Title: Functional differences in face processing between the amygdala and ventrolateral prefrontal cortex in monkeys

Proposed Journal section: Cognitive neuroscience

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Running title: Face processing in amygdala and prefrontal cortex

Keywords: communication; emotion; *Macaca mulatta*; rhesus monkey

Abbreviations: PSTH, Peristimulus time histograms; SD, Standard deviation; vlPFC, Ventrolateral prefrontal cortex.

Abstract

The ability to categorize social information is essential to survive in a primate's social group. In the monkey brain, there are neural systems to categorize social information. Among these, the relationship between the amygdala and the ventrolateral prefrontal cortex (vlPFC) has recently gained focus with regard to emotion regulation. However, the processing of facial information and the functional differences in these two areas remain unclear. Thus, in this study, we examined the response properties of single neurons in the amygdala and vlPFC while presenting video clips of three types of facial emotions (aggressive threat, coo, and scream) in *Macaca mulatta*. Neurons in the amygdala were preferentially activated upon presentation of a scream facial expression, which is strongly negative, whereas the neurons in the vlPFC were activated upon presentation of coo, a facial expression with multiple meanings depending on the social context. Information analyses revealed that the amount of information conveyed by the amygdala neurons about the type of emotion transiently increased immediately after stimulus presentation. In contrast, the information conveyed by the vlPFC neurons showed sustained elevation during stimulus presentation. Therefore, our results suggest that the amygdala processes strong emotion roughly but rapidly, whereas the vlPFC spends a great deal of time processing ambiguous facial information in communication, and make an accurate decision from multiple possibilities based on memory.

INTRODUCTION

Because the ability to categorize social information is essential to survive in primate's social group, it is assumed that primates possess neural systems to categorize social information in the brain. Two of several candidates for those systems are located in the amygdala and ventrolateral prefrontal cortex (vlPFC). The primary function of the primate amygdala is emotion processing (LeDoux, 2000; Phelps and LeDoux, 2005), and the amygdala activity is linked with autonomic physiological reactions (Laine et al., 2009). The human amygdala is specifically activated when the subjects see fearful facial expressions (Morris et al., 1996) in addition to body movements of others expressing emotion (Hadjikhani and de Gelder, 2003). Moreover, several researches reported neurons that show different responses to different facial expressions or different directions of gaze of others in the monkey amygdala (Nakamura et al., 1992; Kuraoka and Nakamra, 2006, 2007; Gothard et al., 2007; Hoffman et al., 2007; Tazumi et al., 2010). On the other hand, neurons in the monkey vlPFC have been reported to respond to faces and vocalizations (Ó Scalaidhe et al., 1997; Sugihara et al., 2006; Tsao et al., 2008; Romanski and Diehl, 2011). There are also neurons that show responses to social behaviors of others in the monkey vlPFC (Tsunada and Sawaguchi, 2012).

The interaction between the amygdala and the vlPFC has recently received attention in relation to emotion regulation (Townsend and Altshuler, 2012). Hariri et al. (2000) reported an

increase in regional cerebral blood flow in the right vlPFC during a face cognition task, and a decrease in regional cerebral blood flow in the left and right amygdala. These data imply that the vlPFC regulates emotional responses generated by the amygdala in face perception, through conscious evaluation and appraisal (Hariri et al., 2003).

The primate amygdala and vlPFC are closely related with each other as described above. Then, what are the functional differences in categorizing social information between the amygdala and vlPFC? One candidate for the differences is time of processing. The amygdala has been known to process emotion roughly (Vuilleumier et al., 2003) but rapidly (Balderston et al., 2014). In contrast, neurons in the primate vlPFC have been reported to be involved in complex cognitive functions such as memory (Goldman-Rakic, 1995), behavioural planning (Tanji and Hoshi, 2008) and decision-making (Sakagami and Pan, 2007) that require a little time to be accomplished. Thus, we hypothesized that the role of the amygdala is larger than that of the vlPFC at an early stage of the processing of social information, whereas the role of the vlPFC becomes larger at a late stage.

In the present study, we directly compared neuronal activity between the amygdala and vlPFC of *Macaca mulatta* during the presentation of face stimuli under the same experimental conditions to elucidate the role of neurons in these two brain regions in face processing. We found rapid phasic peak of information processing about the type of emotion in the amygdala: the information reached a peak 260 ms after stimulus onset, and maintained more than half of

the peak for 170 ms. We also found long-lasting information processing in the vlPFC: the information reached a peak 630 ms after stimulus onset, and maintained more than half of the peak for 720 ms.

EXPERIMENTAL PROCEDURES

Subjects We used three rhesus monkeys (*Macaca mulatta*, 5–7 kg) for neuron recordings in the amygdala and two rhesus monkeys (5–7 kg) for neuron recordings in the vlPFC. Water was withheld before each daily session and juice was given as a reward in the experimental room. Supplemental water and vegetables were given after the session when needed, and monkey chow was available *ad libitum*. All experiments were carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals' of the National Institute of Health (1996), the 'Guide for Care and Use of Laboratory Primates' published by the Primate Research Institute, Kyoto University (2002, 2010). The research was conducted under experimental license No. 2010-012 approved and issued by Kyoto University, and the 'Guide for Care and Use of Laboratory Primates' published by the National Institute of Neuroscience, National Center of Neurology and Psychiatry (2005) under experimental license No. 002; approved and issued by the NCNP. The research adhered to the legal requirements of Japan.

Behavioural tasks and stimuli: All experiments were performed in a dark, soundproof room where a monkey sat in a primate chair and faced a 21 inch multiscan monitor (GDM-F520;

SONY, Tokyo, Japan, or FlexScan T961; EIZO NANANO, Ishikawa, Japan) placed 30–40 cm from its eyes. When the monkey pressed a lever, a yellow fixation spot appeared at the center of the monitor. After keeping the lever pressed and fixating on the spot for 1000 ms, a test stimulus was presented for 1000 ms behind the fixation spot; thereafter, the yellow fixation spot was replaced with a red spot after 300–1500 ms. If the monkey released the lever within 800 ms of spot replacement, it was rewarded. Eye position was continuously monitored using a charge-coupled device camera system. If the monkey's gaze deviated more than 1.5° from the fixation spot or if it released the lever during a trial, the trial was terminated without providing any reward. The test stimuli were 13 full colour video clips (approximate size was 20 x 15 in degree of visual angle), each lasting 1000 ms, presented on a grey background. All stimuli were from monkey, human, or artefact categories, with nine monkey species-specific facial expressions, two human faces, and two artefacts. The monkey face stimuli consisted of three types of emotion recorded from three model monkeys, who were unfamiliar to the subject monkeys. The three types of facial expressions were aggressive threats, screams, and coos. An *aggressive threat* is often expressed by a dominant individual demonstrating an inclination to attack. A *scream* is often expressed by a subordinate who is attacked or threatened by a dominant individual. A *coo* has multiple meanings, and it is often expressed in response to food or separation from the mother or social group (Hinde and Rowell, 1962; Van Hooff, 1962). The type of video clips was not associated with reward. We separately

presented the visual elements and auditory elements of the video clips. However, we only analysed the data for the visual elements because few neurons recorded from the vlPFC responded to the auditory elements.

Recording procedure: The action potentials of single neurons were recorded extracellularly from the amygdala in four hemispheres of three monkeys and the vlPFC in three hemispheres of two monkeys using a polyurethane-coated tungsten microelectrode (1.5–3.0 MΩ, 0.3 mm in diameter). The tungsten microelectrode was inserted through a guide tube (1.1 mm in diameter) that was fixed to a grid into the brain without distortion. A stainless steel guide tube was inserted through the dura to a depth of ~5 mm above the amygdala or to just below the dura over the vlPFC, as estimated from magnetic resonance images taken in advance of the placement. The electrode was advanced using a hydraulic Microdrive (Narishige, Tokyo, Japan) while neuronal activity was monitored. The action potentials were discriminated and converted into pulses using a window discriminator (Model DIS-1; BAK Electronics, Germantown, MD) or a multi-spike detector (Alpha Omega Engineering, Nazareth, Israel). The timing of action potentials and task events was stored on a personal computer with a time resolution of 0.5 ms in four monkeys or 1.0 ms in one monkey. When the activity of a single neuron was isolated, a recording session was started. We presented the visual stimuli pseudo-randomly until each had appeared 10 times. We tested all stimuli 10 times during a recording session even if a single neuron did not appear to show responses to any stimuli.

After the test, we advanced the electrode further until the activity of the next single neuron was isolated.

Data analysis: The stored data were processed off-line using custom-made MATLAB programs and the statistical analyses were conducted using IBM SPSS Statistics software. A neuron was regarded as responsive to the stimulus if the number of spikes during a 1000 ms period, from 100 to 1100 ms after the onset of a stimulus (stimulation period), was significantly different from the 1000 ms period immediately before the stimulus onset (baseline period) ($P < 0.01$, Wilcoxon signed-ranks test). Next, to construct neuronal population activity curves in response to the stimuli, peristimulus time histograms (PSTH) were sampled in 10 ms non-overlapping bins and convolved with a Gaussian kernel of 30 ms standard deviation.

We have previously reported neuronal discrimination of stimuli among monkey, human, and artefact categories in the amygdala (Kuraoka and Nakamura, 2012). Therefore, in this study, we focused our analyses on neuronal discrimination of the stimuli among the monkey sub-category, i.e., ‘type of emotion’ and ‘identity’. To quantitatively examine the difference in neuronal responses to different emotions (aggressive threat, scream, and coo) and different identities (three model monkeys), we calculated the mutual information about each emotion and identity contained in the neural activity of single neurons using neuron-by-neuron spike counts. We then averaged the mutual information across the population of neurons. We

followed the calculation method reported by Sugase et al. (1999). The information associated with an occurrence of neuronal responses ($I(S; R)$) was quantified as a decrease in entropy of the stimulus occurrence ($H(S)$):

$$I(S; R) = H(S) - H(S|R) = \sum_s -p(s)\log p(s) \; - \left\langle \sum_s -p(s|r)\log p(s|r) \right\rangle_r$$

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 an average of the signal distribution $p(r)$. We evaluated the significance of information using the chi-square test (Kitazawa et al., 1998). We calculated the information about emotion or identity in 50 ms sliding windows that were moved in 10 ms steps for each neuron using the spike counts during the stimulation period minus the firing rate during the baseline period.

We also examined if there was any deviation of the distribution of neurons preferring each emotion type. Firstly, we calculated the centre of balance of all recorded loci, and thereafter we divided our recorded area into quadrants with the centre and the antero-posterior and dorso-ventral axes. Then, we counted the number of neurons preferring each emotion type within each quadrant and compared the number of neurons among quadrants.

RESULTS

We recorded the activity of 227 single neurons from the amygdala and 125 single neurons from the vlPFC. Of these, 77 in the amygdala and 61 in the vlPFC were tested on the visual element condition and showed face-responsiveness. The data set from the amygdala neurons is the data presented in our previous study (Kuraoka and Nakamura, 2006, 2007, and 2012), but only the visual element condition is used in this study.

Figure 1 shows examples of the response to faces of a neuron in the amygdala (Fig. 1a) and vlPFC (Fig. 1b). This amygdala neuron showed the best response to the scream expressed by monkey B and the second best response to the scream expressed by monkey C. The response started just after the stimulus onset but the magnitude of the response gradually decreased during the stimulation. The response magnitude (the number of spikes) during the 1000 ms stimulation period, from 100 to 1100 ms after the stimulus onset, was compared among the nine monkey stimuli. A two-way ANOVA with ‘type of emotion’ and ‘identity’ as main factors revealed that there were significant main effects of both, type of emotion ($F(2,81) = 39.5$, $P < 0.001$) and identity ($F(2,81) = 45.8$, $P < 0.001$) on the activity of this amygdala neuron. The post-hoc analysis revealed that the mean firing rate during the stimulation period in response to the scream stimuli was significantly higher than to the threat and coo stimuli ($P < 0.001$, Tukey least significant difference [LSD] test). The mean firing rate during the stimulation period in response to the coo stimuli was also significantly higher

than to the threat stimuli ($P = 0.001$). With respect to Identity, the mean firing rate during the stimulation period in response to monkey C stimuli was significantly higher than to monkey A ($P < 0.001$) and B ($P = 0.003$) stimuli. The mean firing rate during the stimulation period in response to monkey B stimuli was also significantly higher than to monkey A stimuli ($P < 0.001$). By contrast, the vlPFC neuron showed the best response to the coo stimuli expressed by monkey B. This neuron exhibited a clear response peak just after the stimulus onset and maintained a high firing rate throughout stimulation. This neuron showed the second and third best responses to the coos expressed by monkey C and monkey A, respectively. There were significant main effects of both Type of emotion ($F(2,81) = 307.7$, $P < 0.001$) and Identity ($F(2,81) = 123.9$, $P < 0.001$) on the activity of this vlPFC neuron. The post-hoc analysis revealed that the response to the coo stimuli was significantly higher than to the threat and scream stimuli ($P < 0.001$, Tukey LSD test). The mean firing rates during the stimulation period in response to the threat and scream stimuli were not significantly different ($P = 0.96$, Tukey LSD test). With respect to Identity, the mean firing rate during the stimulation period in response to monkey B stimuli was significantly higher than to monkey A ($P < 0.001$) and C ($P < 0.001$) stimuli. The mean firing rate during the stimulation period in response to monkey C stimuli was also significantly higher than to monkey A stimuli ($P = 0.031$).

We examined the optimal stimulus, which elicited maximal neuronal firing during the stimulation period, for each neuron among the nine monkey faces for the 77 and 61

monkey-responsive neurons in the amygdala and the vlPFC, respectively. In the amygdala, about half of the monkey-responsive neurons (38/77; 49%) showed a maximal response to the scream stimuli, 29% (22/77) showed a maximal response to the coo stimuli, and the remaining 22% (17/77) showed a maximal response to the aggressive threat stimuli. Thus, the monkey-responsive amygdala neurons tended to 'prefer' the screams to the other types of emotion (P = 0.009, chi-square test, Fig. 2a). On Identity, monkeys A, B, and C elicited the maximal responses of 27% (21), 34% (26), and 39% (30) in the 77 monkey-responsive neurons, respectively. There was no significant tendency for any particular monkey model to elicit maximal responses more frequently from the amygdala neurons (P = 0.45, chi-square test, Fig. 2a). In the vlPFC, about half of the monkey-responsive neurons (28/61; 46%) showed a maximal response to the coo stimuli, 36% (22/61) showed a maximal response to the aggressive threat, and the remaining 18% (11/61) showed a maximal response to the scream. The monkey-responsive vlPFC neurons tended to "prefer" the coos to the other types of emotion (P = 0.026, chi-square test, Fig. 2b). On identity, monkeys A, B, and C showed maximal responses of 34% (21), 38% (23), and 28% (17) in the 61 monkey-responsive neurons, respectively. Therefore, like the amygdala neurons, there was no significant tendency for any particular monkey model elicit the maximal responses more frequently from vlPFC neurons (P = 0.63, chi-square test, Fig. 2b). Thus, both the amygdala and vlPFC neurons preferred a specific facial expression, irrespective of the identity of the monkey model; the

scream stimuli were optimal for amygdala neurons whereas the coo stimuli were optimal for the vlPFC neurons. It is noteworthy that screams have a strong emotional meaning, whereas the coos are often used in social communication.

We then examined and compared the response profiles of the neuronal population in the amygdala and vlPFC. Figure 3 shows temporal changes in the mean neuronal response curves of the neuronal population in the amygdala (Fig. 3a) and vlPFC (Fig. 3b). The mean ± standard deviation (SD) of firing rate during the baseline period was 7.21 ± 7.76 and 6.82 ± 8.83 spikes/s in the amygdala and vlPFC, respectively. A one-way ANOVA revealed that the magnitude of the baseline activity of the amygdala neurons was significantly higher than the vlPFC neurons ($F(1,12395) = 6.93$, $P = 0.008$). The mean ± SD of the firing rate during the stimulation period was 14.2 ± 15.5 and 10.1 ± 10.4 spikes/s in the amygdala and the vlPFC, respectively. The firing rate of the response of the amygdala neurons was also significantly higher than the vlPFC neurons ($F(1,12395) = 274.8$, $P < 0.001$). Interestingly, the response of the amygdala neurons showed a high peak just after stimulus onset and rapid decrease after this, whereas vlPFC neurons tend to show a sustained response during the stimulation period.

As shown in Figure 3, the magnitude of the population response of the amygdala and vlPFC neurons varied, to some extent, among the three facial expressions (threat, coo, or scream) and the three monkey identities (monkey A, B, or C). Thus, to quantitatively evaluate the ability of neuronal responses in the amygdala and the vlPFC to discriminate different

types of emotion (aggressive threat, scream, and coo) and different identities (three model monkeys), we calculated the information about the type of emotion or identity contained in the neural activity of each single neuron (see Materials and Methods). For example, information about the type of emotion and identity contained in the activity of the amygdala neuron in Figure 1a during the stimulation period was 0.094 ± 0.37 bits (mean ± SD) and 0.061 ± 0.30 bits, respectively. The information about type of emotion and identity contained in the activity of the vlPFC neuron in Figure 1b during the stimulation period was 1.4 ± 0.51 and 0.49 ± 0.72 bits (mean ± SD), respectively. Figure 4 shows the distribution of the information about identity against that of emotion for each neuron in the amygdala (Fig. 4a) and vlPFC (Fig. 4b). Thirty-three neurons in the amygdala had more information about type of emotion than identity (below the diagonal line in Fig. 4a), while 37 neurons had more information about identity than emotion (above the diagonal line in Fig. 4a). On the other hand, 36 neurons in the vlPFC had more information about type of emotion than identity (below the diagonal line in Fig. 4b), whereas only 16 neurons had more information about identity than type of emotion (above the diagonal line in Fig. 4b). In the amygdala, the number of neurons conveying more information about type of emotion than identity was not significantly different from the number of neurons conveying more information about identity than type of emotion ($P = 0.63$, binomial test). However, in the vlPFC, the number of neurons conveying more information about type of emotion than identity was significantly higher than

the number of neurons conveying more information about identity than type of emotion (P = 0.006, binomial test). We directly compared the amount of information about the type of emotion with that of identity conveyed by neuronal populations in the amygdala and vlPFC. In the amygdala, the amount of information about type of emotion was not significantly different from identity (P = 0.89, Wilcoxon signed-ranks test). However, in the vlPFC, the amount of information about type of emotion during the stimulation period was greater than about identity (P = 0.004, Wilcoxon signed-ranks test).

Figure 6 shows temporal changes in the average information about the type of emotion (Fig. 5a) and identity (Fig. 5b) conveyed by neuronal population responses in the amygdala and vlPFC, respectively. As shown in Figure 5a, information about type of emotion showed a sharp increase just after the stimulus onset and then decreased immediately in the amygdala. The information reached a peak 260 ms after stimulus onset, and maintained more than half of the peak from 210 to 380 ms after stimulus onset. By contrast, information about type of emotion showed a gradual increase after the stimulus onset and remained high until the stimulus offset in the vlPFC. The information reached a peak 630 ms after stimulus onset, and maintained more than half of the peak from 270 to 990 ms after stimulus onset. The information about identity was highest just after the stimulus onset in both the amygdala and vlPFC (Fig. 5b). The information in the amygdala reached a peak 250 ms after stimulus onset, and maintained more than half of the peak from 200 to 310 ms after stimulus onset. By

contrast, the information in the vlPFC reached a peak 190 ms after stimulus onset, and maintained more than half of the peak from 160 to 410 ms after stimulus onset. We next compared the information about type of emotion and identity between the amygdala and vlPFC in each 50 ms sliding window (see Materials and Methods). Immediately after the stimulus onset, the information about both emotion and identity conveyed by the amygdala neurons was significantly higher ($P < 0.05$, Mann-Whitney test) than the information conveyed by vlPFC neurons. By contrast, in the middle and latter period of stimulation, the information, in particular about the type of emotion, conveyed by vlPFC neurons became significantly higher ($P < 0.05$, Mann-Whitney test) than the information conveyed by amygdala neurons. Thus, as with population activity, the information conveyed by amygdala neurons showed a high peak just after stimulus onset and a rapid decrease thereafter in relation to the type of emotion, whereas the information conveyed by vlPFC neurons tended to show high levels during the stimulation period. In contrast, the information about identity conveyed by both the amygdala and vlPFC neurons showed peaks just after stimulus onset, although the amygdala peak was higher than the vlPFC peak.

As stated in our previous study (Kuraoka and Nakamura, 2007), face-responsive neurons in the amygdala were mainly recorded from the lateral, basal, and central nuclei. In this study, we also examined the locations of the recorded vlPFC neurons, and found that face-responsive neurons in the vlPFC were mainly recorded from areas 45 and 12 (Fig. 6),

where previous studies have also reported visual responsiveness (Ó Scalaidhe et al., 1997; Sugihara et al., 2006; Romanski and Diehl, 2011). Our recording sites were mainly located in the caudal part of the vlPFC. This result corresponds to the fact that only a few recorded neurons responded to the auditory element of the video stimuli because the auditory responsive region of the vlPFC was located in more rostral region (Romanski, 2007). In fact, 5 neurons that responded to the auditory element of the stimuli were recorded from the rostral part of our recording area. Moreover, analysing distribution of neurons within the recording area in the vlPFC resulted that there was no significant deviation of the distribution of neurons preferring each emotion type ($P = 0.099$, chi-square test).

DISCUSSION

Neuronal activity is known to encode information about the identity of faces in both the amygdala and the vlPFC (Ó Scalaidhe et al., 1997; Kuraoka and Nakamura, 2006; Gothard et al., 2007; Romanski and Diehl, 2011). However, the neuronal code for different types of emotion has not been examined in the vlPFC, although many studies have reported it in the amygdala (Nakamura et al., 1992; Kuraoka and Nakamura, 2006; Gothard et al., 2007; Hoffman et al., 2007). In this study, we first explicitly report that the population activity of neurons in the vlPFC showed discriminative responses to different type of emotion.

Many previous studies have investigated the role of the monkey vlPFC in representation of

vocalization (Romanski and Goldman-Rakic, 2002; Romanski et al., 2005; Averbeck and Romanski, 2006; Cohen et al., 2007; Tunada et al., 2011). Neurons responding to the auditory stimuli have been mainly found in the anterior part of the vlPFC (Romanski, 2007). In fact, we also found neurons that showed auditory responsiveness at the anterior part of the recording area (Fig. 6). However, those neurons were small in number because our recording chamber centered at the caudal part of the vlPFC. Thus, we focused attention on the analysis of the neuronal responses to the face stimuli in this study.

We found that there were mainly two differences in the characteristics of responses to face stimuli between the amygdala and the vlPFC. Firstly, the difference was observed in the preference for type of facial expressions. Amygdala neurons were preferentially activated during presentation of scream expressions, whereas vlPFC neurons were preferentially activated during the presentation of coo expressions. Secondly, the difference also appeared in the temporal characteristics of the neuronal activity. Although timing of the increase in the neuronal activity in response to face stimuli was not so different between the amygdala and vlPFC, the information analysis revealed that the information about emotion conveyed by amygdala neurons showed a phasic peak immediately after the face-stimulus onset, whilst information conveyed by vlPFC neurons showed a continuous elevation throughout facial stimulation. These results indicate that the difference in temporal characteristics of the neuronal activity between the monkey amygdala and vlPFC results not from detection of

social information but from discrimination of type of social information.

Processing of facial expressions in the amygdala

In our previous study (Kuraoka and Nakamura, 2007), we discussed a fear-detecting system in the monkey amygdala, based on the fact that the majority of amygdala neurons are preferentially activated during presentation of a scream expression. The population activity data in the present study also supported this notion. As shown in Fig. 3a, the population activity in response to the scream, exhibiting strong fear in the amygdala, was the highest among the three types of emotion. The fearful expressions of others provides information to the viewer about the presence of threat (Whalen et al., 2001), and a fight or flight response to a threat should be elicited rapidly for survival. Thus, the processing of discriminating facial expressions in the amygdala might be directly related to strong emotional responses. In fact, the amygdala projects to brain areas closely related to the production of emotional responses, such as the thalamus, hypothalamus, and brain stem (Amaral et al., 1992).

The present study also revealed that information about the type of emotion in the amygdala showed a high peak more rapidly than in the vlPFC. The evidence that emotion discrimination in the amygdala precedes that in the vlPFC has been also reported in the recent human study (Kohno et al., 2015). The human amygdala has been known to process emotion roughly (Vuilleumier et al., 2003) but rapidly (Balderston et al., 2014). Rapid processing of

facial expressions is also known in the human amygdala (Sato et al., 2011). However, the timing of peak processing of facial expressions in the monkey amygdala in our present study (about 260 ms after stimulus onset) was later than that found in humans (about 135 ms after stimulus onset). The stimuli of facial expressions in the present study were dynamic picture images, whereas Sato et al. (2011) used static face photos. Our dynamic stimuli of facial expressions started from a frame showing subtle emotion. Consequently, emotional intensity was not very high in the first frame of the stimulus video clips. In contrast, representative facial expressions, i.e., a frame showing the maximal emotional intensity, appeared in the middle of our 1-second stimulus. These kinds of stimuli may delay the timing of peak processing of facial expressions when compared with the static images used in Sato et al. (2011). Nevertheless, it is interesting that the timing of peak processing for discriminating different facial expressions in the amygdala is more rapid than in the vlPFC. Moreover, as shown in Figure 5a, the rapid increase of the information about type of emotion in the amygdala ceased in a brief period (about 170 ms in half-value width of the amount of peak information). It is also noteworthy that the information about type of emotion was lower in the amygdala than in the vlPFC for a long time during stimulation period (Fig. 5a). These results suggest that the amygdala processes the information about type of emotion more roughly than the vlPFC does.

Taken these evidences together, it is implicated that the amygdala processes strong

emotion roughly but rapidly for rapid reactions to the presence of threat even though the reactions could be inefficient in practice.

Processing of facial expressions in the vlPFC

Unlike the amygdala, the facial expression preferentially activating vlPFC neurons was the coo. The population activity of vlPFC neurons was highest in response to the coo throughout stimulation. The coo is not directly related to an emotion; rather it is often expressed in multiple context such as separation from a mother or social group, and is occasionally accompanied by a clear call, which provokes the response of a group member (Van Hooff, 1962). Therefore, the present data suggest that vlPFC neurons are involved in the processing of information that is difficult to be categorized to a specific meaning in social communication.

Nakamura et al. (1999) reported that the human inferior frontal cortex was associated with the processing of facial expressions. The human inferior frontal cortex and the macaque vlPFC are known to have comparable cytoarchitecture (Petrides and Pandya, 2002). The study of Nakamura et al. (1999) compared the brain activity of human participants when they were assessing facial expressions or attractiveness. The results showed that the right inferior frontal cortex, but not the amygdala, was activated during the assessment of facial expressions. Moreover, another human study reported that damage to the left vlPFC and adjacent anterior

insula, was associated with a specific difficulty in discriminating different subtle facial expressions (Tsuchida and Fellows, 2012). These studies suggest that the human vlPFC processes communicative information conveyed by slight and ambiguous changes in facial features but is not related to the induction of strong emotional reactions like the amygdala. The data in our present study support these suggestions.

In our study, information analysis clarified that there was a greater mean amount of information about type of emotion during the stimulus period than identity in the vlPFC (Fig. 4b). This result indicates that discriminating the type of emotion of other conspecifics forms an important part of face processing in the vlPFC. Additionally, time series analysis of information about the type of emotion revealed that this information conveyed by neurons in the vlPFC showed a gradual increase that continued after a sharp decrease in emotional information was seen in the amygdala (Fig. 5a). This result means that neurons in the vlPFC continue to process the type of emotion at a higher level, and for a longer period, than neurons in the amygdala.

The monkey vlPFC is known to be involved in cognitive processing such as memory of communicative information (Hwang and Romanski, 2015), behavioural planning (Tanji and Hoshi, 2008) and decision-making (Sakagami and Pan, 2007). Thus, the continuous retention of information about the type of emotion in the vlPFC is useful to determine an appropriate response to a conspecific. This is because they should react adaptively and flexibly to a coo

expression, with multiple meanings, depending on the situation. In human, the vlPFC is reported to be involved in working memory of nonspatial content (Nee et al., 2013), and is also engaged in retrieval of mnemonic context especially in multiple context (Chapados and Petrides, 2015). Therefore, the vlPFC might spend a great deal of time processing ambiguous information in social communication, and try to make an accurate decision against multiple possibilities based on memory.

The monkey area 45, which is a subdivision of the vlPFC, is reported to be activated when monkeys observe actions performed by others (Nelissen et al., 2005). Area 45 is connected to area 12 (Gerbella et al., 2010), which is another subdivision of the vlPFC, and area 12 projects to area F5 (Borra et al., 2011), which is the ventral premotor cortex. Consequently, the monkey vlPFC sends output to area F5. Neurons in area F5 have been known to be activated not only during the execution of actions, but also during the observation of actions performed by others, i.e., mirror neurons (Rizzolatti and Fabbri-Destro, 2008). Interestingly, action recognition is strongly related to communication (Rizzolatti and Arbis, 1998). Moreover, in area F5, mirror neurons responding to the observation of communicative mouth actions are also found (Ferrari et al., 2003). Taken above evidences and our results together, social information represented in the vlPFC might be a source of communicative responses generated by the processing in the ventral premotor cortex.

Connections between the amygdala and vlPFC

Previous studies regarding neural connections between the amygdala and the vlPFC have mainly reported indirect connections via the orbitofrontal or medial prefrontal cortex. A direct neural connection between the amygdala and vlPFC is thought to be weak (Barbas et al., 2011); in contrast, the neural connection between the orbitofrontal cortex and amygdala is quite strong (Barbas et al., 2011). In addition, the neural connection between the orbitofrontal cortex and vlPFC is also strong (Borra et al., 2011). The medial prefrontal cortex is also known to have neural connections with the amygdala and vlPFC (Barbas et al., 2011; Borra et al., 2011). Recently, a direct connection between the amygdala and vlPFC has received a great deal of attention. Gerbella et al. (2014) reported reciprocal neural connections between the amygdala and vlPFC (mainly area 45). Thus, the different processing of facial expressions in the amygdala and vlPFC might be affected through both direct and indirect neural connections between the two areas.

Conclusion

In the present study, we elucidated the functional differences in processing facial expressions between the amygdala and vlPFC in monkeys. In summary, the amygdala neurons rapidly but roughly discriminated the type of facial expressions, and preferentially responded to the strong negative emotional face. By contrast, the vlPFC neurons gradually but stably

discriminated the type of facial expressions, and preferentially responded to the face that is difficult to be categorized to a specific meaning in social communication. These results suggest that the amygdala is involved in the rapid detection of emotion in faces for immediate emotional reactions even though the reactions could be inefficient in practice, whereas the vlPFC spends a great deal of time processing ambiguous information of facial expression, and make an accurate decision from multiple possibilities to communicate with conspecifics.

Acknowledgements

This work is supported by JSPS KAKENHI Grant Number 24700423, Kinki University Research Grant 2013 (to KK) and a Grant-in-Aid for Scientific Research on Innovative Areas "Neural creativity for communication (No.4103)" (21120009) of MEXT, Japan (to KN).

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Figure Legends

Figure 1. Response profiles of an amygdala neuron (**a**) and vlPFC neuron (**b**) showing visual responsiveness. Each diagram consists of a representative still image of a facial expression, raster display, and peristimulus time histogram, from top to bottom. The light gray area in each diagram indicates the period when the stimulus was presented. The first character of each monkey stimulus label corresponds to the monkey's identity. For example, B_coo indicates the coo expression of monkey B.

Figure 2. Frequency histogram for 77 amygdala neurons (**a**) and 61 vlPFC neurons (**b**) showing their optimal response to a stimulus among nine monkey stimuli. Coo, coo; Scr, scream; Thr, aggressive threat. The amygdala neurons showed greater response to the scream (dark gray) than to the coo and aggressive threat (white and black, respectively), regardless of monkey identity. The vlPFC neurons showed greater response to the coo than to the scream and aggressive threat, regardless of monkey identity.

Figure 3. Population average of neuronal activity of amygdala (**a**) and vlPFC (**b**) neurons showing differential responses to different facial expressions (upper row) and different identities (lower row). To construct population-average neuronal activity curves, peristimulus

time histograms (PSTHs) were sampled in 10 ms non-overlapping bins and convolved with a Gaussian kernel of 30 ms standard deviation (SD).

Figure 4. Individual neuron information regarding identity plotted against emotion. Histograms show the frequency distribution of neurons across a diagonal.

Figure 5. Temporal changes for average information about emotion (**a**) and identity (**b**). The information was calculated using 50 ms sliding windows that were moved in 10 ms steps. Cross and circle marks denote bins showing a significant difference in magnitude of information between the amygdala and the vlPFC.

Figure 6. Recording sites in the right vlPFC of one monkey. Dots denote locations of penetration. Circles and rectangles denote locations of visual and auditory responsive neurons, respectively. PS, principal sulcus; AS, arcuate sulcus.

Figure 1

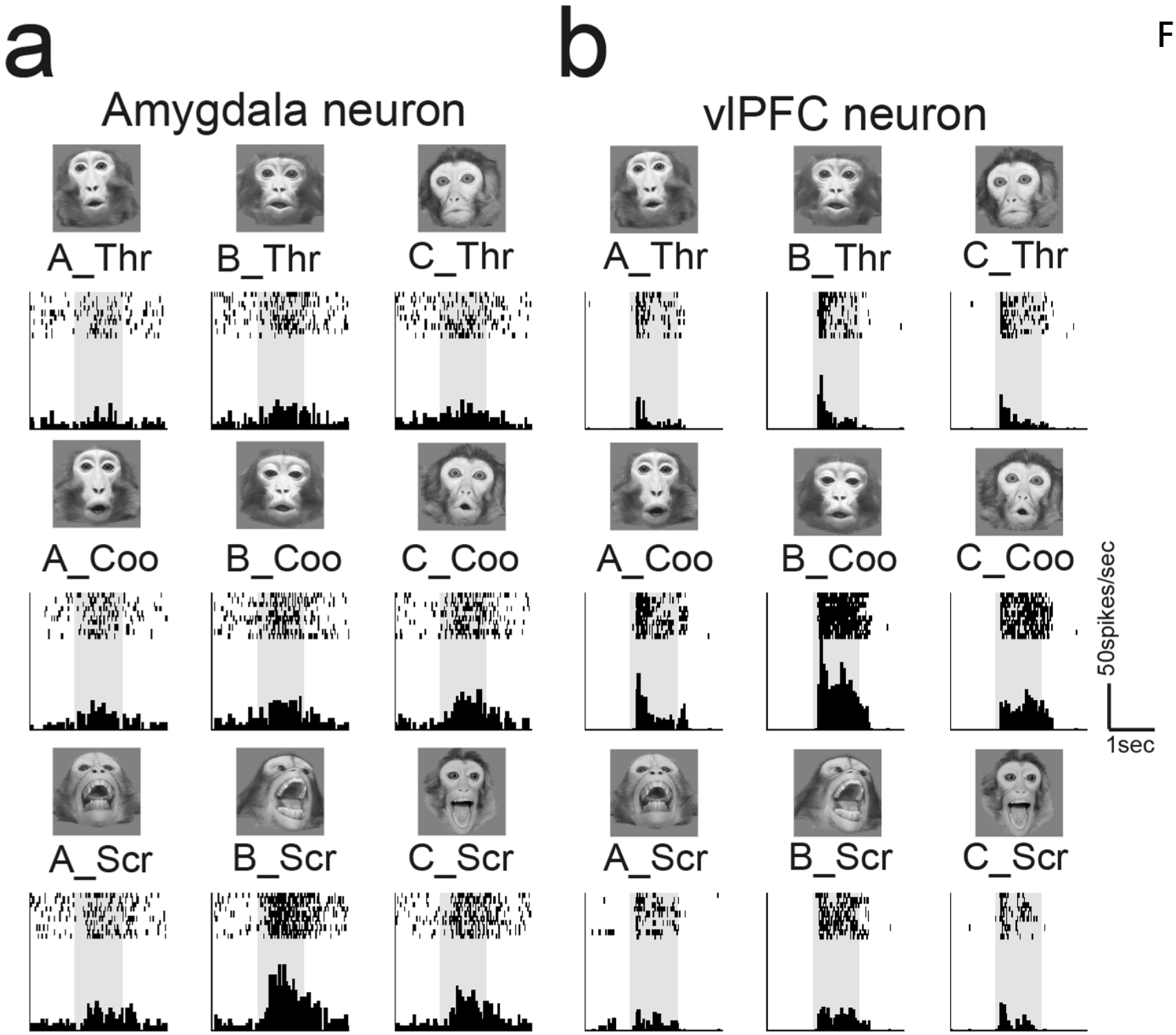
a
Amygdala neuron
A_Thr
B_Thr
C_Thr
A_Coo
B_Coo
C_Coo
A_Scr
B_Scr
C_Scr
b
vlPFC neuron
A_Thr
B_Thr
C_Thr
A_Coo
B_Coo
C_Coo
A_Scr
B_Scr
C_Scr
50spikes/sec
1sec

Figure 2

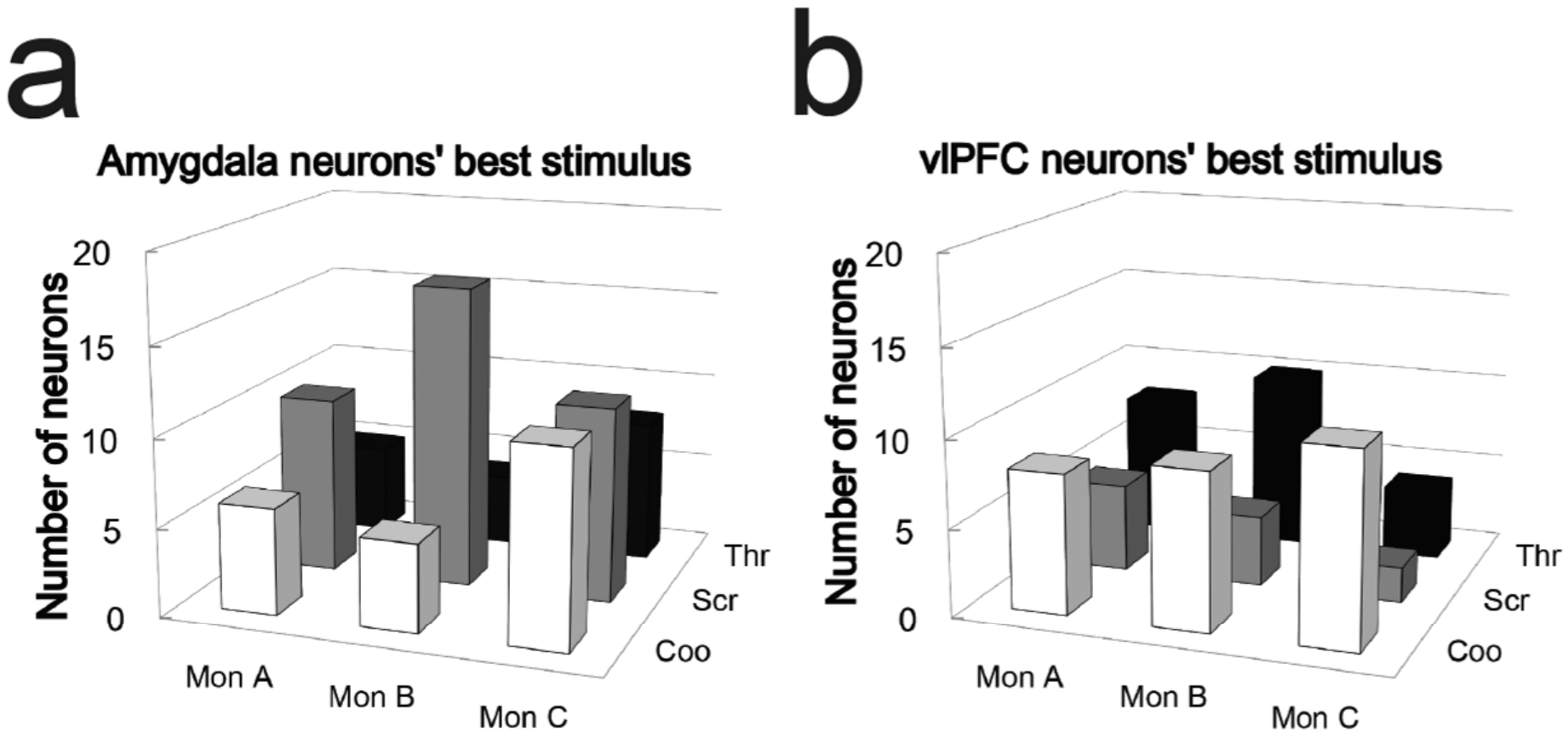
a
Amygdala neurons' best stimulus
Number of neurons
20
15
10
5
0
Mon A
Mon B
Mon C
Thr
Scr
Coo
b
vlPFC neurons' best stimulus
Number of neurons
20
15
10
5
0
Mon A
Mon B
Mon C
Thr
Scr
Coo

Figure 3

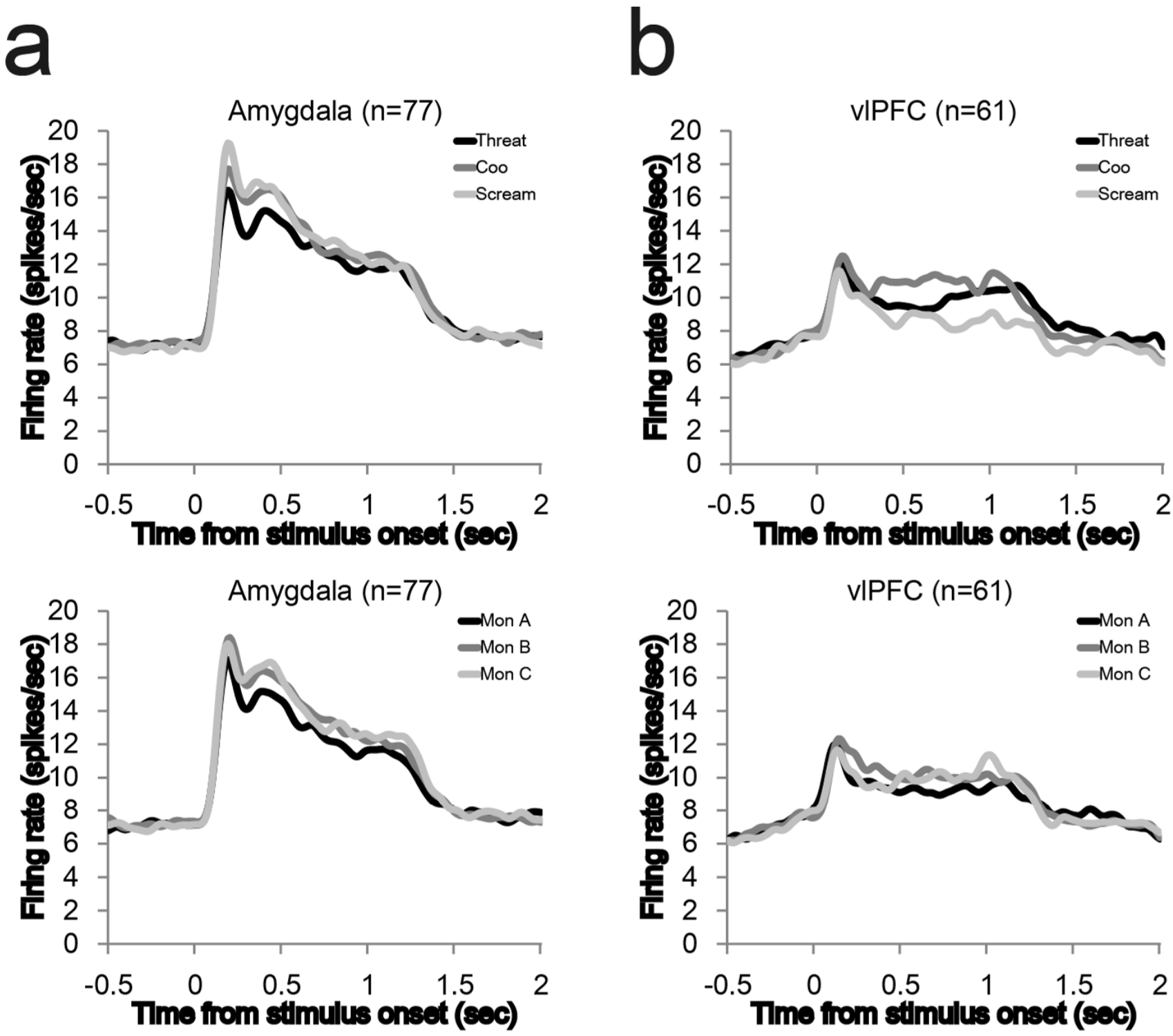
a
b
Amygdala (n=77)
vlPFC (n=61)
Threat
Coo
Scream
Mon A
Mon B
Mon C
Firing rate (spikes/sec)
Time from stimulus onset (sec)
0
2
4
6
8
10
12
14
16
18
20
-0.5
0
0.5
1
1.5
2

Figure 4

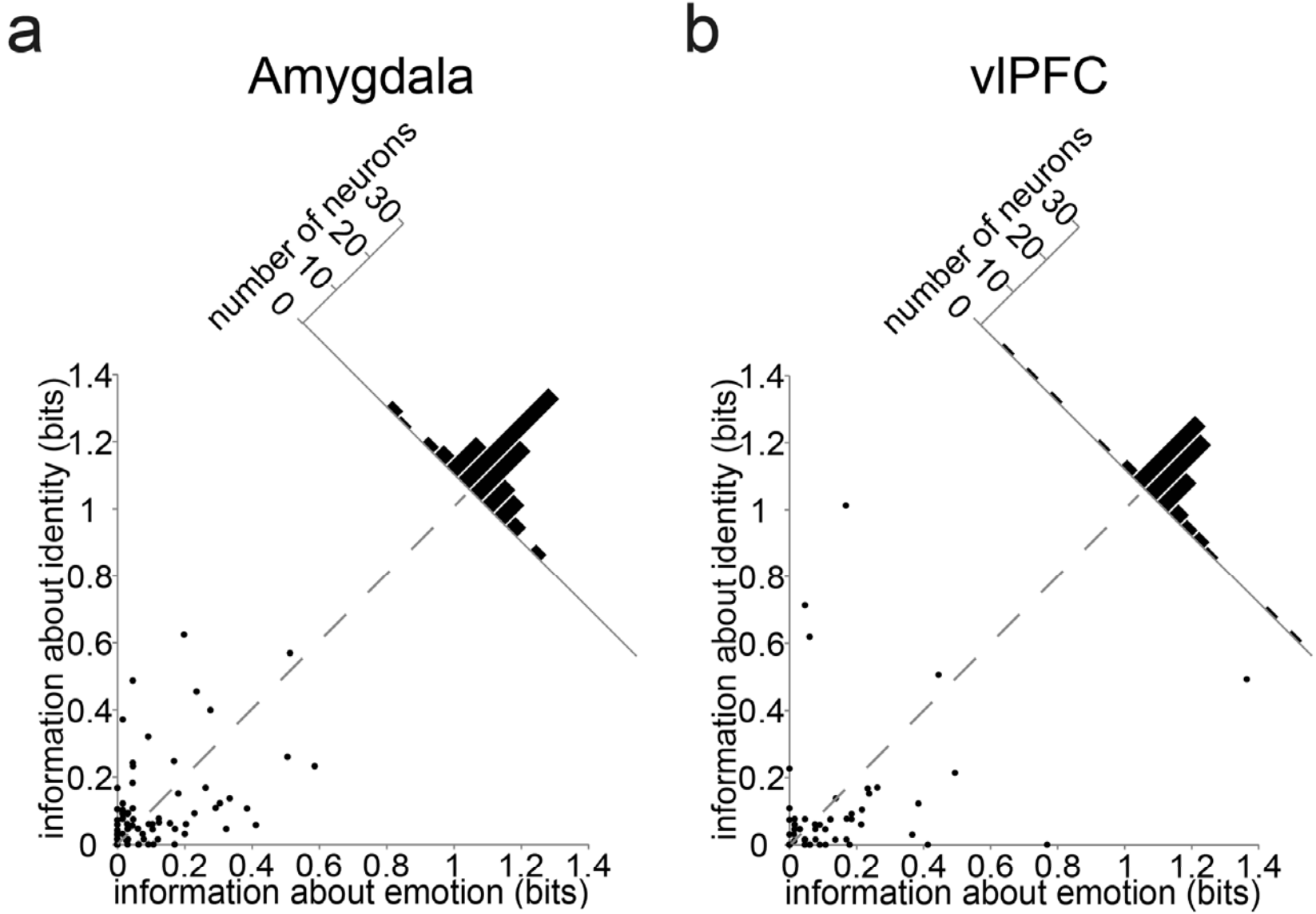
a
Amygdala
number of neurons
0
10
20
30
information about identity (bits)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
information about emotion (bits)
b
vlPFC
number of neurons
0
10
20
30
information about identity (bits)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
information about emotion (bits)

Figure 5

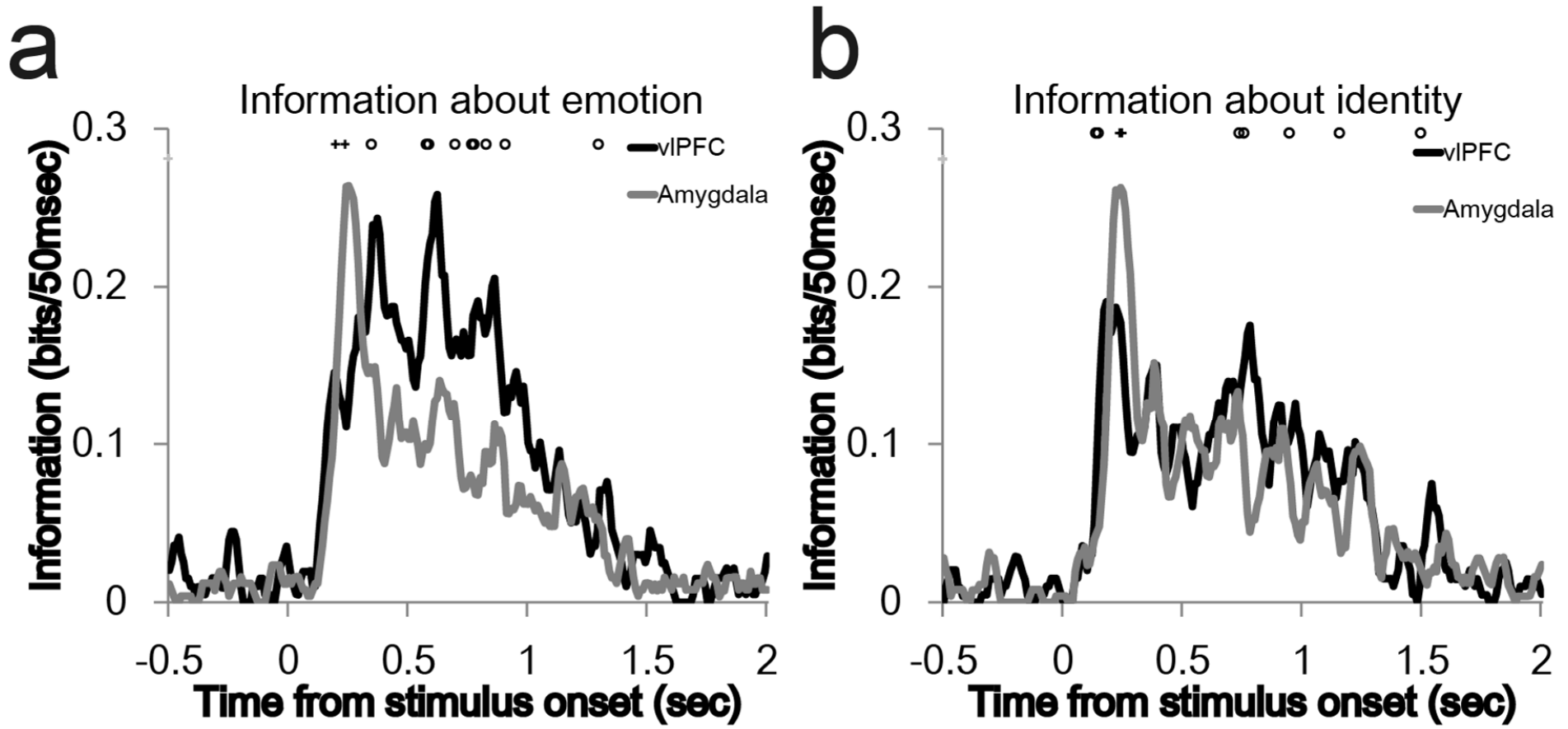
a
Information about emotion
vlPFC
Amygdala
Information (bits/50msec)
0.3
0.2
0.1
0
-0.5
0
0.5
1
1.5
2
Time from stimulus onset (sec)
b
Information about identity
vlPFC
Amygdala
Information (bits/50msec)
0.3
0.2
0.1
0
-0.5
0
0.5
1
1.5
2
Time from stimulus onset (sec)

Figure 6