Dopamine neurons projecting to the posterior striatum reinforce avoidance of threatening stimuli

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Midbrain dopamine neurons are well known for their role in reward-based reinforcement learning. We found that the activity of dopamine axons in the posterior tail of the striatum (TS) scaled with the novelty and intensity of external stimuli, but did not encode reward value. We demonstrated that the ablation of TS-projecting dopamine neurons specifically inhibited avoidance of novel or high-intensity stimuli without affecting animals' initial avoidance responses, suggesting a role in reinforcement rather than simply in avoidance itself. Furthermore, we found that animals avoided optogenetic activation of dopamine axons in TS during a choice task and that this stimulation could partially reinstate avoidance of a familiar object. These results suggest that TS-projecting dopamine neurons reinforce avoidance of threatening stimuli. More generally, our results indicate that there are at least two axes of reinforcement learning using dopamine in the striatum: one based on value and one based on external threat.

Early electrophysiological recordings in monkeys and rodents revealed that many dopamine neurons are excited by unpredictable rewards or by reward-predicting stimuli. Conversely, these neurons are inhibited by the omission of expected reward and by aversive events. Transient activation of dopamine neurons can substitute for positive reward, whereas transient suppression of dopamine neurons can mimic negative outcomes. These results led to the proposal that dopamine acts as a bidirectional reinforcement signal used by the brain to maximize the value of future outcomes. However, multiple studies have found that at least some dopamine neurons are activated by nonrewarding events. For instance, some dopamine neurons in the substantia nigra pars compacta (SNC) are activated by both rewarding and aversive stimuli. This led to the proposal that these dopamine neurons signal 'motivational salience' (the absolute value of value) and facilitate a behavioral reaction when an important stimulus (whether it is good or bad) is detected. However, the function of these dopamine neurons remains unknown. Additionally, multiple studies have reported that some dopamine neurons have larger responses to novel stimuli than to familiar stimuli. This has been interpreted as a 'novelty bonus' because novelty may be rewarding itself or may signal potential reward.

To more clearly understand the diversity of dopamine signals, recent studies have focused on the projection targets of dopamine neurons and have shown that different regions of the striatum receive distinct dopamine signals. Dopamine neurons projecting to the ventral striatum (VS) display patterns of activity consistent with the value-prediction error seen in canonical dopamine neurons, those projecting to the TS are activated by aversive and neutral stimuli. A previous study demonstrated that dopamine responses to novel stimuli are localized in TS dopamine axons and do not coincide with value-related dopamine signals in VS, suggesting that these signals are unlikely to function as a novelty bonus for value learning and instead could have a different function. In this study, we investigate the functional significance of responses to nonrewarding stimuli in dopamine axons in TS.

Results
Dopamine axons in TS encode external stimulus intensity but not value. Previous studies of projection-specified populations have not examined the covariation of dopamine activity with value or salience, which are fundamental characteristics for evaluating the function of each population. To better understand the nature of dopamine signals in the striatum, we first characterized the activity of dopamine axons by presenting an array of stimuli to head-fixed mice. We monitored the activity of dopamine axons at their projection targets using fiber fluorometry (also called photometry; Fig. 1 and Supplementary Figs. 1 and 2). To do that, a calcium indicator (GCaMP) was expressed specifically in dopamine neurons. To mask any potential responses related to the auditory detection of nonauditory stimuli (for example, water delivery), training and experiments were performed with constant background noise (see Methods).

We first examined how dopamine axon activity in VS or TS covaried with outcome value. Consistent with previous results, dopamine axons in VS responded strongly to water but only weakly to a neutral tone, whereas dopamine axons in TS responded strongly to tones and airpuffs but only weakly to water. The activity of dopamine axons in VS scaled with the volume of the reward (Fig. 1a), consistent with the idea that dopamine signals encode reward value in this area. Unexpectedly, responses of dopamine axons in TS to water delivery were not significantly modulated by reward volume (Fig. 1b), indicating that the small responses to water observed here and in previous studies were potentially caused by primitive sensory information such as the water valve click rather than reward value. Unlike dopamine axons in VS, the responses in TS scaled with the intensity of the tone (Fig. 1b) or airpuff (Supplementary Fig. 3).

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Fig. 1 | Signals from dopamine axons in TS scale with external stimulus intensity but not with reward volume. Fluorescence was collected from GCaMP-expressing dopamine axons and cell bodies using optic fibers while animals received different reward volumes (blue) or tone volumes (orange). a. Signals from dopamine axons in VS (mean ± s.e.m. across n = 11 animals) in response to different volumes of reward (F_w = 3.91, P = 0.0072, one-way ANOVA). b. Signals from dopamine axons in TS (mean ± s.e.m. across n = 9 animals) in response to different volumes of reward (F_w = 2.05, P = 0.1057, one-way ANOVA) or tones (F_w = 3.05, P = 0.0275, one-way ANOVA). c. Signals from dopamine neurons in SNL (mean ± s.e.m. across n = 9 animals) in response to different volumes of reward (F_w = 0.68, P = 0.61, one-way ANOVA) or tones (F_w = 3.93, P = 0.0088, one-way ANOVA). VTA, ventral tegmental area. d. Signals from TS-projecting dopamine neurons in SNL (mean ± s.e.m. across n = 6 animals) in response to different volumes of reward (F_w = 0.35, P = 0.84, one-way ANOVA) or tones (F_w = 5.13, P = 0.0037, one-way ANOVA). e. Left: TS dopamine axon responses to a variety of external stimuli (n = 9 animals); solid line is the mean and transparent area is s.e.m.). Responses to unexpected airpuff (magenta), unexpected bitter water (green), and omission of expected water (blue) dopamine axons in (middle) VS (water omission: t = -4.98, P = 0.00076, n = 10 animals; airpuff: t = -2.95, P = 0.015, n = 10 animals; quinine: t = -1.30, P = 0.000028, n = 7 animals; paired t test) and (right) TS (water omission: t = -0.83, P = 0.43, n = 11 animals; airpuff: t = 4.93, P = 0.000081, n = 10 animals; quinine: t = 0.90, P = 0.40, n = 8 animals; paired t test). Solid line is mean and transparent area is s.e.m. *P < 0.05, ANOVA with post hoc two-sided t test and Tukey correction for multiple comparisons. **P < 0.01, two-sided t test (peak signal in 1 s following stimulus × peak signal during 1 s within the intertrial interval).
Because high-intensity external stimuli (such as tones or air-puffs) are potentially aversive, we next examined whether dopamine axons in TS respond to all events with negative value. We found that, whereas dopamine axon signals in VS were inhibited by every negative stimulus that we tested (Fig. 1e), dopamine axons in TS did not respond to some types of negative stimuli such as bitter taste (quinine) or the omission of expected water (Fig. 1e and Supplementary Fig. 4). Instead, dopamine axons in TS were strongly activated by high-intensity stimuli of multiple modalities including somatosensory, auditory, visual, and olfactory stimuli (Fig. 1e and Supplementary Fig. 4). Further, consistent with a previous study (Fig. 1e and Supplementary Fig. 4), dopamine axon responses in TS scaled with the novelty of each stimulus, and the signals decayed differently depending on the stimulus intensity and type (Supplementary Fig. 3). Together, these data demonstrate that dopamine axons in TS do not respond to all forms of reward and/or punishment, indicating that they do not simply encode positive and/or negative value. Instead, these data indicate that dopamine axons in TS respond specifically to novel or high-intensity external stimuli.

Because dopamine signals are potentially modulated at axon terminals, we also recorded activity at the cell bodies of midbrain dopamine neurons. To target TS-projecting dopamine neurons, we first examined their distribution within the midbrain by retrogradely infecting their axons in TS with rabies virus expressing GFP (Fig. 2 and Supplementary Fig. 5). We found that TS-projecting dopamine neurons were concentrated in the lateral part of substantia nigra, especially the most lateral part, called substantia nigra pars lateralis (SNL). We found that most of their axons were located within TS, with no other region containing fluorescence that differed significantly from baseline (Fig. 2b,c), indicating that TS-projecting dopamine neurons send very few collaterals to other regions. We recorded the population activity of dopamine neurons in SNL, the primary location of TS-projecting dopamine neurons (Fig. 2 and Supplementary Fig. 5), using fiber photometry. Like dopamine axon signals in TS, signals from dopamine neurons in SNL covaried with tone intensity (Fig. 1c), and their small response to reward was not significantly modulated by reward volume (Fig. 1c).

Finally, we recorded specifically from retrogradely labeled TS-projecting dopamine neurons using self-inactivating rabies virus (Fig. 1d and see Methods). Consistent with the activity we observed from dopamine axons in TS, we found that activity in TS-projecting dopamine neurons in SNL covaried with tone intensity (Fig. 1d) but not with water reward value (Fig. 1d). These neurons displayed increased responses to novel external stimuli as well, consistent with the idea that the signal was modulated by both external stimulus intensity and novelty (Supplementary Fig. 6). These results indicate that the unique activity observed in dopamine axons in TS was not due to local modulation at the axon level in TS, but rather reflected the activity at the cell bodies of this unique population in SNL.

Stimulation of dopamine axons in TS causes avoidance and lesion of TS-projecting dopamine neurons reduces avoidance in a choice task. It is widely accepted that the activation of dopamine neurons is positively reinforcing. In other words, dopamine release increases the frequency of actions or decisions that elicit dopamine release. Our finding that the activity of TS-projecting dopamine neurons differs fundamentally from the activity of VS-projecting dopamine neurons suggests that TS-projecting dopamine neurons could have a different function. Therefore, we tested whether direct

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**Fig. 2 | TS-projecting dopamine neurons are mainly localized in SNL and do not send substantial collaterals to other regions.**

a. Midbrain dopamine neurons labeled with anti-TH antibody (magenta) and TS-projecting dopamine neurons labeled with GFP (green). b. Forebrain dopamine axons (magenta) and the axons of TS-projecting dopamine neurons (green). DMS, dorsomedial striatum; DLS, dorsolateral striatum; LHb, lateral habenula; Ce, central amygdala; BLA, basolateral amygdala. c. Schematic for labeling TS-projecting dopamine neurons and their axons. d. Distribution of GFP-labeled (green) cell bodies and TH-labeled cell bodies in the midbrain (mean ± s.e.m. across n = 6 animals; SNC: t = 5.042, P = 0.0040, n = 6 animals, t test; SNL: t = 9.36, P = 0.00023, n = 6 animals, t test). e. Distribution of GFP-labeled axons in the forebrain and distribution of TH-labeled axons (mean ± s.e.m. across n = 8 animals; TS: t = 7.46, P = 0.00029, n = 8 animals, t test with Tukey correction for multiple comparisons). **P < 0.005, two-sided t test with Tukey correction for multiple comparisons**

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stimulation of dopamine axons in TS is reinforcing in a choice task (Fig. 3a). First, mice were trained to enter a central port to initiate a trial and to then choose one of two side ports associated with different outcomes (Fig. 3b). In this task, mice preferred the port associated with a large amount of water over a smaller amount of water and avoided the port associated with airpuff or bitter taste (quinine; Fig. 3c). In short, mice learned to develop choice biases according to the outcomes within a session.

Next, we examined the effect of optogenetic stimulation of dopamine axons in TS in a choice task. After mice learned the choice task, the axons of VS-projecting or TS-projecting dopamine neurons were optogenetically activated using a light-gated ion channel, channelrhodopsin-2, in one of the two choice ports while mice received reward (Fig. 3d and Supplementary Fig. 7). The stimulation port was pseudorandomly assigned in each session. Stimulation in VS biased the animals’ choices toward the port associated with stimulation (Fig. 3d), consistent with the idea that dopamine acts as positive reinforcement. On the other hand, stimulation in TS caused a bias against the port associated with stimulation (Fig. 3d). These results demonstrate that the optogenetic activation of dopamine axons in VS and TS have opposite effects on animals’ behavioral choices. Optogenetic activation of dopamine axons in TS caused avoidance of a behavioral choice associated with activation, instead of acting as a positive reinforcer of the choice.

Next, we tested whether ablation of TS-projecting dopamine neurons using a selective neurotoxin, 6-hydroxydopamine (6-OHDA), had an effect on avoidance behavior (Fig. 4). The 6-OHDA injections in TS specifically reduced dopamine innervation of TS and dopamine cell bodies in SNL without affecting neighboring dopamine axons in the striatum or amygdala and without affecting noradrenaline neurons in the locus coeruleus (Fig. 4a and Supplementary Fig. 8), consistent with our rabies-based axon-labeling data (Fig. 2). The lesioned mice did not exhibit differences in general locomotor activity (Supplementary Fig. 9).

We examined choice bias using different outcomes at two water ports (Fig. 4 and Supplementary Fig. 10). When choosing between different volumes of water reward, both control and lesioned mice showed a clear preference for the larger volume of water (Fig. 4c). However, when given a choice between water and water + airpuff, lesioned mice did not show systematic bias away from the side associated with airpuff as a population (Fig. 4c and Supplementary Fig. 10a,b) and received more airpuffs total in a session (Fig. 4d). Of note, we show here results of the first session in which animals experienced airpuff (see Methods), and during this single session individual lesioned mice displayed a variety of port biases, consistent with the idea that random choice (i.e., choosing both ports equally when both have the same value) is not necessarily a default strategy when random choice is not advantageous\(^2,26\). Notably, choice stickiness, or a ‘win–stay’ strategy, was observed in a similar frequency in a control session (choosing between two water ports) between control and lesioned mice (Supplementary Fig. 10c). However, in an airpuff session, whereas control mice repeated the same choice (stay) more often after the choice of a water port than after the choice of an airpuff port, lesioned mice showed a similar frequency of stay after the choice of either port (Supplementary Fig. 10c), indicating that lesioned mice did not acquire different choice preference even right after experiencing water + airpuff versus only water.

Unexpectedly, the lesioned animals’ immediate behavioral responses to airpuff remained largely unimpaired. Lesioned mice still displayed an immediate retreat with a similar retreat distance as controls in response to the first airpuff (Fig. 5a–c and Supplementary Fig. 10d). Over the course of the session, however, the lesioned mice responded very differently. While control mice continued to retreat from airpuffs over many trials, lesioned mice retreated significantly less after a few trials (Fig. 5a–c and Supplementary Fig. 11). Thus, whereas detection of airpuff and initial retreat responses to airpuff itself were intact, lesioned mice did not maintain the retreat responses in subsequent trials. These results suggest that there is a system responsible for the initial retreat behaviors (‘fixed reaction’\(^21\)), independent of TS-projecting dopamine neurons, and that TS-projecting dopamine neurons are responsible for maintenance of the retreat. Consistent with a role in maintenance or reinforcement of avoidance, dopamine axons in TS (in intact mice) responded strongly to airpuffs and the signals remained high during this task (Fig. 5d–f and Supplementary Fig. 10e), much like the signals we observed in headfixed mice (Supplementary Fig. 3).

Notably, lesioned mice were able to learn from other negative events such as bitter taste and water reduction similarly to control mice (Fig. 4c). This suggests that TS-projecting dopamine neurons were not responsible for learning from all types of negative events.
This is consistent with our observation that the activity of dopamine axons in TS increased in response to external stimuli such as tone and airpuff, but not in response to the bitter taste or omission of water (Fig. 1e). To determine whether the effects of lesion on choice preference were dependent on dopamine, we pharmacologically inhibited D1 dopamine receptors in TS and observed effects similar to those obtained in 6-OHDA-lesioned mice (Fig. 4e,f and Supplementary Fig. 12). Thus, dopamine in TS is critical for avoiding airpuff, and this is mediated at least partially by D1 receptor signaling.

**Midbrain dopamine neurons projecting to TS reinforce avoidance of novel objects.** Our results demonstrate that dopamine neurons in TS are important for learning to avoid airpuff punishment. However, TS-projecting dopamine neurons are excited not only by airpuff, but also by seemingly neutral novel stimuli of multiple modalities (Supplementary Fig. 3). It is not immediately clear what airpuff and novelty could have in common. To understand the function of novelty signals in TS-projecting dopamine neurons, we first examined animals’ behavioral responses to a novel object (Fig. 6). When animals encounter novelty, they typically display elevated exploration, orientation, or approach to the novel stimulus compared to a familiar one (Fig. 6a). Consistent with these observations, our mice approached novel objects more frequently than familiar objects (Fig. 6a). However, novel objects caused a more intricate behavior than simple approach. Mice frequently performed ‘bouts’ of investigation, in which they approached the novel object and then quickly retreated (Figs. 6a,b and 7a,b, Supplementary Fig. 13, and Supplementary Video 1). The mice repeated these short bouts multiple times. The bouts became gradually longer, and the mice spent more time at the vicinity of the novel object over days. These approach–avoidance conflicts have been observed across various animal species, including humans, and have been interpreted as an unstable equilibrium of curiosity and timidity (described as “weal or woe” by William James)5, or a sense of potential fear.

To examine the role of TS-projecting dopamine neurons in this approach–retreat behavior, we recorded signals from dopamine axons in TS as mice interacted with a novel object in a familiar environment. Signals in TS increased as mice reached the closest point of approach to the novel object in the bout (as they began to retreat), but not as they approached it (Fig. 6b–c). The responses in TS were significantly larger when mice retreated from a novel object than from a familiar object or from the same location in an open field (Fig. 6c). The signals in TS were not correlated with the animals’ velocity or with the initiation of movement, indicating that the signal may not be directly related to motor activity (Supplementary Fig. 14). To control for motion artifacts, we recorded from control mice expressing GFP instead of GCaMP and observed no obvious artifacts that could explain the GCaMP signals (Supplementary Fig. 14).

Next, we tested whether and how TS-projecting dopamine neurons regulate animals’ responses to novelty by lesioning TS-projecting dopamine neurons using 6-OHDA. When the lesioned mice first encountered a novel object, they exhibited behavior similar to that of control mice: an approach followed by a quick retreat (Fig. 7a,b, Supplementary Fig. 13, and Supplementary Video 2). Strikingly, after a few bouts, the lesioned animals began to spend a much longer time near the novel object per bout (Fig. 7a,b and Supplementary Videos 3 and 4). In total, lesioned mice spent a significantly longer time near the object (Fig. 7b), but they did not approach the object more frequently than control mice (Fig. 7b). In short, mice with an ablation of TS-projecting dopamine neurons showed relatively normal novelty responses (approach–retreat) at first, but the avoidance component of this response to the novel object quickly diminished.

**Fig. 4 | Ablation of TS-projecting dopamine neurons eliminates choice bias against a threatening stimulus.** a, Schematic of ablation of TS-projecting dopamine neurons and examples of the effect of saline (top) or 6-OHDA (bottom) on dopamine axons in TS (‘tail’), but not more anterior striatum (‘anterior’). Axons labeled with anti-TH antibody (magenta). b, Experimental design. c, Normalized choice bias (mean ± s.e.m. across n = 10 animals per group) for airpuff, quinine, and the reduction of water in saline (blue); airpuff: t = −4.13, P = 0.0026, n = 10 animals per group; quinine: t = −10.34, P = 0.00000272, n = 10 animals per group; water reduction: t = 3.045, P = 0.014, n = 10 animals per group). 6-OHDA (red); airpuff: t = 0.31, P = 0.77, n = 10 animals per group; quinine: t = −7.78, P = 0.0000028, n = 10 animals per group; water reduction: t = −2.48, P = 0.035, n = 10 animals per group) animals. Saline × 6-OHDA: airpuff: t = 2.59, P = 0.0034, n = 10 animals per group; quinine: t = 1.40, P = 0.18, n = 10 animals per group; water reduction: t = 0.059, P = 0.95, n = 10 animals per group. Solid dots indicate mean and transparent dots indicate each animal. d, Total number of ‘incorrect’ choices (mean ± s.e.m. across n = 10 animals per group) of water + X in saline (blue) and 6-OHDA (red) animals in a session (saline × 6-OHDA: t = 2.29, P = 0.0341, n = 10 animals per group). Solid dots indicate mean and transparent dots indicate each animal. e, Schematic of D1 antagonist application. f, Choice bias (mean ± s.e.m. across 6 animals) for airpuff following acute application of saline (blue) or D1 antagonist (green). Saline × D1 antagonist: t = 4.44, P = 0.0068, n = 6 animals, paired t test. Solid dots indicate mean and transparent dots indicate average across sessions for each animal. *P < 0.05, **P < 0.005, n/s, nonsignificant; two-sided t test
Notably, like retreat from airpuff, initial retreat from a novel object was independent of TS-projecting dopamine neurons. This suggests that dopamine in TS has a role in maintaining or reinforcing avoidance of objects based on their novelty. The retreats eventually diminished over days, consistent with recording data showing that responses in dopamine axons in TS decayed slowly during the first session but were much smaller in later sessions (Fig. 6c and Supplementary Fig. 15).

Conversely, closed-loop optogenetic stimulation of dopamine axons in TS while animals approached a familiar object reduced the duration of mice’s interaction with the object, which could be interpreted as a reinstatement of avoidance. Bout lengths became shorter following the activation of dopamine axons in TS (Fig. 7c) and remained shorter after the stimulation had ended (Fig. 7c). Because some dopamine neurons express a synaptic vesicular transporter for glutamate, Vglut2, which allows co-release of glutamate, we examined the function of glutamate co-release from dopamine neurons during novelty responses. We genetically knocked out Vglut2 in dopamine neurons (See Methods) and found that mice with no Vglut2 in dopamine neurons showed approach–retreat behaviors similar to those of control littermates (Supplementary Fig. 15), suggesting that glutamate release from dopamine neurons is not essential for novel object avoidance behavior.

Discussion

When comparing inputs to dopamine neurons with different projection targets, we previously identified TS-projecting dopamine neurons based on their unique set of inputs: they receive relatively little input from VS and more input from dorsolateral-shifted regions such as the subthalamus and globus pallidus. By recording activity from dopamine axons in VS, dorsomedial striatum, dorsolateral striatum, and TS, we found that the activity of dopamine axons in TS is also distinctively different. We showed that dopamine axons in TS are excited by both aversive and neutral stimuli and that they have some characteristics of prediction error: they signal the prediction of a stimulus and their responses to the stimulus itself are suppressed by prediction. In the present study, we more systematically and parametrically examined dopamine responses in TS (focusing on unconditioned stimuli) and examined the function of this signal as a reinforcer. We found that dopamine axons in TS were monotonically modulated by the intensity and novelty of external stimuli, reminiscent of a previous proposal that the early phase of dopamine signals encodes stimulus intensity, but not aversiveness itself. In a sense, TS-projecting dopamine neuron activity could be interpreted as the extreme case of having only this intensity-encoding phase without encoding value, while value-encoding is the main phase in canonical dopamine neurons. In addition, our results indicated that dopamine axons in TS were not excited by all stimuli. Instead, we found that they were specifically excited by external stimuli, but not by ingestion-related stimuli (for example, bitter taste). Further, we found that TS-projecting dopamine neurons encoded both the novelty and intensity of stimuli of multiple modalities. Consistent with their activity, we found that TS-projecting dopamine neurons were important for both avoidance of airpuff...
punishment and avoidance of a novel object, but not for avoidance of all types of negative outcomes. Finally, we found that stimulation of TS-projecting dopamine neurons facilitated avoidance and that ablation of TS-projecting dopamine neurons reduced avoidance.

Combining these insights, we propose that dopamine axons in TS encode the physical salience (such as intensity and novelty) of external stimuli to signal potential external threats in the environment. Of note, threat and aversiveness have been distinguished in many studies, and for good reason. For example, a previous study found a behavioral difference between responses to external threats and aversive taste, showing that visual stimuli associated with external threats, but not aversive taste, draw attention in monkeys. These differences are potentially connected to the difference between fear and disgust caused by these stimuli. Consistent with these differences, our data showed that different brain systems underlie learning from different types of punishments and that dopamine in TS is specifically involved in avoidance of external threats.

Dopamine in TS reinforces threat avoidance by signaling external threat. Striatal dopamine has previously been proposed to be involved in avoidance within the framework of the canonical view of dopamine function. A series of studies showed that dopamine neurons are excited by the prediction of successful avoidance of aversive events (‘safety’) and that this safety signal functions to promote successful avoidance. This theory beautifully incorporates the function of dopamine in active avoidance into the framework of value-coding: dopamine neurons signal both reward and safety.

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**Fig. 6** | Dopamine axons in TS are active as animals retreat from novel objects but not as they approach novel objects. **a**, Left: mice exhibit bouts of approach and retreat when investigating novel objects. Right: frequency of approach to objects (mean ± s.e.m. across n = 10 animals; novel × familiar: t = 3.67, P = 0.001, n = 10 animals, paired t test; novel × no object: t = 4.41, P = 0.0017, n = 10 animals, paired t test). Solid dots indicate mean and transparent dots indicate each animal. **b**, Example GCaMP trace from dopamine axons in TS (green) aligned to animal’s distance from the novel object (black), with dotted lines indicating nearest approach per bout. **c**, Left: example GCaMP signals aligned to onset of retreat. Bouts were sorted based on time of approach, shown as a gray line. Right: average GCaMP responses across n = 6 animals to novel object (cyan), familiar object (magenta), and no object control (black; novel × familiar: t = 3.04, P = 0.021, n = 6 animals, paired t test; novel × no object: t = 3.65, P = 0.015, n = 6 animals, paired t test). Solid dots indicate mean and transparent dots indicate each animal. *P < 0.05, **P < 0.01, two-sided t test.

**Fig. 7** | Ablation of TS-projecting dopamine neurons reduces retreat from novel objects and stimulation partially reestablishes avoidance of novel objects. **a**, Example paths from saline (blue) and 6-OHDA (red) mice during the first 5 min of a 10-min novel object interaction. No-object control paths also shown for comparison (black). **b**, Left: time-course of bout duration (mean ± s.e.m. across n = 10 animals per group) in saline (blue) or 6-OHDA (red) mice during two 10-min sessions denoted by dotted lines (trial 1: saline × 6-OHDA: t = 1.67, P = 0.11, n = 10 animals per group, unpaired t test; all trials: saline × 6-OHDA: t = 4.16, P = 0.0016, n = 10 animals per group, unpaired t test). Right: number of approaches to (saline × 6-OHDA: t = 0.665, P = 0.514, n = 10 animals per group, unpaired t test) and fraction of time spent near (saline × 6-OHDA: t = 5.77, P = 0.000018, n = 10 animals per group, unpaired t test) a novel object (mean ± s.e.m. across n = 10 animals per group). Solid dots indicate mean and transparent dots indicate each animal. **c**, Stimulation of TS dopamine axons during interaction with a familiar object (control: first 15 min × stimulation: t = 0.40, P = 0.69, n = 16 sessions from 8 animals, paired t test; first 15 min × last 15 min: t = 0.031, P = 0.96, n = 16 sessions from 8 animals, paired t test; ChR2: first 15 min × stimulation: t = 3.40, P = 0.0039, n = 16 sessions from 8 animals, paired t test; first 15 min × last 15 min: t = 2.25, P = 0.040, n = 16 sessions from 8 animals, paired t test). Solid dots indicate mean and transparent dots indicate each session. *P < 0.05, **P < 0.01, n/s, nonsignificant; two-sided t test.
Fig. 8 | Separate axes for dopamine-based reinforcement learning. Top: a model of dopamine-based reinforcement learning using value prediction error (PE) signals conveyed to VS primarily by VTA dopamine neurons. Bottom: a model of dopamine-based reinforcement learning using threat signals conveyed to TS primarily by SNL dopamine neurons.

as positive value. However, this rule does not apply to dopamine in TS. Contrary to this idea, dopamine axons in TS do not encode outcome values, and this system operates differently from the canonical dopamine system.

Although they encode different information, dopamine in TS may function, like canonical dopamine, as a reinforcement signal: primarily affecting future behaviors. Alternatively, dopamine in TS may regulate ongoing behaviors on a moment-by-moment basis. Our results suggest a role in reinforcement. First, in our choice tasks, the timing of optogenetic activation (when mice entered a reward port) and subsequent choice on the next trial were well separated: avoidance behavior manifested in subsequent trials without concurrent optogenetic activation of TS-projecting dopamine neurons. Similarly, in novel object exploration, the effect of optogenetic activation lingered even after the activation ended. Additionally, the initial retreats from airpuff and novel objects were intact in lesioned animals, and only subsequent responses were affected by lesions. These results suggest that, in our tasks, dopamine in TS primarily functioned as a reinforcement signal, although we cannot formally exclude the possibility that it also affects ongoing behavior.

Optogenetic activation of dopamine axons in TS caused choice bias against the port associated with stimulation. In theory, there are at least two ways to explain this effect. One possibility is that a dopamine increase in TS may suppress preceding behaviors (i.e. it may be ‘inhibitory’ or ‘weakening’), in a manner opposite to canonical dopamine. Alternatively, a dopamine increase may reinforce an avoidance behavior or a threat prediction by Pavlovian association.

Our observations favor the latter hypothesis. After the ablation of TS-projecting dopamine neurons, mice displayed a similar number of approaches to novel objects, while retreat from novel objects differed compared to control mice. This suggests that dopamine plays a role in maintaining a high level of novelty avoidance or threat prediction, rather than discouraging approach. We therefore propose that dopamine in TS reinforces a specific type of behavior (the avoidance of threatening stimuli) by updating or maintaining a high level of threat prediction based on evidence acquired about threatening stimuli.

The amygdala has long been studied in relation to fear and fear-based learning. Although they are neighboring regions, TS and amygdala are often contrasted in functional experiments. For example, even the ablation of all medium spiny neurons in the striatum hardly affects fear conditioning by electric shock, which is the most commonly used model for amygdala studies. Two possibilities are that TS is more specialized for behaviors based on ‘threat prediction’ without experiencing actual pain (see below) or that TS is especially important when animals consider both reward and threat. Accumulating studies show that amygdala is important for learning from reward as well as from threat. Consistent with this broad function, amygdala projects to both VS and TS and receives dopaminergic projections from a wide range of nuclei in supramammillary areas, ventral tegmental area, SNC, and SNL.

By contrast, TS receives dopamine innervation primarily from SNL. How these structures collaborate during avoidance is an important question to address in the future.

Similarities between trial-and-error learning and novel object exploration. Although performing a choice task with a rigid trial-based structure and self-directed investigation of novel objects are seemingly very different behaviors, we found a potentially unifying explanation for dopamine function across them. Animals typically show ‘unstable equilibrium’ of approach–retreat to novelty. Multiple studies have found that dopamine in other brain areas, such as dorsomedial striatum and dorsolateral striatum, is important for exploration triggered by novelty. Our findings, together with results from these studies, suggest that dopamine plays a role on both sides of this equilibrium. Approach–retreat behaviors in foraging have been interpreted as ‘risk assessment’. In this sense, the animals’ novelty exploration can be viewed as a sequential process of learning and decision based on value and threat (similar to sequential choice behavior). The distortion of equilibrium in such a situation potentially causes maladaptive novelty-seeking or excess fear of the strange in development, in psychiatric conditions such as addiction and autism, and under stress. Our data suggests that dopamine may function in event-by-event behavioral updating, not only in value-based decision-making but also in a broader range of behaviors than was previously thought.

Conclusion

While our findings indicate that dopamine in TS and canonical dopamine systems serve different functions, we propose that these dopamine systems may function through a similar mechanism using Pavlovian prediction. Thus, a functional difference between these systems may come from the information that these dopamine signals convey and the behavioral outcomes owing to this difference of information, rather than in their algorithms. Whereas canonical dopamine in VS provides bidirectional signals along the axis of value, dopamine in TS provides information along a different dimension, which includes the intensity and novelty of external stimuli (Fig. 8). Notably, because TS is a sensory region of the striatum, receiving inputs mainly from thalamus and sensory cortices such as visual and auditory cortex, threat information carried by dopamine potentially directly impacts stimulus representation in TS.

Notably, the dynamics of dopamine responses in these systems are quite different. A previous study found that dopamine responses in VS and TS are initialized in an opposite manner: responses to a novel stimulus in dopamine axons in VS are initialized at zero, whereas responses in dopamine axons in TS are initialized at a high level, although it was not clear why their dynamics are opposite. Combined with the present finding that TS-projecting dopamine neurons reinforce threat avoidance, the functional significance of
these dynamics begins to make sense. Dopamine- striatum systems seem to have cautious initialization: high for threat and low for value. A threat-prediction system with high initialization may be critical to learning to avoid unknown threats, which are potentially harmful, without experiencing actual outcomes such as pain or death. Further, dopamine axons in TS do not show inhibition ('dip') with omission of the outcome15, possibly indicating that the threat prediction system does not use active weakening but rather gradually erases the prediction.

The existence of separate systems for different types of reinforcement signals resembles the anatomically segregated reward and punishment dopamine systems observed in fruit flies17, although the nature of the reinforcement signals are not identical in nonhuman primates, a recent study reported that dopamine neurons in caudolateral SNC that project to caudate tail also did not respond to reward but instead responded to salient visual stimuli18, suggesting a degree of similarity of dopamine in mouse TS and primate caudate tail, although more comparative studies are needed in the future to confirm these similarities. These multiple reinforcement systems may function in parallel or cooperate, but they may also compete when animals decide between options that contain both potential value and potential threat, such as during interactions with novel stimuli. If this is the case, understanding the balance or equilibrium between these systems will be crucial for understanding the rich behavioral dynamics that animals display in natural environments.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-018-0222-1.

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Author contributions
W.M.: conceptualization, investigation, formal analysis, writing (original draft); K.A.: methodology (novel object); R.A.: methodology (rabies virus); N.U.: conceptualization, supervision, writing (review and editing); M.W.-U.: conceptualization, supervision, writing (original draft).

Competing interests
The authors declare no competing interests.

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Methods

Animals. For this study, 90 mice between 3 and 6 months old were used. Dopamine transporter (DAT)-cre (B6.129S7-Tph1tm1Myr/Mmnb/J, Jackson Laboratory; RRID:IMSR_JAX:005680) heterozygous mice were used for recording signals from dopamine axons expressing GCaMP6f for stimulation of dopamine axons expressing ChR2, and for anatomical examination of TS-projecting dopamine neurons. All mice were backcrossed with C57BL/6J (Jackson Laboratory). C57BL/6J mice were used for ablation of TS-projecting dopamine neurons using 6-OHDA. Ioxp-flanked Vglut2 (Vglut2loxP/loxP; Slc17a6tm1(Cre)Bkn/J, Jackson Laboratory; RRID:IMSR_JAX:006660) heterozygous mice were used for recording from dopamine neurons, we unilaterally performed a single surgery with two key components: (i) AAV-DIO-ChR2-YFP surgical procedure. To express ChR2 specifically in dopamine neurons, we unilaterally injected 250 nL of AAV5-FLEX-TC (1.0×1012 particles/mL, UNC Vector Core) into the VTA and SNC (500 nL total). AAV1-CAG-FLEX-GFP (4.5×1012 particles/mL, UNC Vector Core) was injected as a control. Virus injection lasted several minutes, and then the injection pipette was then slowly removed over the course of several minutes to prevent damage to the tissue. We then performed optic fiber implants into VS and TS. To do this, we first slowly lowered optical fibers (200-µm diameter, Doric Lenses) into the striatum. Once fibers were lowered, we first attached them to the skull with UV-curing epoxy (Thorlabs, NOA81) and then a thick layer of black Ortho-Jet dental adhesive (Lang Dental). After waiting 15 min for this to dry, we applied a very small amount of rapid-curing epoxy (Devcon, A00254) to attach the fiber cannulas even more firmly to the underlying adhesive. After waiting 15 min for the epoxy to dry, the surgery was complete.

Rabies surgical procedure for anatomical examination. To label TS-projecting dopamine neurons and their axons, we first injected 250 nL of AAV5-FLEX-TGa (1.0×1012 particles/mL, UNC vector core) into the VTA and SNC of mice expressing Cre in dopamine neurons (DAT-cre animals). This would allow EnvA-pseudotyped rabies virus to infect these cells. After waiting 3 weeks for AAV viral expression, we injected 500 nL of SADdG-GFP(EnvA) (5×10^7 pfu/mL, Salk Institute Vector Core) into TS. This virus retrogradely infected dopamine neurons projecting to TS, brightly labeling their cell bodies and axons throughout the brain due to the high viral copy number. After waiting 1 week for rabies viral expression, animals were killed and brains were sectioned for analysis. Notably, this method is extremely sensitive compared to AAV-based approaches due to the high copy number of rabies virus.

Surgical procedures. All surgeries were performed under aseptic conditions with animals anesthetized with isoflurane (1–2% at 0.5–1.0 L/min). Analgesia (ketoprofen, 5 mg/kg, I.P.; buprenorphine, 0.1 mg/kg, I.P.) was administered postoperatively. We used the following coordinates to target our injections and implants:

- (VTA) bregma: −3.0 mm, lateral: 0.6 mm, depth: 4.5–4.0 mm;
- (SNc) bregma: −3.3 mm, lateral: 1.6 mm, depth: 3.5–4.0 mm;
- (SNL) bregma: −3.2 mm, lateral: 2.0 mm, depth: 3.5 mm;
- (VS) bregma: 1.0 mm, lateral: 1.25 mm, depth: 3.85 mm; and
- (TS) bregma: −1.0 mm, lateral: 3.25 mm, depth: 2.5 mm.

GCaMP surgical procedure. To prepare animals for GCaMP recording in the striatum, we performed a single surgery with three key components: (i) AAV-FLEX-GCaMP6f virus injection into the midbrain, (ii) headplate implantation, and (iii) one or more optic fiber implants into the striatum. To express GCaMP6f specifically in dopamine neurons, we unilaterally injected 250 nL of AAV5-CAG-FLEX-GCaMP6f (1.0×10^12 particles/mL, Penn Vector Core) into both the VTA and SNC (500 nL total). AAV1-CAG-FLEX-GFP (4.5×10^12 particles/mL, UNC Vector Core) was injected as a control. Virus injection lasted several minutes, and then the injection pipette was slowly removed over the course of several minutes to prevent damage to the tissue. So that mice could be headfixed during recording, we installed a headplate onto each mouse with C and B Metabond adhesive cement (Parkell, Edgewood, NY). We used circular headplates to ensure that the skull above the striatum would be accessible for fiber implants. Finally, during the same surgery, we also implanted optic fibers (200-µm diameter, Doric Lenses, Canada) into the VS and/or TS (or two one fiber per mouse). To do this, we first slowly lowered optical fibers into the striatum. Once fibers were lowered, we first attached them to the skull with UV-curing epoxy (NOA81, Thorlabs, NJ), and then a layer of black dental adhesive (Ortho-Jet, Lang Dental). After waiting 15 min for this to dry, we applied a very small amount of rapid-curing epoxy (Devcon, A00254) to attach the fiber cannulas even more firmly to the underlying glue and headplate. We used magnetic fiber cannulas (Doric, MFC_200/245) and the corresponding patch cords to allow for recordings in freely moving animals. After waiting 15 min for the epoxy to dry, the surgery was complete.

To record from dopamine neurons in SNL regardless of projection site, we injected GCaMP6f into the midbrain (as above), but also implanted the optic fiber into the midbrain. To record from dopamine neurons in SNL that project to TS, we performed two surgeries. In the first surgery, we injected 250 nL of AAV5-FLEX-TGa (1.0×10^12 particles/mL, UNC vector core) and 250 nL of AAV8-FLEX(Dfr)-GCaMP6f (1.0×10^12 particles/mL, UNC Vector Core) into the midbrain. After waiting 2 weeks to allow AAV expression, we then performed a second surgery and injected 500 nL of SiR-FLPo(EnvA) (1.1×10^7 plaque-forming units (pfu)/mL) into TS and implanted an optic fiber into SNL.

ChR2 surgical procedure. To prepare animals for unilateral ChR2 stimulation, we performed a single surgery with two key components: (i) AAV-DIO-ChR2-YFP virus injection into the midbrain, and (ii) one or two optic fiber implants into the striatum. To express ChR2 specifically in dopamine neurons, we unilaterally injected 250 nL of AAV5-EF1α-DIO-ChR2-YFP (1.1×10^12 particles/mL, UNC Vector Core) into both the VTA and SNC (500 nL total). AAV1-CAG-FLEX-GFP (4.5×10^12 particles/mL, UNC Vector Core) was injected as a control. Virus injection lasted several minutes, and then the injection pipette was then slowly removed over the course of several minutes to prevent damage to the tissue. We then performed optic fiber implants into TS and VS. To do this, we first slowly lowered optical fibers (200-µm diameter, Doric Lenses) into the striatum. Once fibers were lowered, we first attached them to the skull with UV-curing epoxy (Thorlabs, NOA81) and then a thick layer of black Ortho-Jet dental adhesive (Lang Dental). After waiting 15 min for this to dry, we applied a very small amount of rapid-curing epoxy (Devcon, A00254) to attach the fiber cannulas even more firmly to the underlying adhesive. After waiting 15 min for the epoxy to dry, the surgery was complete.

Behavioral experiments. Headfixed classical conditioning behavioral experiment. After surgery, mice were given 3 weeks to recover and become habituated to the installed headplate and implanted optic fibers. Additionally, this allowed time for viral expression. After this recovery period, mice were handled for 2–3 d and water deprived. Then, mice were habituated to being headfixed for 3 d. During these days, mice were headfixed for 5–10 min and given unexpected water at random intervals (drawn between 1 and 20 s, with a mean of 10 s and a normal distribution). This allowed mice to become habituated to being headfixed. After this, mice were trained to perform a task with four trial types: (i) Odor A → reward (90%; immediately) Odor A → reward omission (10%); (ii) Odor B → no outcome; and (iii) free water (Supplementary Fig. 4). Mice were trained in this task for 10 d before recording neuronal responses to unexpected stimuli of different modalities and intensities. To observe GCaMP signals elicited by unexpected stimuli of different modalities and intensities, we interspersed these outcomes within the context of classical conditioning.

To record from each day, either unexpected water (of varying volumes), unexpected tone (of varying frequencies), unexpected airpuff (of varying pressures), or unexpected quinine (0.001 M) were interspersed with the other trial types. To ensure that the order of this
stimulus presentation did not impact the data, different mice received the stimuli in a different order. To minimize the animals’ ability to use auditory or visual information, such as the click of the valve that released water or air puff (~45 dB measured from position of the animal with valve outside of the behavioral setup and mask), the training was done in darkness with a constant background noise of ~50 dB.

We used a range of 5 kHz tones: 50 dB, 65 dB, 75 dB, 90 dB, and 100 dB. For water volume manipulation, we used 1 µL, 3 µL, 5 µL, 10 µL, and 20 µL. For air puff pressure manipulation, we used 0.2 atm, 0.4 atm, 0.6 atm, 0.8 atm, and 1.0 atm. For quinine, we used a concentration of 0.001 M. On trials with expected water, 10 µL of water was delivered.

Each session consisted of approximately 250 trials and took approximately 45 min. During these sessions, GCaMP signal was continuously sampled and the excitation laser was constantly on at approximately 0.05 mW, measured at the tip of the patch cord outside of the animal. Because unexpected outcomes came at a low frequency, we used the same order of all trials in the session. We defined normalized choice (%) as the percentage of times the mouse chose the port in the total number of choice minus general choice-bias (the percentage of times the mouse chose the same port on the control day without outcome manipulation minus 50). We measured reward distances using the total distance traveled (based on the center of mass of the mouse, extracted from video) in the 1 s period following air puff.

**Novel object interaction.** Mice were first habituated to a box of roughly the same dimensions as their home cage with white floor, white walls, and no objects. This habituation was done for 30 min per day for 5–10 d. Mice with fiber implants were connected to optical recording capable during these sessions so that they would become habituated to freely moving while recordings took place. The recording cable was hung from the ceiling such that it would be able to reach all corners of the cage with minimum weight on the animal. General locomotor activity was measured after these habituation sessions.

After these habituation sessions, a novel object (a Lego toy block) was placed in the center of the box and mice were observed on the next day for all the animals’ interactions with the object in one 10-min session every day for 1 week. We compared GCaMP responses on the first day to the last day to compare responses during interactions with a novel object or a familiar object. We compared these signals to signals observed when the mouse was freely moving in the same box with no object in the center and happened to move from the center to the periphery.

**Familiar object interaction with stimulation.** After habituation to a novel object (30 min per day 5–10 d), we termed it a ‘familiar’ object. We placed mice into a behavioral arena with a familiar object and recorded behavior for 15 min as a baseline. Then, during the next 15 min block, we performed closed-loop stimulation of dopamine axons in TS anytime mice were within a 50-mm radius of the object. As in the choice task, light was delivered in 10 pulses of 10 ms in duration, with 40 ms between pulses (i.e., 20 Hz stimulation for 500 ms). Stimulation was performed with a 473-nm laser at 20 mW. This stimulation was repeated until mice left the imaginary radius around the object. After this 15-min block, we stopped stimulating when mice interacted with the object, to observe whether any changes in behavior persisted.

**GCaMP detection and analysis.** Fiber photometry allows for recording of the activity of genetically defined neural populations in mice by expressing a genetically encoded calcium indicator and chronically implanting an optic fiber13,53,64–67. The optic fiber (200-µm diameter, Doric Lenses) allowed chronic, stable, minimally disruptive access to deep brain regions and interfaced with a flexible patch cord (Doric Lenses) on the skull surface to simultaneously deliver excitation light (473 nm, LaserGlow Technologies) and collect GCaMP53,67 or GFP fluorescence emission. Activity-dependent fluorescence emitted by cells in the vicinity of the implanted fiber’s tip was spectrally separated from the excitation light using a dichroic, passed through a single-band filter, and focused onto a photodetector connected to a current preamplifier (SR570, Stanford Research Systems).

During recording, optic fibers were connected to patch cables, which delivered excitation light (473 nm) and collected all emitted light. The emitted light was subsequently beam filtered using a 556-nm beam-splitter followed by a 600-nm bandpass filter and collected by a photodetector (FDS10x10 silicone photodiode, Thorlabs) connected to a current preamplifier (SR570, Stanford Research Systems). This preamplifier output a voltage signal that was collected by a NIDAQ board (National Instruments). The NIDAQ board was connected to the same computer that was used to control odor, water, tone, and air puff delivery with LabView (National Instruments), so GCaMP signals could be readily aligned to task events such as reward delivery or tone delivery.

**GCaMP signals or GFP control signals were collected as voltage measurements from current preamplifiers.** The \( \Delta F/F \) measurement was calculated using a running median of 100 s, such that the voltage measurement at any point in time \( (F_t) \) was subtracted from the running median voltage \( (\bar{F}) \). Then divided by the running median voltage to find \( \Delta F/F = (F_t - \bar{F})/\bar{F} \). To quantify responses, we looked for ‘peak responses’ (negative or positive) by finding the point with the maximum absolute value during the 1-s window following the stimulus onset in each trial. To compare the response and baseline, baseline peak was obtained during 3–4 s before stimulus onset.
During novel object interaction, we defined ‘bouts’ of investigation by measuring distance traveled (using the center of mass) in the 1-s period following airpuff. These were thresholded such that the outline of the mouse could be identified, and then were processed in Matlab (MathWorks, Natick, MA, USA). Briefly, the videos captured using the FlyCap2 software that accompanies the camera, and videos from Backfly at 60 frames per s (fps) with H.264 video compression. The video was illuminated with infrared (IR) light to detect the animals. Video detection and analysis were used as the data in each figure. GCaMP signals or GFP control signals were measured in each animal, and the average signal was used to calculate the signal-to-noise ratio. For each animal, the total signal was normalized to the expected level (i.e., in the case of low signal-to-noise ratio, the denominator would be larger), allowing for comparison across animals with different baseline fluorescence. Additionally, this normalization corrected for the small amount of bleaching we observed over the course of the trial in the raw fluorescence (Supplementary Fig. 2). The average responses to a stimulus type were averaged within each animal, and these averages were used as the data in each figure. GCaMP signals or GFP control signals were collected through LabView during the training for offline analysis.

**Video detection and analysis.** We used infrared (IR) light to illuminate the arena and recorded video using a camera (BFLY-U3-0352M, Point Grey Research Backfly) at 60 frames per s (fps) with H.264 video compression. The video was captured using the FlyCap2 software that accompanies the camera, and videos were processed in Matlab (MathWorks, Natick, MA, USA). Briefly, the videos were thresholded such that the outline of the mouse could be identified, and then the center of mass, nose position, and minimum distance from the novel object were extracted for further analysis. To quantify movement velocity, we took the derivative of the position of the center of mass. Therefore, this measurement was sensitive only to large movements such as running and not sensitive to small movements such as grooming.

In the choice task, we extracted retreats from the reward port by finding the distance traveled (using the center of mass) in the 1-s period following airpuff. During novel object interaction, we defined ‘bouts’ of investigation by measuring when the mouse entered a 50-mm radius of the object. The beginning of the ‘approach’ was defined as when the animal began moving toward the object (first increase of velocity relative to the object directly preceding crossing into 50-mm radius), regardless of the animal’s distance from the object at that time. The beginning of ‘retreat’ was defined as when the animal began moving away from the object (first point of negative velocity relative to object after crossing into the 50-mm radius), regardless of the animal’s distance from the object at that time. The ‘-’ duration (of the bout) was defined as the duration of the time the animal remained within 50 mm of the object.

We compared the number of such bouts that animals made within the first 10-min session of novel object investigation (Fig. 6). To determine the fraction of time spent near the object, we simply measured how much of the 10-min session each animal spent within 50 mm of the object. For this and other measurements, we required only that the mouse’s nose crossed within 50 mm of the object, and not the center of mass. Because mice investigate by extending their nose toward a stimulus, their center of mass was often much further from the object than their nose.

**Histology and fluorescence analysis.** Animals were perfused using 4% paraformaldehyde and then their brains were sliced into 100-μm thick coronal sections using a vibratome and stored in PBS. These slices were then stained with rabbit anti-tyrosine hydroxylase (TH; AB152, EMD Millipore) at 4 °C for 2 d to reveal dopamine axons in the striatum, dopamine cell bodies in the midbrain, and other neurons expressing TH throughout the brain (such as noradrenaline neurons in the LC). These slices were then stained with fluorescent secondary antibodies and fluorescent Nissl at 4 °C for 1 d. Slices were then mounted in antifade solution (Vectashield antifade mounting medium, Vector Laboratories, H-1000) and imaged using a Zeiss LSM 700 inverted confocal microscope. The same laser settings and gain were used for all samples so that fluorescent signals could be compared across animals, and brightness was adjusted in ImageJ for figures.

To quantitatively compare across brains, we used two methods. (i) For cell body comparison, we manually counted the number of labeled cell bodies in the VTA, SNC, or SNL in each optical section. The boundaries were drawn manually for each section. (ii) For axon density comparison, we first defined areas for analysis using anatomical landmarks visible by autofluorescence or fluorescent Nissl. Then, we measured the average fluorescence intensity of rhod- GFP or anti-TH antibody in each region. We divided this by the average fluorescence intensity from an unlabelled brain (in the case of rhod-GFP) or from an unstained slice of the same brain (in the case of anti-TH antibody staining).

**Randomization, blinding, and data exclusion.** Randomization. For the GCaMP recording experiments, GFP control animals and GCaMP experimental animals were selected at random by the experimenter. Trial structure was pseudorandom (trials were randomly shuffled in blocks of 200 to ensure that all trial types would be presented in each session). For the 6OHDA lesion experiment, another lab member randomly selected mice to assign to either saline or 6OHDA groups. The experimenter did not know the identity of each animal until analysis was complete. For the D1 antagonist experiments, saline and 6OHDA sessions were done in a random order (randomized separately for each mouse).

**Blinding.** For the 6OHDA lesion studies, the experimenter was blind to the animals’ identities (control or lesion) during data collection and analysis. The identities of the animals were revealed to the experimenter only after analysis was complete. For the D1 antagonist experiments, the experimenter was not blinded and knew which solution was being infused (i.e., saline or D1 antagonist) at the time of the experiment.

**Data exclusion.** No animals were excluded from the study: all analysis includes data from all animals. However, after applying D1 receptor antagonist, we limited our analysis to the first 60 trials performed by the mice in each session to prevent off-target effects of the drug, due to potential spread from injection site.

**Statistical analyses.** Data analysis was performed using custom software written in Matlab (MathWorks, Natick, MA, USA). All code used for analysis is available on request. All statistical tests were two-sided. For statistical comparisons of the mean, we used one-way ANOVA and two-sample Student’s t tests, unless otherwise noted. Paired t tests were conducted only when the same mouse’s performance was being compared across multiple drug conditions (Fig. 4e,f) or different sessions (novel object versus familiar object sessions in Fig. 6). The significance level was corrected for multiple comparisons using Holm–Sidak’s tests unless otherwise indicated. All error bars in the figures are s.e.m.

No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. To be conservative, in most cases, we compared across animals rather than across trials or across sessions. Data distribution was assumed to be normal, but this was not formally tested.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability and code availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request. Similarly, any code used for analysis (Matlab scripts) is also available from the corresponding author upon reasonable request. More information regarding the statistical tests and methods is also available online in the Nature Research Reporting Summary.

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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  State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection

Labview 2010 was used to collect data both from head fixed mice (primarily fiber photometry signals from GCaMP-expressing mice) and to collect choice data during various choice tasks. FlyCapture2 was used to capture video of mice during choice tasks and novel object exploration.

Data analysis

MATLAB R2017B was used to analyze all data (photometry, choice data, and video data). Any codes used are available upon request.

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Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data and any code used for analysis are available upon request.
Life sciences study design

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Sample size
No formal power analysis was carried out. In all cases, we aimed for a sample size typical of similar studies in the field. In the 6OHDA lesion experiment, we aimed for a sample size large enough to demonstrate behavioral differences between groups. We analyzed the data by animal (n = number of animals) rather than by session or trial to be conservative.

Data exclusions
No animals were excluded from the study: all analysis includes data from all animals. However, after applying D1 receptor antagonist, we limited our analysis to the first 60 trials performed by the mice in each session to prevent off-target effects of the drug, due to potential spread from injection site.

Replication
Animals were not formally divided into separate groups for "proof of principle" and "replication". Instead, we pooled all data, and displayed data from each animal (along with the average and standard error) as much as possible.

Randomization
For the GCaMP recording experiments, GFP control animals and GCaMP experimental animals were selected at random by the experimenter. For the 6OHDA lesion experiments, another lab member randomly selected mice to be in either Saline or 6OHDA groups. For the D1 antagonist experiments, saline and 6OHDA sessions were done in a random order (randomized separately for each mouse).

Blinding
For the 6OHDA lesion studies, the experimenter was blind to the animals’ identities (control or lesion) during data collection and analysis. The identities of the animals were revealed to the experimenter only after analysis was complete. For the D1 antagonist experiments, the experimenter was not blind, and knew which solution was being infused (i.e. saline or D1 antagonist).

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ✗   | Unique biological materials |
| ✗   | Antibodies |
| ✗   | Eukaryotic cell lines |
| ✗   | Palaeontology |
| ✗   | Animals and other organisms |
| ✗   | Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ✗   | ChIP-seq |
| ✗   | Flow cytometry |
| ✗   | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials
All materials used are commercially available, and vendors are listed in the relevant Methods sections.

Antibodies

Antibodies used
We used a rabbit anti-tyrosine hydroxylase (TH) antibody (AB152, EMD Millipore). The lot number for this antibody was 2458991. The dilution used was 1:500 and staining was performed for 2 days at 4 degrees Celsius.

Validation
The specificity of this antibody has been verified by the company (http://www.emdmillipore.com/US/en/product/Anti-Tyrosine-Hydroxylase-Antibody-MM_NF-AB152#overview).
Animals and other organisms

Policy information about [studies involving animals](https:// ARRIVE guidelines) recommended for reporting animal research

| Laboratory animals | 96 male mice between 3 and 6 months old were used for this study. Surgeries were performed when mice were 3 months old so that data could be collected when mice were between 4-6 months old. Dopamine transporter (DAT)-cre (B6.SJL-Slc6a3tm1.1(cre)Bkmn/J, Jackson Laboratory; RRID:MSR JAX:006660) Heterozygous mice were used for recording signals from dopamine axons expressing GCaMP, for stimulation of dopamine axons expressing ChR2 and for histological examination of TS-projecting dopamine neurons using rabies virus. All mice were backcrossed with C57BL/6J (Jackson Laboratory) for many generations. C57BL/6J mice were used for ablation of TS-projecting dopamine neurons using 6-OHDA. Vglut2floX (Slc17a6tm1low/J, Jackson Laboratory 012898)63 homozygous/DAT-cre heterozygous mice and their littermates (Vglut2floX homozygous mice) were used for novelty exploration behavioral tests. Animals were housed on a 12 hour dark/12 hour light cycle (dark from 07:00 to 19:00) and performed a task at the same time each day. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Harvard Animal Care and Use Committee. |
| Wild animals | No wild animals were used. |
| Field-collected samples | No field-collected samples were used. |