this analysis (see Materials and Methods). $^{**}p < 0.01$; $^{*}p < 0.05$, Wilcoxon.

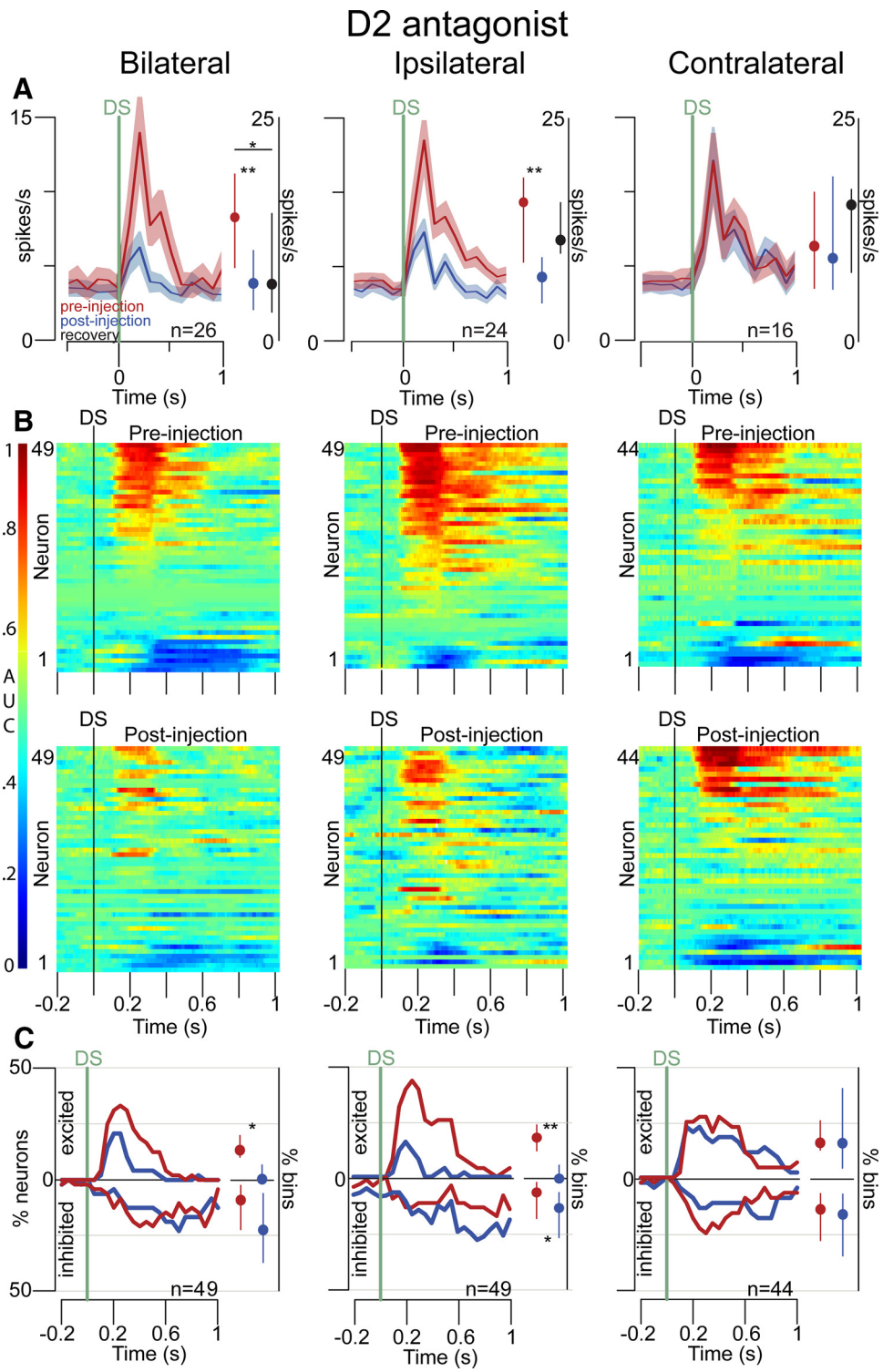

Figure 6. D2 receptor activation is necessary for DS-evoked excitation. ***A***, Peri-event time histograms aligned to DS onset for neurons with significant DS-evoked excitation in the period before raclopride injection. DS-evoked excitations were reduced by bilateral and ipsilateral injections, but not by contralateral injections. Format and conventions as in Figure 5*A*. ***B***, Data are presented in the same format as in Figure 5*B*, but for all neurons recorded during D2 antagonist injection. The conclusions are also similar: DS-evoked excitations were consistently reduced after bilateral and ipsilateral, but not contralateral, D2 antagonist injections. There were some emergent inhibitions at long post-DS latency after ipsilateral (but not bilateral or contralateral) injection. ***C***, Fraction of neurons showing significant excitation and inhibition in 50 ms bins aligned to DS onset. Format and conventions as in Figure 5*C*.

7*B*,*C*). In contrast, D2 antagonist injection reduced both the magnitude and number of NS-evoked excitations (Fig. 8). NS-evoked inhibitions were not reduced by either antagonist (Figs. 7*B*,*C*, 8*B*,*C*). Therefore, under these conditions D1 receptor activation is required for NAc neurons to produce large-magnitude excitations in response to salient reward-predictive stimuli, whereas D2 receptor activation is required for responses to both reward-predictive and neutral stimuli.

We considered the possibility that reduced reward-seeking behavior after bilateral infusions could have been due to in-

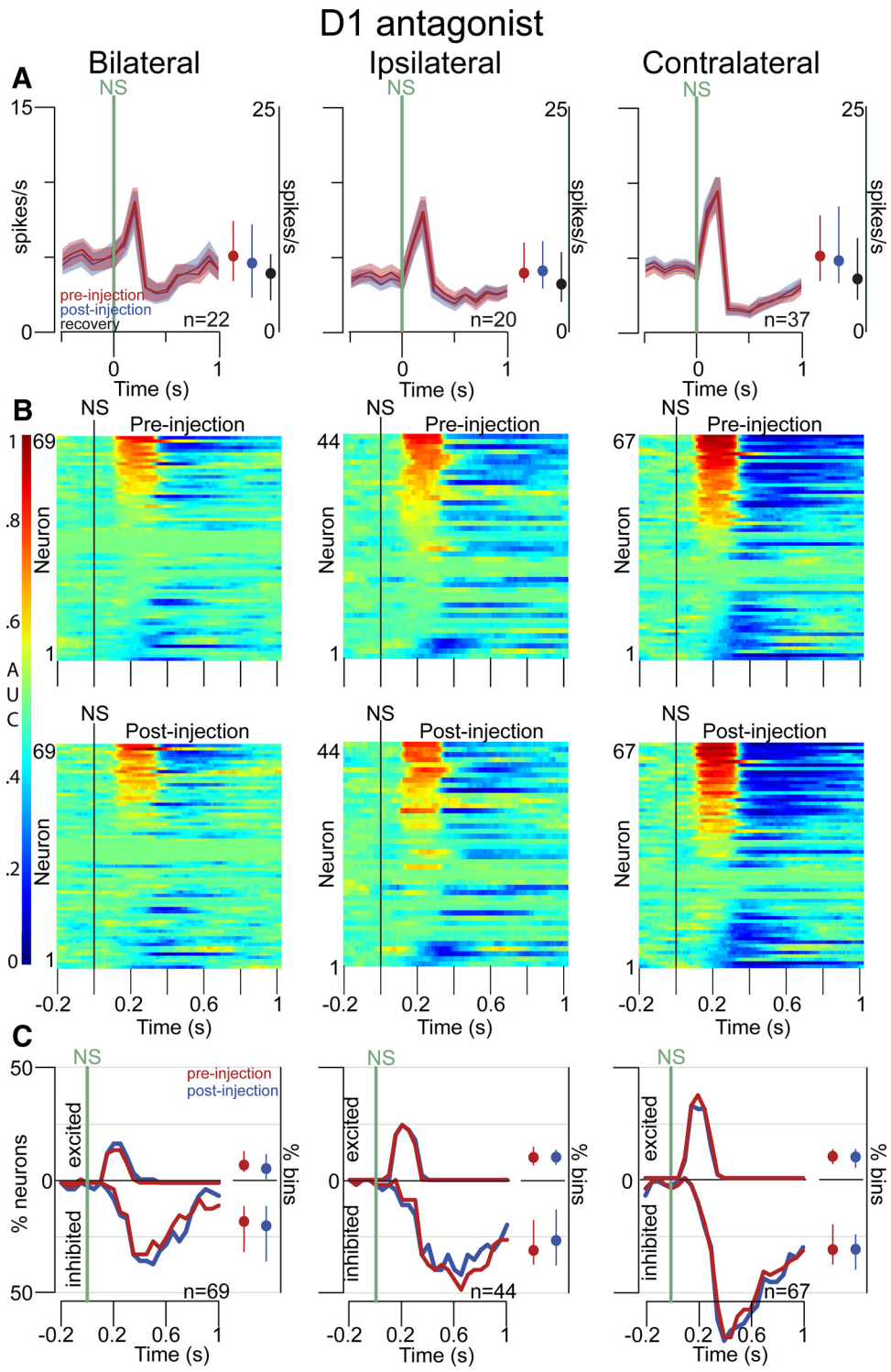

Figure 7. D1 receptor activation is not required for NS-evoked excitation. ***A***, Peri-event time histograms aligned to NS onset for neurons with significant DS-evoked excitation in the preinjection period. These populations entirely overlap, thus the same neurons were used for the analyses in Figures 5*A* and 6*A*. Plotting conventions are identical to those in Figure 5*A*. $^{**}p < 0.01$; $^{*}p < 0.05$, Wilcoxon. ***B***, Graphs show data from the same neurons, recorded in the same sessions, as in Figure 5*B*; however, the AUC values are aligned to NS onset. The results show that NS-evoked excitations and inhibitions were consistently unaffected by D1 antagonist injection. ***C***, Fraction of neurons showing significant excitation and inhibition in 50 ms bins aligned to NS onset, before and after bilateral (left), ipsilateral (middle), and contralateral (right) infusions. See legend for Figure 5*C* and Materials and Methods.

terruption of a neural process related to reinforcement or to hedonic processing of reward. Such processes may involve the subpopulations of NAc neurons that are inhibited or excited during consumption of sucrose (Nicola et al., 2004b; Roitman et al., 2005; Taha and Fields, 2005). Because animals continued to earn reward after unilateral antagonist infusion, we were able to determine whether neuronal activity related to reward consumption was dependent on dopamine receptor activation. We examined firing during the 5 s after the animal's entry into the reward receptacle, the time period during

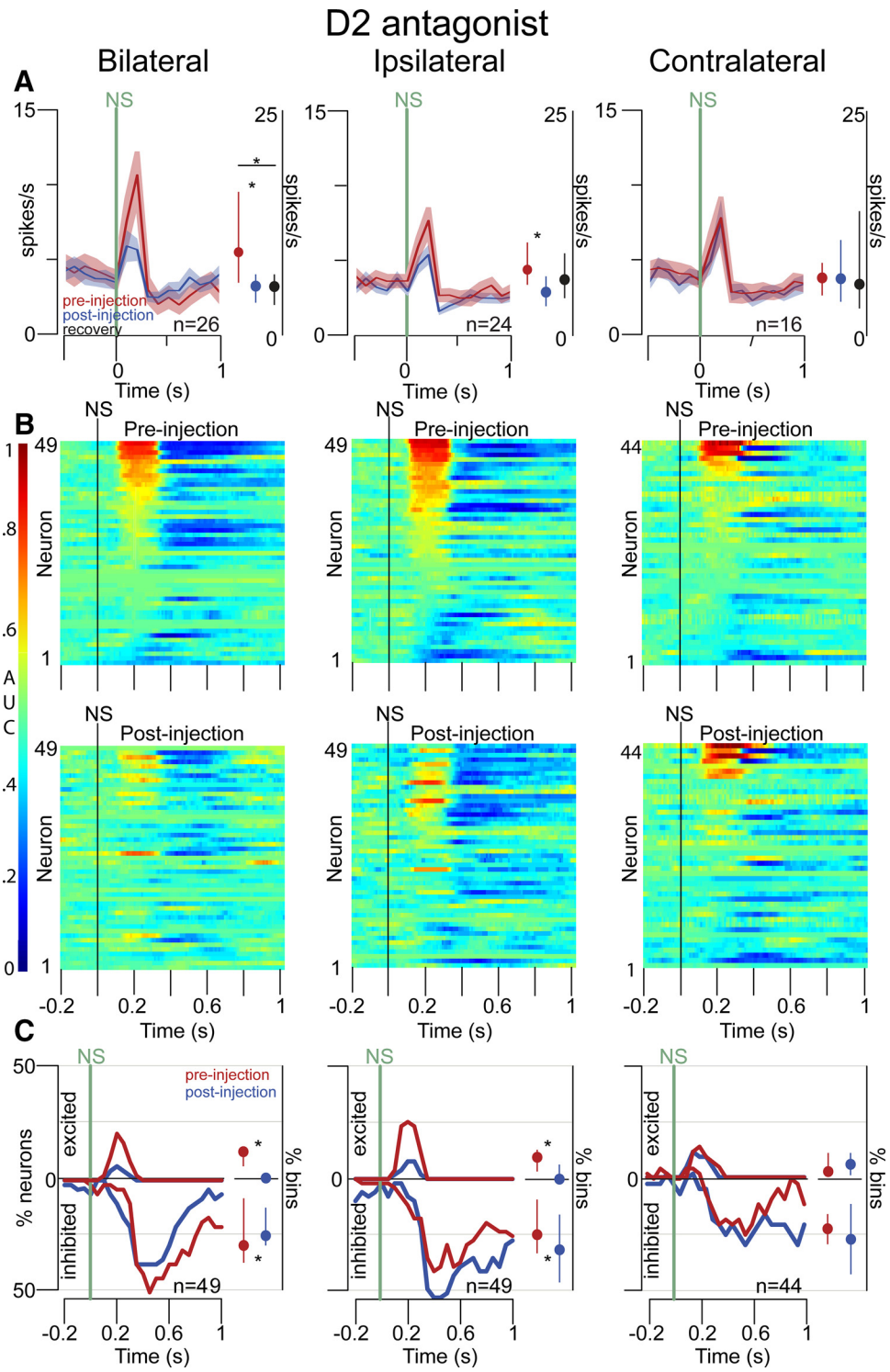

Figure 8. D2 receptor activation is necessary for NS-evoked excitation. ***A***, Peri-event time histograms aligned to NS onset for neurons with significant DS-evoked excitation in the preinjection period. NS excitation was reduced in the bilateral and ipsilateral conditions but not in contralateral neurons. Format and conventions as in Figure 5*A*. ***B***, Graphs show data from the same neurons, recorded in the same sessions, as in Figure 7*B*; however, the AUC values are aligned to NS onset. The results show that NS-evoked excitations were consistently reduced by bilateral and ipsilateral D2 antagonist injection. At long latency after NS onset, some neurons showed emergent inhibition after bilateral and ipsilateral injection. ***C***, Fraction of neurons showing significant excitation and inhibition in 50 ms bins aligned to NS onset. Format and conventions as in Figure 5C.

which reward consumption typically occurs (Nicola, 2010). Using ROC analysis, we compared firing in 200 ms bins within this window to the 10 s precue baseline; heat maps of the resulting AUC values show little effect of antagonist injection either ipsilateral or contralateral to the injection (Fig. 9*A*, *C*). The proportions of excited and inhibited neurons were not affected by the antagonists (Fig. 9*B*,*D*), strongly suggesting that that consumption-related excitations and inhibitions do not depend on dopamine. Similar results were obtained when we performed the same analysis using 50 ms bins (data not shown).

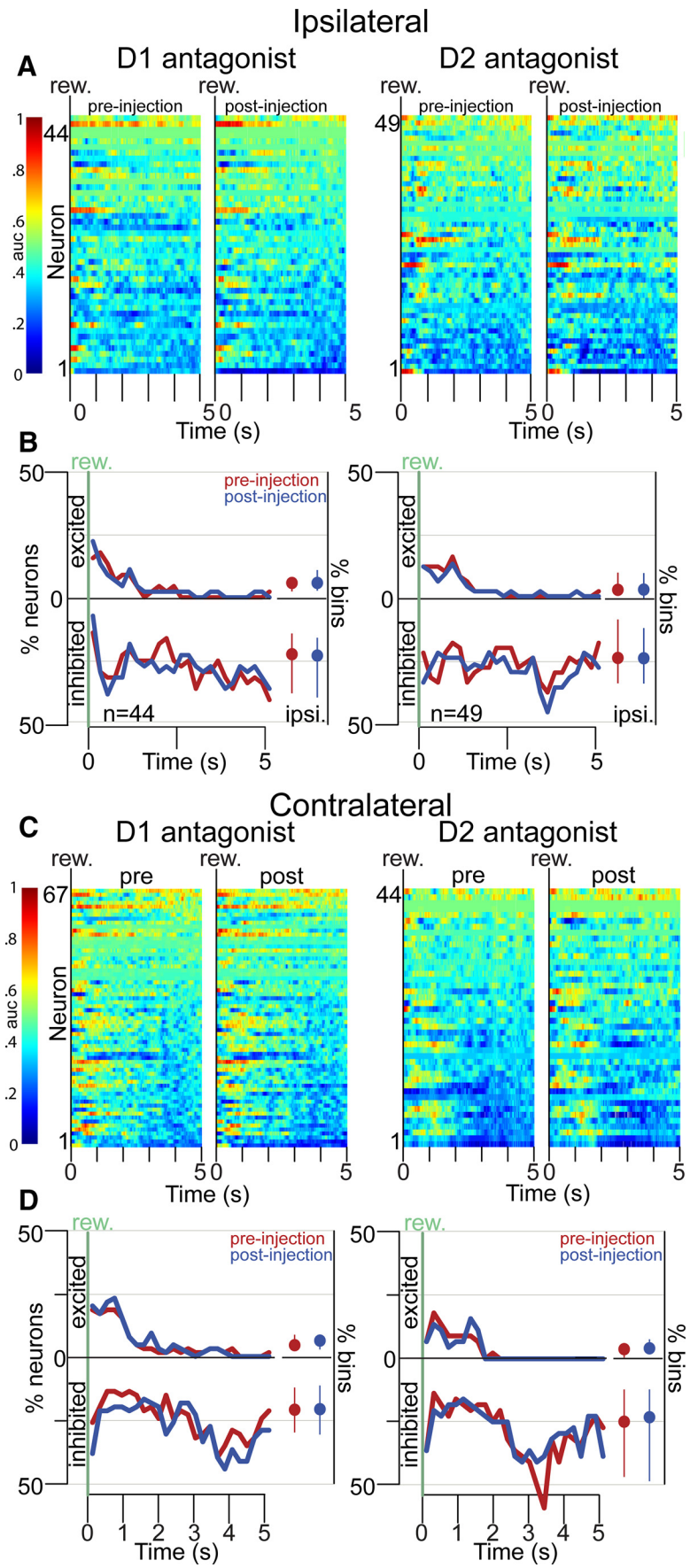

Figure 9. Neural activity aligned to reward receptacle entry is not affected by ipsilateral or contralateral D1 or D2 antagonist injection. ***A***, ***C***, ROC AUC values are calculated and displayed as described in Figure 5*B*, except time bins are longer (200 ms) and aligned to the animal's entry into the reward receptacle after pressing the lever in response to DS presentation. Animals consumed sucrose reward throughout most of the subsequent 5 s period displayed in the graphs. Neurons are sorted by the average AUC value in the last 3 s of reward (rew.) consumption. Firing in the preinjection (pre) and postinjection (post) periods is shown for unilateral injections of the D1 antagonist SCH23390 (left column) and the D2 antagonist raclopride (right column) that were ipsilateral (***A***) or contralateral (***C***) to the recorded neurons. ***B***, ***D***, Fraction of neurons recorded ipsilateral (***A***) or contralateral (***D***) to SCH23390 (left) and raclopride (right) injections that exhibit significant excitations or inhibitions after reward receptacle entry. Lines above and below 0 refer to excitatory and inhibitory neural responses, respectively; red and blue lines correspond to preinjection and postinjection periods, respectively. After injection of either antagonist, there was no significant change in the fraction of bins with significant excitation in the 1.5 s after receptacle entry, or the fraction of bins with significant inhibition in the 5 s after receptacle entry ($p > 0.1$ for both antagonists, Wilcoxon).

To rule out the possibility that the observed results were due to some factor other than the antagonist (e.g., physical disturbance caused by the injection or some component of the drug vehicle) we injected saline in some experiments. As shown by an example neuron (Fig. 10*A*) and by the average excitation across cue-excited neurons (Fig. 10*B*), DS-evoked excitations were not altered by saline injection; NS-evoked excitations were also not affected (Fig. 10*C*). Moreover, saline injection did not influence the proportions of neurons showing significant excitation and inhibition after DS or NS onset (Fig. 10*D*–*G*).

Finally, we asked whether dopamine receptor activation could be permissive for cued approach behavior by contributing to baseline firing rates of NAc neurons. Inconsistent with this hypothesis, there was no significant effect of either the D1 or D2 antagonist on the baseline firing rates of either DS-excited or other NAc neurons (Fig. 10*H*,*I*).

Histology

Nissl-stained sections indicated that probe placements were constrained to the NAc. Figure 11 indicates, for each rat, the approximate locations of the cannulae. Although the NAc core was targeted in all cases, some recorded neurons were likely to have been in the shell.

Discussion

These findings suggest a mechanism whereby NAc dopamine promotes reward-seeking behavior elicited by environmental stimuli: dopamine receptor activation facilitates cue-evoked excitations, which in turn promote short-latency initiation of ap-

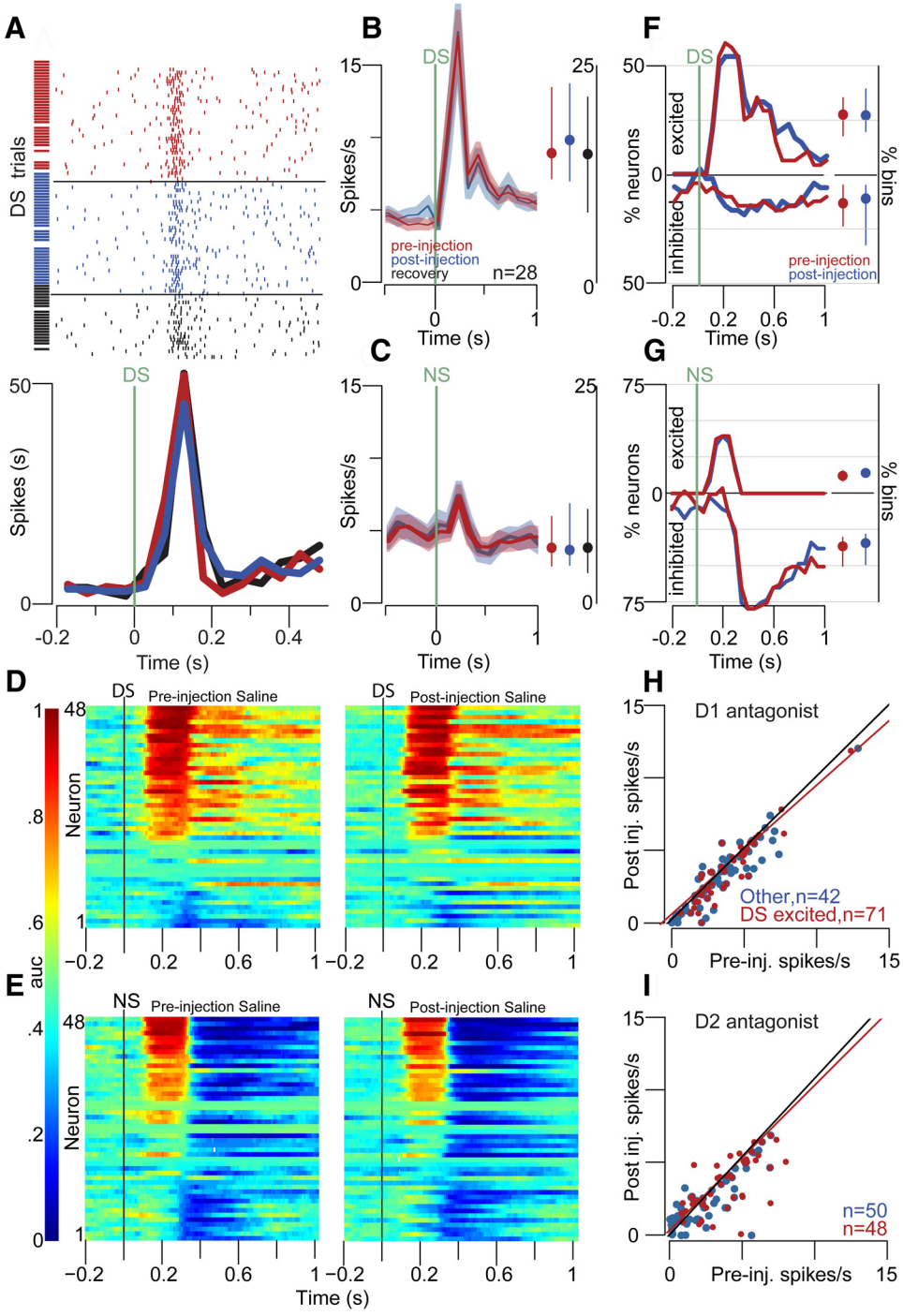

Figure 10. Saline infusion does not affect DS- or NS-evoked excitation and neither D1 nor D2 receptor activation is required for maintenance of baseline firing rates. ***A***, Single DS-excited neuron recorded during saline infusion. Conventions are identical to those used in Figure 3. ***B***, ***C***, Average DS-aligned (***B***) and NS-aligned (***C***) peri-event histogram for neurons exhibiting significant DS-evoked excitation in the preinjection period. The red trace shows data taken from the period before saline injection, and the blue trace shows postsaline injection data. ***D***, ***E***, ROC AUC values were calculated as for Figures 5*B* and 6*B* [50 ms bins, aligned to DS (***D***) or NS (***E***) onset] for neurons recorded during saline injection. During some unilateral experiments, an antagonist was injected into one hemisphere while saline was injected in the other as a control. Therefore, the neurons shown here are a subset of the neurons recorded contralateral to D1 and D2 antagonist infusions. Neurons were sorted by the AUC value at 200 ms after DS onset, and are presented in the same order in the graphs for the preinjection (left) and postinjection (right) periods. Saline had little or no effect on DS-evoked excitations and inhibitions, ruling out the possibility that local infusion destabilized neural recordings or otherwise reduced DS-evoked neural activity. ***F***, ***G***, Fraction of neurons ipsilateral to saline infusions that exhibited significant excitations or inhibitions after DS (***F***) and NS (***G***) onset. Conventions as in Figure 5*C*. There was no significant change in the fraction of bins with significant excitation or inhibition after saline infusion for either cue (red and blue dot plots show preinjection and postinjection fractions, respectively; $p > 0.1$, Wilcoxon). ***H***, ***I***, Average baseline firing rate before (*x*-axis) and after (*y*-axis) SCH23390 (***H***) and raclopride (***I***) injection. Neurons recorded during both bilateral and ipsilateral injections are shown. Red dots indicate neurons that exhibited significant DS-evoked excitation in the preinjection period, and blue dots show baseline firing rates for all other neurons. The black line is the unity line and the red line is a linear fit to baseline firing rates of DS-excited neurons. Slopes of the fitted lines were not significantly different from unity (D1 antagonist, slope $= 0.95 \pm 0.06$, $r^2 = 0.89$; D2 antagonist, slope $= 0.86 \pm 0.19$, $r^2 = 0.44$; errors are SE), indicating that the antagonists did not affect baseline firing rates. Moreover, direct comparison of baseline firing rates before and after injection revealed no significant difference for either antagonist ($p > 0.1$, Wilcoxon).

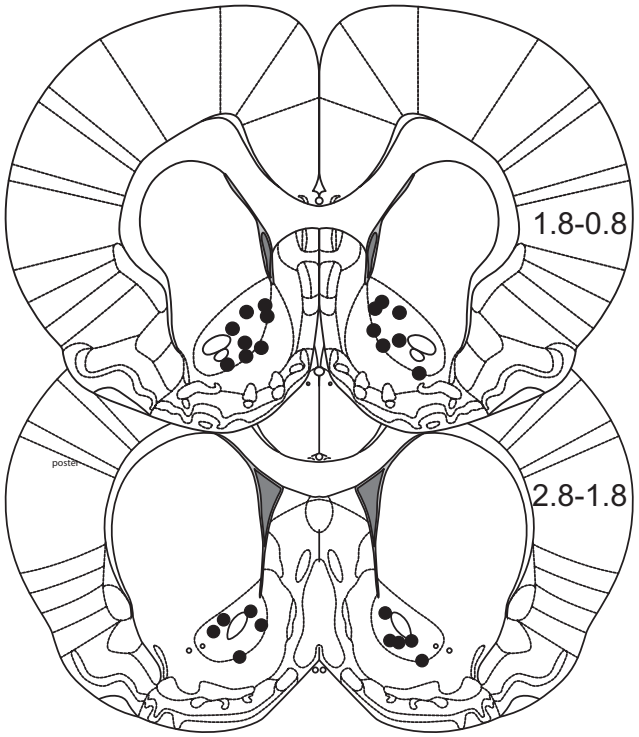

Figure 11. Histological reconstruction of antagonist injection sites. Figure depicts two coronal sections of rat brain that encompass the majority of the anterior–posterior extent of the NAc (0.8 mm–2.8 mm anterior from bregma). Black dots represent estimates of the location of the cannulae (which were located in the center of the recording arrays).

proach to reward-associated objects. This conclusion is strongly supported by the observation that bilateral dopamine antagonist injection both increased the latency to initiate movement (Fig. 1*D*) and reduced the magnitude of cue-evoked excitations (Figs. 3–6). Reduced cue-evoked excitation cannot have been a consequence of impaired behavior because unilateral injections did not change DS-cued behavior (Fig. 1*C–F*), yet profoundly reduced DS-evoked excitation in the injected tissue (Figs. 3*B*,*D*, 5, 6). These excitations were a predominant neural response in the NAc (occurring in 45% of the recorded neurons), and they both preceded movement onset (Fig. 2*B*) and predicted movement initiation latency with greater firing on trials with shorter latency (Fig. 2*D*) (McGinty et al., 2013; Morrison and Nicola, 2014). Therefore, cue-evoked excitation is both dopamine dependent and necessary for vigorous reward seeking.

Our results demonstrate that cue-evoked excitation, and no other form of neural activity in the NAc, is likely a critical signal in the neural circuit that sets the latency of goal-directed movements. This conclusion follows from the observation that the antagonists diminished cue-evoked excitation without reducing cue-evoked inhibitions, reward consumption-associated firing, or baseline firing rates. Furthermore, the trials in which bilateral injections of the antagonists were most effective in reducing the excitation were those in which they caused the greatest behavioral impairment (Fig. 4), strongly arguing against the possibility that some other undetected change in neuronal encoding was responsible for the behavioral effects. Therefore, our data firmly link dopamine receptor activation in the NAc, the magnitude of cue-evoked excitation, and the animal's latency to initiate reward seeking.

Previous work showed that VTA inactivation that reduced NAc cue-evoked excitations and inhibitions also prevented animals from exhibiting cued approach behavior (Yun et al., 2004). However, that study did not eliminate the possibility that these changes were an indirect circuit effect. Here, we demonstrate that dopamine receptors local to the recorded neurons are necessary for cue-evoked excitation, eliminating the possibility that the antagonist effects are due to an action of dopamine upstream of the NAc. In contrast, even though cue-evoked inhibitions were reduced by VTA inactivation (Yun et al., 2004), they were not reduced by local dopamine antagonist injection, and therefore these inhibitions are unlikely to be the result of a direct action of dopamine within the NAc.

The effects of the D1 and D2 antagonists on both DS-evoked approach behavior and DS-evoked firing were remarkably similar. These observations are consistent with a long line of NAc microinjection experiments in which D1 and D2 antagonists produced nearly indistinguishable behavioral effects at doses similar to ours (Hiroi and White, 1991; Ozer et al., 1997; Koch et al., 2000; Eiler et al., 2006; Pezze et al., 2007; Lex and Hauber, 2008; Liao, 2008; Nicola, 2010; Shin et al., 2010; Haghparast et al., 2012). These results, together with the contrast between the antagonist concentration in the injectate that is required to observe effects (mM) and the affinity of the drugs for their targets (nM), call into question whether the drug effects are specific. Although the effective concentration at the receptor is likely to be considerably lower than the injected concentration due to diffusion, metabolism, and oxidation of the drugs, the combined efficacy and time course of these processes is unknown. Therefore, one formal possibility is that both the behavioral and electrophysiological effects of SCH23390 and raclopride are the result of both drugs binding one or more receptors that are not bound by dopamine at all. Several factors argue against this possibility. Cue-evoked approach behavior is blocked not only by SCH23390 and raclopride, but also by injection of the broad-spectrum dopamine receptor antagonist flupenthixol into the NAc (Di Ciano et al., 2001; Saunders and Robinson, 2012), by inactivation of the VTA (Yun et al., 2004) and by lesion of the NAc with 6-hydroxydopamine (Parkinson et al., 2002), which selectively kills catecholaminergic fibers. Moreover, NAc injection of a dopamine reuptake blocker, a D1 or D2 receptor agonist, or the dopamine releaser amphetamine increases the probability of cued approach (Wyvell and Berridge, 2000; Nicola et al., 2005; du Hoffmann and Nicola, 2013). Finally, optogenetic self-stimulation of VTA dopamine neurons (a behavior undoubtedly maintained by dopamine neuron activation) is attenuated by injection of SCH23390 or raclopride into the NAc at doses similar to those used here (Steinberg et al., 2014). It is difficult to conceive of a simple mechanism that could account for each of these results without positing that SCH23390 and raclopride block cued approach by blocking the effects of endogenous dopamine.

An alternative possibility is that the antagonists bind not only their target receptors, but off-target dopamine receptors as well. At concentrations of 10 μM or lower, raclopride does not bind D1-like receptors (Hall et al., 1986); higher concentrations have not been tested. Therefore, raclopride could be specific for D2/D3 receptors even at the mM injectate concentrations used by us and others, particularly after diffusion, metabolism, and oxidation are taken into account. Estimates of the SCH23390 binding constant to D2-like receptors range between 1 and 5 μM (Bourne, 2001; Mottola et al., 2002); although these values suggest that SCH23390 binds D2/D3 receptors at the injected concentrations, the functional efficacy of SCH23390 in blocking activation of D2-like receptors by dopamine is unknown. Our observation that raclopride reduced NS-evoked excitation whereas SCH23390 did not

supports the idea that the drugs acted at different receptors, but does not definitively demonstrate their specificity. Nevertheless, even if one or both drugs blocked both receptor types to reduce DS-evoked excitation, this would be entirely consistent with our conclusion that activation of at least one form of dopamine receptor is required for DS-evoked excitation. Thus, although the question of drug specificity remains unanswered, this question only marginally weakens our main conclusion that dopamine facilitates cued approach by increasing cue-evoked excitation.

If in fact the drugs did act specifically, our findings that D1 and D2/D3 antagonists each reduced cue-evoked firing in the majority of cue-excited neurons suggests that activation of these receptors leads synergistically to excitation in the same neurons. Whereas D1 and D2 receptors are found in largely segregated populations of neurons in the NAc (Albin et al., 1989; Gerfen et al., 1990), substantial proportions of NAc core and shell neurons that express D1 receptors also contain mRNA for D3 receptors (Le Moine and Bloch, 1996), which are blocked by D2 antagonists, including raclopride. Coexpression of D1 and D3 receptors provides a potential mechanism whereby dopamine could promote excitation in NAc neurons by a synergistic effect that would be blocked by either D1 or D2/3 antagonists (Schwartz et al., 1998). Alternatively (or in addition), the interaction between D1 and D2 (and/or D3) receptors may occur at the local circuit level (Goto and Grace, 2005; Gerfen and Surmeier, 2011). For instance, dopamine acts at D1 receptors to reduce GABA release onto NAc neurons (Nicola and Malenka, 1997; Hjelmstad, 2004), an effect that could promote excitation in concert with activation of D2/D3 receptors on spiny neurons (Hopf et al., 2003). Notably, these mechanisms posit that dopamine does not excite NAc neurons directly, but rather increases their excitability in response to glutamatergic input; thus, they could explain why cue-evoked excitations are blocked not just by dopamine antagonists, but also by inactivation of the basolateral amygdala and prefrontal cortex (Ambroggi et al., 2008; Ishikawa et al., 2008), both of which send glutamatergic projections to the NAc (Brog et al., 1993).

The similarities and differences between SCH23390 and raclopride effects may be the result of two contrasting neural mechanisms, involving phasic and tonic dopamine. Because both D1 and D2/D3 antagonists reduced DS-evoked excitation, but the smaller NS-evoked excitation occurring in the same neurons was reduced only by the D2/D3 antagonist (Figs. 8, 9), it appears that dopamine promotes encoding of stimulus value via activation of D1 receptors, but facilitates firing responses to all cues (whether or not they are associated with a valuable outcome) via D2/D3 receptors. This could be due to the greater phasic dopamine transients elicited in the NAc by reward-predictive than neutral cues (Phillips et al., 2003; Roitman et al., 2004). Because D2/3 receptors have a higher affinity for dopamine than D1 receptors, small NS-evoked dopamine transients may be sufficient to activate only D2/3 receptors, whereas reward-predictive DSs may elevate the dopamine concentration to levels high enough to activate D1 receptors (Grace, 1991).

Alternatively, the magnitude of cue-evoked excitation could be regulated by tonic, rather than phasic dopamine. Tonic dopamine levels may reflect the opportunity cost of inaction (Niv et al., 2007), thereby setting the vigor of operant performance. Thus, if sufficiently high tonic dopamine levels are achieved, enough dopamine receptors could become activated to facilitate cue-evoked excitation and decrease the latency of reward-seeking approach. A similar mechanism may also underlie the well known contribution of NAc dopamine to performance of uncued operant tasks that require a high level of effort (Salamone and Correa, 2012), in which dopamine disruption increases latencies to approach the operandum (Nicola, 2010). Implicit external cues (e.g., sight of the lever) or internal cues (e.g., arising from timing or hunger) could trigger approach by exciting NAc neurons to a greater extent when opportunity costs and dopamine levels are high.

In summary, regardless of the specific pharmacological mechanism, our results demonstrate that NAc dopamine promotes reward-seeking behavior by elevating the excitation of NAc neurons to salient environmental stimuli. The magnitude of this excitation sets the latency of the subject to initiate an approach response. Via this mechanism, dopamine regulates both the vigor and probability of cued reward-seeking.

References

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci 12:366–375. CrossRef Medline

Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. Neuron 59:648–661. CrossRef Medline

Ambroggi F, Ghazizadeh A, Nicola SM, Fields HL (2011) Roles of nucleus accumbens core and shell in incentive-cue responding and behavioral inhibition. J Neurosci 31:6820–6830. CrossRef Medline

Bourne JA (2001) SCH 23390: the first selective dopamine D1-like receptor antagonist. CNS Drug Rev 7:399–414. CrossRef Medline

Brog JS, Salyapongse A, Deutch AY, Zahm DS (1993) The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. J Comp Neurol 338:255–278. CrossRef Medline

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. J Neurosci 21:9471–9477. Medline

Drai D, Benjamini Y, Golani I (2000) Statistical discrimination of natural modes of motion in rat exploratory behavior. J Neurosci Methods 96: 119–131. CrossRef Medline

du Hoffmann J, Nicola SM (2013) Increased nucleus accumbens dopamine inhibits satiety in a cued approach task. Soc Neurosci Abstr 39.867.11/LLL22.

du Hoffmann J, Kim JJ, Nicola SM (2011) An inexpensive drivable cannulated microelectrode array for simultaneous unit recording and drug infusion in the same brain nucleus of behaving rats. J Neurophysiol 106: 1054–1064. CrossRef Medline

Eiler WJ 2nd, Masters J, McKay PF, Hardy L 3rd, Goergen J, Mensah-Zoe B, Seyoum R, Cook J, Johnson N, Neal-Beliveau B, June HL (2006) Amphetamine lowers brain stimulation reward (BSR) threshold in alcohol-preferring (P) and -nonpreferring (NP) rats: regulation by D-sub-1 and D-sub-2 receptors in the nucleus accumbens. Exp Clin Psychopharmacol 14:361–376. CrossRef Medline

Gerfen CR, Surmeier DJ (2011) Modulation of striatal projection systems by dopamine. Annu Rev Neurosci 34:441–466. CrossRef Medline

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432. CrossRef Medline

Goto Y, Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. Nat Neurosci 8:805–812. CrossRef Medline

Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41:1–24. CrossRef Medline

Haghparast A, Ghalandari-Shamami M, Hassanpour-Ezatti M (2012) Blockade of D1/D2 dopamine receptors within the nucleus accumbens attenuated the antinociceptive effect of cannabinoid receptor agonist in the basolateral amygdala. Brain Res 1471:23–32. CrossRef Medline

Hall H, Sallemark M, Jerning E (1986) Effects of remoxipride and some related new substituted salicylamides on rat brain receptors. Acta Pharmacol Toxicol 58:61–70. CrossRef Medline

Hiroi N, White NM (1991) The amphetamine conditioned place prefer-

ence: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. Brain Res 552:141–152. CrossRef Medline
Hjelmstad GO (2004) Dopamine excites nucleus accumbens neurons through the differential modulation of glutamate and GABA release. J Neurosci 24:8621–8628. CrossRef Medline
Hopf FW, Cascini MG, Gordon AS, Diamond I, Bonci A (2003) Cooperative activation of dopamine D1 and D2 receptors increases spike firing of nucleus accumbens neurons via G-protein betagamma subunits. J Neurosci 23:5079–5087. Medline
Ishikawa A, Ambroggi F, Nicola SM, Fields HL (2008) Dorsomedial prefrontal cortex contribution to behavioral and nucleus accumbens neuronal responses to incentive cues. J Neurosci 28:5088–5098. CrossRef Medline
Koch M, Schmid A, Schnitzler HU (2000) Role of muscles accumbens dopamine D1 and D2 receptors in instrumental and Pavlovian paradigms of conditioned reward. Psychopharmacology 152:67–73. CrossRef Medline
Le Moine C, Bloch B (1996) Expression of the D3 dopamine receptor in peptidergic neurons of the nucleus accumbens: comparison with the D1 and D2 dopamine receptors. Neuroscience 73:131–143. CrossRef Medline
Lex A, Hauber W (2008) Dopamine D1 and D2 receptors in the nucleus accumbens core and shell mediate Pavlovian-instrumental transfer. Learn Mem 15:483–491. CrossRef Medline
Liao RM (2008) Development of conditioned place preference induced by intra-accumbens infusion of amphetamine is attenuated by co-infusion of dopamine D1 and D2 receptor antagonists. Pharmacol Biochem Behav 89:367–373. CrossRef Medline
McGinty VB, Lardeux S, Taha SA, Kim JJ, Nicola SM (2013) Invigoration of reward seeking by cue and proximity encoding in the nucleus accumbens. Neuron 78:910–922. CrossRef Medline
Morrison SE, Nicola SM (2014) Neurons in the nucleus accumbens promote selection bias for nearer objects. J Neurosci 34:14147–14162. CrossRef
Mottola DM, Kilts JD, Lewis MM, Connery HS, Walker QD, Jones SR, Booth RG, Hyslop DK, Piercey M, Wightman RM, Lawler CP, Nichols DE, Mailman RB (2002) Functional selectivity of dopamine receptor agonists. I. Selective activation of postsynaptic dopamine D2 receptors linked to adenylate cyclase. J Pharmacol Exp Ther 301:1166–1178. CrossRef Medline
Nicola SM (2007) The nucleus accumbens as part of a basal ganglia action selection circuit. Psychopharmacology 191:521–550. CrossRef Medline
Nicola SM (2010) The flexible approach hypothesis: unification of effort and cue-responding hypotheses for the role of nucleus accumbens dopamine in the activation of reward-seeking behavior. J Neurosci 30:16585–16600. CrossRef Medline
Nicola SM, Malenka RC (1997) Dopamine depresses excitatory and inhibitory synaptic transmission by distinct mechanisms in the nucleus accumbens. J Neurosci 17:5697–5710. Medline
Nicola SM, Yun IA, Wakabayashi KT, Fields HL (2004a) Cue-evoked firing of nucleus accumbens neurons encodes motivational significance during a discriminative stimulus task. J Neurophysiol 91:1840–1865. CrossRef Medline
Nicola SM, Yun IA, Wakabayashi KT, Fields HL (2004b) Firing of nucleus accumbens neurons during the consummatory phase of a discriminative stimulus task depends on previous reward predictive cues. J Neurophysiol 91:1866–1882. CrossRef Medline
Nicola SM, Taha SA, Kim SW, Fields HL (2005) Nucleus accumbens dopamine release is necessary and sufficient to promote the behavioral response to reward-predictive cues. Neuroscience 135:1025–1033. CrossRef Medline
Niv Y, Daw ND, Joel D, Dayan P (2007) Tonic dopamine: opportunity costs and the control of response vigor. Psychopharmacology 191:507–520. CrossRef Medline
Ozer H, Ekinci AC, Starr MS (1997) Dopamine D1- and D2-dependent catalepsy in the rat requires functional NMDA receptors in the corpus striatum, nucleus accumbens and substantia nigra pars reticulata. Brain Res 777:51–59. CrossRef Medline
Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, Rudarakanchana N, Halkerston KM, Robbins TW, Everitt BJ (2002) Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. Behav Brain Res 137:149–163. CrossRef Medline
Pezze MA, Dalley JW, Robbins TW (2007) Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. Neuropsychopharmacology 32:273–283. CrossRef Medline
Phillips PE, Stuber GD, Heien ML, Wightman RM, Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. Nature 422:614–618. CrossRef Medline
Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM (2004) Dopamine operates as a subsecond modulator of food seeking. J Neurosci 24:1265–1271. CrossRef Medline
Roitman MF, Wheeler RA, Carelli RM (2005) Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron 587–597.
Salamone JD, Correa M (2012) The mysterious motivational functions of mesolimbic dopamine. Neuron 76:470–485. CrossRef Medline
Saunders BT, Robinson TE (2012) The role of dopamine in the accumbens core in the expression of Pavlovian-conditioned responses. Eur J Neurosci 36:2521–2532. CrossRef Medline
Schoenbaum G, Chiba AA, Gallagher M (1998) Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci 1:155–159. CrossRef Medline
Schwartz JC, Diaz J, Bordet R, Griffon N, Perachon S, Pilon C, Ridray S, Sokoloff P (1998) Functional implications of multiple dopamine receptor subtypes: the D1/D3 receptor coexistence. Brain Res Brain Res Rev 26:236–242. CrossRef Medline
Shin R, Cao J, Webb SM, Ikemoto S (2010) Amphetamine administration into the ventral striatum facilitates behavioral interaction with unconditioned visual signals in rats. PLoS One 5:e8741. CrossRef Medline
Steinberg EE, Boivin JR, Saunders BT, Witten IB, Deisseroth K, Janak PH (2014) Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. PLoS One 9:e94771. CrossRef Medline
Stuber GD, Hnasko TS, Britt JP, Edwards RH, Bonci A (2010) Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. J Neurosci 30:8229–8233. CrossRef Medline
Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9:321–353. CrossRef Medline
Taha SA, Fields HL (2005) Encoding of palatability and appetitive behaviors by distinct neuronal populations in the nucleus accumbens. J Neurosci 25:1193–1202. CrossRef Medline
Van Bockstaele EJ, Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res 682:215–221. CrossRef Medline
Wyvell CL, Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. J Neurosci 20:8122–8130. Medline
Yun IA, Wakabayashi KT, Fields HL, Nicola SM (2004) The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. J Neurosci 24:2923–2933. CrossRef Medline