nature neuroscience

Representation of negative motivational value in the primate lateral habenula

Masayuki Matsumoto & Okihide Hikosaka

An action may lead to either a reward or a punishment. Therefore, an appropriate action needs to be chosen on the basis of the values of both expected rewards and expected punishments. To understand the underlying neural mechanisms, we conditioned monkeys using a Pavlovian procedure with two distinct contexts: one in which rewards were available and another in which punishments were feared. We found that the population of lateral habenula neurons was most strongly excited by a conditioned stimulus associated with the most unpleasant event in each context: the absence of the reward or the presence of the punishment. The population of lateral habenula neurons was also excited by the punishment itself and inhibited by the reward itself, especially when they were less predictable. These results suggest that the lateral habenula has the potential to adaptively control both reward-seeking and punishment-avoidance behaviors, presumably through its projections to dopaminergic and serotonergic systems.

Making an appropriate choice of action requires that the brain computes the value for each action on the basis of both expected rewards and expected punishments. A straightforward way to perform this computation would be to have neurons that represent both kinds of values. But are there such neurons in the brain?

Human imaging studies have reported that activity in several brain areas represents the values of both rewards and punishments[1–4]. However, it is possible that distinct types of single neurons in these areas represent the values separately for rewards and punishments. The only way to answer to this question is to analyze the activity of single neurons while an animal behaves in the expectation of rewards and punishments. To date, several unit-recording studies have found value-coding neurons in several brain areas[5–11]; however, most of these studies were based on the manipulation of reward properties (that is, size and/or probability). A small number of studies used both rewards and punishments as possible outcomes[7,12–14].

The lateral habenula, a brain structure located in the epithalamus, is in a good position to represent emotional and motivational events. It receives inputs from forebrain limbic regions[15,16] and projects to midbrain structures such as the substantia nigra pars compacta and ventral tegmental area, which contain dopamine neurons, and the raphe nuclei, which contain serotonin neurons[17]. Thus, the lateral habenula could control the monoaminergic (especially dopaminergic and serotonergic) systems that influence emotion and motivation[18–20]. Indeed, electrical stimulation of the lateral habenula inhibits dopamine neurons[21,22] and serotonin neurons[23]. Consistent with this view, the lateral habenula has been implicated in many emotional and cognitive functions including anxiety, stress, pain, learning and attention[24,25]. In a recent study, we found that neurons in the lateral habenula respond to rewards and sensory stimuli predicting rewards and that they send these reward-related signals to dopamine neurons in the substantia nigra by inhibiting them[26]. However, this study did not test whether lateral habenula neurons respond to punishments or to sensory stimuli predicting punishments.

To investigate how lateral habenula neurons respond to punishments and their predictors, as well as rewards and their predictors, we recorded the activity of lateral habenula neurons in monkeys while they were conditioned in a Pavlovian procedure with two distinct contexts: one in which rewards were available and another in which punishments were feared. We found that many lateral habenula neurons responded differentially to visual stimuli that indicated rewarding and aversive events and did so in a context-dependent manner.

RESULTS

We conditioned two monkeys using a Pavlovian procedure with an appetitive unconditioned stimulus (liquid reward) and an aversive unconditioned stimulus (airpuff directed at the face). This Pavlovian procedure consisted of two blocks of trials, a reward block (**Fig. 1a**) and a punishment block (**Fig. 1b**). In the reward block, three conditioned stimuli were associated with reward, with probabilities of 100%, 50% and 0%. In the punishment block, three conditioned stimuli were associated with airpuff, with probabilities of 100%, 50% and 0%. Thus, this Pavlovian procedure had two distinct contexts. Each trial in each block started after the presentation of a timing cue (central small spot) on a screen. 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

Laboratory of Sensorimotor Research, National Eye Institute, US National Institutes of Health, 49 Convent Drive, Bethesda, Maryland 20892-4435, USA. Correspondence should be addressed to M.M. (matsumotom@nei.nih.gov).

Received 19 August; accepted 30 October; published online 30 November 2008; doi:10.1038/nn.2233

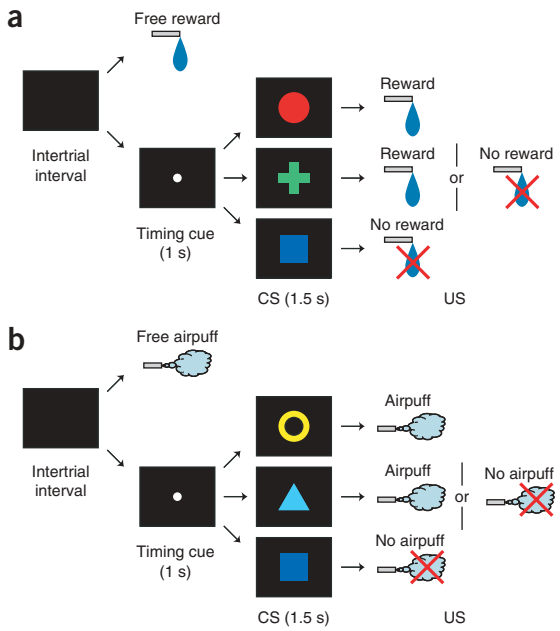

Figure 1 Pavlovian procedure with two distinct contexts. (**a**) Reward block. (**b**) Punishment block. CS, conditioned stimulus; US, unconditioned stimulus.

delivered. In addition to the cued trials, noncued trials were included in which a reward alone (free reward) was delivered during the reward block and an airpuff alone (free airpuff) was delivered during the punishment block. Each block consisted of 42 trials and was repeated twice or more. Notably, the same visual stimulus (blue square) was used as the conditioned stimulus that was associated with no outcome in both blocks, although this conditioned stimulus would be unpleasant in the reward block but pleasant in the punishment block. We monitored anticipatory licking (a type of approach behavior) and blinking (a type of avoidance behavior) of the monkeys during conditioned stimulus presentation. These behavioral data suggested that the monkeys discriminated between the conditioned stimuli (**Supplementary Note** and **Supplementary Figs. 1** and **2** online).

Response to conditioned stimuli

We recorded single-unit activity from 72 lateral habenula neurons (45 in monkey N and 27 in monkey D) using the Pavlovian procedure. These neurons were estimated to be in the lateral habenula by their physiological properties and by magnetic resonance imaging (MRI, see Methods), and we confirmed their localization histologically (**Supplementary Note** and **Supplementary Fig. 3** online). We first examined the response of these neurons to the conditioned stimuli (the activity of an example neuron is shown in **Fig. 2a**,**b**). The activity of the neuron decreased after the appearance of the 100% and 50% reward conditioned stimuli (the conditioned stimuli associated with reward at 100% and 50% probabilities, respectively) and increased after the appearance of the 0% reward conditioned stimulus in the reward block (**Fig. 2a**). The magnitude of the inhibition decreased and reversed to an excitation as the reward probability decreased. In contrast, the activity increased after the appearance of the 100% and 50% airpuff conditioned stimuli and decreased after the appearance of the 0% airpuff conditioned stimulus in the punishment block (**Fig. 2b**). The magnitude of the excitation decreased and reversed to an inhibition as the airpuff probability decreased. Notably, the same blue square that was associated with no outcome elicited an excitation in the reward block and elicited an inhibition in the punishment block.

To characterize the responses to the conditioned stimuli (hereafter called conditioned stimulus responses), we performed a one-way analysis of variance (ANOVA) across the six conditions (that is, 100%, 50% and 0% reward conditioned stimuli and 100%, 50% and 0% airpuff conditioned stimuli) for each neuron. By this analysis, 49 of the 72 neurons showed significantly differential conditioned stimulus responses across the conditions ($P < 0.05$, one-way ANOVA). The averaged activity of these neurons showed the strongest excitation to 0% reward conditioned stimulus in the reward block (**Fig. 2c**) and 100% airpuff conditioned stimulus in the punishment block (**Fig. 2d**). These excitatory responses were graded by the reward probability and the airpuff probability in the opposite directions.

These results suggest that the conditioned stimulus responses of lateral habenula neurons were modulated by the motivational valence that was assigned with the conditioned stimuli. We thus plotted the averaged magnitude of the conditioned stimulus responses according to the objective value of the outcomes (**Fig. 3a**). Because the objective value of a future reward is determined by the multiplicative product of its magnitude and its probability[27], and as we fixed the reward magnitude, the objective reward value should be scaled according to its probability in the positive direction (**Fig. 3a**). It is then natural to scale the objective airpuff value in the same manner, now in the negative direction (**Fig. 3a**). In support of this assumption, the frequency of approach behavior (anticipatory licking) increased as the positive value increased, whereas the frequency of avoidance behavior (anticipatory blinking) increased as the negative value increased (**Supplementary Fig. 1**).

The averaged magnitude of the conditioned stimulus response increased as the objective value decreased in both the reward and punishment blocks (**Fig. 3a**). To examine whether such a response pattern was achieved by single lateral habenula neurons, we calculated the correlation coefficient between conditioned stimulus response and objective value of each neuron separately for the reward and punishment blocks (**Fig. 3b**). Many neurons showed a significant negative correlation in the reward block ($n = 35$) and the punishment block ($n = 30$) ($P < 0.05$). Of these, 23 neurons showed a significant negative correlation in both of them ($P < 0.05$). The mean correlation coefficient was significantly smaller than zero in both blocks ($P < 0.01$, Wilcoxon signed-rank test). These results indicate that many individual neurons increased their conditioned stimulus responses as the objective value decreased in both blocks.

However, the relationship between the conditioned stimulus response and the objective value appears somewhat different between the reward and punishment blocks. In the punishment block, the averaged conditioned stimulus response linearly increased as the objective value decreased (**Fig. 3a**). In the reward block, the increase in the averaged conditioned stimulus response was larger between 0% and 50% reward conditioned stimuli than between 50% and 100% reward conditioned stimuli (**Fig. 3a**). To statistically analyze this trend, we calculated a linearity index (see Methods) for each neuron, separately for the reward and punishment blocks (**Fig. 3c**). Briefly, the linearity index is positive if the response to the 50% conditioned stimulus is larger than the average of the responses to the 0% and 100% conditioned stimuli and negative if the response to the 50% conditioned stimulus is smaller than the average. In the punishment block, the mean of the linearity indices was not significantly different from zero ($P > 0.05$, Wilcoxon signed-rank test), indicating that the conditioned stimulus response linearly increased as the objective value decreased. In the reward block, however, the mean of the linearity

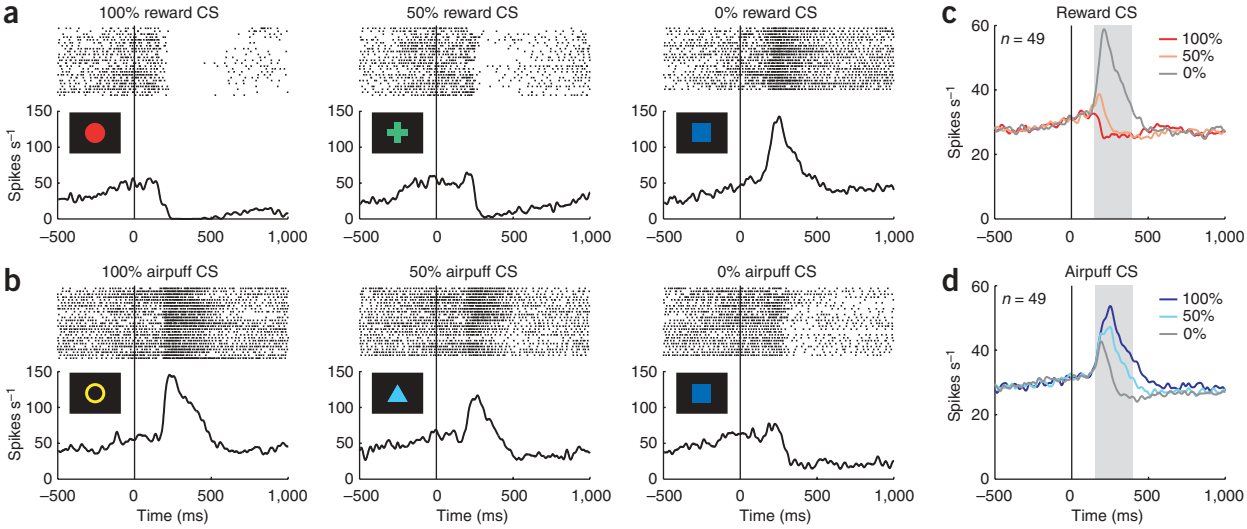

Figure 2 Responses of lateral habenula neurons to conditioned stimuli. (**a**) Activity of an example neuron during the reward block. Rasters and spike density functions (SDFs) are aligned by conditioned stimulus onset and shown for 100%, 50% and 0% reward conditioned stimuli. (**b**) Activity of the neuron shown in **a** during the punishment block. Rasters and SDFs are shown for 100%, 50% and 0% airpuff conditioned stimuli. (**c**) Averaged activity of the 49 neurons during the reward block. SDFs are shown for 100% (dark red), 50% (light red) and 0% reward conditioned stimuli (gray). Gray area indicates the period that was used to analyze conditioned stimulus response. (**d**) Averaged activity of the 49 neurons during the punishment block. SDFs are shown for 100% (dark blue), 50% (light blue) and 0% airpuff conditioned stimuli (gray).

indices was significantly smaller than zero ($P < 0.01$, Wilcoxon signed-rank test), indicating that the conditioned stimulus response increased abruptly from the 50% to the 0% reward conditioned stimuli. This nonlinearity is reminiscent of the change in the number of blinks in the reward block (**Supplementary Fig. 1**). If the number of blinks is related to the unpleasantness, these results may suggest that lateral habenula neurons preferentially represent unpleasant events (for example, 0% reward conditioned stimulus) rather than events that are not unpleasant (for example, 50% and 100% reward conditioned stimuli).

Another notable feature of the relationship between conditioned stimulus response and objective value is the interruption between the reward and punishment blocks. Although the objective values of the 0% reward conditioned stimulus and 0% airpuff conditioned stimulus were identical, the response to the 0% reward conditioned stimulus was significantly larger than the response to the 0% airpuff conditioned stimulus ($P < 0.01$, Wilcoxon signed-rank test; **Fig. 3a**). This interruption indicates the context-dependency of the conditioned stimulus response and has important consequences. That is, lateral habenula neurons were, on average, most strongly and equally excited by a conditioned stimulus that was associated with the most unpleasant event in each context, regardless of whether the event was the absence of reward (0% reward conditioned stimulus) or the presence of airpuff (100% airpuff conditioned stimulus) (**Fig. 3a**). This appears to correspond with the well-known relativity of subjective values[28].

The relativity of the conditioned stimulus response was accomplished by single lateral habenula neurons, as there was a clear correlation in single cellular responses between the most unpleasant events in the two contexts: the response to the 0% reward conditioned stimulus and the response to the 100% airpuff conditioned stimulus for individual neurons ($r = 0.906$, $P < 0.01$; **Supplementary Note** and **Supplementary Fig. 4** online). The same tendency was observed for the most pleasant events in the two contexts: the response to the 100% reward conditioned stimulus and the response to the 0% airpuff conditioned stimulus ($r = 0.729$, $P < 0.01$; **Supplementary Note** and **Supplementary Fig. 4**).

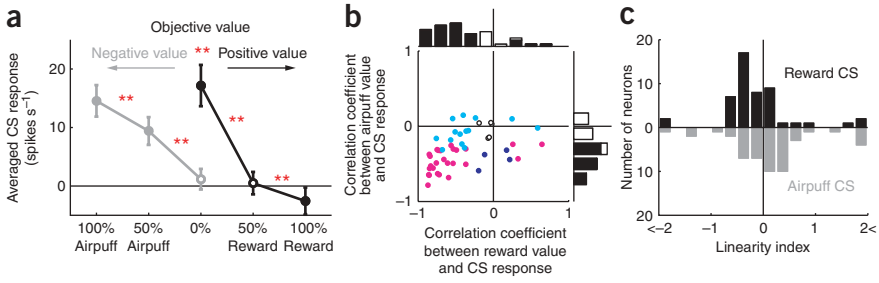

Figure 3 Relationship between objective value and conditioned stimulus response. (**a**) Averaged magnitude of the conditioned stimulus response of the 49 neurons plotted against the objective value of outcome for the reward block (black) and the punishment block (gray). Filled symbols indicate a significant deviation from zero ($P < 0.05$, Wilcoxon signed-rank test). Double asterisks indicate a significant difference between two conditioned stimulus responses ($P < 0.01$, Wilcoxon signed-rank test). Error bars indicate s.e.m. (**b**) Correlation coefficients of the 49 neurons between objective value and conditioned stimulus response. The abscissa indicates correlation coefficient between reward value and conditioned stimulus response. The ordinate indicates correlation coefficient between airpuff value and conditioned stimulus response. Cyan, dark blue and magenta dots indicate neurons with statistically significant correlation between reward value and conditioned stimulus response, between airpuff value and conditioned stimulus response, and both of them, respectively ($P < 0.05$). White dots indicate no significance ($P > 0.05$). The marginal histograms show the distribution of correlation coefficients. Black bars indicate neurons with statistically significant correlation ($P < 0.05$). White bars indicate no significance ($P > 0.05$). (**c**) Distributions of the linearity indices of the 49 neurons. Black bars indicate the distribution of linearity indices in the reward block. Gray bars indicate the distribution in the punishment block.

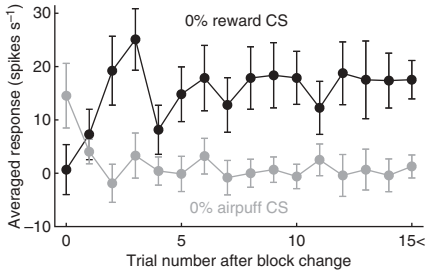

Figure 4 Changes in the averaged responses of the 49 neurons to 0% reward conditioned stimulus (black) and 0% airpuff conditioned stimulus (gray) after the block context was reversed. The abscissa indicates the number of preceding trials (excluding 0% reward conditioned stimulus and 0% airpuff conditioned stimulus trials) in a given block. When either 0% reward conditioned stimulus or 0% airpuff conditioned stimulus was presented on the first trial after block change, the neuronal response was included in the data at trial zero. Error bars indicate s.e.m.

Because the physical properties of the 0% reward conditioned stimulus and 0% airpuff conditioned stimulus were identical, these conditioned stimuli could only be distinguished by the block context (reward block or punishment block). We thus examined how the differential responses to the 0% reward conditioned stimulus and 0% airpuff conditioned stimulus developed after the block context was changed. We plotted the averaged responses to the 0% reward conditioned stimulus and 0% airpuff conditioned stimulus against the number of preceding trials (excluding 0% reward conditioned stimulus and 0% airpuff conditioned stimulus trials) in a given block (**Fig. 4**). The responses at trial zero reflected the previous context, but then changed and reached a plateau after the second or third trials.

Response to unconditioned stimuli and their omission

Many lateral habenula neurons also responded to the unconditioned stimuli. We aligned the activity of an example neuron by unconditioned stimulus onset (**Fig. 5a**,**b**). The neuron showed phasic responses to the unconditioned stimuli: an inhibition to free reward (**Fig. 5a**) and an excitation to free airpuff (**Fig. 5b**). However, these responses were strongly modulated by the preceding conditioned stimuli. The inhibitory response to reward disappeared when the reward was completely predictable following 100% reward conditioned stimulus (100% reward) and decreased when the reward was partially predictable following 50% reward conditioned stimulus (50% reward). The excitatory response to airpuff decreased when the airpuff was completely predictable following 100% airpuff conditioned stimulus (100% airpuff) or partially predictable following 50% airpuff conditioned stimulus (50% airpuff).

These responses were commonly found in lateral habenula neurons. To investigate the response to reward, we analyzed the activity of 51 neurons with a significant response to at least one of the 100% reward, 50% reward or free reward conditions ($P < 0.05$, Wilcoxon signed-rank test). The averaged activity was strongly inhibited by the free reward (**Fig. 5c**). This inhibitory response was decreased by the preceding 50% reward conditioned stimulus and diminished by the preceding 100% reward conditioned stimulus. To investigate the response to airpuff, we analyzed the activity of 60 neurons with a significant response to at least one of the 100% airpuff, 50% airpuff or free airpuff conditions ($P < 0.05$, Wilcoxon signed-rank test). The averaged activity was strongly excited by the free airpuff (**Fig. 5d**). This excitatory response was decreased by the preceding conditioned stimuli.

The omission of the unconditioned stimulus sometimes evoked an opposite response. The neuron described above (**Fig. 5a**,**b**) showed an excitation when reward was partially predicted by the 50% reward conditioned stimulus but did not occur (50% reward omission) (**Fig. 6a**), although it showed neither excitation nor inhibition when reward did not occur as predicted by the 0% reward conditioned stimulus (0% reward omission) (**Fig. 6a**). On the other hand, this neuron did not show a clear response when airpuff was partially predicted by the 50% airpuff conditioned stimulus but did not occur (50% airpuff omission) (**Fig. 6b**), or when airpuff did not occur as

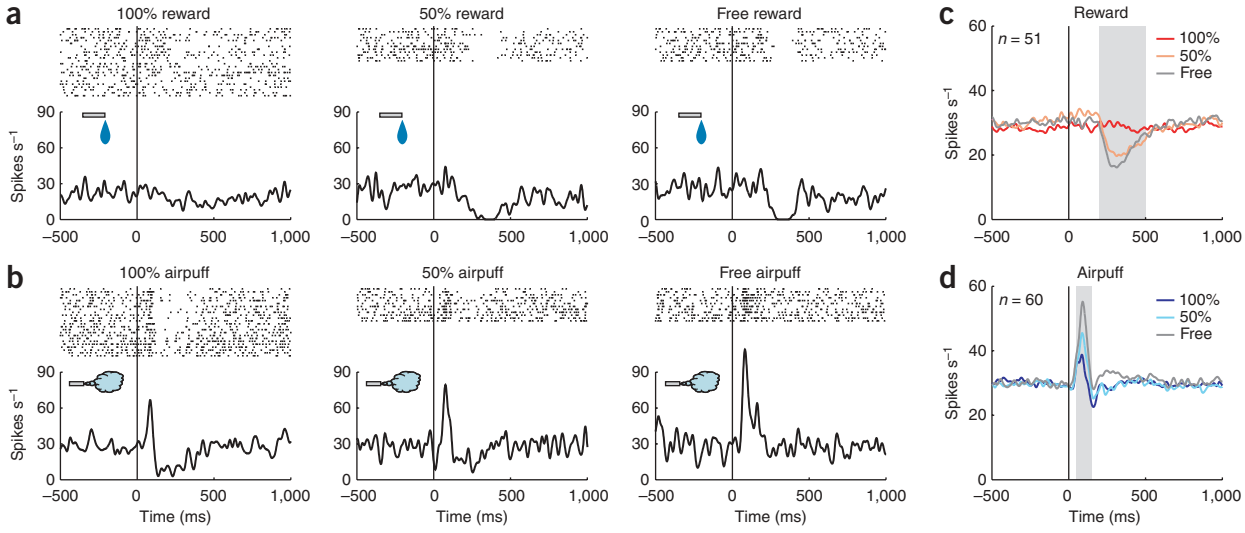

Figure 5 Responses of lateral habenula neurons to unconditioned stimuli. (**a**) Activity of an example neuron during the reward block. Rasters and SDFs are aligned by reward onset and shown for 100% reward, 50% reward and free reward. (**b**) Activity of the neuron shown in **a** during the punishment block. Rasters and SDFs are aligned by airpuff onset and shown for 100% airpuff, 50% airpuff and free airpuff. (**c**) Averaged activity of the 51 neurons showing a significant response to at least one of the 100% reward (dark red), 50% reward (light red) or free reward (gray) ($P < 0.05$, Wilcoxon signed-rank test). Gray area indicates the period that was used to analyze the responses to unconditioned stimuli. (**d**) Averaged activity of the 60 neurons showing a significant response to at least one of the 100% airpuff (dark blue), 50% airpuff (light blue), and free airpuff (gray) ($P < 0.05$, Wilcoxon signed-rank test).

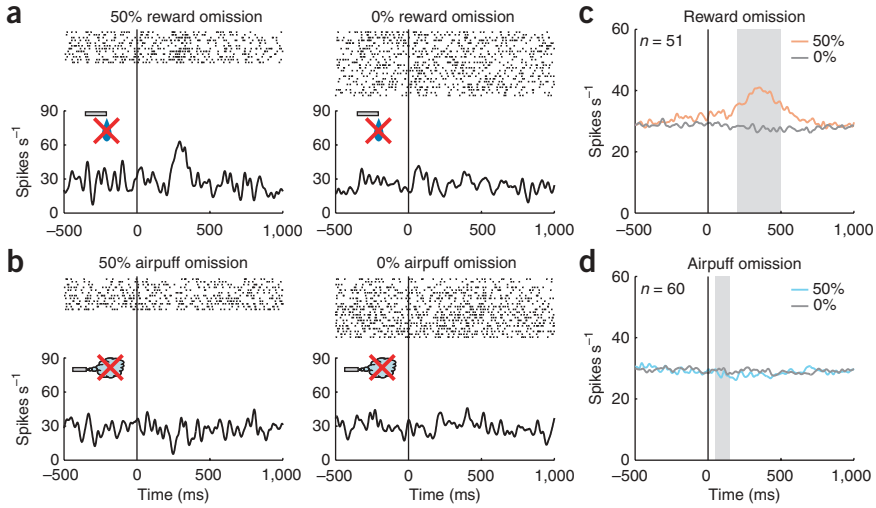

Figure 6 Responses of lateral habenula neurons to unconditioned stimulus omission. (**a**) Activity of the neuron shown in **Figure 5a**,**b** during the reward block. Rasters and SDFs are aligned by conditioned stimulus offset, which occurred simultaneously with reward onset in rewarded trials, and are shown for 50% reward omission and 0% reward omission. (**b**) Activity of the neuron shown in **a** during the punishment block. Rasters and SDFs are aligned by conditioned stimulus offset, which occurred simultaneously with airpuff onset in airpuff trials, and are shown for 50% airpuff omission and 0% airpuff omission. (**c**) Averaged activity of the 51 neurons for 50% (light red) and 0% reward omission (gray). Gray area indicates the period that was used to analyze the response to unconditioned stimulus omission. (**d**) Averaged activity of the 60 neurons for 50% (light blue) and 0% airpuff omission (gray).

predicted by the 0% airpuff conditioned stimulus (0% airpuff omission) (**Fig. 6b**).

The population of lateral habenula neurons had a response pattern that was similar to that of the example neuron (**Fig. 6c**,**d**). The averaged activity was excited by 50% reward omission but not by 0% reward omission (**Fig. 6c**). On the other hand, the averaged activity did not change in response to 50% airpuff omission or 0% airpuff omission (**Fig. 6d**).

The profiles of the responses to unconditioned stimuli and their omission (hereafter referred to as unconditioned stimulus responses) show an interesting parallel with a 'prediction-error signal' that indicates a discrepancy between predicted and actual values of outcomes. We sorted the averaged magnitude of the unconditioned stimulus response by prediction errors for reward (**Fig. 7a**) and airpuff (**Fig. 7b**). A positive value of the prediction error indicates that the outcome was better (more appetitive or less aversive) than predicted by the preceding conditioned stimulus, and a negative value indicates that the outcome was worse (less appetitive or more aversive) than predicted by the conditioned stimulus. We found that the averaged magnitudes of the responses increased as the prediction error became more negative for both reward and airpuff.

To examine whether this response pattern was achieved by single lateral habenula neurons, we calculated the correlation coefficient between prediction error and unconditioned stimulus response for all 72 neurons, separately for reward and airpuff (**Fig. 7c**). Many neurons showed a significant negative correlation for reward (34 out of 72 neurons) and airpuff (17 out of 72 neurons) ($P < 0.05$). The mean correlation coefficient was significantly smaller than zero for both of them ($P < 0.01$, Wilcoxon signed-rank test). Of these, ten neurons showed a significant negative correlation for both reward and airpuff ($P < 0.05$). The frequency of neurons showing a significant negative correlation for both reward and airpuff (that is, 10 out of 72) was not significantly different from the frequency expected by chance under the assumption that the negative correlations for reward and airpuff happened independently (χ^2 test for independence, $P > 0.05$). These results indicate that lateral habenula neurons, as a population, increased their activity as the prediction error became more negative for both reward and airpuff, but this was not necessary true for individual neurons.

Relation between conditioned and unconditioned responses

Next, we examined the relationship between the conditioned stimulus and unconditioned stimulus responses. We compared the responses to 100% reward conditioned stimulus and free reward for each neuron (**Fig. 8a**). A majority of lateral habenula neurons responded to both the reward conditioned stimulus and the reward itself in the same

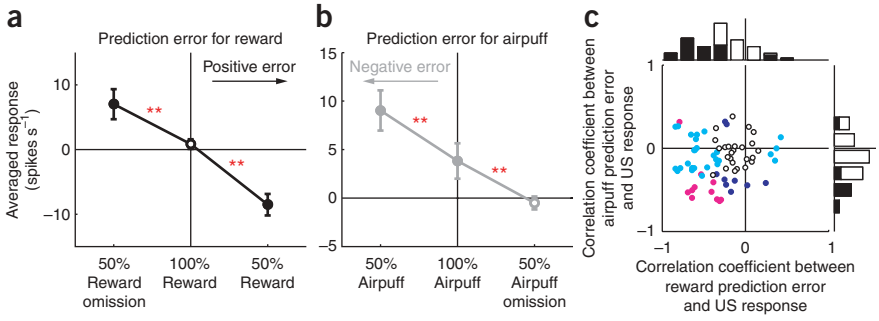

Figure 7 Relationship between prediction error and unconditioned stimulus response. (**a**) Averaged magnitude of the unconditioned stimulus response of the 51 neurons plotted against prediction error for reward. Filled symbols indicate a significant deviation from zero ($P < 0.05$, Wilcoxon signed-rank test). Double asterisks indicate a significant difference between two responses ($P < 0.01$, Wilcoxon signed-rank test). Error bars indicate s.e.m. (**b**) Averaged magnitude of the unconditioned stimulus response of the 60 neurons plotted against prediction error for airpuff. The data are presented as in **a**. (**c**) Correlation coefficients of all 72 neurons between prediction error and unconditioned stimulus response. The abscissa indicates the correlation coefficient between reward prediction error and unconditioned stimulus response. The ordinate indicates the correlation coefficient between airpuff prediction error and unconditioned stimulus response. Cyan, dark blue and magenta dots indicate neurons with statistically significant correlation between reward prediction error and unconditioned stimulus response, between airpuff prediction error and unconditioned stimulus response, and both of them, respectively ($P < 0.05$). White dots indicate no significance ($P > 0.05$). The marginal histograms show the distribution of correlation coefficients. Black bars indicate neurons with statistically significant correlation ($P < 0.05$). White bars indicate no significance ($P > 0.05$).

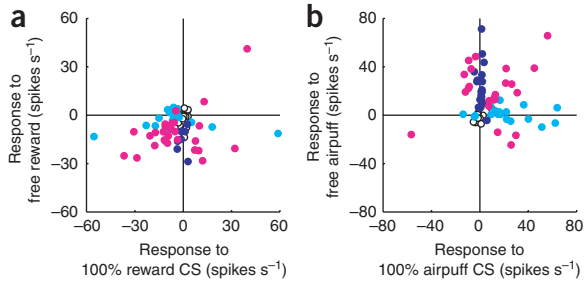

Figure 8 Comparison between conditioned stimulus response and unconditioned stimulus response. (**a**) Comparison between the response to 100% reward conditioned stimulus and the response to free reward for all 72 neurons. Dark blue, cyan and magenta dots indicate neurons with statistically significant responses to free reward, 100% reward conditioned stimulus, and both of them, respectively ($P < 0.05$, Wilcoxon signed-rank test). (**b**) Comparison between the response to 100% airpuff conditioned stimulus and the response to free airpuff for all 72 neurons. Dark blue, cyan and magenta dots indicate neurons with statistically significant responses to free airpuff, 100% airpuff conditioned stimulus, and both of them, respectively ($P < 0.05$, Wilcoxon signed-rank test).

directions (mostly inhibition). Of the 72 neurons, 20 neurons showed significant conditioned stimulus and unconditioned stimulus responses in the same directions, whereas 7 neurons showed significant conditioned stimulus and unconditioned stimulus responses in opposite directions ($P < 0.05$, Wilcoxon signed-rank test). We also compared the responses to 100% airpuff conditioned stimulus and free airpuff (**Fig. 8b**). The responses to the airpuff conditioned stimulus and the responses to the airpuff itself were often expressed by separate groups of neurons; of the 72 neurons, 13 showed significant conditioned stimulus and unconditioned stimulus responses in the same directions, 18 showed a significant response to only 100% airpuff conditioned stimulus and 20 showed a significant response to only free airpuff ($P < 0.05$, Wilcoxon signed-rank test). Thus, although lateral habenula neurons as a population responded both to the conditioned stimuli and the unconditioned stimuli in the same directions, it was not necessarily true for individual neurons. This might suggest that the conditioned stimulus and unconditioned stimulus responses of lateral habenula neurons are mediated by afferents from different brain areas.

Effect of eye position and movement on neural responses

On most of the trials, the monkeys fixated their gaze on the central cue (timing cue) before the conditioned stimulus was presented, even though the central eye fixation was not required. On a small number of trials (1% in monkey N and 4% in monkey D), however, the eye position was away from the central cue when the conditioned stimulus was presented (that is, out of a central eye window, $\pm 2.5 \times 2.5$ degrees). Therefore, it is possible that the responses of lateral habenula neurons to conditioned stimuli, unconditioned stimuli and unconditioned stimulus omissions were influenced by the variation of eye position. To test this possibility, we re-analyzed the entire dataset on the basis of trials that were selected using several criteria of eye fixation during the presentation of the conditioned stimuli (**Supplementary Note** and **Supplementary Fig. 5** online). We found that the effects of eye position were small and did not affect the main results.

DISCUSSION

Using a Pavlovian procedure with two distinct contexts, we found that neurons in the lateral habenula responded to motivational events and their predictors such that their response magnitude was inversely correlated with the associated values. Their population responses were graded for both reward-based and punishment-based values.

Notably, the conditioned stimulus responses of lateral habenula neurons were context-dependent. This context-dependency had two notable consequences. First, lateral habenula neurons were most strongly and equally excited by a conditioned stimulus that was associated with the most unpleasant event in a given context, namely the absence of reward in the reward block and the presence of airpuff in the punishment block. Behavioral studies using blocking procedures have suggested that these kinds of unpleasant experiences are processed by the same neural mechanism[29]. Therefore, the lateral habenula may be part of the unified mechanism implied by these studies. Second, the expectation of no-outcome induced different responses in lateral habenula neurons depending on the context: excitation in the reward block versus no net response in the punishment block. This appears to correspond to the departure of subjective value from objective value, which we experience in everyday life[28].

These profiles of the conditioned stimulus response suggest that the lateral habenula is a unified neural mechanism for representing negative motivational values induced by both rewards and punishments. However, the value coding by lateral habenula neurons was somewhat different between rewards and punishments. The conditioned stimulus response linearly increased as the objective value decreased in the punishment block, but its changes in the reward block were less linear (**Fig. 3a**). These results suggest that lateral habenula neurons represent unpleasant events more precisely than pleasant events.

The unconditioned stimulus responses of lateral habenula neurons were also context-dependent and were modulated by prediction errors for both rewards and punishments. Reward prediction error is thought to be crucial for learning of goal-directed behaviors[30] and its neural correlates have been found in different brain areas. The most notable of these correlates is found in midbrain dopamine neurons[31,32]. However, there have been no reports on prediction error coding for punishments. Our results indicate that the unconditioned stimulus responses of lateral habenula neurons are modulated by the prediction error for punishments. Thus, the unconditioned stimulus responses of lateral habenula neurons to airpuff were weaker when the airpuff was fully expected (that is, 100% airpuff) than when it was partially expected (that is, 50% airpuff). The effect of expectation on neuronal responses was also supported by an additional analysis (**Supplementary Note** and **Supplementary Fig. 6** online). However, the punishment prediction error coding by lateral habenula neurons was not perfect. On average, the neurons were still excited by airpuff even when it was fully expected (that is, 100% airpuff) and did not show either excitation or inhibition to the omission of expected airpuff (that is, 50% airpuff omission). This may suggest that lateral habenula neurons preferentially respond to negative motivational events (for example, 100% airpuff) rather than to positive motivational events (for example, 50% airpuff omission).

In addition to the lateral habenula, other brain areas are considered to represent rewards and punishments. A previous study[12] showed that midbrain dopamine neurons respond to airpuff-predicting stimuli but do so inconsistently, unlike lateral habenula neurons. One hypothesis may be that positive motivational values are preferentially represented by dopamine neurons, whereas negative motivational values are preferentially represented by lateral habenula neurons. However, the inconsistency of the airpuff-related responses in dopamine neurons could be the result of the fact that the monkeys were able to avoid the airpuff by acting quickly in the previous experiment. Another area that is likely to represent both rewards and punishments is the amygdala.

Both reward- and punishment-related values are represented by different groups of amygdala neurons[7]. Many of them respond differently to reward and airpuff themselves, and these responses are frequently modulated by prediction[33]. Possible functional relationships between the lateral habenula and the amygdala will be an important issue, although no direct connection has been shown between them.

The value signals in the lateral habenula would be useful for controlling both reward-seeking behaviors and punishment-avoidance behaviors. These functions might be mediated, at least in part, by dopamine and serotonin neurons, which have been implicated in learning and motivation of goal-directed behaviors[18–20,34–36]. Indeed, several lines of evidence have suggested that the lateral habenula exerts inhibitory control over dopamine neurons[21,22,37] and serotonin neurons[23,38]. Although both dopamine and serotonin neurons encode reward-related signals, albeit in different manners[39], the method by which they encode punishment-related signals remains unknown. To understand the function of the value signals in the lateral habenula, it is important to elucidate how these signals are processed in dopamine and serotonin neurons.

METHODS

Animals. Two adult rhesus monkeys (*Macaca mulatta*; monkey N, female, 6.0 kg; monkey D, male, 11.0 kg) were used for the experiments. All procedures for animal care and experimentation were approved by the Institute Animal Care and Use Committee and complied with the Public Health Service Policy on the humane care and use of laboratory animals. See the **Supplementary Note** for detailed experimental procedures.

Behavioral task. The monkeys were trained in a Pavlovian procedure that consisted of two blocks of trials: a reward block (**Fig. 1a**) and a punishment block (**Fig. 1b**). In the reward block, three conditioned stimuli (a red circle, green cross and blue square for monkey N and a yellow ring, cyan triangle and blue square for monkey D) were associated with a liquid reward as an unconditioned stimulus with 100%, 50% and 0% probability, respectively. In the punishment block, three conditioned stimuli (a yellow ring, cyan triangle and blue square for monkey N and a red circle, green cross and blue square for monkey D) were associated with an airpuff that was directed at the monkey's face as an unconditioned stimulus with 100%, 50% and 0% probability, respectively. The sizes of these conditioned stimuli were 8.6 × 8.6 to 10 × 10 degrees. The liquid reward was delivered through a spout that was positioned in front of the monkey's mouth. The airpuff (20–30 psi) was delivered through a narrow tube placed 6–7 cm from the face. Each trial started after the presentation of a timing cue (size, 2.6 × 2.6 degrees) on a screen (the monkeys were not required to fixate it) for both blocks. 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, noncued trials were included in which a reward alone (free reward) was delivered during the reward block or an airpuff alone (free airpuff) was delivered during the punishment block. All trials were presented with a random intertrial 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; noncued, 6 trials). For 50% trials, the conditioned stimulus was followed by the unconditioned stimulus on six trials and was not followed by the unconditioned stimulus on the other six trials. The block changed without any external cue. For each neuron, we collected data by repeating the reward and punishment blocks twice or more.

We monitored licking and blinking of the monkeys. To monitor licking, we attached a strain gauge to the spout that was positioned in front of the monkey's mouth and measured strains of the spout caused by licking. To monitor blinking, we used a magnetic search coil technique[40]. A small, Teflon-coated stainless-steel wire (<5-mm diameter, five or six turns) was taped to an eyelid. Eye closure was identified by the vertical component of the eyelid coil signal.

Localization of the lateral habenula. We used the same technique to localize the lateral habenula that we used in a previous study[26]. We estimated the position of the lateral habenula by obtaining MRIs (4.7 T, Bruker) that were based on the coordinates of the recording chamber, whose inner walls were visualized with an enhancer (betadine ointment). On MRIs parallel to the recording chamber, the habenulae appeared as two round structures that were located about 4 mm anterior to the superior colliculi. The localization of the lateral habenula was then achieved by electrophysiological recording and verified by histological examination at the end of the experiments. As shown in our previous study[26], the firing patterns and spike shapes of lateral habenula neurons were distinctly different from those of neurons in the surrounding thalamic area (mediodorsal thalamus). Lateral habenula neurons fired tonically with relatively high background rates, whereas mediodorsal thalamus neurons showed irregular and burst firing with lower background rates, and their action potentials were much broader than those of lateral habenula neurons. Furthermore, most of the lateral habenula neurons, but none of the mediodorsal thalamus neurons, were sensitive to reward outcome.

Data analysis. We analyzed anticipatory licking, anticipatory blinking and neuronal activity during the Pavlovian procedure described above.

To evaluate the frequency and strength of anticipatory licking, we used the strain gauge signal. We first calculated the velocity of the strain of the spout and then 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 normalized magnitude was determined by $(X - Min) / (Max - Min)$, where 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, we used the vertical component of the eyelid signal. We first calculated the downward velocity of eyelid movement. We set a threshold and counted how many times the velocity crossed that 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 150–400-ms period after conditioned stimulus onset minus the background discharge rate during the 250-ms period preceding conditioned stimulus onset. Responses to reward and reward omission were defined as the discharge rate during the 200–500-ms period after reward onset minus the background discharge rate during the 250-ms period preceding reward onset. Responses to airpuff and airpuff omission were defined as the discharge rate during the 50–150-ms period after airpuff onset minus the background discharge rate during the 250-ms period preceding airpuff onset. These time windows were determined on the basis of the averaged activity of lateral habenula neurons. Specifically, we set the time windows such that they included major parts of the excitatory and inhibitory responses of lateral habenula neurons.

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

To examine the linearity between the objective value and the magnitude of the conditioned stimulus response, we calculated a linearity index for each neuron, separately for the reward and punishment blocks. The linearity index was calculated by the following equation:

$$\text{Linearity index} = (R_{50CS} - \frac{R_{100CS}+R_{0CS}}{2})/|R_{100CS} - R_{0CS}|$$

where R_{100CS}, R_{50CS} and R_{0CS} indicate the response magnitudes for 100% , 50% and 0% conditioned stimuli, respectively. The linearity index is zero if the relationship is perfectly linear (that is, if the response to 50% conditioned

stimulus is equal to the average of the responses to 0% and 100% conditioned stimuli), is positive if the response to 50% conditioned stimulus is larger than the 0–100% average and negative if the response to 50% conditioned stimulus is smaller than the 0–100% average.

To calculate spike density functions (SDFs), each spike was replaced by a Gaussian curve ($\sigma = 10$ ms).

Histology. After the end of the recording sessions in monkey N, we selected representative locations for electrode penetrations into the lateral habenula. When typical single- or multi-unit activities were recorded, we made electrolytic micro-lesions at the recording sites (12 μA and 30 s). Monkey N was then deeply anesthetized with an overdose of pentobarbital sodium and perfused with 10% formaldehyde (wt/vol). The brain was blocked and equilibrated with 10% sucrose (wt/vol). Frozen sections were cut every 50 μm in the coronal plane and stained with cresyl violet.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We thank S. Hong, M. Yasuda and E. Bromberg-Martin for valuable discussion, and M.K. Smith, J.W. McClurkin, A.M. Nichols, T.W. Ruffner, A.V. Hays and L.P. Jensen for technical assistance. 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 analyzed 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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