Systems/Circuits

Neuronal Encoding of Emotional Valence and Intensity in the Monkey Amygdala

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Neuropsychological and neuroimaging studies have suggested that the primate amygdala plays an essential role in processing the emotional valence and intensity of visual stimuli, which is necessary for determining whether to approach or avoid a stimulus. However, the neuronal mechanisms underlying the evaluation of emotional value remain unknown. In the present study, we trained male macaque monkeys to perform an operant conditioning task in which fractal visual patterns were associated with three different amounts of air puff delivered to the cheek (negative) or liquid reward (positive). After confirming that the monkeys successfully differentiated the emotional valence and intensity of the visual stimuli, we analyzed neuronal responses to the stimuli in the amygdala. Most amygdala neurons conveyed information concerning the emotional valence and/or intensity of the visual stimuli, and the majority of those conveying information about emotional valence responded optimally to negative stimuli. Further, some amygdala neurons conveyed information related to both emotional valence and intensity, whereas a small portion conveyed information related to emotional intensity alone. These results indicate that the primate amygdala encodes both emotional valence and intensity, highlighting its important role in the avoidance of dangerous stimuli and animal survival.

Key words: **amygdala; emotion; intensity; primates; single-unit recording; valence**

Significance Statement

Evaluating the emotional value of visual stimuli is essential for animal survival, especially in primates. Emotional value is estimated from the emotional valence and intensity of stimuli, and evidence indicates that the amygdala is likely to play a major role in processing these types of information. To our knowledge, the current study is the first to examine the responses of neurons in the monkey amygdala to visual stimuli that differ in emotional valence and intensity simultaneously. Our data suggest that the amygdala plays an important role in the evaluation of emotional stimuli and in the decision to escape negative and harmful stimuli.

Introduction

Animal survival depends directly on the ability to avoid harmful stimuli and approach rewarding stimuli. As vision is the dominant sensory modality for most primate species, including humans, we often receive emotional information from visual stimuli, which must then be rapidly and appropriately assessed for emotional value to enable selection of the optimal response. Two types of information are reportedly required to evaluate the emotional value of a stimulus: emotional valence and intensity (Russell, 1980; Lang, 1995). Emotional valence is a qualitative concept referring to pleasantness or unpleasantness and can therefore be described as negative or positive. In contrast, emotional intensity is a quantitative concept referring to the degree of saliency and is expressed as large or small. The brain integrates these two types of emotional information to assess the emotional value of a particular visual stimulus, determine whether it is better to escape from or approach the stimulus, and choose the optimal reaction.

The primate amygdala, a brain structure located in the medial temporal lobe, is considered a key structure involved in processing the emotional value of sensory stimuli (Ball et al., 2009). Some lesion studies have reported that damage to the amygdala diminishes the fear response to aversive stimuli (e.g., snakes) and alters preferences for appetitive stimuli (Klüver and Bucy, 1938; Weiskrantz, 1956; Murray et al., 1996; Baxter and Murray, 2002). Several electrophysiological studies in monkeys have also suggested that the amygdala plays a key role in emotional evaluation. Early studies demonstrated that some amygdala neurons respond to rewards (Sanghera et al., 1979; Nishijo et al., 1988; Nakamura et al., 1992). More recently, Salzman's group reported that activity in amygdala neurons changes

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depending on whether the valence of the stimulus is negative or positive (Paton et al., 2006; Belova et al., 2007). In their study concerning the responsiveness of amygdala neurons to emotional valence, Paton et al. (2006) first trained monkeys to learn which stimuli were associated with a negative outcome (i.e., an air puff) and which were associated with a positive outcome (i.e., a reward). They then reversed the stimulus–reward association. Some amygdala neurons responded selectively to negative or positive stimuli, and reversal of the stimulus–reward association also reversed the type of neuronal response. These results clearly demonstrate that some neurons in the amygdala encode emotional valence.

In addition to the neurons that encode valence, some amygdala neurons exhibit changes in activity depending on the amount of reward the visual stimulus is associated with (Bermudez and Schultz, 2010; Leathers and Olson, 2017). These neurons convey information regarding the emotional intensity of the stimulus. However, to our knowledge, no neurophysiological studies have investigated the representation of emotional intensity in the amygdala for negative stimuli or the representation of both emotional valence and intensity. Therefore, the complete neuronal mechanisms underlying the evaluation of the emotional valence and intensity remain unclear.

To fully understand the neuronal mechanism underlying emotional evaluation, we believe it is important to confirm the emotional significance of behavior and to elucidate the relationship between representations of emotional valence and intensity. Therefore, in this study, we carefully prepared visual stimuli as follows: First, we chose fractal patterns as emotionally neutral visual stimuli to exclude the influence of the original emotional values on the results. Second, for emotional valence, we associated neutral visual stimuli with a negative outcome and others with a positive outcome (Paton et al., 2006). Third, for emotional intensity, the stimuli were associated with three different amounts of liquid reward or air puff. Finally, and most important, we confirmed that the monkeys could differentiate between multiple levels of intensity. Using this design, we aimed to investigate neuronal encoding of the emotional valence and intensity of visual stimuli in the monkey amygdala, as well as the functional differences among locations within the amygdala.

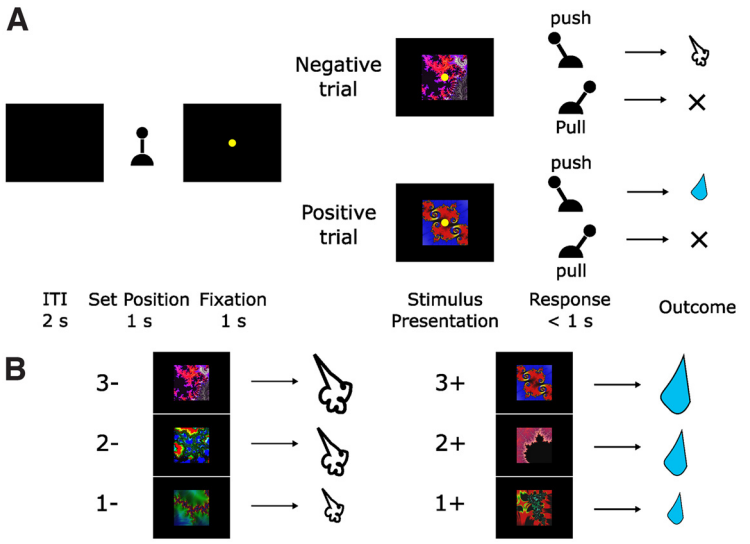

Figure 1. ***A***, Flow of the conditioning task. When the monkey placed the joystick at the center set position and waited for 1 s, a yellow fixation point appeared at the center of the monitor. After the monkey gazed at the fixation point for 1 s, a visual stimulus was presented. The monkey was required to pull or push the joystick within 1 s after stimulus onset to obtain an optimal outcome (i.e., avoidance of an air puff or receipt of a liquid reward) in a negative or a positive trial, respectively. ***B***, Task conditions. Each stimulus set consisted of three different air puff durations and three different amounts of liquid reward. The air puff durations for stimuli with valences of −3, −2, and −1 were 24, 32, and 40 ms for monkey I and 18, 24, and 30 ms for monkey T. When delivering air puffs, a pressure of 29.0 psi was used in both monkeys. The reward amounts for stimuli with valences of +3, +2, and +1 were 0.4, 0.8, and 1.2 ml for monkey I and 0.2, 0.6, and 1.0 ml for monkey T.

Materials and Methods

Subjects. Two male Japanese monkeys (*Macaca fuscata*) were used in this study (monkey I, 8.6 kg, 7 years old; monkey T, 7.9 kg, 8 years old). Both monkeys were born and raised at the Primate Research Institute at Kyoto University and housed in individual cages and placed on water control for the duration of the experiment. All experimental procedures were approved by the Animal Welfare and Animal Care Committee of Kyoto University (license nos. 2017-173, 2018-033, 2019-083, 2020-093, and 2021-050) and were conducted in accordance with the *Guide for Care and Use of Laboratory Primates* published by the Primate Research Institute of Kyoto University in 2010.

Task procedures. During the experiment, which was conducted in a dark soundproof room, each monkey sat in a primate chair with its head fixed and performed an operant conditioning task (Fig. 1*A*). The monkey used a joystick placed at waist level to perform the task. Fractal patterns (8 × 8° in visual angle) were used as visual stimuli, and the average luminance of the stimuli was adjusted to 32 cd/m^2. The stimuli were presented on a CRT monitor placed 40 cm from the eyes of the monkey. An air puff and a liquid reward were used as negative and positive outcomes, respectively. For negative trials, a puff of compressed air (29.0 psi) was generated by a compressor (Tools Island) and delivered to the left cheek of the monkey. The amount of air or reward was controlled by solenoid valves (VT317, SMC). The duration of the air puff and the amount of reward were adjusted for each monkey. The durations of the air puff were 24, 32, and 40 ms for monkey I and 18, 24, and 30 ms for monkey T. The reward amounts were 0.4, 0.8, and 1.2 ml for monkey I and 0.2, 0.6, and 1.0 ml for monkey T.

The stimulus set included six visual stimuli (Fig. 1*B*), each of which was associated with three different durations of air puff and three different amounts of liquid reward. Stimuli associated with the long, medium, and short air puffs were abbreviated as −3, −2, and −1, respectively. Stimuli associated with large, medium, and small rewards were abbreviated as +3, +2, and +1, respectively.

As shown in Figure 1*A*, the monkey first placed the joystick at the center set position and waited for 1 s. Then, a yellow fixation point appeared at the center of the monitor. When the monkey gazed at an area within 3° of the fixation point for 1 s, a visual stimulus was presented for 1 s. In negative trials, the air puff could be avoided by pulling the joystick within 1 s of the stimulus presentation, whereas pushing or nonresponse resulted in delivery of the air puff. Each trial was followed by a 2 s intertrial interval (ITI). In positive trials, the reward could be obtained by pushing the joystick within 1 s of the stimulus presentation, whereas pulling or nonresponse resulted in no reward. The monkeys were required to fixate on the fixation point for 300 ms after the stimulus onset as well as during the 1 s fixation period. If the monkey failed to gaze within 3° of the fixation point for the 1300 ms period, the trial was regarded as erroneous, and an ITI started. The task was controlled by a custom code written in MATLAB (MathWorks), and eye movements were recorded using an infrared eye-tracking system (iRecHS2, https://staff.aist.go.jp/k.matsuda/eye/indexj.html). Before starting electrophysiological recordings, we confirmed that the monkeys had learned the relationship between the visual stimuli and the outcome, and correct rates in both monkeys were more than 90%.

Electrophysiological recording. To record the activity of single neurons, a stainless steel chamber was implanted on the skull and oriented

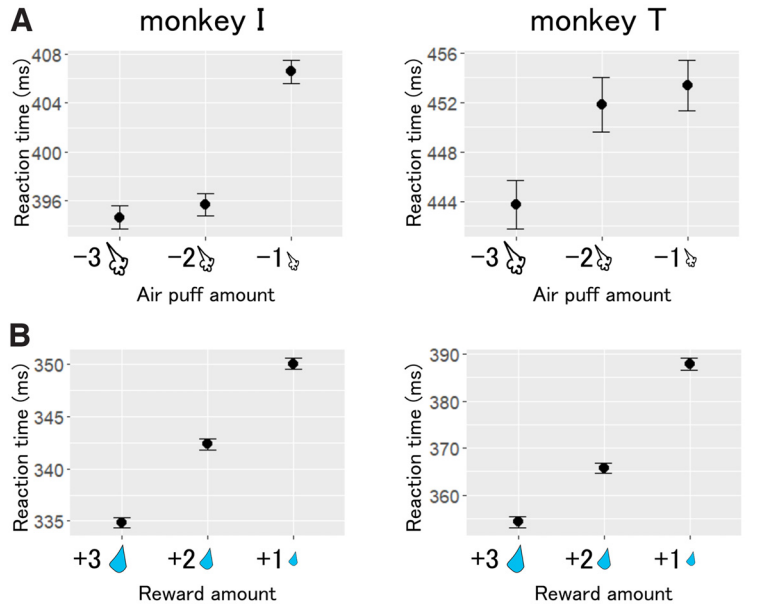

Figure 2. ***A***, Response times for negative visual stimuli. The vertical axis represents the reaction time (ms), whereas the horizontal axis represents the amount of air puff, with larger values on the left (−3, −2, and −1). Left, Plot shows findings for monkey I, in whom the mean response times for stimuli with valences of −3, −2, and −1 were 395, 396, and 407 ms, respectively. Right, Plot shows findings for monkey T, in whom the mean response times for stimuli with valences of −3, −2, and −1 were 444, 452, and 454 ms, respectively. Error bars indicate ±1 SEM. ***B***, Response times for positive visual stimuli. The vertical axis represents the reaction time (ms), whereas the horizontal axis represents the amount of liquid reward, with larger values on the left (+3, +2, and +1). Left, Plot shows findings for monkey I, in whom the mean response times for stimuli with valences of +3, +2, and +1 were 335, 342, and 350 ms, respectively. Right, Plot shows findings for monkey T, in whom the mean response times for stimuli with valences of +3, +2, and +1 were 354, 366, and 388 ms, respectively. Error bars indicate ±1 SEM.

vertically to the amygdala using structural MR images (0.3 T; voxel size, 1.5 mm) of each monkey brain for guidance. Detailed localization of the target was performed using an application (MRIcro, https://people.cas.sc.edu/rorden/mricro/mricro.html) designed for the visualization of MR images.

Neuronal activity was recorded extracellularly using tungsten electrodes (0.25 mm in diameter, FHC) and linear electrode arrays with 16 equidistant contacts on a shaft with a diameter of 236 μm (S-probe, Plexon). The impedance for each electrode ranged from 0.5 to 1.5 MΩ. During the conditioning task, the electrodes were lowered to the target through a guide tube with a diameter of 1.1 mm using a hydraulic micromanipulator (MO-95, Narishige). Spike activity was recorded and monitored using a neural data acquisition system (OmniPlex, Plexon), and single-neuron activities were sorted off-line (Offline Sorter, Plexon). Each stimulus was presented 10 times to ensure the reproducibility and stability of neuronal responses. We used three different stimulus sets in each recording session to ascertain that the observed responsiveness was related to the emotional valence and/or intensity of the stimulus and not to its physical features (see below, Experimental design and statistical analyses). Thus, the monkeys completed at least 180 correct trials in each recording session [3 stimulus sets × 2 emotional valences (reward or air puff) × 3 intensities (quantity) × 10 repetitions]. New stimulus sets containing 18 new visual stimuli were introduced every month.

Histologic analysis. After the final recording session, small electrolytic lesions (4 μA for 150 s or 20 μA for 30 s) were made using a lesion maker (Digital Midgard Precision Current Source, Stoelting), after which the monkeys were deeply anesthetized via an intravenous injection of an overdose of thiopental sodium. After disappearance of the nociceptive reflex, the animals were perfused with 0.5 M PBS followed by 8% formalin in 0.5 M PBS. The lesions were identified in 100-μm-thick coronal sections cut on a freezing microtome, and these lesions were used as reference points to determine the recording sites.

Experimental design and statistical analysis . All data were analyzed using custom codes written in MATLAB and R software for statistical computing and graphics, version 4.0.4. To ensure that the monkeys recognized the emotional valence of the visual stimuli, we confirmed that the correct rate for the conditioning task was more than 90%. Further, to ascertain whether the monkeys had differentiated the emotional intensity of visual stimuli, we compared the reaction time to the visual stimuli, that is, the time from stimulus onset to joystick manipulation (Kruskal–Wallis test and Steel–Dwass multiple comparison test, $p < 0.05$).

In the analysis of neuronal data, we first identified responses to the visual stimuli in each amygdala neuron by comparing the number of spikes between a baseline period (−200 to −1 ms before the stimulus onset) and a stimulation period (1–200 ms after stimulus onset). If the number of spikes during the stimulation period differed significantly from that during the control period (Wilcoxon rank sum test, $p < 0.05$), we regarded the neuron as responsive. For responsive neurons, we examined whether the response patterns were stable or variable across the three stimulus sets (Kruskal–Wallis test, $p < 0.05$). If the response pattern varied among the three stimulus sets, we considered the neuron responsive to some physical features of visual stimuli, such as local shape and color, rather than to their emotional value, and they were not included in further analyses. Second, to quantitatively examine the information conveyed by each neuron, we calculated the mutual information concerning emotional valence (negative or positive) and emotional intensity (large, medium, or small) for each neuron using the calculation method reported by Sugase et al. (1999). The information associated with the occurrence of neuronal responses *[I(S; R)]* was quantified as the decrease in the entropy of the stimulus occurrence *[H(S)]* as follows:

$$I(S;R) = H(S) - H(S|R) = \sum_s -p(s)\log p(s) - \left[\sum_s -p(s|r)\log p(s|r)_r\right],$$

where S is the set of stimuli s, R is the set of signals r (counts of a spike), $p(s|r)$ is the conditional probability of stimulus s given an observed spike count r, and $p(s)$ is the prior probability of stimulus s. The brackets indicate the average of the signal distribution, which is designated as $p(r)$. We calculated the information using a 50 ms sliding window that moved in 10 ms steps between the baseline period and the stimulation period. Third, we classified the responsive neurons into four types based on mutual information concerning valence and intensity. If the mutual information concerning emotional valence was significantly larger during the stimulation period than during the baseline period (Wilcoxon rank sum test, $p < 0.05$), the neuron was considered to convey emotional valence information. Similarly, if the mutual information concerning emotional intensity was significantly larger during the stimulation period than during the baseline period, the neuron was considered to convey emotional intensity information.

In addition, for neurons encoding information related to emotional valence, we identified the optimal stimulus (i.e., that inducing the greatest number of spikes) and examined whether it was negative or positive. We examined response latency of the neurons that showed stronger responses to negative or positive stimuli in the stimulation using the bootstrap method. The response latency was defined as a time point that first exceeded the mean spontaneous rate by 2.33 SDs of the baseline period in the stimulation period in the population level. These processes were repeated 1000 times by the bootstrap method and computed the 95% confidence intervals.

Results

Behavioral data

In the conditioning task, the monkeys were required to pull the joystick to avoid the air puff in negative trials and push it to

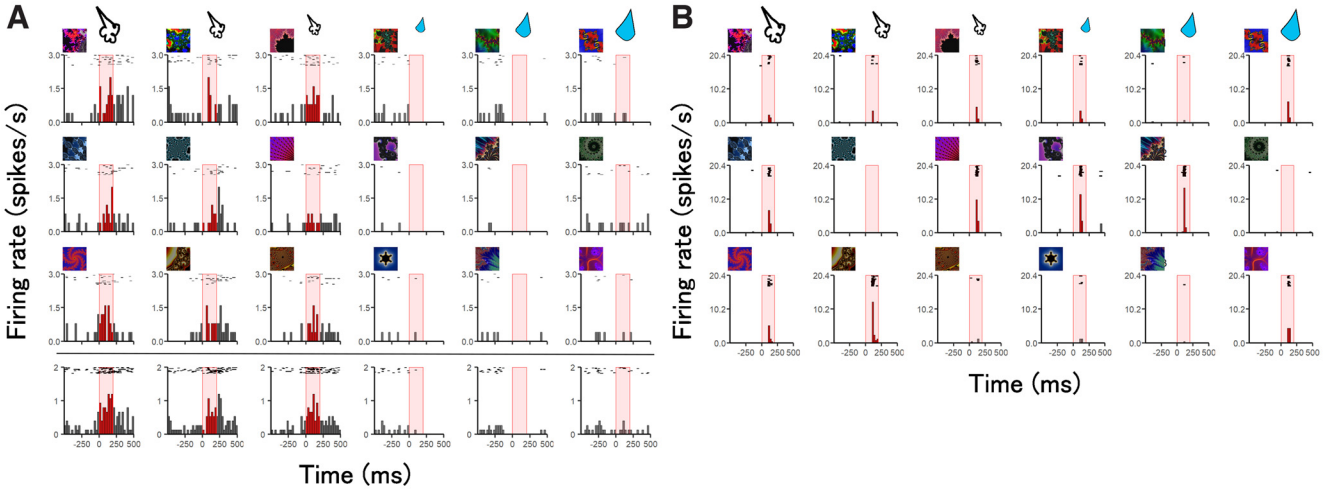

Figure 3. ***A***, An example of neuronal responsiveness in a analyzed neuron. The top three rows show the responses in each stimulus set, whereas the bottom row shows the overall responses. ***B***, An example of neuronal responsiveness in a neuron excluded from the database. The raster plot and peristimulus time histograms of a single neuron were aligned to the timing of stimulus presentation, which was considered time zero. The red shaded areas indicate the stimulation period (200 ms), and the red colored bars indicate the significant responses to the visual stimuli (Wilcoxon rank sum test, $p < 0.05$). We used three different stimulus sets in each recording session to ensure the reproducibility of responses to the emotional valence and/or intensity.

obtain the reward in positive trials. The mean rate of correct responses during the recording sessions was 99.6% (monkey I, 99.9%; monkey T, 98.6%), indicating that the monkeys correctly recognized the emotional valence of visual stimuli. Then, to confirm that the monkeys could differentiate the emotional intensity associated with each visual stimulus (i.e., the amount of air puff or reward), we compared the reaction times for three negative (−3, −2, −1) or positive stimuli (+3, +2, +1). The monkeys responded more quickly to positive stimuli than to negative stimuli (Wilcoxon rank sum test, $p < 0.001$). More important, in both monkeys, more rapid responses were associated with greater emotional intensity of the stimulus (Fig. 2). For monkey I, the mean reaction times for valences of −3, −2, and −1 were 395, 396, and 407 ms, respectively, whereas those for valences of +3, +2, and +1 were 335, 342, and 350 ms, respectively. For monkey T, the mean reaction times for valences of −3, −2, and −1 were 444, 452, and 454 ms, respectively, whereas those for valences of +3, +2, and +1 were 354, 366, and 388 ms, respectively. There were significant differences in reaction time for both negative and positive stimuli (Kruskal–Wallis test; for monkey I, negative valence, $\chi^2_{(2)} = 239.43$, $p < 0.001$; positive valence, $\chi^2_{(2)} = 982.15$, $p < 0.001$; for monkey T, negative valence, $\chi^2_{(2)} = 33.47$, $p < 0.001$; positive valence, $\chi^2_{(2)} = 642.66$, $p < 0.001$). *Post hoc* analyses revealed that reaction times differed significantly for all combinations except one (Steel–Dwass multiple comparison test; for monkey I, negative condition −3 vs −2, $t = 4.18$, $p < 0.001$; −3 vs −1, $t = 15.00$, $p < 0.001$; −2 vs −1, $t = 10.79$, $p < 0.001$; positive valence, +3 vs +2: $t = 16.01$, $p < 0.001$; +3 vs +1, $t = 30.75$, $p < 0.001$; +2 vs +1, $t = 16.10$, $p < 0.001$; for monkey T, negative valence, −3 vs −2, $t = 3.76$, $p < 0.001$; −3 vs −1, $t = 5.65$, $p < 0.001$; −2 versus −1, $t = 1.96$, $p = 0.12$; positive valence, +3 vs +2, $t = 11.03$, $p < 0.001$; +3 vs +1, $t = 24.87$, $p < 0.001$; +2 vs +1, $t = 14.60$, $p < 0.001$). These data indicate that the monkeys differentiated the emotional intensity of visual stimuli according to the amount of reward or air puff.

Neuronal responses in the amygdala

Extracellular activity was recorded from 505 single neurons in the amygdala while the monkeys performed the conditioning task (121 recording sessions from monkey I and 51 recording sessions from monkey T, 386 neurons from monkey I and 119 neurons from monkey T). Among the 505 neurons, 441 were responsive. Figure 3, *A* and *B*, show two examples of the responses, where three rows correspond to the responses to three different stimulus sets. The neuron shown in Figure 3*A* exhibited strong responses to all three stimuli associated with a negative valence (−3, −2, and −1) in all the stimulus sets. The response pattern was stable across the three stimulus sets (Kruskal–Wallis test, $p < 0.05$). These responses could be explained mainly by the emotional value associated with the stimuli, but not by the physical features of visual stimuli. Of the 441 responsive neurons, 297 neurons (67%) showed stable responses across the three stimulus sets just like this neuron. On the other hand, the neuron shown in Figure 3*B* exhibited a clear response to the stimulus associated with large reward (+3) in the stimulus set 1 (top row). However, the neuron did not respond to the +3 stimulus, whereas it showed strong responses to the stimuli associated with small and middle reward (+1 and +2) and clear responses to the stimuli associated with a long and short air puff (−3 and −1) in the stimulus set 2 (middle row). It strongly responded to the stimulus associated with a middle air puff (−2) in the stimulus set 3 (bottom row). The response pattern varied across the three stimulus sets (Kruskal–Wallis test, $p > 0.05$). These responses were probably explained mainly by the physical features of visual stimuli rather than emotional value. Of the responsive neurons, 144 neurons (33%) showed response patterns varied across the stimulus sets. The data of these 144 neurons were not included in the database given the instability of the response among the three stimulus sets. Thus, for the 297 neurons, we pooled all the data from recording sessions using the three stimulus sets (like the fourth row in Fig. 3*A*), and the data were further analyzed.

Based on the mutual information conveyed by each neuron, we classified the 297 neurons into four types. Figure 4*A* shows an example of a neuron conveying information concerning emotional valence. As clearly shown, this neuron responded to visual stimuli with a negative valence regardless of the intensity but not to those with a positive valence. In contrast, the neuron shown in Figure 4*B* responded only to visual stimuli with a positive valence regardless of the intensity but not to those with a negative valence. These neurons were considered to convey information

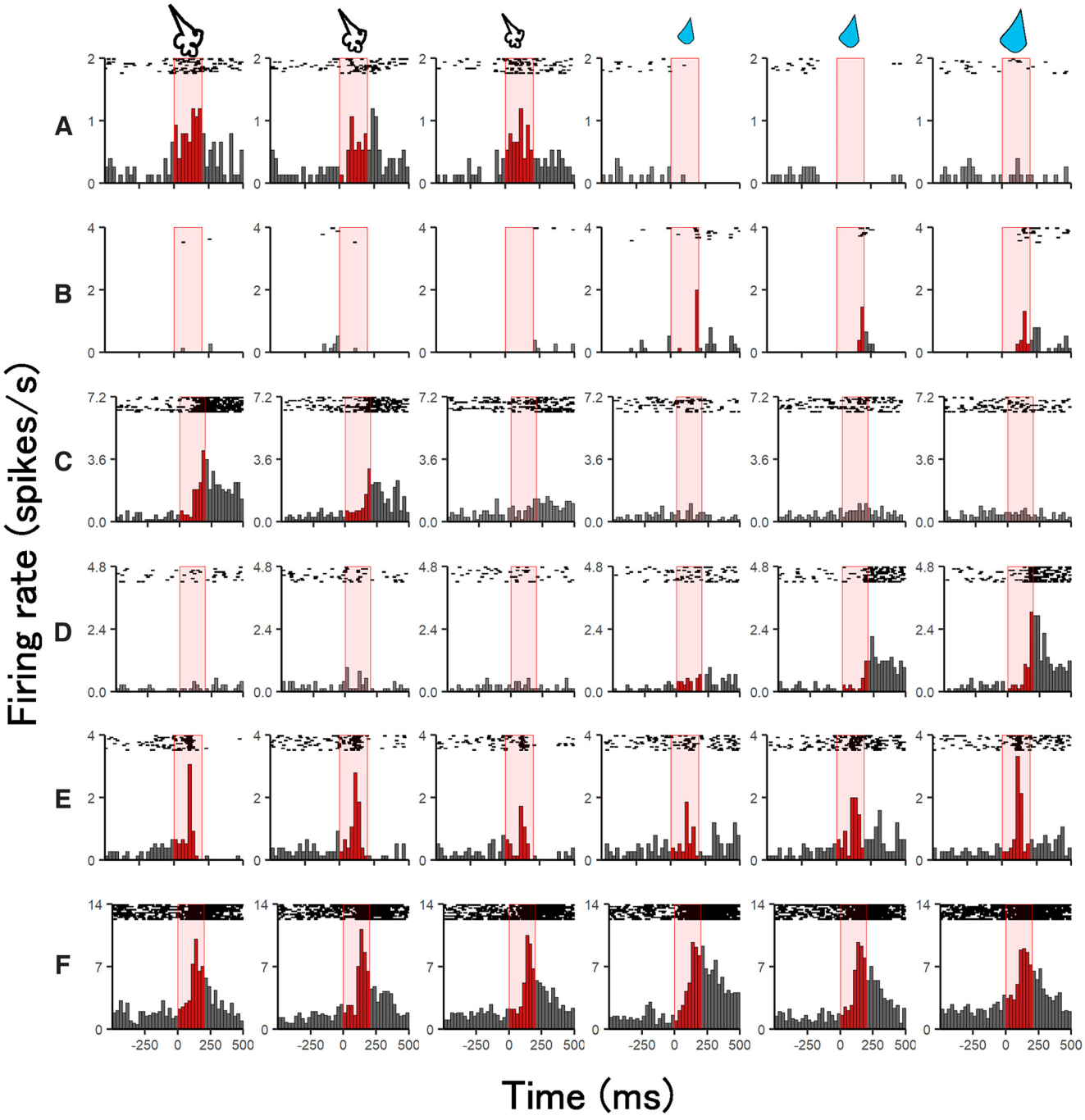

Figure 4. Examples of responses in amygdala neurons. ***A***, Responses of a neuron that encoded information related to valence only, which responded only to visual stimuli with a negative valence and not to the intensity of a negative valence (Val neuron). ***B***, Responses of a neuron that responded only to visual stimuli with a positive valence and not to the intensity of positive valence (Val neuron). ***C***, Responses of a neuron that encoded both valence and intensity information and responded only to visual stimuli with a negative valence (Val & Int neurons). ***D***, Responses of a neuron that responded only to visual stimuli with a positive valence (Val & Int neurons). ***E***, Responses of a neuron that encoded intensity information only (Int neuron). ***F***, Responses of a neuron that responded to visual stimuli but did not contain information related to either emotional valence or intensity (NE neuron). The red shaded areas indicate the stimulation period (200 ms), and the red colored bars indicate the significant responses to the visual stimuli (Wilcoxon rank sum test, $p < 0.05$).

about emotional valence only and were designated as emotional valence (Val) neurons. The neuron shown in Figure 4*C* also responded only to visual stimuli with a negative valence as shown in Figure 4*A*, but the magnitude of the response changed depending on the intensity. This neuron exhibited stronger responses to visual stimuli associated with a large air puff (−3), weaker responses to those associated with medium air puffs (−2), and no response to those associated with a small air puff (−1). Thus, the neuron conveyed information regarding both emotional intensity and emotional valence. The neuron shown in Figure 4*D* conveys information regarding both positive emotional valence and intensity, exhibiting stronger responses to visual stimuli associated with a large reward (+3) and weaker responses to those associated with medium and small rewards (+2 and +1). Neurons conveying information about both emotional valence and intensity were designated as emotional valence and intensity (Val & Int) neurons. Figure 4*E* shows an example of a neuron conveying

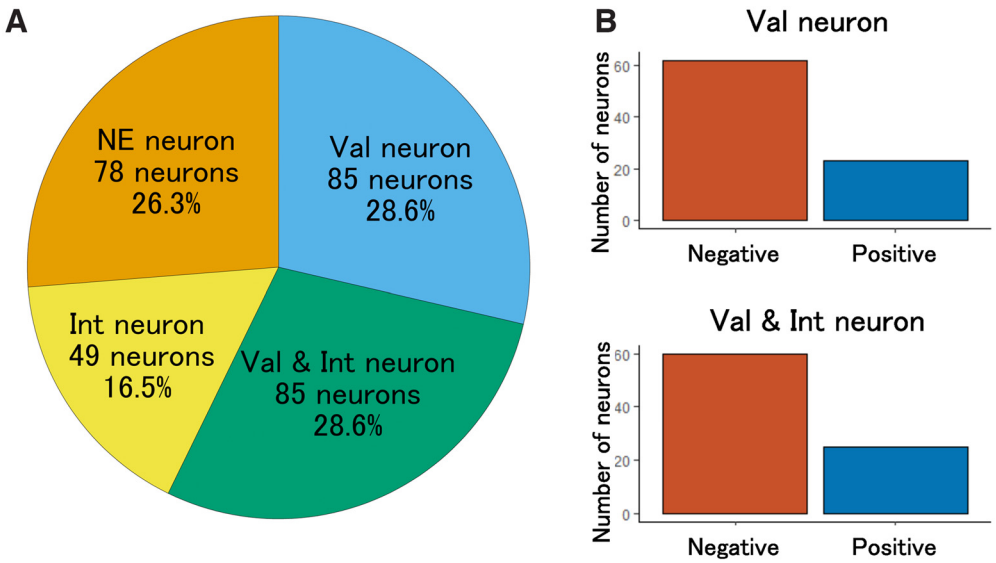

Figure 5. ***A***, The number and proportion of each type of neuron. The pie chart shows response types (blue, Val neuron; green, Val & Int neurons; yellow, Int neuron; orange, NE neuron), the number of neurons exhibiting each response type (85, 85, 49, and 78 neurons), and the percentage of neurons for each type (28.6, 28.6, 16.5, and 26.3%). ***B***, The number of neurons responding to positive and negative valence in Val neurons (top, negative, 62 neurons; positive, 23 neurons) and Val & Int neurons (bottom, negative, 60 neurons; positive, 25 neurons).

information concerning emotional intensity only. This neuron exhibited stronger responses to both (−3) and (+3), weaker responses to both (−2) and (+2), and the weakest responses to both (−1) and (+1). These neurons were designated as emotional intensity (Int) neurons. Figure 4*F* shows an example of a neuron that responded similarly to all visual stimuli. This neuron conveyed no statistically significant mutual information regarding emotional valence or intensity. Neurons of this type were designated as nonemotional (NE) neurons.

We further performed a regression analysis of the firing rate of each Int neuron separately for stimuli with a negative or positive valence, which further placed Int neurons into four classes based on the regression coefficients (Fig. 5). The neurons shown in Figure 5*A* exhibited stronger responses to a stronger intensity like the neuron shown in Figure 4*E*. This class of neurons could represent relative strength among stimuli regardless of valence. By contrast, the neurons shown in Figure 5*B* exhibited stronger responses to weaker intensity. This class of neurons could represent relative weakness. On the other hand, the neurons shown in Figure 5*C* exhibited stronger responses to a stronger intensity of positive valence, whereas these neurons exhibited stronger responses to weaker intensity of negative valence. In other words, these neurons responded more vigorously to more positive and less negative stimuli. The response pattern of the neurons shown in Figure 5*D* were just the opposite. They exhibited stronger responses to a weaker intensity of positive valence, whereas these neurons exhibited stronger responses to a stronger intensity of negative valence. These neurons responded more vigorously to less positive and more negative stimuli. These results indicated that there are two major classes of Int neurons—those encoding the relative intensity of emotional value (Fig. 5*A*,*B*) and those encoding the relative betterness of emotional value (Fig. 5*C*,*D*).

We designated 85, 85, 49, and 78 neurons as Val, Val & Int, Int, and NE neurons, respectively. Figure 6 summarizes the number and proportion of each type of neuron. More than half of the neurons (Val neurons and Val & Int neurons, 170/297, 57%) conveyed information regarding emotional valence, whereas approximately half (Val & Int neurons and Int neurons, 134/297, 45%) conveyed information regarding emotional intensity. In total, approximately three-fourths of the neurons analyzed (219/297) were considered to represent the emotional valence and/or intensity of visual stimuli in the monkey amygdala.

For Val and Val & Int neurons, we investigated whether the optimal stimulus was positive or negative. Our findings indicated that 71.8% of neurons (122/170 neurons) preferred negative stimuli over positive stimuli (binomial test, $p < 0.001$). This stronger response for negative stimuli was consistent when we investigated the responses of Val and Val & Int neurons separately (Val neurons, stronger negative responses in 62/85 neurons, binomial test, $p < 0.001$; Fig. 6*B*, top; Val & Int neurons, stronger negative response in 60/85 neurons, binomial test, $p < 0.001$; Fig. 6*B*, bottom). This stronger response for negative stimuli was also consistent when examined separately in each monkey (70% for monkey I and 80% for monkey T). This result indicates that the amygdala is more involved in processing negative valence than positive valence. There was no difference in the response latency between neurons that responded more strongly to negative and positive stimuli. The mean response latencies to negative and positive stimuli were 81.8 and 80.1 ms, respectively.

Localization of neurons in the amygdala

We also examined the locations of the neurons from which recordings were obtained. As shown in Figure 7, we recorded the activity of single neurons in the dorsal portion of the amygdala. Responsive neurons were mainly located in the central, medial, accessory basal, and basal nuclei, although there was a small number of responsive neurons in the lateral nucleus. Neurons conveying information about emotional valence (Val and Val & Int neurons) or emotional intensity (Val & Int and Int neurons) accounted for 60% (51/85) and 48.2% (41/85) of neurons in the central nuclei, 53.6% (30/56) and 41.1% (23/56) of neurons in the medial nuclei, 56.6% (43/76) and 43.4% (33/76) of neurons in the accessory basal nuclei, 57.8% (37/64) and 40.6% (26/64) of neurons in the basal nuclei, and 56.3% (9/16) and 68.8% (11/16) of neurons in the lateral nuclei, respectively. Among Val and Val & Int neurons,

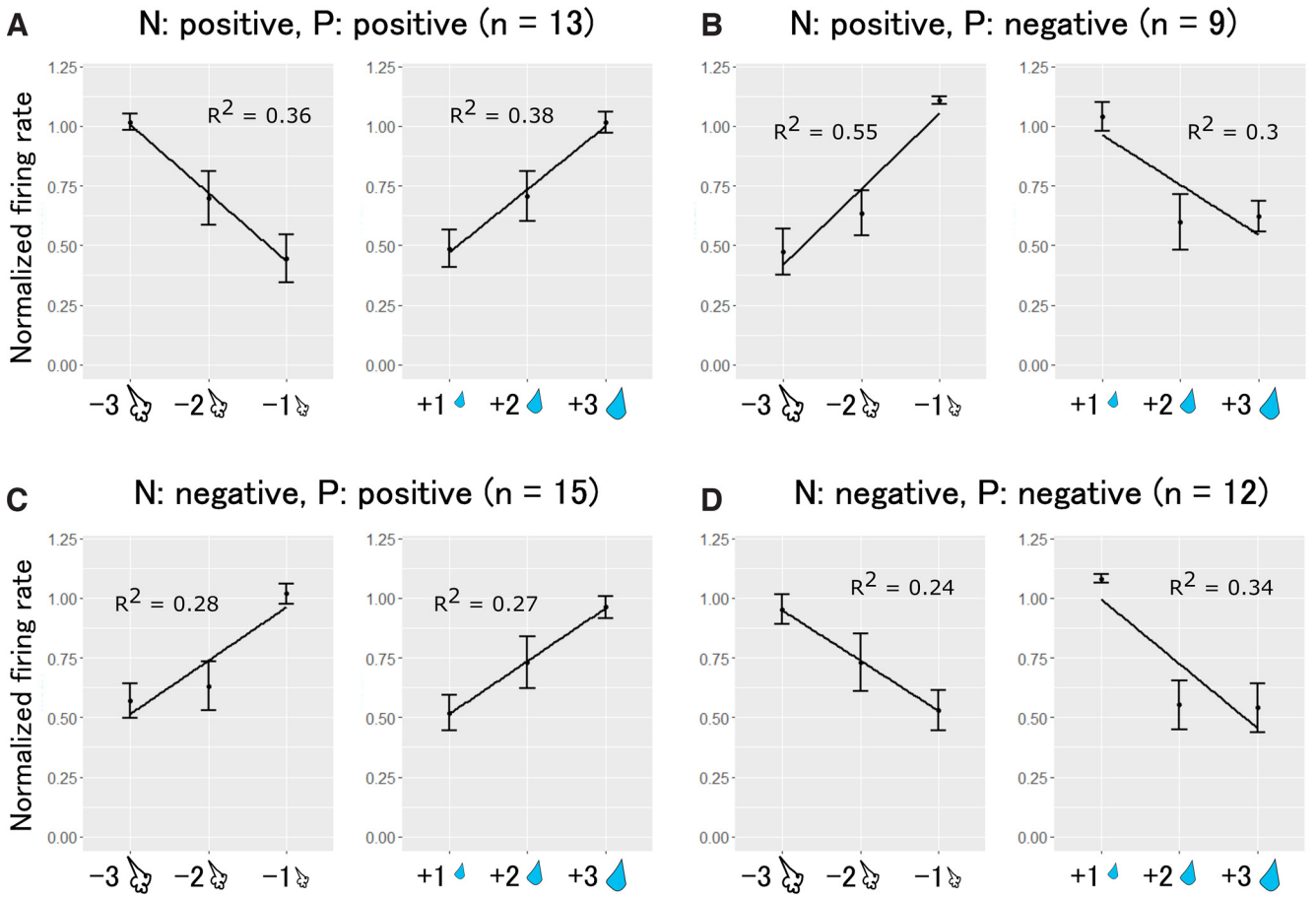

Figure 6. Int neurons were classified into two types based on the regression analysis. ***A–D***, One type is the neurons that encode the emotional intensity of the whole stimulus state used for the task (***A***, ***B***), and the other encodes the relative emotional intensity in negative and positive stimuli, respectively (***C***, ***D***). The regression lines fitted to the firing rates for negative and positive stimuli ($p < 0.05$). The responsiveness of neurons that are positively correlated to the intensity of both negative and positive stimuli ($n = 13$; N, negative stimuli; P, positive stimuli) is shown in ***A***. The figure illustrates the linear regression lines, the mean of the normalized firing rates, the error bars, and the coefficient of determination (R^2). The vertical axis indicates the mean of the normalized firing rate, and the horizontal axis indicates the intensity of negative and positive stimuli. Error bars represent ±1 SEM. The responsiveness of neurons that are negatively correlated to the intensity of negative stimuli and negatively correlated to the intensity of positive stimuli ($n = 9$) is shown in ***B***. The responsiveness of neurons that are negatively correlated to the intensity of negative stimuli and positively correlated to the intensity of positive stimuli ($n = 15$) is shown in ***C***. The responsiveness of neurons that are positively correlated to the intensity of negative stimuli and negatively correlated to the intensity of positive stimuli ($n = 12$) is shown in ***D***.

neurons in which optimal responses were observed following the presentation of negative stimuli accounted for 74.5% (38/51), 56.7% (17/30), 83.7% (36/43), 67.6% (25/37), and 66.7% (6/9) of neurons in the central, medial, accessory basal, basal, and lateral nuclei, respectively. Although we recorded neuronal activity from the dorsal half of the amygdala, we observed no statistically significant differences in this region. These data suggest that the four types of neurons are intermingled in the amygdala.

Discussion

In the current study, we examined the responses of neurons in the monkey amygdala to visual stimuli associated with three different durations of air puff (negative valence) and three different volumes of liquid reward (positive valence). To our knowledge, this study is the first to investigate the neuronal representation of emotional valence and intensity simultaneously. Accordingly, our results are the first to demonstrate that most amygdala neurons convey information concerning the emotional valence and/or intensity of visual stimuli, independent of physical features (73.7%, 219/297 neurons). Our findings also indicated that the majority of the amygdala neurons conveying information about emotional valence responded optimally to negative stimuli (71.8%, 122/170). Further, some amygdala neurons conveyed information related to both emotional valence and intensity (28.6%, 85/297), whereas a small portion conveyed information related to emotional intensity alone (16.5%, 49/297).

In this study, fractal patterns that the monkeys had never seen before the experiment were used as visual stimuli. Therefore, all visual stimuli were emotionally neutral for the monkeys at the beginning of the experiment. Then, every time new visual stimuli were introduced, they were associated with three amounts of air puff or reward. To exclude visual responses to some physical features of the stimuli, we used three different stimulus sets to examine the reproducibility of responses. Consequently, one-third of the neurons responded to specific stimuli, and the response profile varied across the three stimulus sets, suggesting that these neurons exhibited pure visual responses depending on the physical features of the stimuli. On the other hand, approximately two-thirds of the responsive neurons exhibited consistent responses across the three stimulus sets, suggesting that the responses could not be explained by the physical features of the

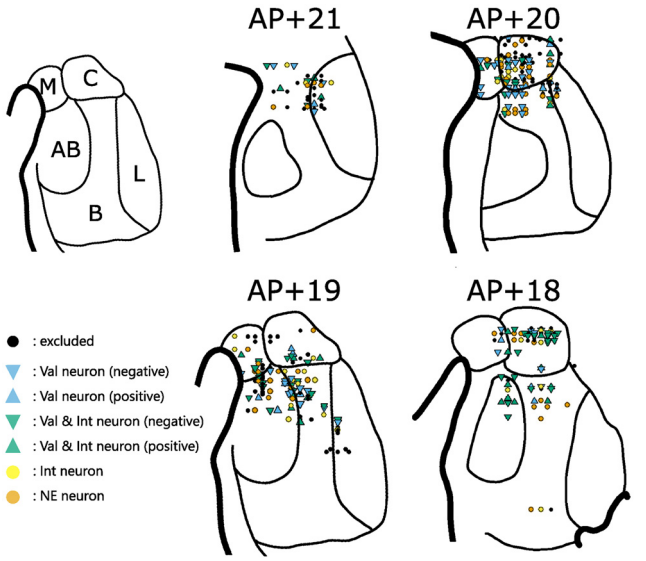

Figure 7. The locations of recorded neurons in monkey I. Neurons exhibiting no response to visual stimuli and those excluded because of instability of the response among the three stimulus sets are indicated in black dots. Neurons exhibiting responses of Val, Val & Int, Int, and NE neurons are represented in blue, green, yellow, and orange, respectively. Inverse triangles indicate neurons that responded optimally to negative stimuli. Triangles indicate neurons that responded optimally to positive stimuli. Circles indicate neurons that did not encode valence information. AB, accessory basal nucleus; B, basal nucleus; C, Central nucleus; L, lateral nucleus; M, medial nucleus.

stimuli. Among the neurons exhibiting consistent responses, one-fourth responded similarly to all six stimuli (−3, −2, −1, +1, +2, and +3), indicating that they may simply transfer information regarding the appearance of emotional stimuli. In contrast, the remaining three-fourths conveyed information related to emotional valence and/or intensity. If the ratio is calculated based on the total number of recorded neurons, our results indicate that >40% of amygdala neurons (219/505) were involved in evaluating the emotional value of the visual stimuli. These results are consistent with those of previous neuronal studies (Paton et al., 2006; Belova et al., 2007) and some imaging studies (Hariri et al., 2002; Zald, 2003).

According to previous studies involving humans (Adolphs et al., 1994; Scott et al., 1997; Terburg et al., 2018) and monkeys (Klüver and Bucy, 1938; Weiskrantz, 1956; Dal Monte et al., 2015), damage to the amygdala induces deficits in the evaluation of negative valence. However, Paton et al. (2006) reported that fewer amygdala neurons responded to negative stimuli (39/100 neurons) than to positive stimuli (61/100 neurons), in contrast to our findings. Indeed, our analysis indicated that the majority of amygdala neurons (66.5%) that conveyed information about emotional valence responded optimally to negative stimuli. In the present study, from an ethical viewpoint, we used relatively mild air puffs as the aversive stimuli and controlled the emotional intensity by the duration of the air puff rather than the air pressure. In addition, the fractal patterns that we used as conditioned stimuli are different from real snakes, food, and other stimuli that have psychological and ecological values. Probably because of these experimental factors, there was no difference in the response latency between negative and positive stimuli. However, the fact that more neurons responded to negative stimuli even under this situation, consistent with previous studies, indicates that the amygdala neurons encode a more negative valence.

Paton et al. (2006) used a trace conditioning paradigm to associate visual stimuli with outcomes. In their task, the monkeys were not required to respond to escape from the negative visual stimulus (i.e., a passively received air puff). However, in the current study, we used an operant conditioning paradigm in which monkeys were required to respond within 1 s of visual stimulation to avoid an air puff, which may have resulted in amygdala activation. Terburg et al. (2018) reported that patients with damage to the amygdala exhibit impairments in the ability to execute rapid escape from an electrical shock in response to visual stimuli, emphasizing the relationship between the amygdala and rapid escape behavior. This is consistent with the results of our study. Together, these data suggest that the monkey amygdala places an emphasis on negative valence to ensure escape from the stimulus, thereby increasing the chance for survival.

Our findings indicated that some single neurons in the amygdala can convey information related to both emotional valence and intensity. As mentioned in the Introduction, we believe that both parameters are important for evaluating emotional value. For example, a mild air puff such as that associated with a tender breeze may be interpreted as neutral or even positive. However, a strong air puff such as that associated with a storm is likely to be interpreted as negative, prompting the decision to escape the situation. This is also true for the intensity of touch on the skin. Thus, information regarding both emotional valence and intensity represented by these neurons can aid in producing emotional responses that are both rapid and accurate.

Interestingly, we observed that some neurons conveyed information regarding the emotional intensity of the visual stimulus only (Int neurons). Some neurons responded optimally to stimuli associated with large amounts of air or reward in the negative and positive conditions, respectively. Air puffs induce sensation in the skin, whereas liquid rewards induce sensations in the oral cavity. Despite these differences in sensory channel and valence, the neurons seemed to encode the intensity associated with the small, medium, and large stimuli. We found two classes of Int neurons conveying different intensity information. One class of neurons encoded the relative intensity among the stimuli with each valence (Fig. 5*A*,*B*). In other words, for these neurons, the stronger (or weaker) the intensity of the stimulus, the larger the response. The information of these neurons would directly affect the quickness of behavior of the animal; that is, the information indicates whether the animal had a better prompt approach to or escape from the stimulus. The other class of neurons encoded the relative betterness in each valence (Fig. 5*C*,*D*). For these neurons, the relatively better (or less worse) the stimulus, the larger the response. The information of these neurons could be useful for calculating the difference in emotional value leading to positive and/or negative reinforcement. The information encoded by these neurons would help the choice of behavior of the animal based on the relative emotional value of the stimulus.

As mentioned in the preceding paragraph, a visual stimulus can be judged based on its emotional intensity. The central nucleus of the amygdala, which included most of the single neurons whose activity was recorded in our study, projects to subcortical regions such as the thalamus, hypothalamus, and brainstem (Price and Amaral, 1981; Amaral, 1992). These subcortical regions are directly related to emotional responses (Holstege, 2009; Buhle et al., 2013; Sieger et al., 2015; Venkatraman et al., 2017; Montardy et al., 2021). Neurons encoding information related to emotional intensity may aid the animal in preparing for a quick emotional response.

In the present study, we were unable to identify any patterns in the localization of the four types of neurons among the

amygdala subnuclei, although we recorded neuronal activity only from the dorsal half of the amygdala. Paton et al. (2006) have also reported that neurons responding to negative and positive valence are anatomically intermingled in the amygdala. Our study replicated this result. Together, these data suggest that the primate amygdala processes the emotional value of a visual stimulus in a distributed manner.

In the present study, we aimed to provide a more complete picture of the neuronal mechanisms underlying the evaluation of the emotional valence and intensity of a visual stimulus. The amygdala is well known to receive multimodal sensory input (Amaral and Price, 1984; Ghashghaei and Barbas, 2002; Morrow et al., 2019; Gothard, 2020). Imaging studies in humans have suggested that the amygdala is involved in the evaluation of emotional valence and/or intensity for both olfactory and gustatory stimuli (Anderson et al., 2003; Small et al., 2003; Winston et al., 2005; Jin et al., 2015). For example, Winston et al. (2005) reported changes in amygdala activity depending on the intensity of positive and negative smells. How amygdala neurons represent and/or integrate emotional information from different sensory modalities and whether emotional valence and emotional intensity can be coded in the amygdala beyond sensory modalities remains unknown. Further studies are required to elucidate the complete neuronal mechanisms involved in the processing of emotional value.

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