Representation of negative motivational value in the primate lateral habenula

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SUPPLEMENTARY NOTES

A. Experimental procedure

A plastic head holder and plastic recording chamber were fixed to the skull under general anesthesia and sterile surgical conditions. The recording chamber was placed over the midline of the parietal cortex and was aimed at the lateral habenula. 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 by acrylic screws.

Single-unit recordings were performed using tungsten electrodes (Frederick Haer Company, Bowdinham, ME) that were advanced by an oil-driven micro-manipulator (MO-97A, Narishige, Japan). The recording sites were determined using a grid system which allowed recordings at every 1 mm between penetrations. The electrode was introduced into the brain through a stainless steel guide tube which was inserted into one of the grid holes and then into the brain via the dura. For finer mapping of neurons, we also used a complementary grid which allowed electrode penetrations between the holes of the original grid. Single neurons were isolated on-line using a custom voltage-time window discrimination software [MEX, Laboratory of Sensorimotor Research, National Eye Institute, National Institutes of Health (LSR/NEI/NIH), Bethesda, MD].

The behavioral task was under the control of a QNX-based real-time experimentation data acquisition system (REX, LSR/NEI/NIH, Bethesda, MD). The monkeys sat in a primate chair, facing a frontoparallel screen 33 cm from the monkey's eyes in a sound-attenuated and electrically shielded room. Stimuli generated by an active matrix liquid crystal display projector (PJ550, ViewSonic) were rear-projected on the screen.

B. Anticipatory licking and blinking

We analyzed anticipatory licking and blinking during CS presentation. As shown in Supplementary Fig. 1a and b, anticipatory licking in the reward block increased as the probability of reward increased, though monkey N showed slightly greater licking during the presentation of 0% reward CS than 50% reward CS. On the other hand, as shown in Supplementary Fig. 1c and d, anticipatory blinking in the punishment block increased as the probability of airpuff increased. These results suggest that the monkeys discriminated the CSs behaviorally.

Interestingly, the magnitude of licking was generally larger in the reward block than in the airpuff block (Supplementary Fig. 1a and b). In particular, it was larger for 0% reward CS than 0% airpuff CS ($P < 0.01$, Wilcoxon rank-sum test), even though the CSs were physically identical. Supplementary Fig. 2 indicates how quickly this difference developed after the block context was changed. The licking at trial zero reflected the previous context, but then quickly changed and reached a plateau. We found, however, that the difference in the licking magnitude was present even before the CSs were presented (during timing cue presentation). The licking magnitude before CS presentation

was significantly larger in the reward block (monkey N, mean ± s.d. = 0.62 ± 0.19; monkey D, mean ± s.d. = 0.40 ± 0.22) than in the punishment block (monkey N, mean ± s.d. = 0.37 ± 0.19; monkey D, mean ± s.d. = 0.13 ± 0.14) ($P < 0.01$, Wilcoxon rank-sum test). This difference in the licking magnitude before CS presentation could reflect a change in the internal state of the monkey between the reward and punishment blocks, not CS-specific reward expectation. Therefore, the larger licking level for 0% reward CS than 0% airpuff CS could be explained by the change in the internal state of the monkey.

C. Recording sites of lateral habenula neurons

After the end of the recording sessions in monkey N, we made electrolytic microlesions at the representative recording sites. The representative recording sites in the left and right lateral habenulae are visible in Supplementary Fig. 3.

D. Responses to the most unpleasant CSs and the most pleasant CSs in each block

As shown in Fig. 2a and b, the example neuron was excited by both of 0% reward CS and 100% airpuff CS which were associated with the most unpleasant event in each block. To examine whether other individual neurons were excited similarly by these CSs, we compared CS responses between 0% reward CS and 100% airpuff CS for each neuron. As shown by the scatter plot in Supplementary Fig. 4a, a clear correlation was observed ($r = 0.906$, $P < 0.01$), indicating that many neurons were excited similarly by these CSs. Thus, many lateral habenula neurons represented the CSs associated with the most unpleasant event in each block in a similar way. We also compared CS responses between 100% reward CS and 0% airpuff CS which were associated with the most

pleasant event in each context. Again, a significant correlation was observed ($r = 0.729$, $P < 0.01$) and many neurons responded similarly to these CSs (Supplementary Fig. 4b). Thus, many lateral habenula neurons represented the CSs associated with the most pleasant event in each block in a similar way.

E. Effect of eye position on CS, US and US omission responses

Because the monkeys were not required to fixate, they occasionally did not look at the presented CS. Therefore, it is possible that the responses of lateral habenula neurons to CSs, USs and US omissions were influenced by the variation of eye position. To examine the effect of eye position, we re-analyzed the entire data using trials in which monkey's eye position was within a central eye window (± 2.5 × 2.5 deg) when the CS was presented. Supplementary Fig. 5 indicates the averaged responses to CSs (Supplementary Fig. 5a and b), USs (Supplementary Fig. 5c and d), and US omissions (Supplementary Fig. 5e and f) on the selected trials. These response profiles do not differ from the original response profiles shown in Fig. 2c and d, Fig. 5c and d, and Fig. 6c and d.

However, even a smaller deviation of eye position (i.e., within ± 2.5 × 2.5 deg) might affect the CS, US and US omission responses. To examine this possibility, we used a smaller eye window (± 0.5 × 0.5 deg) and separated trials into two groups: one in which eye position was within the small eye window (i.e., ± 0.5 × 0.5 deg) at the CS onset, the other in which eye position was out of the small eye window but within the large eye window (i.e., ± 2.5 × 2.5 deg). We then compared the averaged responses of lateral habenula neurons to CSs, USs and US omissions (i.e., responses to 100%, 50% and 0%

reward CSs, 100%, 50% and 0% airpuff CSs, 100% and 50% reward, 100% and 50% airpuff, 50% reward omission, and 50% airpuff omission) between the two groups of trials. As the result, we did not find a significant difference in the averaged responses between the two groups ($P > 0.05$, Wilcoxon signed-rank test) except the response to 50% airpuff CS ($P = 0.024$, Wilcoxon signed-rank test). However the difference in the response to 50% airpuff CS between the two groups was very small (mean difference ± s.d. across 49 neurons = 2.74 ± 8.41 spikes/s). Thus, we concluded that eye position did not affect the results of analyses for CS, US and US omission responses.

F. Effect of eye movement on CS, US and US omission responses

Even if the monkeys looked at a CS when it was presented, they occasionally looked away from the CS during its presentation. Such eye movement might affect the CS, US and US omission responses. To exclude this possibility, we separated trials into two groups: one in which the monkeys made eye movement(s) during the 400 ms after the CS onset (400 ms is the end of the window to analyze CS responses), the other in which the monkeys did not make eye movements during the period. We then compared the CS, US and US omission responses between the two groups of trials. The result was that we did not find a significant difference in the averaged responses between the two groups ($P > 0.05$, Wilcoxon signed-rank test) except the responses to 0% reward CS ($P = 0.011$, Wilcoxon signed-rank test) and 50% airpuff CS ($P = 0.037$, Wilcoxon signed-rank test). However the difference in the response to 0% reward CS (mean difference ± s.d. across 48 neurons = 2.18 ± 7.22 spikes/s) and 50% airpuff CS (mean difference ± s.d. across 49 neurons = 2.72 ± 8.51 spikes/s) were very small. Thus, we concluded that eye

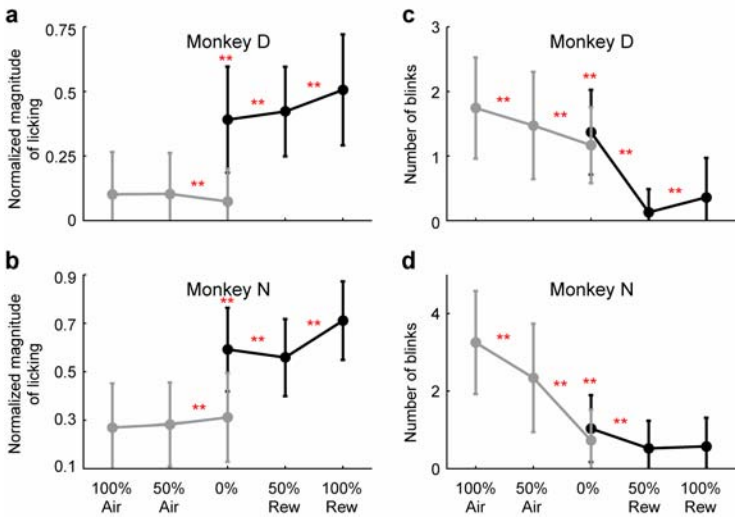
movement did not affect the results of the analyses for CS, US and US omission responses.

G. Stronger responses to unexpected CSs and USs

Our results indicated that the US responses of lateral habenula neurons were modulated by the prediction error for both reward and punishment. To further examine this issue, we analyzed CS and US responses on the first trials after the block context was changed. Because our Pavlovian procedure consisted of two blocks with distinct contexts, the monkeys could predict the kind of CSs (i.e., related either to reward or to airpuff) based on the block context. However, the CS that was presented on the first trial after the block change was less predictable (e.g., airpuff-related instead of reward-related) because the block context was changed without any instruction. We thus compared in Supplementary Fig. 6 the neuronal responses on the first trials (colored SDFs) with the responses in the rest of the trials (gray SDFs) for 100% reward CS (a), 100% airpuff CS (b), 50% reward CS (c), and 50% airpuff CS (d). In addition, we present the same comparison for free reward (e) and free airpuff (f) because they were also less predictable if they occurred on the first trial after the block change. The responses on the first trials were generally larger than the responses on the other trials. The difference was statistically significant ($P < 0.05$, Wilcoxon signed-rank test), except the responses to 50% airpuff CS and free reward ($P > 0.05$, Wilcoxon signed-rank test). These results indicate that CS and US responses of lateral habenula neurons were enhanced when the CS and US were less predictable. This response profile of lateral habenula neurons is consistent with our hypothesis that lateral habenula neurons encode prediction error

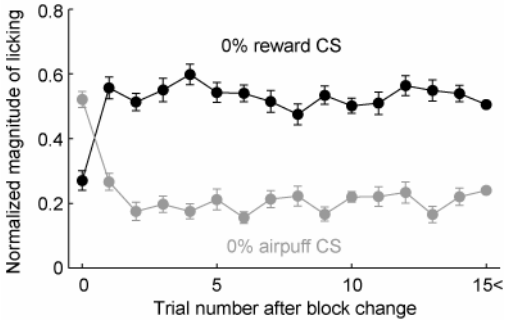
signals.

Supplementary Figure 1



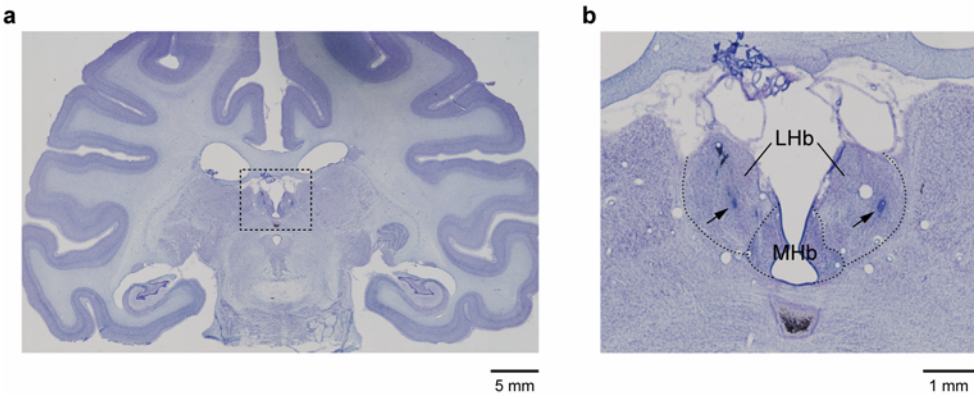
Anticipatory licking and blinking. (**a, b**) The average normalized magnitude of anticipatory licking during the presentation of reward CSs (black) and during the presentation of airpuff CSs (gray) for monkey D (**a**) and monkey N (**b**). Double asterisks indicate a significant difference between two points ($P < 0.01$, Wilcoxon rank-sum test). Error bars indicate s.d. (**c**, **d**) The average number of anticipatory blinks during the presentation of reward CSs (black) and during the presentation of airpuff CSs (gray) for monkey D (**c**) and monkey N (**d**).

Supplementary Figure 2



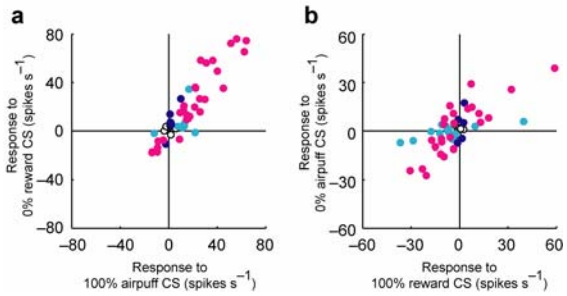
Changes in the normalized magnitude of licking during the presentation of 0% reward CS (black) and 0% airpuff CS (gray) after the block context was reversed. The data from monkey N and monkey D were combined. Conventions are the same as Fig. 4.

Supplementary Figure 3



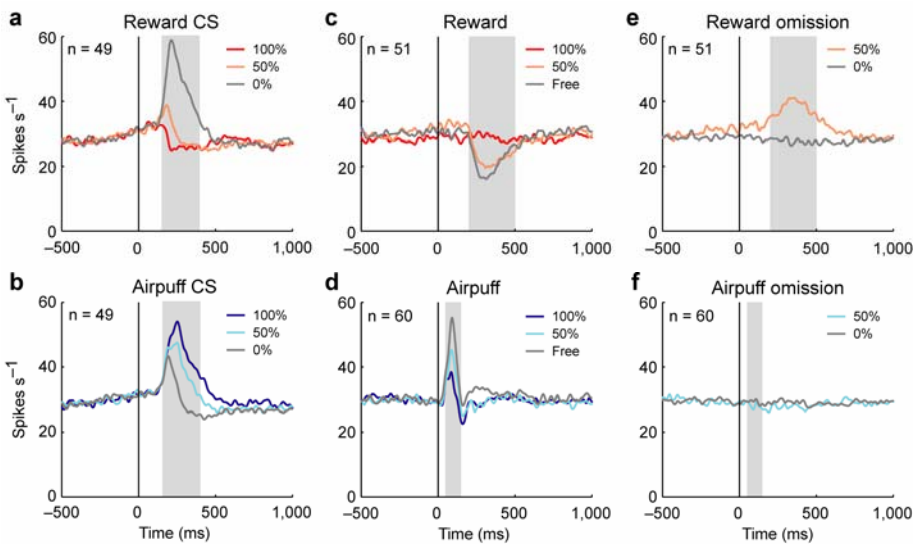
Recording sites of lateral habenula neurons in monkey N. **b** represents parts of **a** (indicated by dashed rectangle). Arrows indicate electrolytic microlesions at the representative recording sites. LHb, lateral habenula; MHb, medial habenula.

Supplementary Figure 4



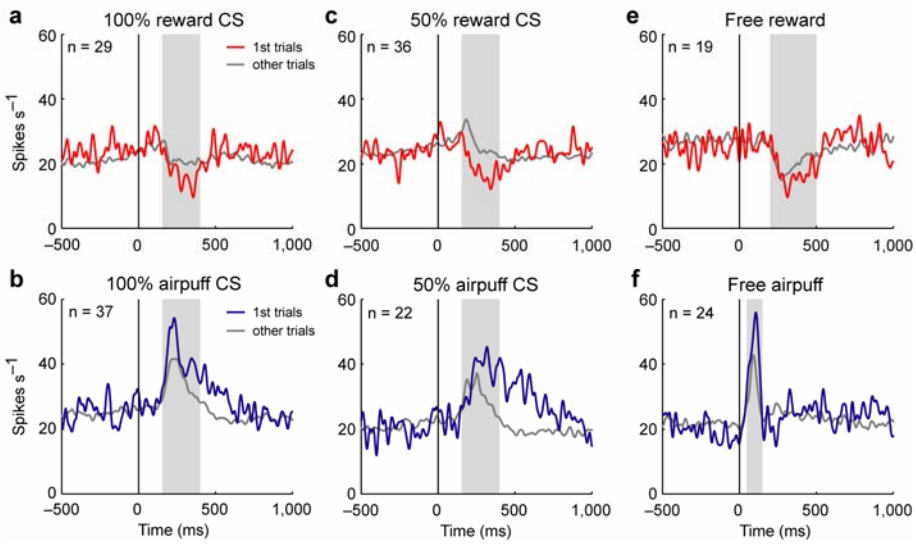
Comparison of CS responses. (**a**) Comparison of CS responses between 0% reward CS and 100% airpuff CS for the 49 neurons. Dark blue, cyan and magenta dots indicate neurons with statistically significant CS response to 0% reward CS, 100% airpuff CS, and both of them, respectively ($P < 0.05$, Wilcoxon signed-rank test). (**b**) Comparison of CS responses between 0% airpuff CS and 100% reward CS for the 49 neurons. Dark blue, cyan and magenta dots indicate neurons with statistically significant CS response to 0% airpuff CS, 100% reward CS, and both of them, respectively ($P < 0.05$, Wilcoxon signed-rank test).

Supplementary Figure 5



Averaged CS, US and US omission responses for the selected trials in which monkey's eye position was within a central eye window (± 2.5 × 2.5 deg) when the CS was presented. (**a**, **b**) The averaged CS responses of the 49 neurons for the reward and airpuff CSs. Conventions are the same as Fig. 2c and d. (**c**, **d**) The averaged US responses of the 51 neurons for reward and of the 60 neurons for airpuff. Conventions are the same as Fig. 5c and d. (**e**, **f**) The averaged US omission responses of the 51 neurons for reward omission and of the 60 neurons for airpuff omission. Conventions are the same as Fig. 6c and d.

Supplementary Figure 6



Enhancement of CS and US responses on first trials after the block context was changed. (**a**-**f**) Averaged activity aligned by 100% reward CS (**a**), 100% airpuff CS (**b**), 50% reward CS (**c**), 50% airpuff CS (**d**), free reward (**e**) and free airpuff (**f**). Colored SDFs indicate the activity on the first trials. Gray SDFs indicate the activity on the other trials. The numbers of neurons sampled are different across conditions (shown at the left-upper corner) because a given CS or a free reward/airpuff sometimes never appeared on the first trial after the block change while a single neuron was recorded due to the pseudo-random schedule. Gray area indicates the period that was used to analyze the magnitudes of neuronal responses.