LETTERS

Two types of dopamine neuron distinctly convey positive and negative motivational signals

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Midbrain dopamine neurons are activated by reward or sensory stimuli predicting reward[1–4]. These excitatory responses increase as the reward value increases[5]. This response property has led to a hypothesis that dopamine neurons encode value-related signals and are inhibited by aversive events. Here we show that this is true only for a subset of dopamine neurons. We recorded the activity of dopamine neurons in monkeys (*Macaca mulatta*) during a Pavlovian procedure with appetitive and aversive outcomes (liquid rewards and airpuffs directed at the face, respectively). We found that some dopamine neurons were excited by reward-predicting stimuli and inhibited by airpuff-predicting stimuli, as the value hypothesis predicts. However, a greater number of dopamine neurons were excited by both of these stimuli, inconsistent with the hypothesis. Some dopamine neurons were also excited by both rewards and airpuffs themselves, especially when they were unpredictable. Neurons excited by the airpuff-predicting stimuli were located more dorsolaterally in the substantia nigra pars compacta, whereas neurons inhibited by the stimuli were located more ventromedially, some in the ventral tegmental area. A similar anatomical difference was observed for their responses to actual airpuffs. These findings suggest that different groups of dopamine neurons convey motivational signals in distinct manners.

If midbrain dopamine neurons actually encode value-related signals, their activity should be inhibited by aversive stimuli because aversive stimuli have negative motivational values. However, the results are inconsistent, some studies showing inhibitions[6] and others showing both inhibitions and excitations[7–11] by aversive stimuli. Few of these studies examined the effects of rewards on the same dopamine neurons[12,13], partly because the animals were anaesthetized.

To test whether dopamine neurons encode motivational values, we conditioned two monkeys using a Pavlovian procedure with two distinct contexts (Fig. 1): one in which a liquid reward was expected (appetitive block; Fig. 1a) and one in which an aversive airpuff was anticipated (aversive block; Fig. 1b). In each block, three conditioned stimuli were associated with the unconditioned stimulus (reward or airpuff) with 100%, 50% and 0% probability, respectively. These three conditioned stimuli were considered to convey three different levels of motivational value. In the appetitive block, anticipatory licking increased as the probability of reward increased (Fig. 1c). In the aversive block, anticipatory blinking increased as the probability of airpuff increased (Fig. 1d).

While the monkeys were conditioned using the Pavlovian procedure, we recorded single-unit activity from 103 putative dopamine neurons (68 in monkey N and 35 in monkey D) in and around the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). Their electrophysiological properties were distinctly different from other neurons in the SNc and VTA (Supplementary Fig. 1), and we henceforth call them dopamine neurons.

Most previous studies on midbrain dopamine neurons have characterized dopamine neurons as a functionally homogeneous population[1]. We found that this is not true. In Fig. 2a, e, we show the activity of two dopamine neurons, separately for different conditioned stimuli. Their

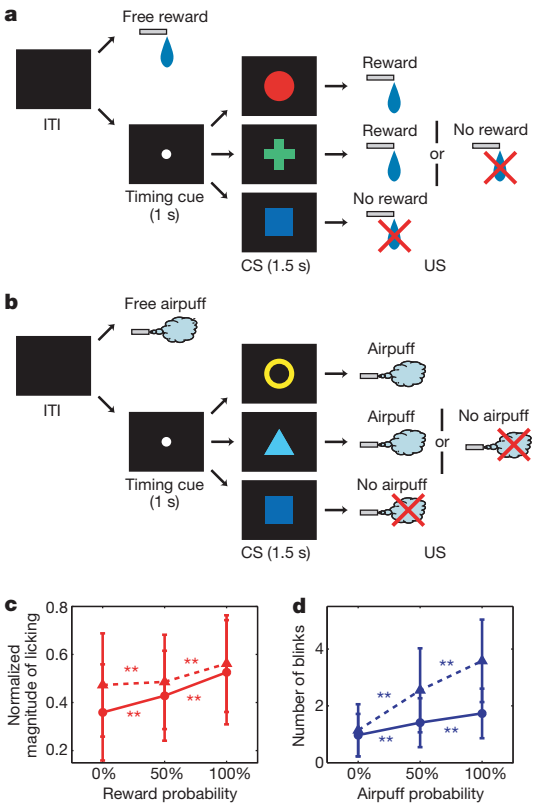

Figure 1 | Pavlovian procedure. **a**, Appetitive block. Three conditioned stimuli were associated with apple juice with 100%, 50% and 0% probability, respectively. **b**, Aversive block. Three conditioned stimuli were associated with an aversive airpuff with 100%, 50% and 0% probability, respectively. In both blocks, each trial started after the presentation of a timing cue (central small spot) on the screen. After 1 s, the timing cue disappeared and one of the three conditioned stimuli was presented. After 1.5 s, the conditioned stimulus disappeared and the unconditioned stimulus (reward or airpuff) was delivered. In addition to the cued trials, uncued trials were included in which a reward alone (free reward) was delivered during the appetitive block and an airpuff alone (free airpuff) was delivered during the aversive block. **c**, Average normalized magnitude of anticipatory licking during the presentation of the reward-predicting conditioned stimuli for monkey D (solid line) and monkey N (dashed line). **d**, Average number of anticipatory blinks during the presentation of the airpuff-predicting conditioned stimuli for monkey D (solid line) and monkey N (dashed line). Double asterisks indicate a significant difference between two data points ($P < 0.01$, Wilcoxon rank-sum test). Error bars, s.d. ITI, inter-trial interval; CS, conditioned stimulus; US, unconditioned stimulus.

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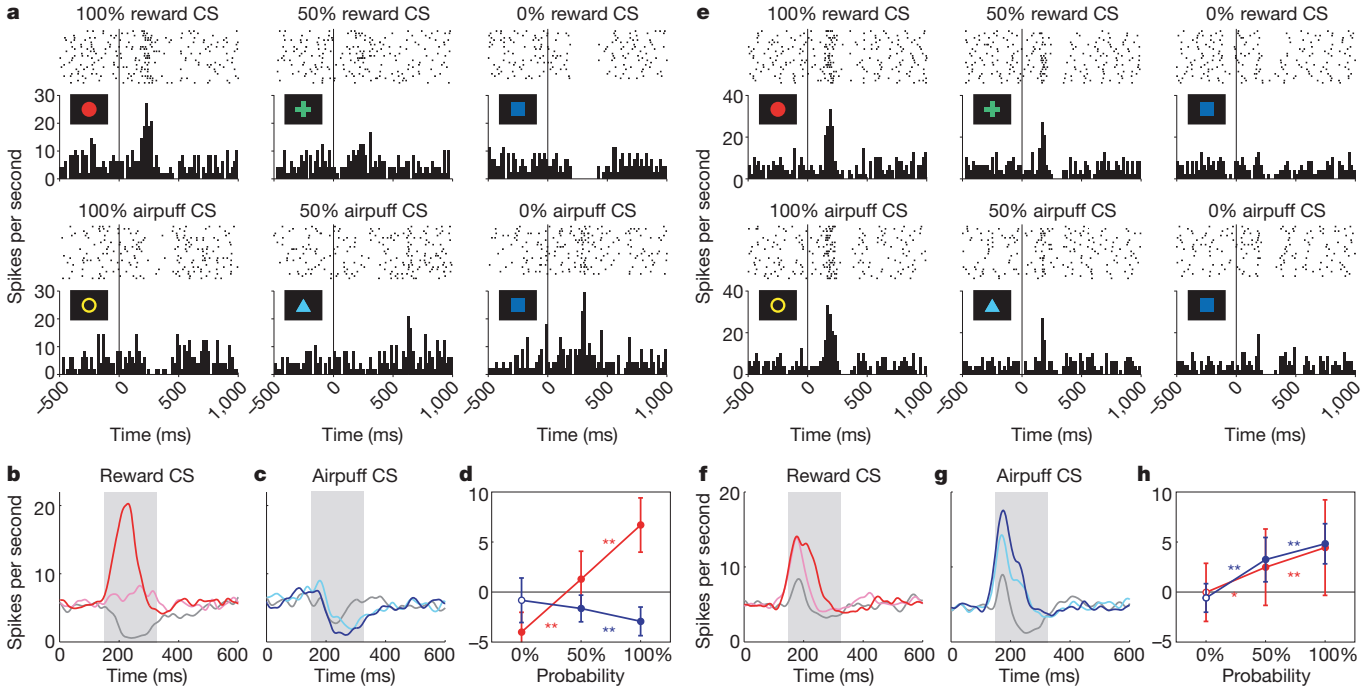

Figure 2 | Responses of dopamine neurons to conditioned stimuli. **a**, **e**, Activity of two example neurons in the appetitive block (top row) and aversive block (bottom row), which were classified as ACS-inhibited type (**a**) and ACS-excited type (**e**). Histograms (20-ms bins) and rasters are aligned at the start of the conditioned stimulus and are shown for 100% reward CS, 50% reward CS, 0% reward CS, 100% airpuff CS, 50% airpuff CS and 0% airpuff CS. **b**, **c**, Averaged activity of 24 ACS-inhibited neurons. **f**, **g**, Averaged activity of 38 ACS-excited neurons. Spike density functions are shown for 100% reward CS (red), 50% reward CS (pink) and 0% reward CS (grey) in the appetitive block (**b**, **f**), and for 100% airpuff CS (dark blue), 50% airpuff CS (light blue) and 0% airpuff CS (grey) in the aversive block (**c**, **g**). Grey areas indicate the period that was used to analyse responses to conditioned stimuli. **d**, **h**, The magnitudes of the responses of the ACS-inhibited neurons (**d**) and ACS-excited neurons (**h**) to the reward-predicting conditioned stimuli (red) and airpuff-predicting conditioned stimuli (blue). Filled symbols indicate a significant deviation from zero ($P < 0.05$, Wilcoxon signed-rank test). Red and blue asterisks indicate a significant difference between two responses for the reward-predicting and airpuff-predicting conditioned stimuli, respectively (double asterisk, $P < 0.01$; single asterisk, $P < 0.05$; Wilcoxon signed-rank test). Error bars, s.d.

activities were similar in the appetitive block (top row). Both of them were excited by 100% reward conditioned stimulus (the conditioned stimulus associated with reward with 100% probability). This excitation decreased in response to 50% reward conditioned stimulus, and changed to an inhibition in response to 0% reward conditioned stimulus. However, the dopamine neurons showed completely different responses in the aversive block (bottom row). In response to 100% airpuff conditioned stimulus, the neuron shown in Fig. 2a was inhibited whereas the neuron shown in Fig. 2e was excited. Furthermore, as the probability of airpuff decreased, their response magnitudes were graded in opposite directions.

To characterize the responses to conditioned stimuli, we classified the 103 neurons into three groups based on the response to 100% airpuff conditioned stimulus (Supplementary Table 1). Neurons showing a significant inhibition and excitation were classified as airpuff conditioned stimulus (ACS)-inhibited type ($n = 24$) and ACS-excited type ($n = 38$), respectively ($P < 0.05$, Wilcoxon signed-rank test). Neurons showing no significant response were classified as ACS-non-responsive type ($n = 41$, $P > 0.05$, Wilcoxon signed-rank test). The responses of individual neurons to conditioned stimuli are shown in Supplementary Fig. 2 and Supplementary Table 2. In the following, we will focus on the ACS-inhibited and ACS-excited neurons (see Supplementary Fig. 3 for ACS-non-responsive neurons; see also Supplementary Note A and Supplementary Table 3 for the electrophysiological properties of each type).

The averaged activity of the ACS-inhibited neurons was modulated by the reward probability (Fig. 2b) and airpuff probability (Fig. 2c) in opposite directions. The excitatory response to the reward-predicting conditioned stimuli decreased and became an inhibition as the reward probability decreased (Fig. 2b, red line in Fig. 2d). By contrast, the inhibitory response to the airpuff-predicting conditioned stimuli decreased as the airpuff probability decreased (Fig. 2c, blue line in Fig. 2d). The same trend was found in individual ACS-inhibited neurons (Supplementary Note B and Supplementary Fig. 4a). These results suggest that the ACS-inhibited neurons encode motivational value on a single scale, and are most strongly excited in response to the most positive stimulus (100% reward conditioned stimulus) and most strongly inhibited in response to the most negative stimulus (100% airpuff conditioned stimulus).

The averaged activity of the ACS-excited neurons was also modulated by the reward probability (Fig. 2f) and airpuff probability (Fig. 2g), but in the same direction. The excitatory response decreased as the outcome probability decreased for both reward-predicting and airpuff-predicting conditioned stimuli (Fig. 2h; see also Supplementary Note B and Supplementary Fig. 4b for individual neurons). These results suggest that the ACS-excited neurons do not encode motivational value.

Previous studies have repeatedly shown that dopamine neurons are excited by reward when it is unexpected[1]. However, it is still debatable whether they are excited or inhibited by aversive stimuli and, if so, in what context. Figure 3a shows the responses to reward and airpuff of the same neuron shown in Fig. 2a. This neuron was strongly excited when reward was presented without preceding conditioned stimulus (free reward) and inhibited when airpuff was presented without preceding conditioned stimulus (free airpuff), consistent with value coding. By contrast, the neuron shown in Fig. 3e was excited by both free reward and free airpuff.

We then reclassified the 103 neurons into three groups on the basis of the response to free airpuff (Supplementary Table 1). Neurons showing significant inhibition and excitation were classified as airpuff unconditioned stimulus (AUS)-inhibited type ($n = 47$) and AUS-excited type ($n = 11$), respectively ($P < 0.05$, Wilcoxon signed-rank test). Neurons showing no significant response were classified as AUS-non-responsive type ($n = 45$, $P > 0.05$, Wilcoxon signed-rank test). The

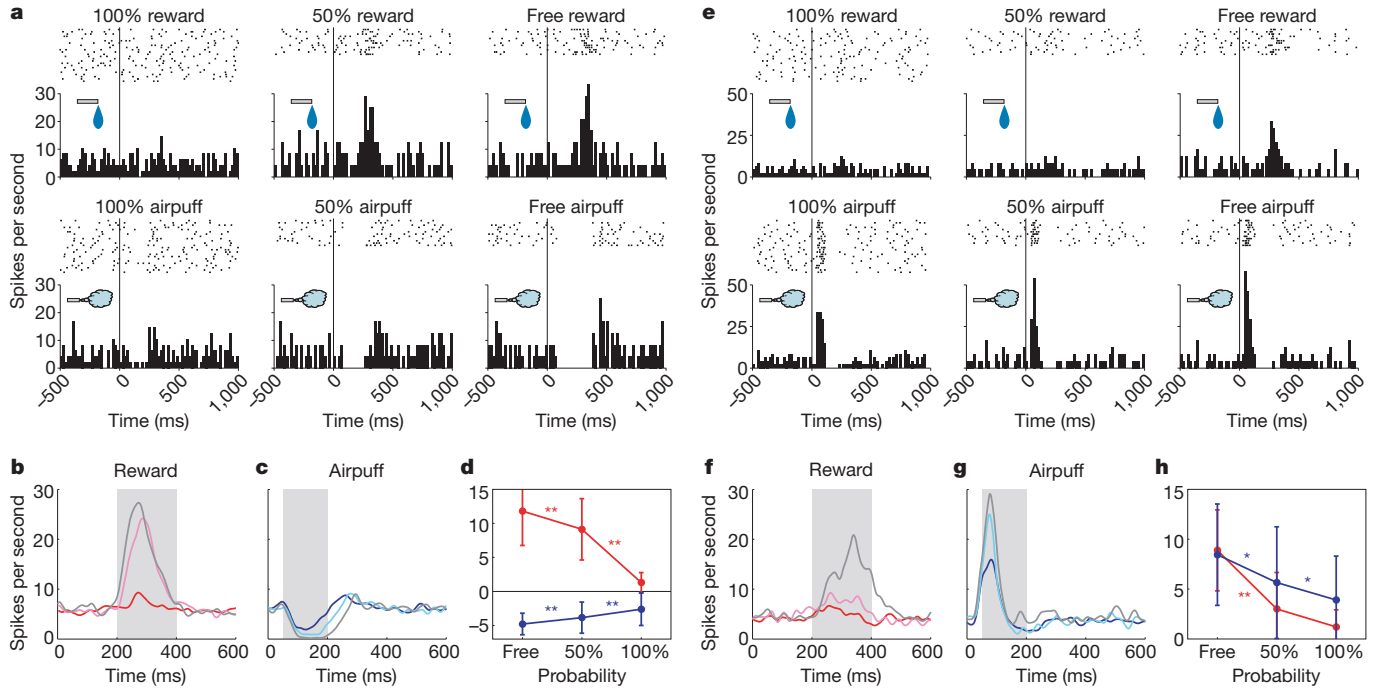

Figure 3 | Responses of dopamine neurons to unconditioned stimuli. **a**, **e**, Activity of two example neurons in the appetitive block (top row) and aversive block (bottom row), which were classified as AUS-inhibited type (**a**) and AUS-excited type (**e**). Histograms and rasters are aligned at the start of the unconditioned stimulus and are shown for 100% reward, 50% reward, free reward, 100% airpuff, 50% airpuff and free airpuff. **b**, **c**, Averaged activity of 47 AUS-inhibited neurons. **f**, **g**, Averaged activity of 11 AUS-excited neurons. Spike density functions are shown for 100% reward (red), 50% reward (pink) and free reward (grey) in the appetitive block (**b**, **f**), and for 100% airpuff (dark blue), 50% airpuff (light blue) and free airpuff (grey) in the aversive block (**c**, **g**). Grey areas indicate the period that was used to analyse responses to unconditioned stimuli. **d**, **h**, The magnitudes of the responses of the AUS-inhibited neurons (**d**) and AUS-excited neurons (**h**) to reward (red) and airpuff (blue). Significance measures and error bars are the same as Fig. 2d, h.

responses of individual neurons to unconditioned stimuli are shown in Supplementary Fig. 5 and Supplementary Table 2. We note that this classification differs from that based on the response to 100% airpuff conditioned stimulus. In the following, we will focus on the AUS-inhibited and AUS-excited neurons (see Supplementary Figs 6 and 7 for AUS-non-responsive neurons, see also Supplementary Note C and Supplementary Table 4 for the electrophysiological properties of each type).

The averaged responses to the reward and airpuff are shown for the AUS-inhibited neurons in Fig. 3b, c and for the AUS-excited neurons in Fig. 3f, g. In both types, the excitatory response to reward disappeared when the reward was completely predictable by following 100% reward conditioned stimulus, and decreased when the reward was partly predictable by following 50% reward conditioned stimulus (Fig. 3b, f). This is consistent with the reward-prediction-error hypothesis that the activity of dopamine neurons represents the difference between the expected and actual values of reward[14,15].

The AUS-inhibited neurons appeared to encode prediction error even for aversive outcomes, albeit partly, because these neurons were inhibited by an unexpected aversive airpuff (free airpuff; Fig. 3c) and this inhibitory response decreased monotonically as the airpuff became more predictable (Fig. 3d; see Supplementary Note D and Supplementary Fig. 8a for individual neurons). We note that the excitatory response of the AUS-excited neurons to the airpuff also decreased as the airpuff became more predictable (Fig. 3g, h; see Supplementary Note D and Supplementary Fig. 8b for individual neurons).

The prediction-error hypothesis predicts that when an outcome is unexpectedly omitted, neurons should respond in the direction opposite to that in which they respond when the same outcome is unexpectedly delivered[14,15]. We found that AUS-inhibited neurons tended to show this kind of response to both reward omission and

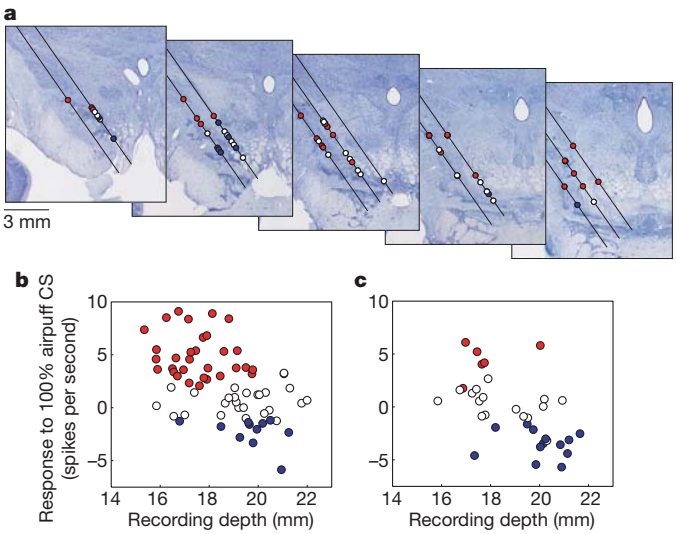

Figure 4 | Locations of dopamine neurons in relation to their responses to airpuff-predicting conditioned stimulus. **a**, Recording sites of 68 dopamine neurons in monkey N are plotted on five coronal sections shown rostrocaudally from left to right (interval, 1 mm). Red circles indicate neurons showing significant excitations to 100% airpuff CS (that is, ACS-excited neurons). Blue circles indicate neurons showing significant inhibitions to 100% airpuff CS (that is, ACS-inhibited neurons). White circles, no significance (that is, ACS-non-responsive neurons). Black lines indicate electrode penetration tracks, which were tilted laterally by 35°. **b**, **c**, Relation between recording depth and the response to 100% airpuff CS for monkey N (**b**) and monkey D (**c**). Red, blue and white circles indicate ACS-excited, ACS-inhibited and ACS-non-responsive neurons, respectively. The recording depth was measured from a reference depth set by a manipulator to advance the recording electrode.

airpuff omission, whereas AUS-excited neurons showed no response to the omission of the outcome (Supplementary Note E and Supplementary Fig. 9).

The current consensus, that dopamine neurons carry reward-related information, is thought to hold for all dopamine neurons located in the midbrain, including both the SNc and the VTA[1]. Because we have found types of dopamine neuron that differ with regard to their responses to aversive events, we now ask whether they were located in different regions in the midbrain. Figure 4a shows the recording sites of the 68 dopamine neurons in monkey N in relation to the response to 100% airpuff conditioned stimulus. Neurons showing a significant excitation (that is, ACS-excited neurons; red circles) tended to be located in the more dorsolateral part, and neurons showing a significant inhibition (that is, ACS-inhibited neurons; blue circles) tended to be located in the more ventromedial part. To test this trend statistically, we examined the relation between the recording depth and the response to 100% airpuff conditioned stimulus for monkey N (Fig. 4b) and monkey D (Fig. 4c). As shown by the scatter plots, a significant negative correlation was found for both monkeys (monkey N: correlation coefficient $r = -0.50$, $P < 0.01$; monkey D: $r = -0.57$, $P < 0.01$). This negative correlation confirmed the dorsolateral–ventromedial differentiation of the excitatory and inhibitory responses evoked in dopamine neurons by the airpuff-predicting conditioned stimulus. Similar location differences were found in relation to response to airpuff itself (Supplementary Note F and Supplementary Fig. 10).

It has generally been assumed that midbrain dopamine neurons form a unified functional group, all representing reward-related signals in a similar manner[1]. Our results are roughly consistent with this idea as far as the reward-related signals are concerned. However, clear heterogeneity was revealed when we examined their responses to aversive events. We found two types of dopamine neuron, one inhibited and the other excited by airpuff or its predictor. This suggests that the unified concept of dopamine neurons needs to be changed (see Supplementary Note G for the relationship between our findings and previous studies).

We propose that there are at least two functional groups of dopamine neurons. Dopamine neurons in the first group (airpuff-inhibited type, that is, ACS- and AUS-inhibited types) would represent motivational value. Their responses co-varied with prediction errors associated with both reward and airpuff, and therefore would be useful in learning to approach rewards and avoid aversive stimuli. The function of the second group (airpuff-excited type, that is, ACS- and AUS-excited types) is not immediately clear, but we found that their response to the conditioned stimulus was correlated with the latency of the monkey's orienting response (gaze shift) to the conditioned stimulus and that this correlation appeared only after the conditioned stimulus was paired with reward or airpuff (Supplementary Note H and Supplementary Fig. 11). These results raise the possibility that the responses of the airpuff-excited dopamine neurons to a conditioned stimulus reflect the motivational salience of the conditioned stimulus. However, this interpretation may not be valid for the responses of these neurons to unconditioned stimulus or its omission.

We note that the two types of dopamine neuron were distributed differently, the airpuff-excited type in the dorsolateral region in the SNc and the airpuff-inhibited type in the ventromedial region in the SNc as well as the VTA (see Supplementary Note I for details). In monkeys[16] and rats[17], dopamine neurons in the dorsolateral SNc project mainly to the dorsal striatum, whereas those in the ventromedial SNc and VTA project mainly to the ventral striatum. The airpuff-inhibited dopamine neurons in the ventromedial region in the SNc and VTA may thus transmit value-related information to the ventral striatum, which is thought to process reward values[18–20]. On the other hand, the airpuff-excited dopamine neurons in the dorsolateral region in the SNc respond to motivationally salient stimuli, whether they are appetitive or aversive, and send the signal to the dorsal striatum, which is related to orienting behaviour[21–23]. This may be part of the mechanism by which orienting behaviour such as saccadic eye movement is induced by motivationally salient stimuli[24].

The two types of dopamine neuron may receive inputs from different sources. The airpuff-excited dopamine neurons may receive inputs from areas such as the basal forebrain, in which neurons also show excitatory responses to both appetitive and aversive events[25,26] (see Supplementary Note J for further discussion). The airpuff-inhibited dopamine neurons may receive inputs, at least partly, from the lateral habenula. Using the same Pavlovian procedure, we have shown that lateral habenula neurons are excited by the airpuff-predicting conditioned stimulus and inhibited by the reward-predicting conditioned stimulus, indicating that they encode motivational value similarly to the airpuff-inhibited dopamine neurons, but in the opposite manner[27]. The value signals in the lateral habenula would then be transmitted to the dopamine neurons by inhibiting them[28], and this effect was stronger on dopamine neurons located in the ventromedial SNc or the VTA, where the airpuff-inhibited type dominates (Supplementary Note K and Supplementary Fig. 12).

So far, we have classified dopamine neurons into two types. However, the real picture is more complex. First, the difference between the two types was not distinct; there was another group of dopamine neurons that did not belong to either type (that is, the type non-responsive to airpuff or its predictor). Second, the classification was different for conditioned and unconditioned stimuli (Supplementary Note L, Supplementary Table 1 and Supplementary Fig. 13b). More neurons were excited by the airpuff-predicting conditioned stimulus, whereas more neurons were inhibited by the airpuff itself. This might indicate flexible operation of the dopamine system. If a salient stimulus (that is, a conditioned stimulus) is presented, it would be beneficial to orient attention to the stimulus and judge whether it predicts a rewarding event or an aversive event. This is the time when a majority of dopamine neurons are excited, thus promoting the orienting behaviour. If an aversive event occurs (that is, unconditioned stimulus), it would be crucial to learn to avoid the action that led to the aversive event. This is the time when a majority of dopamine neurons are inhibited, thus promoting avoidance learning.

METHODS SUMMARY

Two adult rhesus monkeys (*Macaca mulatta*) were used for the experiments. All procedures for animal care and experimentation were approved by the Animal Care and Use Committee of the National Eye Institute and complied with the Public Health Service Policy on the humane care and use of laboratory animals.

A plastic head holder and plastic recording chamber were fixed to the skull under general anaesthesia and sterile surgical conditions. The recording chamber was placed over the frontoparietal cortex, tilted laterally by 35°, and aimed at the SNc and VTA. Two search coils were surgically placed under the conjunctiva of the eyes. The head holder, the recording chamber and the eye coil connectors were all embedded in dental acrylic that covered the top of the skull, and were connected to the skull using acrylic screws.

We conditioned two monkeys using a Pavlovian procedure with an appetitive unconditioned stimulus (liquid reward) and an aversive unconditioned stimulus (airpuff). During the Pavlovian procedure, we recorded the activity of dopamine neurons in and around the SNc and VTA. We estimated the position of the SNc and VTA by magnetic resonance imaging and identified dopamine neurons by their electrophysiological properties. After the end of recording sessions in one monkey, we confirmed the recording sites histologically. We analysed anticipatory licking, anticipatory blinking and neuronal responses during the Pavlovian procedure. We focused on three kinds of neuronal responses: (1) responses elicited by conditioned-stimulus presentation, (2) responses elicited by unconditioned-stimulus delivery and (3) responses elicited by unconditioned-stimulus omission. Details of the Pavlovian procedure, identification of dopamine neurons, analysis methods, and histological procedure can be found in the full Methods.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions M.M. designed the Pavlovian procedure, performed the experiments and analysed the data. O.H. supported all of these processes. M.M. and O.H. discussed the results and wrote the manuscript.

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METHODS

Pavlovian procedure. Our Pavlovian procedure consisted of two blocks of trials, an appetitive block (Fig. 1a) and an aversive block (Fig. 1b). In the appetitive block, three conditioned stimuli (red circle, green cross and blue square for monkey N; yellow ring, cyan triangle and blue square for monkey D) were associated with a liquid reward (apple juice) as an unconditioned stimulus with 100%, 50% and 0% probability, respectively. In the aversive block, three conditioned stimuli (yellow ring, cyan triangle and blue square for monkey N; red circle, green cross and blue square for monkey D) were associated with an airpuff directed at the monkey's face as an unconditioned stimulus with 100%, 50% and 0% probability, respectively. The liquid reward was delivered through a spout that was positioned in front of the monkey's mouth. The airpuff (20–30 p.s.i.) was delivered through a narrow tube placed 6–7 cm from the face.

Each trial started after the presentation of a timing cue for both blocks. The monkeys were not required to look at the timing cue. After 1 s, the timing cue disappeared and one of the three conditioned stimuli was presented pseudo-randomly. After 1.5 s, the conditioned stimulus disappeared and the unconditioned stimulus was delivered. In addition to the cued trials, uncued trials were included in which a reward alone (free reward) was delivered during the appetitive block and an airpuff alone (free airpuff) was delivered during the aversive block. All trials were presented with a random inter-trial interval that averaged 5 s (3–7 s) for monkey N and 4.5 s (3–6 s) for monkey D. One block consisted of 42 trials with fixed proportions of trial types (100%, 12 trials; 50%, 12 trials; 0%, 12 trials; uncued, 6 trials). For 50% trials, the conditioned stimulus was followed by the unconditioned stimulus in six trials and was not followed by the unconditioned stimulus in the other six trials. The block changed without any external cue. For each neuron, we collected data by repeating the appetitive and aversive blocks twice or more.

We monitored licking and blinking of the monkeys. To monitor licking, we attached a strain gauge to the reward spout and measured strains on the spout resulting from licking. To monitor blinking, a magnetic-search-coil technique was used. A small Teflon-coated stainless-steel wire (<5 mm in diameter, five or six turns) was taped to an eyelid. Eye closure was identified by the vertical component of the eyelid-coil signal.

Identification of dopamine neurons. We searched for dopamine neurons in and around the SNc and VTA. Dopamine neurons were identified by their irregular firing, tonic baseline activity around five spikes per second, broad spike potential and phasic excitation to free reward.

Data analysis. We analysed anticipatory licking, anticipatory blinking and neuronal activity during the Pavlovian procedure.

To evaluate the frequency and strength of anticipatory licking, the strain-gauge signal was used. We first calculated the velocity of the signal change under licking. Then we integrated the absolute velocity during conditioned-stimulus presentation for each trial. This integrated velocity becomes larger if the monkeys more frequently and strongly lick the spout. We defined this value as the magnitude of anticipatory licking in the trial. The magnitude was normalized according to the following formula: normalized magnitude equals $(X - \mathrm{Min})/(\mathrm{Max} - \mathrm{Min})$. Here X is the magnitude of anticipatory licking in the trial, Max is the maximum magnitude in the recording session and Min is the minimum magnitude in the recording session.

To count the number of anticipatory blinks during conditioned-stimulus presentation, the vertical component of the eyelid signal was used. We first calculated the downward velocity of eyelid movement. We set a threshold and counted how many times the velocity crossed the threshold during conditioned-stimulus presentation for each trial. This count was defined as the number of anticipatory blinks in the trial.

In analyses of neuronal activity, responses to each conditioned stimulus were defined as the discharge rate during the interval 150 to 325 ms after conditioned stimulus onset minus the background discharge rate during the 250 ms before conditioned stimulus onset. Response to reward was defined as the discharge rate during the interval 200 to 400 ms after reward onset minus the background discharge rate during the 250 ms before reward onset. Response to airpuff was defined as the discharge rate during the interval 50 to 200 ms after airpuff onset minus the background discharge rate during the 250 ms before airpuff onset. Response to reward omission was defined as the discharge rate during the interval 200 to 500 ms after the conditioned stimulus ended minus the background discharge rate during the 250 ms before the conditioned stimulus ended. Response to airpuff omission was defined as the discharge rate during the interval 150 to 350 ms after the conditioned stimulus ended minus the background discharge rate during the 250 ms before the conditioned stimulus ended. These time windows were determined on the basis of the averaged activity of dopamine neurons. Specifically, we set the time windows such that they include major parts of the excitatory and inhibitory responses.

Because the 0% reward conditioned stimulus and 0% airpuff conditioned stimulus were physically identical, they could only be distinguished by the block context (appetitive block or aversive block). Therefore, to analyse responses to 0% reward conditioned stimulus and 0% airpuff conditioned stimulus, we excluded all trials with the 0% reward conditioned stimulus or the 0% airpuff conditioned stimulus that were presented before the block context could be known, that is, before the block's first presentation of 100% conditioned stimulus, 50% conditioned stimulus or free outcome.

We characterized the electrophysiological properties of recorded neurons by (1) baseline firing rate, (2) irregularity of firing pattern and (3) spike waveform. Baseline firing rate is the mean firing rate during the 250 ms before the onset of the timing cue. To quantify irregularity of firing pattern, we used an irregularity metric introduced in ref. 29 and called 'IR'. First, interspike interval (ISI) was computed as follows: if spike $i-1$, spike i and spike $i+1$ occurred in this order, the interval between spike $i-1$ and spike i corresponds to ISI_i and the interval between spike i and spike $i+1$ corresponds to ISI_{i+1}. Second, the difference between adjacent ISIs was computed as $|\log(\mathrm{ISI}_i/\mathrm{ISI}_{i+1})|$. This value was then assigned to the time spike i occurred. Thus, small IR values indicate regular firing and large IR values indicate irregular firing. We then computed a median of all IR values during the inter-trial interval (during the 1,000 ms before timing-cue onset). To quantify spike waveform, we measured the spike duration of 67 dopamine neurons (whose spike waveforms were successfully recorded). The typical spike consisted of the following waves: first, sharp negative; second, sharp positive; third, slow negative; fourth, slow positive. We measured the spike duration from the peak of the first wave (sharp negative) to the peak of the third wave (slow negative).

Histology. After the end of the recording session in monkey N, we selected representative locations for electrode penetration. When typical dopamine activity was recorded, we made electrolytic microlesions at the recording sites (12 μA and 30 s). Then monkey N was deeply anaesthetized using pentobarbital sodium, and perfused with 10% formaldehyde. The brain was blocked and equilibrated with 10% sucrose. Frozen sections were cut every 50 μm in the coronal plane. The sections were stained with cresyl violet.

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A. Electrophysiological properties of ACS-inhibited, ACS-excited and ACS-non-responsive neurons

Supplementary table 3 shows the electrophysiological properties of the three types of dopamine neurons (ACS-inhibited, ACS-excited and ACS-non-responsive types). There was no significant difference in either baseline firing rate, irregularity of firing pattern, or spike duration among the three types ($P > 0.05$, Wilcoxon rank-sum test). In each type of neurons the baseline firing rate was not significantly different between the appetitive block and the aversive block ($P > 0.05$, Wilcoxon signed-rank test).

We also compared the electrophysiological properties of the putative dopamine neurons we examined with 37 putative non-dopamine neurons recorded in the same regions (SNc and VTA). Supplementary Fig. 1a shows the averaged spike shapes of ACS-inhibited type, ACS-excited type, ACS-non-responsive type, and non-dopamine neurons. The spike shapes of the three types of dopamine neurons were very similar to each other, and were broader than the spike of non-dopamine neurons. The scatter plot in Supplementary Fig. 1b shows the relationship between spike duration and baseline firing rate for individual neurons. The three types of putative dopamine neurons make up one cluster which was clearly separated from a loose cluster of the putative non-dopamine neurons. Although a few of the putative non-dopamine neurons showed spike durations and baseline firing rates similar to the putative dopamine neurons, they did not show an excitatory response to free reward (open circles).

B. Correlation between outcome probability and CS-evoked response for individual neurons

As shown in the averaged response magnitude of ACS-inhibited neurons (Fig. 2d), the CS (conditioned stimulus)-evoked excitation increased as the reward probability increased (red line) while the CS-evoked inhibition increased as the airpuff probability increased (blue line). To examine whether such a response pattern was achieved by individual neurons, we calculated the correlation coefficient between CS-evoked response and outcome probability for each ACS-inhibited neuron, separately for reward probability (abscissa in Supplementary Fig. 4a) and airpuff probability (ordinate in Supplementary Fig. 4a). A majority of the ACS-inhibited neurons showed a significant positive correlation between CS-evoked response and reward probability (n = 23/24) ($P < 0.05$). Some of them showed a significant negative correlation between CS-evoked response and airpuff probability (n = 7/24) ($P < 0.05$); none of them showed a significant positive correlation. Of these, 7 neurons showed a significant positive correlation for reward probability as well as a significant negative correlation for airpuff probability ($P < 0.05$). The mean correlation coefficient was significantly larger than zero for reward probability and significantly smaller than zero for airpuff probability ($P < 0.01$, Wilcoxon signed-rank test). These results indicate the tendency that ACS-inhibited neurons increased their CS-evoked excitatory response as the reward was more likely and increased the CS-evoked inhibitory response as the airpuff was more likely.

The same correlation analysis was performed for ACS-excited and ACS-non-responsive neurons. Many of the ACS-excited neurons (Supplementary Fig. 4b) showed a significant positive correlation between CS-evoked response and reward probability (n =

20/38) or between CS-evoked response and airpuff probability (n = 30/38) ($P < 0.05$). Of these, 14 neurons showed a significant positive correlation in both cases ($P < 0.05$). The mean correlation coefficient was significantly larger than zero for both reward probability and airpuff probability ($P < 0.01$, Wilcoxon signed-rank test). These results indicate the tendency that ACS-excited neurons increased their CS-evoked excitatory response as the reward was more likely and as the airpuff was more likely.

Many of the ACS-non-responsive neurons (Supplementary Fig. 4c) showed a significant positive correlation between CS-evoked response and reward probability (n = 31/41) and between CS-evoked response and airpuff probability (n = 16/41) ($P < 0.05$). Of these, 13 neurons showed a significant positive correlation in both cases ($P < 0.05$). The mean correlation coefficient was significantly larger than zero for both reward probability and airpuff probability ($P < 0.01$, Wilcoxon signed-rank test).

C. Electrophysiological properties of AUS-inhibited, AUS-excited and AUS-non-responsive neurons

Supplementary table 4 shows the electrophysiological properties of the three types of dopamine neurons (AUS-inhibited, AUS-excited and AUS-non-responsive types). The baseline firing rate was significantly higher in the AUS-inhibited neurons than in the AUS-excited neurons ($P < 0.01$, Wilcoxon rank-sum test). There was no significant difference in spiking regularity or spike duration ($P > 0.05$, Wilcoxon rank-sum test). In each type of neurons the baseline firing rate was not significantly different between the appetitive block and the aversive block ($P > 0.05$, Wilcoxon signed-rank test).

D. Correlation between outcome probability and US-evoked response for individual neurons

We calculated the correlation coefficient between US (unconditioned stimulus)-evoked response and outcome probability for AUS-inhibited, AUS-excited and AUS-non-responsive neurons. For this analysis, we assumed that the probability of free outcome is zero.

A majority of AUS-inhibited neurons (Supplementary Fig.8a) showed a significant negative correlation between US-evoked response and reward probability (n = 45/47) ($P < 0.05$). Some of them showed a significant positive correlation between US-evoked response and airpuff probability (n = 15/47) ($P < 0.05$); only one of them showed a significant negative correlation. Of these, 14 neurons showed a significant negative correlation for reward probability as well as a significant positive correlation for airpuff probability ($P < 0.05$). The mean correlation coefficient was significantly smaller than zero for reward probability and significantly larger than zero for airpuff probability ($P < 0.01$, Wilcoxon signed-rank test). These results indicate the tendency that many AUS-inhibited neurons decreased their US-evoked excitatory response as the reward was more predictable and decreased the CS-evoked inhibitory response as the airpuff was more predictable.

Many of the AUS-excited neurons (Supplementary Fig. 8b) showed a significant negative correlation between US-evoked response and reward probability (n = 10/11) and between US-evoked response and airpuff probability (n = 6/11) ($P < 0.05$). Of these, 5 neurons showed a significant negative correlation in both cases ($P < 0.05$). The mean correlation coefficient was significantly smaller than zero for both reward probability and

airpuff probability ($P < 0.01$, Wilcoxon signed-rank test). These results indicate the tendency that many AUS-excited neurons decreased their US-evoked excitatory response as the reward was more predictable and as the airpuff was more predictable.

Many of the AUS-non-responsive neurons (Supplementary Fig. 8c) showed a significant negative correlation between US-evoked response and reward probability (n = 44/45) ($P < 0.05$). On the other hand, there is no clear tendency between US-evoked response and airpuff probability (3 neurons showed a significant positive correlation, 5 neurons showed a significant negative correlation, $P < 0.05$). The mean correlation coefficient was significantly smaller than zero for reward probability ($P < 0.01$, Wilcoxon signed-rank test) but not significantly different from zero for airpuff probability ($P > 0.05$, Wilcoxon signed-rank test).

E. Responses to reward omission and airpuff omission

We found that AUS-inhibited neurons, but not AUS-excited neurons, responded when reward or airpuff was unexpectedly omitted. Supplementary Fig 9a indicates the activity of the same neuron shown in Fig. 3a, which was classified into AUS-inhibited type. This neuron was inhibited when the partially predicted reward did not occur (50% reward omission), but showed no response when the partially predicted airpuff did not occur (50% airpuff omission). The averaged activity of the AUS-inhibited neurons showed a clear inhibition to 50% reward omission (Supplementary Fig. 9b) and a weak but significant excitation to 50% airpuff omission ($P < 0.01$, Wilcoxon signed-rank test) (Supplementary Fig. 9c). These omission responses support the hypothesis that the AUS-inhibited neurons convey prediction error signals for both positive and negative outcomes.

In contrast, the AUS-excited neurons did not show a clear response to reward omission or airpuff omission (Supplementary Fig. 9d, e and f).

F. Recording sites of dopamine neurons in relation to response to airpuff itself

We found that dopamine neurons excited by the airpuff-predicting CS were located more dorsolaterally, whereas neurons inhibited by the CS were located more ventromedially. Similar location differences were found in relation to response to airpuff itself (Supplementary Fig. 10a). Neurons showing a significant excitation to free airpuff (i.e., AUS-excited neurons, red circles) tended to be located more dorsolaterally than neurons showing a significant inhibition (i.e., AUS-inhibited neurons, blue circles). There was a trend for a negative correlation between recording depth and response to free airpuff (Supplementary Fig. 10b, monkey N, $r = -0.44$, $P < 0.01$; Supplementary Fig. 10c, monkey D, $r = -0.22$, $P = 0.19$).

G. Relationship between our findings and preceding studies

It has been shown using anesthetized animals that some dopamine neurons were excited by aversive stimuli[1-4]. However, because the animals were anesthetized, it is difficult to interpret the data in terms of learning and expectation. On the other hand, Mirenowicz and Schultz[5] used awake monkeys and showed that dopamine neurons responded to appetitive CSs, but they were generally unresponsive to aversive CSs. In this experiment, however, the aversive CSs may not have induced strong negative motivation because the monkey was allowed to avoid aversive stimulation (airpuff) by reacting to the CSs. Joshua et al.[6] also used awake monkeys and showed that dopamine

neurons, on average, were strongly excited by appetitive CSs and weakly excited by aversive CSs. In this experiment, however, the excitatory response of dopamine neurons to the aversive CSs was not modulated by outcome probability. Therefore, it is unclear whether these neurons encode motivational value. Guarraci and Kapp[7] used awake rabbits and showed that among dopamine neurons some were excited by aversive CSs and others were inhibited, but the effect of appetitive CSs was not examined.

It has been also suggested that the release of dopamine can increase when animals experience stress or pain, especially in the nucleus accumbens and the prefrontal cortex[8, 9]. This increase in dopamine release has been considered to be caused by slow or sustained increases in dopamine neuron firing. This is based on the observation that the dopamine concentration increased slowly after repeated aversive stimulation (see Schultz[10] for review). Aided by these observations, a theory suggests that such a sustained increase in dopamine transmission underlies the opponent process of motivation which explains history-dependent motivational changes such as the positive value induced by the cessation of aversive stimuli[11]. These ideas predict that the background firing rate of dopamine neurons should increase while the animal is under stress. But our results do not support this hypothesis: the background firing rate was not significantly different between the appetitive block (when the monkey was presumably not under stress) and the aversive block (when the monkey was presumably under stress) in either the airpuff-excited neurons (i.e., ACS-excited or AUS-excited neurons) or airpuff-inhibited neurons (i.e., ACS-inhibited or AUS-inhibited neurons) (Supplementary tables 3 and 4) ($P > 0.05$, Wilcoxon signed-rank test). The airpuff-excited neurons, however, were excited phasically by airpuff or its predictor. These results suggest that the stress- or pain-induced

increase in dopamine release is caused by the phasic (not tonic) increase in firing of a group of dopamine neurons.

H. Orienting response to salient stimuli

When an object is physically salient (e.g., brigher or larger than other objects) or visually salient (e.g., red among green objects), it captures attention and induces an orienting eye movement (saccade) through bottom-up processes[12, 13]. In other words, the salience of a visual object is indexed by the probability or reaction time of the orienting saccade[14, 15].

However, even if an object is not physically or visually salient, attention or eye movement can be drawn to the object if it is associated with a reward or aversive stimulus (motivationally salient)[16]. To test if the reward- and airpuff-predicting CSs are actually salient, we performed a behavioral experiment using a new set of six CSs (from The Amsterdam Library of Object Images[17]) in monkey D (Supplementary Fig. 11). The procedure was basically the same as that used in the main experiment (Fig. 1), except that the CS was presented at the right or left side of the timing cue (Supplementary Fig. 11a). On most trials the monkey fixated the timing cue and then made a saccade to the CS, even though neither fixation nor saccade was required. As a behavioral measure of salience, we measured the gaze latency (i.e., time from the CS onset to the time when the monkey's gaze was directed to the CS), following the above discussion.

The experiment consisted of two stages. In the first stage the all CSs were followed by an equal amount of reward (apple juice). There was no significant difference in the gaze latency among the CSs (dashed lines in Supplementary Fig. 11d and e) ($P > 0.01$,

Wilcoxon rank-sum test). This result indicates that the CSs were not different from each other in terms their physical or visual salience.

In the second stage of experiment, we trained the monkey in the same way as the main experiment, three of the CSs associated with reward with different probabilities (Supplementary Fig. 11b) and the other three CSs associated with airpuff with different probabilities (Supplementary Fig. 11c). There were now significant differences in the gaze latency among the CSs (solid lines in Supplementary Fig. 11d and e). In the appetitive block the gaze latency monotonically decreased as the reward probability increased (Supplementary Fig. 11d). In the aversive block the latency also decreased as the airpuff probability increased (Supplementary Fig. 11e), although the latency was not significantly different between 100% airpuff CS and 50% airpuff CS ($P > 0.01$, Wilcoxon rank-sum test). Therefore, it is plausible that the CS is more salient if the CS predicts an outcome more reliably, regardless of whether it is a reward or an aversive stimulus. This conclusion, together with the neuronal data shown in Fig. 2, suggests that the response of the ACS-excited neurons to the CS was larger if the CS was more salient.

Since the differences in the gaze latency were initially absent but emerged after the conditioning, the salience of the CS was based not on the bottom-up process but on the learned association of the CS with the motivational outcomes (reward or airpuff). These observations raise the possibility that the ACS-excited neurons encode motivational salience. However, it is still debatable whether motivational salience can be assessed only by orienting behavior.

I. Localization of dopamine neurons

According to the reconstructed histology, most neurons were located in the SNc, but some neurons that were intermingled with the fiber bundles of the oculomotor nerve were judged to be in the VTA (e.g., the ventral neurons in the medial penetrations in the two anterior sections).

The airpuff-excited neurons were located in the dorsolateral part of the SNc, probably including both the dorsal tier and the ventral tier of the SNc. Such laterally located dopamine neurons in the monkey are known to project to the sensorimotor (putamen) or associative (caudate) parts of the striatum in addition to other brain areas[18].

Some of the airpuff-inhibited neurons were located in the medial parts of the SNc, but others were judged to be within the VTA, probably in the parabrachial pigmented area or paranigral nucleus[19]. Such medially located dopamine neurons project mainly to the limbic part of the striatum (ventral striatum including the nucleus accumbens)[18]. However, the midline portion of the VTA (rostral and central linear nuclei) was not examined.

Note, however, that Fig. 4 or Supplementary Fig. 10 may not indicate the exact locations of the neurons we recorded from, because electrolytic microlesions were made for a selected set of electrode penetrations near the end of the experiment and slight misalignment or shrinkage of brain sections may have occurred during the histological procedure.

J. Inputs to the airpuff-excited dopamine neurons

The latency of the excitatory response to 100% reward CS was shorter among the ACS-excited neurons (114 ms) than among the ACS-inhibited neurons (144 ms) (the

latencies were calculated by a bootstrap method which we used in our previous study[20]). This might suggest that the source of the excitatory response, at least its initial part, was different between these types of neurons. Among the ACS-excited neurons, there was only a small difference in latency between the excitatory response to 100% reward CS (114 ms) and the excitatory response to 100% airpuff CS (111 ms). It is thus possible that they share common inputs. On the other hand, the response to 100% airpuff CS occurred earlier among the ACS-excited neurons (as an excitation) (111 ms) than among the ACS-inhibited neurons (as an inhibition) (179 ms). The response to the airpuff itself (free airpuff) also occurred earlier among the AUS-excited neurons (as an excitation) (20 ms) than among the AUS-inhibited neurons (as an inhibition) (51 ms).

There are several brain areas that might provide the ACS-excited neurons with the excitatory signals. In the basal forebrain some neurons are excited by sensory stimuli predicting reward and those predicting aversive stimulus as well as reward and aversive stimulus themselves[21, 22]. Novel sensory stimuli activate neurons in the superior colliculus, and this signal is transmitted to dopamine neurons in the SNc[23]. Further, many superior colliculus neurons are activated by aversive stimuli (footshock), and this occurs before SNc dopamine neurons are inhibited or excited by the same stimuli[4]. The central nucleus of the amygdala may also be involved. Discussing the functions of the amygdala, Holland and Gallagher[24] suggested that the pathway from the central amygdala nucleus to the dorsolateral striatum through the SNc contributes to attentional functions in conditioning.

K. Inhibitory effects of the lateral habenula on dopamine neurons

In our first study on the lateral habenula using a visually guided saccade task with positionally biased reward outcomes[20], we found that a majority of dopamine neurons were inhibited by a weak electrical stimulation of the lateral habenula, but the magnitude of the inhibition varied across the dopamine neurons (Fig. 4 in the previous study). We reanalyzed the electrical stimulation data in relation to the locations of the dopamine neurons (Supplementary Fig. 12), and found that the dopamine neurons located more ventromedially tended to be inhibited more strongly by the lateral habenula stimulation. This result suggests that the airpuff-inhibited dopamine neurons (i.e., ACS-inhibited type and AUS-inhibited type) receive stronger inhibitory inputs from the lateral habenula, compared with the airpuff-excited dopamine neurons (i.e., ACS-excited type and AUS-excited type).

This result is consistent with our second study on the lateral habenula[25] which showed that lateral habenula neurons encode motivational value, excited by negative values and inhibited by positive values, in the manner opposite to the airpuff-inhibited dopamine neurons in this study. There are additional findings that support this conclusion. In our first habenula study[20], the dopamine neurons that were inhibited strongly by the lateral habenula stimulation tended to show stronger inhibitory responses to the saccade target that indicated no reward. The no-reward-indicating target in the previous study seems functionally equivalent to the 0% reward CS in the current study. Since the 0% reward CS induced stronger inhibitions in ACS-inhibited neurons (gray line in Fig. 2b) than in ACS-excited neurons (gray line in Fig. 2f), the dopamine neurons that were strongly inhibited by the lateral habenula stimulation may correspond to ACS-inhibited neurons which are located ventromedially.

To summarize, the value signal in the lateral habenula seems to be transmitted preferentially to the ventromedial SNc and the VTA. Weaker inputs from the lateral habenula to dopamine neurons in the dorsolateral SNc, if any, may be overshadowed by salience-related excitatory inputs originating from somewhere else.

L. Relationship between CS- and US-evoked responses

We have classified dopamine neurons based on their responses to two kinds of aversive events, the predictor of an aversive stimulus (airpuff CS) and the aversive stimulus itself. However, these classifications did not match with each other completely. That is, in response to airpuff CS more neurons were excited (n=38) than inhibited (n=24), whereas in response to airpuff itself more neurons were inhibited (n=47) than excited (n=11) (Supplementary table 1). In particular, some dopamine neurons showed opposite responses between airpuff CS and airpuff itself (n=14); common among them were neurons that were excited by airpuff CS but were inhibited by airpuff itself (n=13). The scatter plots of Supplementary Fig. 13 compare the responses to 100% CS and free outcome for each neuron.

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Supplementary table 1

Appetitive block

		100% reward CS			
		Excitation	Inhibition	No response	All
Free reward	Exc.	75	1	27	103
	Inh.	0	0	0	0
	No	0	0	0	0
	All	75	1	27	103

Aversive block

		100% airpuff CS			
		Excitation	Inhibition	No response	All
Free airpuff	Exc.	8	1	2	11
	Inh.	13	15	19	47
	No	17	8	20	45
	All	38	24	41	103

Shown are the numbers of dopamine neurons exhibiting a significant excitation, significant inhibition, or no significant response ($P < 0.05$, Wilcoxon signed-rank test) for the responses to 100% reward CS and free reward (top) and the responses to 100% airpuff CS and free airpuff (bottom).

Supplementary table 2

CS-evoked response

		100% reward CS			
		Excitation	Inhibition	No response	All
100% airpuff CS	Exc.	24	0	14	38
	Inh.	22	0	2	24
	No	29	1	11	41
	All	75	1	27	103

US-evoked response

		Free reward			
		Excitation	Inhibition	No response	All
Free airpuff	Exc.	11	0	0	11
	Inh.	47	0	0	47
	No	45	0	0	45
	All	103	0	0	103

Shown are the numbers of dopamine neurons exhibiting a significant excitation, significant inhibition, or no significant response ($P < 0.05$, Wilcoxon signed-rank test) for the responses to 100% reward CS and 100% airpuff CS (top) and the responses to free reward and free airpuff (bottom).

Supplementary table 3

	ACS-inhibited type	ACS-excited type	ACS-non-responsive type
Baseline firing rate (spks/s)	5.4 ± 1.7	4.8 ± 1.5	5.1 ± 1.7
Baseline firing rate in the appetitive block (spks/s)	5.3 ± 1.8	4.9 ± 1.6	5.0 ± 1.6
Baseline firing rate in the aversive block (spks/s)	5.5 ± 1.7	4.7 ± 1.6	5.3 ± 1.8
Irregularity of firing pattern	0.51 ± 0.13	0.62 ± 0.22	0.57 ± 0.16
Spike duration (ms)	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1

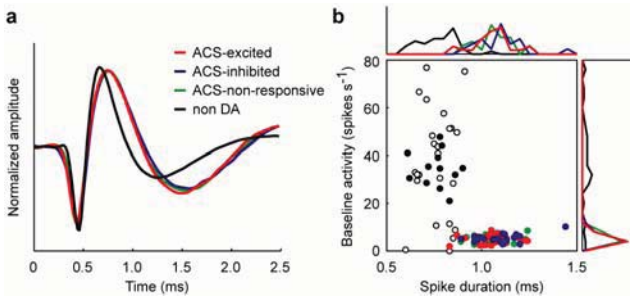
Electrophysiological properties for ACS-inhibited, ACS-excited, and ACS-non-responsive neurons (mean ± s.d.). A significant difference between ACS-inhibited and ACS-excited neurons is indicated by a single asterisk ($P < 0.05$, Wilcoxon rank-sum test) or a double asterisk ($P < 0.01$).

Supplementary table 4

	AUS-inhibited type	AUS-excited type	AUS-non-responsive type
Baseline firing rate (spks/s)	5.7 ± 1.6**	4.0 ± 1.6**	4.7 ± 1.4
Baseline firing rate in the appetitive block (spks/s)	5.6 ± 1.7*	4.1 ± 1.7*	4.7 ± 1.4
Baseline firing rate in the aversive block (spks/s)	5.9 ± 1.6**	3.9 ± 1.6**	4.6 ± 1.5
Irregularity of firing pattern	0.55 ± 0.15	0.66 ± 0.26	0.58 ± 0.19
Spike duration (ms)	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1

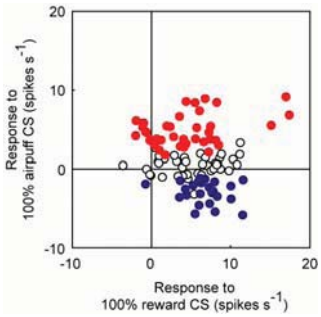
Electrophysiological properties for AUS-inhibited, AUS-excited, and AUS-non-responsive neurons (mean ± s.d.). A significant difference between AUS-inhibited and AUS-excited neurons is indicated by a single asterisk ($P < 0.05$, Wilcoxon rank-sum test) or double asterisk ($P < 0.01$).

Supplementary Figure 1



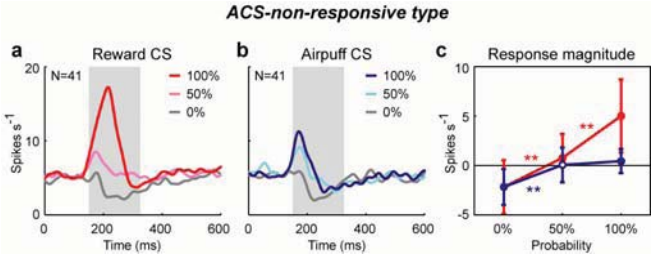
Spike shape and baseline firing rate of dopamine and non-dopamine neurons. a, Averaged spike shapes of dopamine neurons [ACS-excited type (red), ACS-inhibited type (blue), ACS-non-responsive type (green)], and non-dopamine neurons (black). **b**, Relationship between spike duration and baseline firing rate for dopamine neurons [ACS-excited type (red), ACS-inhibited type (blue), ACS-non-responsive type (green)] and non-dopamine neurons [neurons with excitatory responses to free reward (black), neurons without excitatory responses to free reward (white)]. The marginal line graphs show the distributions of baseline activity and spike duration. For recording of spikes, extracellular potentials have been bandpass-filtered (200Hz - 10kHz).

Supplementary Figure 2



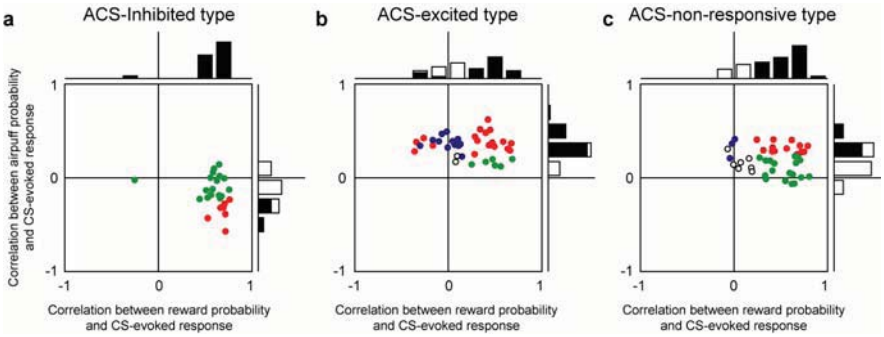
Comparison of reward- and airpuff-CS responses. Data for each neuron are shown by a dot indicating the magnitude of the response to 100% reward CS (abscissa) and the magnitude of the response to 100% airpuff CS (ordinate). Blue dots indicate neurons with statistically significant inhibition to 100% airpuff CS (i.e., ACS-inhibited neurons) ($P < 0.05$, Wilcoxon signed-rank test). Red dots indicate neurons with statistically significant excitation to 100% airpuff CS (i.e., ACS-excited neurons). White dots, no significance (i.e., ACS-non-responsive neurons). Data were obtained from all dopamine neurons (n=103) we examined.

Supplementary Figure 3



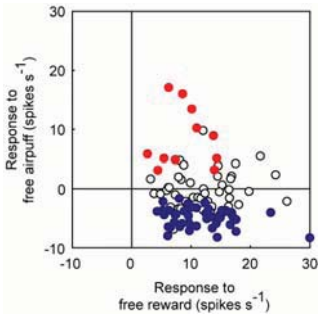
Responses of the ACS-non-responsive neurons to CSs. a, **b**, Averaged activity of 41 ACS-non-responsive neurons aligned at CS onset in the appetitive (**a**) and aversive blocks (**b**). Conventions are the same as Fig. 2b and c. **c**, Response magnitude of the ACS-non-responsive neurons to the reward (red) and airpuff CSs (blue). Conventions are the same as Fig. 2d.

Supplementary Figure 4



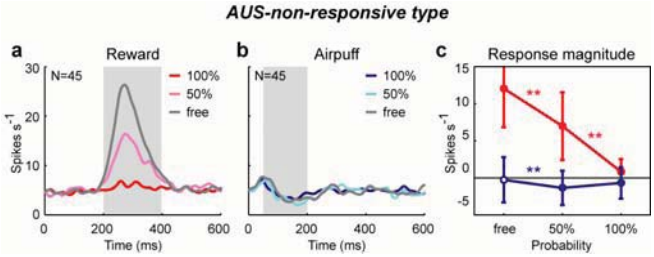
Correlation between outcome probability and CS-evoked response for individual neurons. a, Correlation coefficients for ACS-inhibited neurons (n = 24). Data for each neuron are shown by a dot indicating the correlation coefficient between reward probability and CS-evoked response (abscissa) and the correlation coefficient between airpuff probability and CS-evoked response (ordinate). Green, blue and red dots indicate neurons with a statistically significant correlation between reward probability and CS-evoked response, between airpuff probability and CS-evoked response, and both of them, respectively ($P < 0.05$). White dots, no significance. The marginal histograms show the distributions of correlation coefficients. Black bars indicate neurons with a statistically significant correlation ($P < 0.05$). White bars, no significance. **b**, Correlation coefficients for ACS-excited neurons (n = 38). **c**, Correlation coefficients for ACS-non-responsive neurons (n = 41).

Supplementary Figure 5



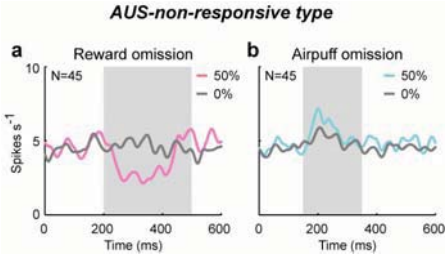
Comparison of reward- and airpuff-US responses. Data for each neuron are shown by a dot indicating the magnitude of the response to free reward (abscissa) and the magnitude of the response to free airpuff (ordinate). Blue dots indicate neurons with statistically significant inhibition to free airpuff (i.e., AUS-inhibited neurons) ($P < 0.05$, Wilcoxon signed-rank test). Red dots indicate neurons with statistically significant excitation to free airpuff (i.e., AUS-excited neurons). White dots, no significance (i.e., AUS-non-responsive neurons). Data were obtained from all dopamine neurons (n=103) we examined.

Supplementary Figure 6



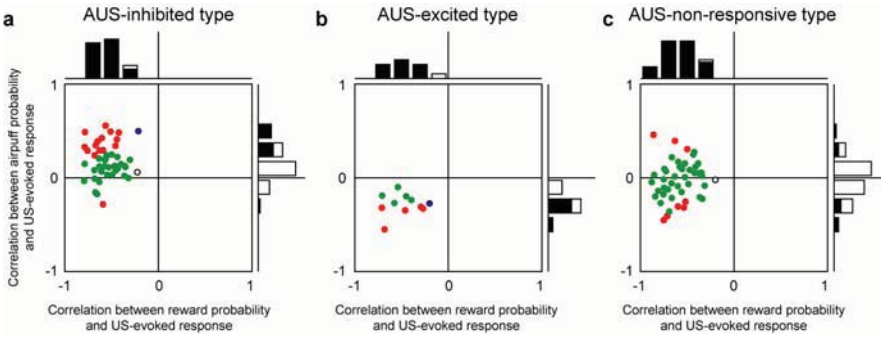
Responses of the AUS-non-responsive neurons to USs. a, **b**, Averaged activity of 45 AUS-non-responsive neurons aligned at US onset in the appetitive (**a**) and aversive blocks (**b**). Conventions are the same as Fig. 3b and c. **c**, Response magnitude of AUS-non-responsive neurons to the reward (red) and airpuff (blue). Conventions are the same as Fig. 3d.

Supplementary Figure 7



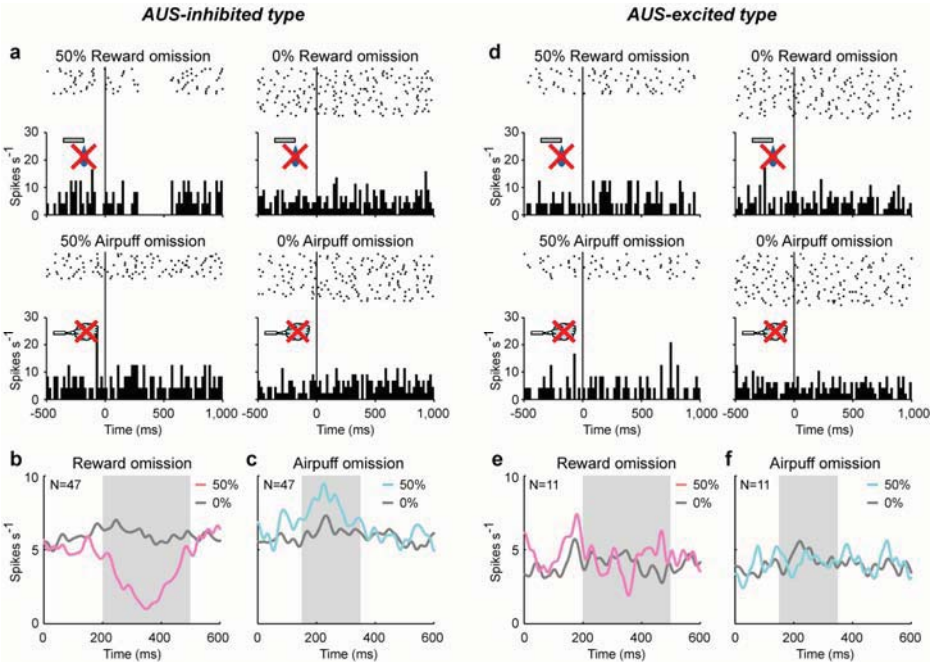
Responses of the AUS-non-responsive neurons to US omission. a, **b**, Averaged activity of 45 AUS-non-responsive neurons aligned at CS offset in the appetitive (**a**) and aversive blocks (**b**). Conventions are the same as Supplementary Fig. 9b and c.

Supplementary Figure 8



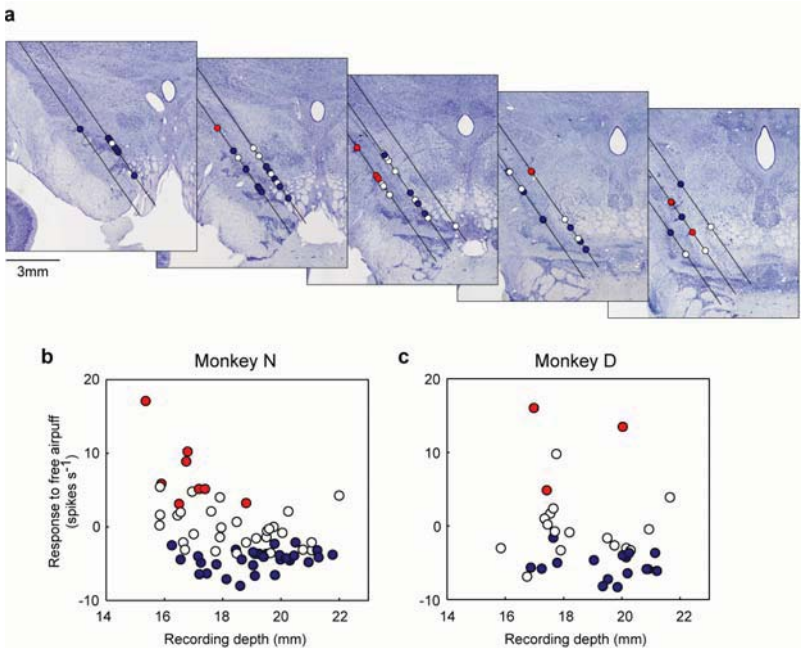
Correlation between outcome probability and US-evoked response for individual neurons. a, Correlation coefficients for AUS-inhibited neurons (n = 47). Data for each neuron are shown by a dot indicating the correlation coefficient between reward probability and US-evoked response (abscissa) and the correlation coefficient between airpuff probability and US-evoked response (ordinate). Green, blue and red dots indicate neurons with a statistically significant correlation between reward probability and US-evoked response, between airpuff probability and US-evoked response, and both of them, respectively ($P < 0.05$). White dots, no significance. The marginal histograms show the distributions of correlation coefficients. Black bars indicate neurons with a statistically significant correlation ($P < 0.05$). White bars, no significance. **b**, Correlation coefficients for AUS-excited neurons (n = 11). **c**, Correlation coefficients for AUS-non-responsive neurons (n = 45).

Supplementary Figure 9



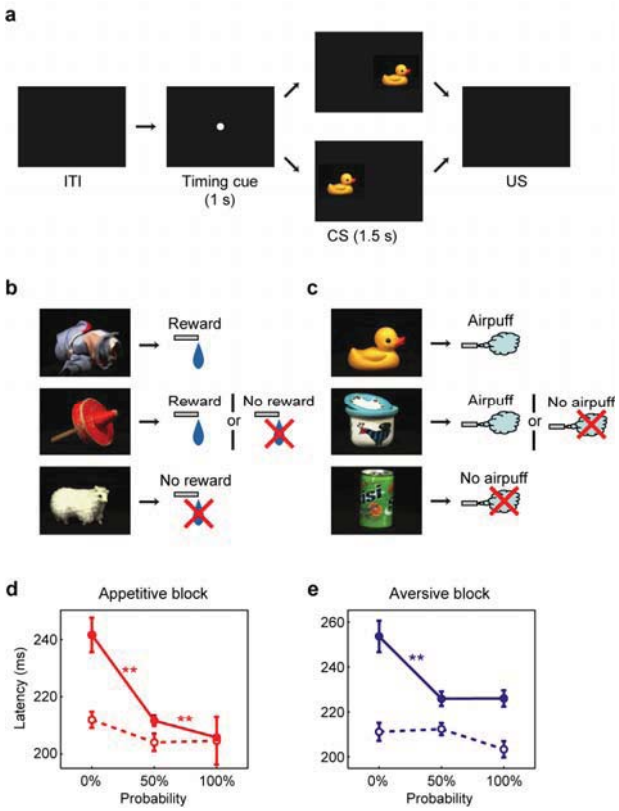
Responses of dopamine neurons to US omission. a, **d**, Activity of the same neurons shown in Fig. 3a and e, respectively, in the appetitive block (top row) and aversive block (bottom row). Histograms and rasters are aligned at the end of the CS when the US would have started (but did not occur), and are shown for 50% reward omission, 0% reward omission, 50% airpuff omission, and 0% airpuff omission. **b**, **c**, Averaged activity of 47 AUS-inhibited neurons. **e**, **f**, Averaged activity of 11 AUS-excited neurons. SDFs are shown for 50% reward omission (light red) and 0% reward omission (gray) in the appetitive block (**b**, **e**), and for 50% airpuff omission (light blue) and 0% airpuff omission (gray) in the aversive block (**c**, **f**). Gray area indicates the period that was used to analyze US omission-evoked response.

Supplementary Figure 10



Locations of dopamine neurons in relation to their responses to airpuff itself. a, Recording sites of the same 68 dopamine neurons in monkey N are plotted on the same five coronal sections in Fig. 4a. Red circles indicate neurons showing significant excitations to free airpuff (i.e., AUS-excited neurons). Blue circles indicate neurons showing significant inhibitions to free airpuff (i.e., AUS-inhibited neurons). White circles, no significance (i.e., AUS-non-responsive neurons). **b**, **c**, Relation between the recording depth and the response to free airpuff for monkey N (**b**) and monkey D (**c**). Conventions are the same as Fig. 4.

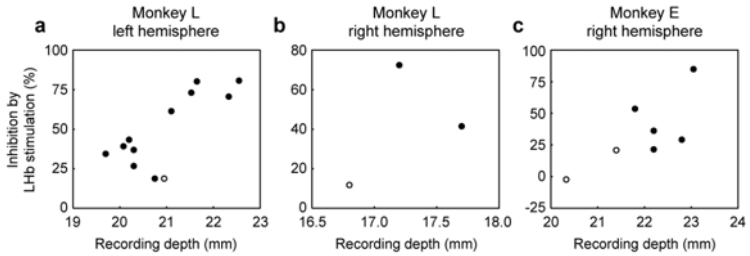
Supplementary Figure 11



Pavlovian procedure to test the effect of motivational salience on orienting behavior. **a**, Sequence of the Pavlovian procedure. After random ITI (3 – 6 s), a timing cue was presented. After 1 s, the timing cue disappeared and one of six CSs was presented at the right or left side of the timing cue. After 1.5 s, the CS disappeared and the US was delivered. **b**, Association between three CSs and outcome in the appetitive block. **c**, Association between three CSs and outcome in the aversive block. **d**, **e**, Gaze latency (i.e., time from the CS onset to the time when the monkey's gaze was directed to the CS) in the appetitive block (**d**) and aversive block (**e**). Dashed line indicates the latency before the CSs were associated with reward or airpuff; all of the CSs were associated with

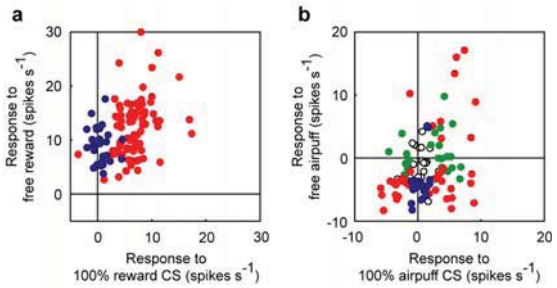
reward with 100% probability. Solid line indicates the latency after the CSs were associated with reward or airpuff. Double asterisks indicate a significant difference in the gaze latency between two data points ($P < 0.01$, Wilcoxon rank-sum test). Error bars indicate s.e.m.

Supplementary Figure 12



Relationship between recording depth and habenula-induced inhibition of dopamine neurons. For each neuron (denoted by a dot), the magnitude of inhibition elicited by the electrical stimulation of the lateral habenula (LHb; 100 μA, single pulse) (ordinate) is plotted against the recording depth in the SNc-VTA region (abscissa). The recording depth was measured from a reference depth set by a manipulator to advance the recording electrode. The data were obtained in our previous study[20], and are shown separately for three hemispheres in two monkeys. There was a significant positive correlation or its trend (**a**, $r = 0.76$, $P < 0.01$; **b**, $r = 0.43$, $P > 0.05$; **c**, $r = 0.74$, $P = 0.059$). Black dots indicate dopamine neurons with a statistically significant inhibition by LHb stimulation ($P < 0.01$, Wilcoxon signed-rank test). White dots, no significance.

Supplementary Figure 13



Comparison between CS- and US-evoked responses. a, Data for each neuron are shown by a dot indicating the magnitude of the response to 100% reward CS (abscissa) and the response to free reward (ordinate). Blue dots indicate neurons with statistically significant response to free reward. Red dots indicate neurons with statistically significant responses to both free reward and 100% reward CS, respectively ($P < 0.05$, Wilcoxon signed-rank test). **b**, Data for each neuron are shown by a dot indicating the magnitude of the response to 100% airpuff CS (abscissa) and the response to free airpuff (ordinate). Blue, green and red dots indicate neurons with statistically significant response to free airpuff, 100% airpuff CS, and both of them, respectively ($P < 0.05$, Wilcoxon signed-rank test). White dots, no significance. Data were obtained from all dopamine neurons (n=103) we examined.