LETTERS

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

Masayuki Matsumoto[1] & Okihide Hikosaka[1]

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

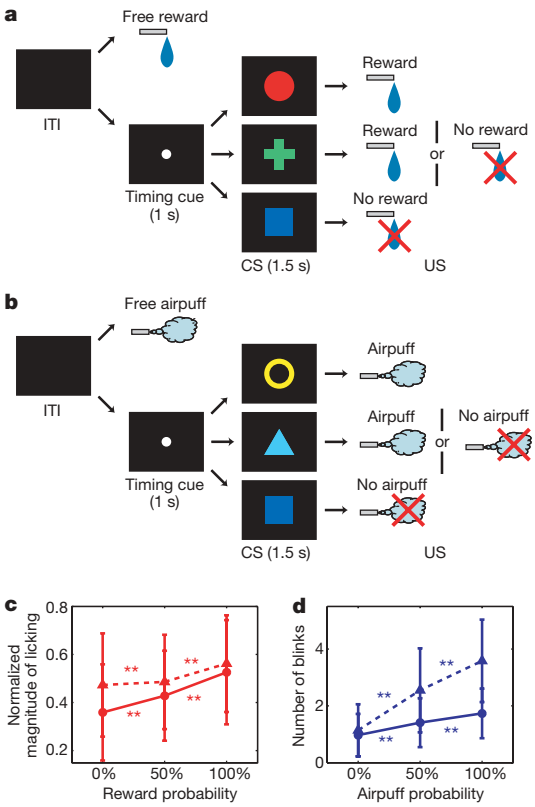

Figure 1 | Pavlovian procedure. **a**, Appetitive block. Three conditioned stimuli were associated with apple juice with 100%, 50% and 0% probability, respectively. **b**, Aversive block. Three conditioned stimuli were associated with an aversive airpuff with 100%, 50% and 0% probability, respectively. 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.

[1]Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892-4435, USA.

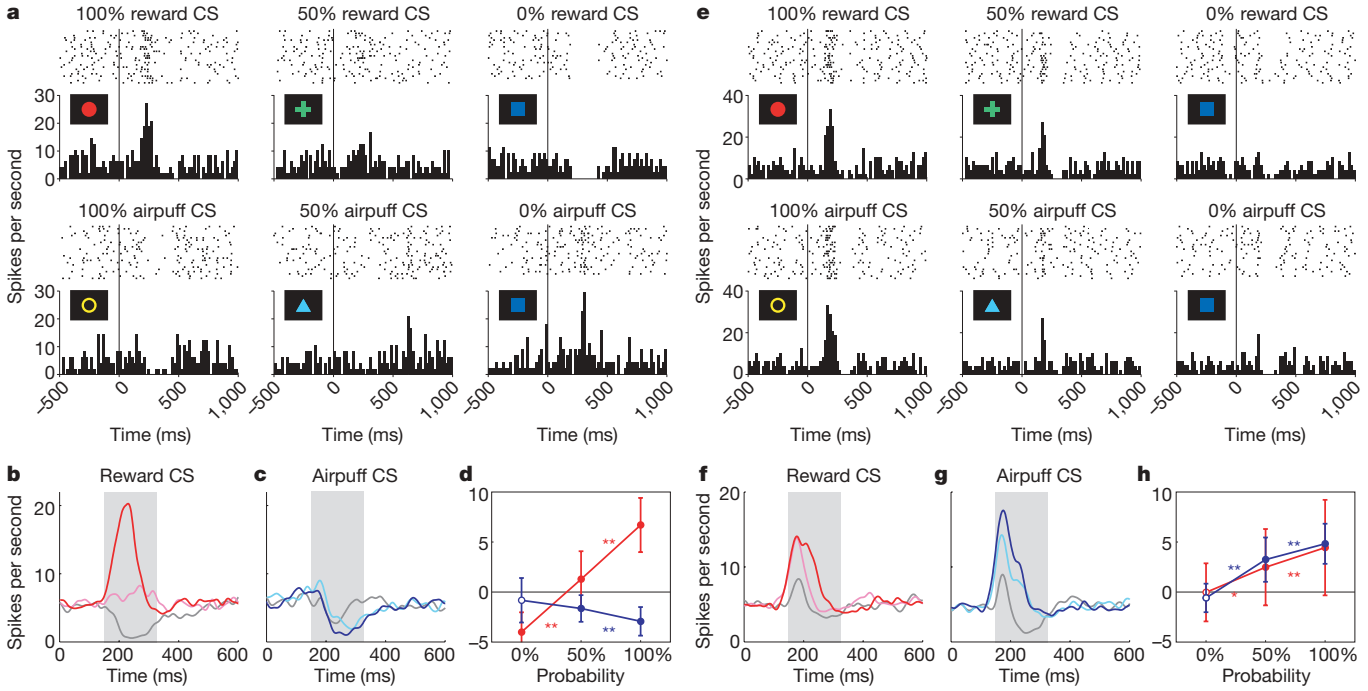

Figure 2 | Responses of dopamine neurons to conditioned stimuli. **a**, **e**, Activity of two example neurons in the appetitive block (top row) and aversive block (bottom row), which were classified as ACS-inhibited type (**a**) and ACS-excited type (**e**). Histograms (20-ms bins) and rasters are aligned 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

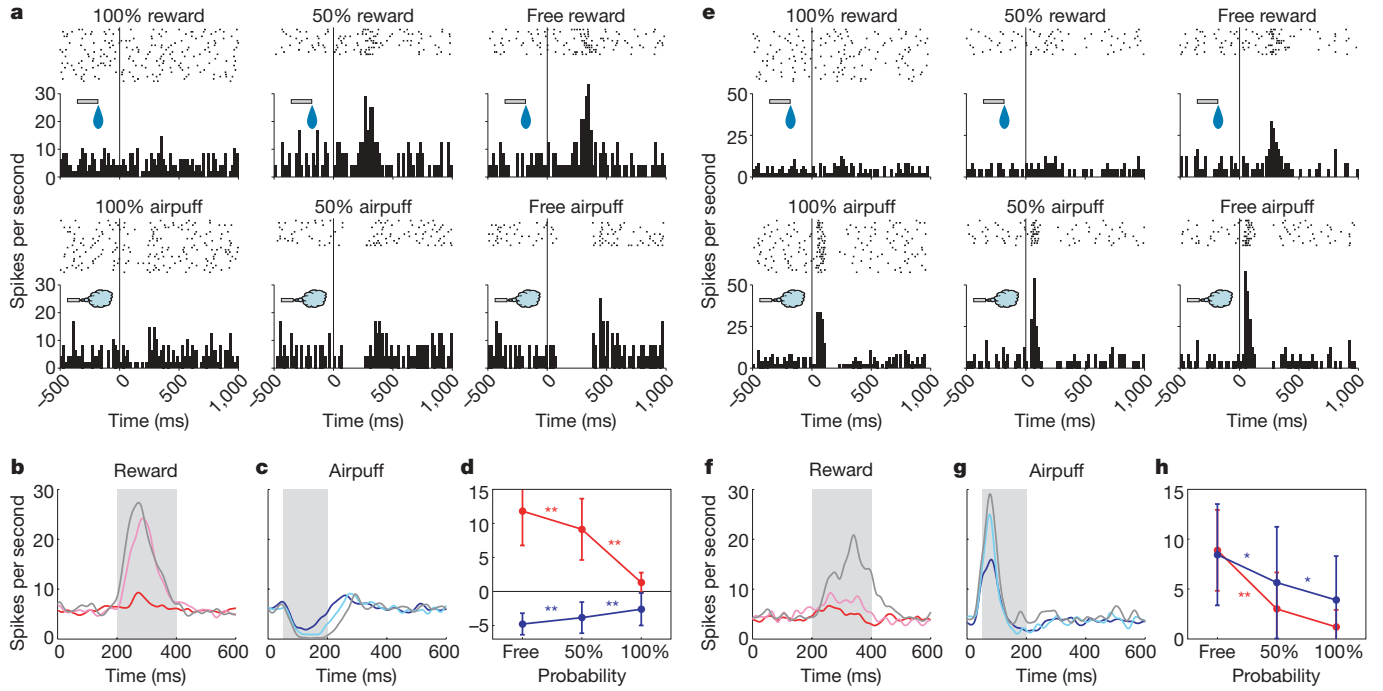

Figure 3 | Responses of dopamine neurons to unconditioned stimuli. **a**, **e**, Activity of two example neurons in the appetitive block (top row) and aversive block (bottom row), which were classified as AUS-inhibited type (**a**) and AUS-excited type (**e**). Histograms and rasters are aligned at the 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

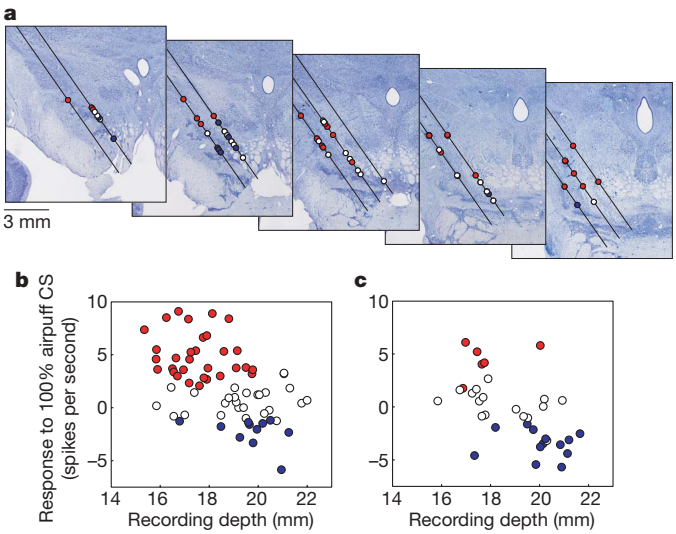

Figure 4 | Locations of dopamine neurons in relation to their responses to airpuff-predicting conditioned stimulus. **a**, Recording sites of 68 dopamine neurons in monkey N are plotted on five coronal sections shown rostrocaudally from left to right (interval, 1 mm). Red circles indicate neurons showing 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.

Received 26 January; accepted 27 March 2009.
Published online 17 May 2009.

1. Schultz, W. Predictive reward signal of dopamine neurons. *J. Neurophysiol.* **80**, 1–27 (1998).

2. Satoh, T., Nakai, S., Sato, T. & Kimura, M. Correlated coding of motivation and outcome of decision by dopamine neurons. *J. Neurosci.* **23,** 9913–9923 (2003).
3. Takikawa, Y., Kawagoe, R. & Hikosaka, O. A possible role of midbrain dopamine neurons in short- and long-term adaptation of saccades to position-reward mapping. *J. Neurophysiol.* **92,** 2520–2529 (2004).
4. Morris, G., Arkadir, D., Nevet, A., Vaadia, E. & Bergman, H. Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron* **43,** 133–143 (2004).
5. Tobler, P. N., Fiorillo, C. D. & Schultz, W. Adaptive coding of reward value by dopamine neurons. *Science* **307,** 1642–1645 (2005).
6. Ungless, M. A., Magill, P. J. & Bolam, J. P. Uniform inhibition of dopamine neurons in the ventral tegmental area by aversive stimuli. *Science* **303,** 2040–2042 (2004).
7. Chiodo, L. A., Antelman, S. M., Caggiula, A. R. & Lineberry, C. G. Sensory stimuli alter the discharge rate of dopamine (DA) neurons: evidence for two functional types of DA cells in the substantia nigra. *Brain Res.* **189,** 544–549 (1980).
8. Coizet, V., Dommett, E. J., Redgrave, P. & Overton, P. G. Nociceptive responses of midbrain dopaminergic neurones are modulated by the superior colliculus in the rat. *Neuroscience* **139,** 1479–1493 (2006).
9. Schultz, W. & Romo, R. Responses of nigrostriatal dopamine neurons to high-intensity somatosensory stimulation in the anesthetized monkey. *J. Neurophysiol.* **57,** 201–217 (1987).
10. Mantz, J., Thierry, A. M. & Glowinski, J. Effect of noxious tail pinch on the discharge rate of mesocortical and mesolimbic dopamine neurons: selective activation of the mesocortical system. *Brain Res.* **476,** 377–381 (1989).
11. Guarraci, F. A. & Kapp, B. S. An electrophysiological characterization of ventral tegmental area dopaminergic neurons during differential Pavlovian fear conditioning in the awake rabbit. *Behav. Brain Res.* **99,** 169–179 (1999).
12. Mirenowicz, J. & Schultz, W. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature* **379,** 449–451 (1996).
13. Joshua, M., Adler, A., Mitelman, R., Vaadia, E. & Bergman, H. Midbrain dopaminergic neurons and striatal cholinergic interneurons encode the difference between reward and aversive events at different epochs of probabilistic classical conditioning trials. *J. Neurosci.* **28,** 11673–11684 (2008).
14. Schultz, W., Dayan, P. & Montague, P. R. A neural substrate of prediction and reward. *Science* **275,** 1593–1599 (1997).
15. Montague, P. R., Dayan, P. & Sejnowski, T. J. A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *J. Neurosci.* **16,** 1936–1947 (1996).
16. Lynd-Balta, E. & Haber, S. N. The organization of midbrain projections to the striatum in the primate: sensorimotor-related striatum versus ventral striatum. *Neuroscience* **59,** 625–640 (1994).
17. Ikemoto, S. Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. *Brain Res. Rev.* **56,** 27–78 (2007).
18. Knutson, B., Adams, C. M., Fong, G. W. & Hommer, D. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J. Neurosci.* **21,** RC159 (2001).
19. Cromwell, H. C. & Schultz, W. Effects of expectations for different reward magnitudes on neuronal activity in primate striatum. *J. Neurophysiol.* **89,** 2823–2838 (2003).
20. Schultz, W., Apicella, P., Scarnati, E. & Ljungberg, T. Neuronal activity in monkey ventral striatum related to the expectation of reward. *J. Neurosci.* **12,** 4595–4610 (1992).
21. Kitama, T., Ohno, T., Tanaka, M., Tsubokawa, H. & Yoshida, K. Stimulation of the caudate nucleus induces contraversive saccadic eye movements as well as head turning in the cat. *Neurosci. Res.* **12,** 287–292 (1991).
22. Hikosaka, O., Takikawa, Y. & Kawagoe, R. Role of the basal ganglia in the control of purposive saccadic eye movements. *Physiol. Rev.* **80,** 953–978 (2000).
23. Carli, M., Evenden, J. L. & Robbins, T. W. Depletion of unilateral striatal dopamine impairs initiation of contralateral actions and not sensory attention. *Nature* **313,** 679–682 (1985).
24. Holland, P. C. & Gallagher, M. Amygdala circuitry in attentional and representational processes. *Trends Cogn. Sci.* **3,** 65–73 (1999).
25. Lin, S. C. & Nicolelis, M. A. Neuronal ensemble bursting in the basal forebrain encodes salience irrespective of valence. *Neuron* **59,** 138–149 (2008).
26. Richardson, R. T. & DeLong, M. R. Electrophysiological studies of the functions of the nucleus basalis in primates. *Adv. Exp. Med. Biol.* **295,** 233–252 (1991).
27. Matsumoto, M. & Hikosaka, O. Representation of negative motivational value in the primate lateral habenula. *Nature Neurosci.* **12,** 77–84 (2009).
28. Matsumoto, M. & Hikosaka, O. Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* **447,** 1111–1115 (2007).

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

Acknowledgements We thank S. Hong, E. Bromberg-Martin, M. Yasuda, S. Yamamoto and Y. Tachibana for discussion, M. K. Smith for his histological expertise, J. W. McClurkin, A. M. Nichols, T. W. Ruffner, A. V. Hays and L. P. Jensen for technical assistance, and G. Tansey, D. Parker and B. Nagy for animal care. This research was supported by the Intramural Research Program at the National Institutes of Health, National Eye Institute.

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.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to M.M. (matsumotom@nei.nih.gov).

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.

29. Davies, R. M., Gerstein, G. L. & Baker, S. N. Measurement of time-dependent changes in the irregularity of neural spiking. *J. Neurophysiol.* **96**, 906–918 (2006).