Title:

Neural Correlates to seen gaze-direction and head orientation in the macaque monkey amygdala.

他個体の視線と顔方向に対するマカクザル扁桃体ニューロンの応答性

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ABSTRACT

Human neuropsychological studies suggest that the amygdala is implicated in social cognition, in which cognition of seen gaze-direction, especially the direct gaze, is essential, and that the perception of gaze direction is modulated by the head orientation of the facial stimuli.   However, neural correlates to these issues remain unknown.   In the present study, neuronal activity was recorded from the macaque monkey amygdala during performance of a sequential delayed non-matching-to-sample task based on gaze direction.   The facial stimuli consisted of two head orientations (frontal; straight to the monkey, profile; 30 degrees rightwards from the front) with different gaze directions (directed toward and averted to the left or right of the monkey).   Of the 1091 neurons recorded, 61 responded to more than one facial stimulus.   Of these face-responsive neurons, 44 displayed responses selective to the facial stimuli (face neurons).   Most amygdalar face neurons discriminated both gaze direction and head orientation, and exhibited a significant interaction between the two types about information. Furthermore, factor analysis on the response magnitudes of the face neurons to the facial stimuli revealed that two factors derived from these facial stimuli were correlated with two head orientations.   The overall responses of the face neurons to direct gazes in the profile and frontal faces were significantly larger than that to averted gazes.   The results suggest that information of both gaze and head direction is integrated in the amygdala, and that the amygdala is implicated in detection of direct gaze.

Key words:

amygdala, social cognition, face neurons, gaze direction, head orientation, monkey

INTRODUCTION

In primates, the gaze direction of other individuals is an important communicative signal in social interaction (Argyle and Cook , 1976; Emery, 2000). This signal can be used to infer the mental state, intention, and attentional direction of other individuals (Baron-Cohen, 1995; Emery, 2000; Hori et al., 2005). Psychological and psychiatric studies have reported that people with autism display deficits in cognition of gaze direction (Pelphrey et al., 2005), and inappropriate behaviors in social interaction (Attwood et al., 1988; Kobayashi and Murata, 1998). In neuroanatomical studies, autistic people were also found to display anatomical abnormalities in the amygdala (Bauman and Kemper, 1988; Abell et al., 1999; Schumann and Amaral, 2006). These findings suggest that the amygdala plays an important role in the cognition of gaze direction in social interaction.

Consistent with this suggestion, functional imaging studies have indicated an increase of regional cerebral blood flow in the human amygdala during presentation of animation, in which the gaze of a stimulus model is directed toward the subject (Kawashima et al., 1999), facial photo of fear expression with gaze directed to the subject (Adams et al., 2003), and during a task in which the subjects were required to infer mental states from photographs of the eye region (Baron-Cohen et al., 1999). Human neuropsychological studies reported that patient D.R. with bilateral amygdalar ablation showed profound impairment in discriminating between direct and averted gazes (Young et al., 1995), and that patient S.M. with bilateral amygdalar lesions displayed deficits in directing her gaze (i.e., attention) to the eye region of the facial photos (Adolphs et al., 2005). These results suggest that the amygdala is critical in processing gaze directions, which are important information for social cognition (Allison et al., 2000).

However, neurophysiological evidence relating the primate amygdala to cognition of gaze direction is lacking, although behavioral studies have also

reported that monkeys could follow the seen gaze-direction and head orientation of other individuals including humans (see a review by Emery, 2000).   Brothers and Ring (1993) found two amygdala neurons that were responsive when the gaze of a conspecific monkey was directed at the subject, but not when the monkey averted its gaze away from the subject.   Since the responses of these neurons were not tested further with other control stimuli, it remains unclear whether the monkey amygdala can code the gaze direction of other individuals.   The first aim of the present study was to investigate neurophysiologically the role of the primate amygdala in cognition of gaze direction, especially the direct gaze.

Second, previous psychological studies have reported that perception of gaze direction was significantly modulated by head orientation (Hietanen, 1999; Langton, 2000; Langton et al., 2004), even though subjects were not required to discriminate head orientations.   This suggests that both kinds of information interact in some areas of the brain.   Consistently, imaging studies have reported that the activity of face-related brain regions is modulated by both gaze direction and head orientation (George et al., 2001; Pageler et al., 2003; Garrett et al., 2004). The second aim of the present study was to examine whether gaze direction-related responses in the amygdala were modulated by head direction although the monkeys were not required to discriminate head orientations.   To address these two issues we recorded and analyzed the neural activity of the monkey amygdala during discrimination of gaze direction.

EXPERIMENTAL PROCEDURES

Animals and behavioral-task apparatus

Two adult monkeys (*Macaca fuscata*), weighing 5.8-8.0 kg, were used. Each monkey was individually housed with food available *ad libitum*.   The monkeys were deprived of water in their cages and obtained juice as reward during training and recording sessions.   Supplemental water and vegetables were given

after each day's session.　To check the monkey's health, his weight was routinely monitored.　The monkeys were treated in strict compliance with the policies of the National Institutes of Health on the Care of Humans and Laboratory Animals, and the Guidelines for the Care and Use of Laboratory Animals at the University of Toyama.

The monkey sat in a monkey chair 30 cm away from the center of a 19-inch computer display for behavioral tasks during the training and recording sessions in a shielded room.　The cathode ray tube (CRT) monitor was set so that its center was on the same horizontal plane as the monkey's eyes.　The monkey chair was equipped with a responding button, which was positioned so that the monkey could easily manipulate it.　An infrared charge-coupled device (CCD) camera for eye-movement monitoring was firmly attached to the chair by a steel rod.　During both training and recording sessions, the monkey's eye position was monitored with 33-ms time resolution by an eye-monitor system (Matsuda, 1996).　Juice reward was accessible to the monkey through a small spout controlled by an electromagnetic valve.　A Psyscope system (Carnegie Mellon University, Pittsburgh, PA, USA) controlled the timing for outputs to the CRT monitor, the electromagnetic valve and sound signal.

Facial stimuli

Figure 1A and B show the stimulus set used in the present study.　Facial stimuli of two persons including one of the experimenters consisted of two head orientations, straight ahead (frontal face) and 30 degrees to the right (profile face). The frontal faces consisted of three gaze directions, directed toward, and averted to the left or right of the monkey; and profile faces had two gaze directions, directed toward and averted to the left of the monkey.

The facial stimuli were 256 digitized color-scale images.　The faces with averted gaze directions were artificially created from the faces with directed gaze by replacing direct gazes in the eye region with the averted gazes, so that only

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difference was a change in gaze direction. In addition, as control stimuli, 256 digitized gray-scale images of two geometric patterns were used. The visual stimuli were displayed on a CRT monitor with a resolution of 640×480 pixels and the size of the stimulus area was $15–20 \times 15–20°$.

It is noted that we did not use the facial stimuli with combination of the profiles rotated by 30 degrees to the right and the gaze direction averted to the left. In these facial stimuli, it is hard to recognize the dark iris; only the white sclera could be seen. In monkey faces, the iris can be always recognized since it occupies the major part of the visible eye. Therefore, this kind of the human facial stimuli seems to be unusual to monkeys. Second, the iris can be recognized in all of the frontal faces regardless of gaze direction. Furthermore, we chose not to include those profiles in which the iris could not be recognized. The lack of the iris induces a qualitative difference among the facial stimuli. According to these two reasons, we avoided those profiles without the iris.

Behavioral paradigms

The monkeys were trained to perform a sequential delayed non-matching-to-sample task that requires discrimination of gaze direction (G-DNMS) (Fig. 1C). The task was initiated by a buzzer tone. Then, a fixation cross appeared on the display for 2 sec, followed by a sample stimulus for 2 sec (sample phase). The control phase was defined as the period for 1 sec before the sample phase. When facial photos were used as sample stimuli, gaze directions of the stimuli were either directed to or averted from the monkey. Then, after an interval of 2.0 sec, the same stimulus appeared again for 500 msec from one to four times (comparison phase). The number of the comparison phase was selected randomly from trial to trial. Finally, a new stimulus with different gaze direction was presented (target phase). When the target appeared, the monkey was required to press a button within 2 sec to acquire a juice reward (0.2 ml). When the monkey failed to respond correctly during the target phase, or press the

button before the target phase, the trials were aborted and a buzzer tone was presented.　Inter-trial intervals (ITI) were 15-25 sec.

　　In the G-DNMS, the monkey compared a pair of the two stimuli in each trial (i.e., sample and target stimuli).　Stimulus pairs consisted of the same category of the stimuli; only pairs of the facial stimuli and those of the geometric patterns were used, and pairs of the facial stimuli and geometric patterns were not used.　In the facial pairs, the averted gazes were always paired with the directed gazes; stimulus pairs of gazes averted to the left and the right were not used.　Furthermore, in the target phase of the facial trials, the same facial stimuli as those in the comparison phase except gaze direction were presented (i.e., same model and same head orientation) so that the monkeys were required to detect a difference in gaze direction (directed vs. averted gazes).　Thus, a total of 14 stimulus pairs (the control stimuli, two pairs; each model, four pairs in the frontal faces and two pairs in the profile faces) were used.　These procedures facilitated monkeys' learning that a shift of gaze direction was an important clue to solve the task.

Training and surgery

　　The monkey was trained with the G-DNMS task for 3 h/day, 5 days/week. The monkey required about 11 months of training to reach a 97% correct-response rate.　After completion of this training period, a head-restraining device (a U-shaped plate made of epoxy resin) was attached to the skull under aseptic conditions and sodium pentobarbital anesthesia (35 mg/kg, i.m., see Nishijo et al., 1988a,b).　The plate was anchored with dental acrylic to titanium bolts inserted in keyhole slots in the skull.　During the surgery, heart and respiratory functions and rectal temperature were monitored on a polygraph system (Nihon Kohden, Tokyo, Japan).　The rectal temperature was controlled at 37 ± 0.5 °C by a blanket heater. Antibiotics were administrated topically and systemically for 1 week to protect against infection.　Two weeks after surgery, the monkey was retrained.　The performance criterion was again attained within 10 days.　All experimental

protocols were approved by the Animal Care and Use Committee of University of Toyama.   Every effort was made to minimize the number of animals used and their suffering.

Stereotaxic localization of the amygdala for recording and histology

Before recording from the amygdala in each hemisphere, a marker consisting of a tungsten wire (diam., 500 μm) was inserted nearby the target area under anesthesia, and the 3-dimensional magnetic resonance imaging (3-D MRI) scans of the monkey head were performed.   The 3-D pictures of the monkey brain with the marker were reconstructed by computer rendering using software for image guided neurosurgical navigation system (Evans; Tomiki Medical Instruments, Kanazawa, Japan).   The 3-D stereotaxic coordinates of the target area were determined in reference to the marker in the 3-D reconstructed brain (Asahi et al., 2003, 2006).

After the last recording session, several small marking lesions were made in the amygdala by passing 20–30 μA of anodal current for 30 sec through an electrode placed stereotaxically and monitored by X-ray.   Subsequently the monkeys were deeply anesthetized with an overdose of sodium pentobarbital (50 mg/kg, i.m.) and perfused transcardially with 0.9% saline followed by 10% buffered formalin.   The brains were removed from the skulls and cut into 50 μm sections through the amygdala.   Sections were stained with Cresyl Violet.   The sites of electrical lesions were determined microscopically.   The location of each recording site was then calculated by comparing the stereotaxic coordinates of recording sites with those of lesions, and plotted on the real tissue sections. Locations of face-responsive neurons in the two monkeys were compared on the basis of the shapes of the amygdalar nuclei, and re-plotted on the serial sections of the amygdala of one monkey from 21 mm (AP21) to 18 mm anterior (AP18) to the interaural line.

There have been some variations in terminology of the intra-amygdalar

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nuclei.　We adopted the atlas of Kusama and Mabuchi (1970) for *Macaca fuscata* based on Johnston's classification (1923) as well as Nishijo et al. (1988a, b). According to this atlas, the amygdala is divided into two groups: one is the basolateral group, which includes the lateral (AL), basolateral (ABl), and basomedial (ABm) nuclei of the amygdala; and the other is the corticomedial (CM) group, which includes the central, medial, and cortical nuclei and the anterior amygdaloid area.

Electrophysiological procedures and data acquisition

　　　Neuronal activity was recorded from each hemisphere in the both subjects. A glass-insulated tungsten microelectrode (0.5-1.5 MΩ at 1 kHz) was stereotaxically inserted vertically into the amygdala on a plane vertical to the orbito-meatal plane in a stepwise fashion by a pulse motor-driven manipulator (SM-21; Narishige, Tokyo, Japan).　The analog signals of neuronal activities, triggers for visual stimuli, juice reward, and button pressing, and the X- Y coordinates of the eye position were digitized and stored in a computer via a Multichannel Acquisition Processor (MAP, Plexon Inc., Dallas TX, USA) system. They were also recorded on a data recorder (RT-145T, TEAC, Tokyo).　The digitized neuronal activities were isolated into single units by their waveform components using the Offline Sorter program (Plexon).　Superimposed waveforms of the isolated units were drawn to check the variability throughout the recording sessions, and then were transferred to the NeuroExplorer program (Plexon) for further analysis.　If the monkey exhibited signs of fatigue, such as closing its eyes for several seconds or moving its eyes or hand slowly, the experimental session was stopped immediately.　In most cases, the unit recording experiment was terminated within 2–3 h.

Data analysis of individual neurons

　　　We analyzed the single neuronal activity during two periods of 500 msec

before (pre) and 500 msec after (post) the onset of a stimulus presented in the sample, comparison or target phase. For the comparison phase, the data in the first comparison phase were discarded from the analyses since the same stimuli as the preceding sample always appeared in the first comparison phase. Nevertheless, each stimulus was presented at least more than four times in each phase for data analysis. For each trial, the firing rate in each period was calculated as the spikes per second. Significant excitatory or inhibitory responses to each stimulus were defined by a paired t-test ($p<0.05$) of neuronal activity between the pre and post 500 msec. Neurons that exhibited significant responses to more than one facial stimulus (paired t-test, $p<0.05$) were defined as face-responsive neurons. Of the face-responsive neurons, neurons that exhibited significant responses only to the facial stimuli, but not to the geometric patterns were defined as face-selective neurons. In the remaining face-responsive neurons that responded to both the facial stimuli and geometric patterns, neuronal responses during the post period to the best facial stimulus were compared with those to the best geometric pattern by a Student t-test ($p<0.05$). Face neurons in the present study included both the face-selective neurons and neurons that exhibited significantly larger responses to the best facial stimulus than to the best geometric pattern. Neuronal responses in the sample and target phases were similarly analyzed. Neuronal activity in the delayed period for 2 sec (inter-stimulus interval) was compared with that in the control phase for 1 sec before the sample phase. Significant excitatory or inhibitory responses in the delayed period were defined by a Student t-test ($p<0.05$) of neuronal activity between these phases.

Neuronal responses in the three phases (sample, comparison, and target phases) to the visual stimuli were separately analyzed in each phase since conditions associated with the visual stimuli (e.g., repetition of stimuli, reward contingency, etc.) were different among the three phases. Since the stimuli in the target phase were always associated with juice rewards, and association of rewards

with the stimuli affected neuronal responses to those stimuli in the amygdala (Nishijo et al., 1988a,b), the responses in the target phase were not analyzed in detail in the following analyses.   Furthermore, the same stimuli were presented in the sample and first comparison phases, and these stimuli were always not associated with the rewards in the present study.   Since this specific reward contingency in these phases might also affect responsiveness to the visual stimuli, the responses in theses phases were also not analyzed in detail in the following analyses.   Thus, the responses only in the comparison phase were analyzed in detail in the following analyses to investigate responsiveness to gaze direction and head orientation.

In order to examine whether the activity of the face neurons was influenced by gaze direction and head orientation, the mean response magnitudes were analyzed by a repeated measures 3-way analysis of variance (ANOVA) based on the general linear mixed model (GLMM) using Statistical Analysis System (SAS) with face model, gaze direction (directed toward and averted to the left of the monkey) and head orientation as factors.   The response magnitude was defined as the difference in neuronal activity between the post and pre 500 msec periods (i.e., activity in the pre period was subtracted from that in the post period).   Response characteristics of the face neurons were defined based on the results of the repeated measures 3-way ANOVA; (1) neurons that exhibited a significant main effect of gaze direction ($p<0.05$) were defined as gaze direction-differential face neurons, (2) head orientation-differential neurons were defined as those that displayed a significant main effect of head orientation ($p<0.05$), and (3) identity-differential neurons were defined as those that displayed a main effect of model.   Interactive response characteristics between the head orientation and gaze direction of the face neurons were also defined based on the results of significant interaction of the 3-way ANOVA: interaction-sensitive neurons were defined as those that displayed significant interactions, at least, between gaze direction and head orientation.   These interaction-sensitive neurons were further categorized

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based on the responsiveness to combination of these two factors; frontal faces with directed gaze, profile faces with directed gazes, frontal faces with averted gazes, and profile faces with averted gazes.   Post-hoc multiple comparisons after the above ANOVAs were performed by Bonferroni's method ($p<0.05$).

Group analyses of the face neurons

In order to examine whether the activity of amygdalar face neurons was modulated by gaze direction and head orientation, the ratio of the face neurons in the above different subcategories was analyzed by chi-squared test ($p<0.05$). Furthermore, to examine what characteristics (i.e., factors) of the face stimuli explained variance of all face neuronal activity, factor analysis using the iterated principal method and Varimax orthogonal rotations (Bieber and Smith, 1986) was accomplished using the response magnitudes of the 44 face neurons to the eight facial stimuli.   The number of factors was determined according to the scree method (Bieber and Smith, 1986).   The facial stimuli were classified based on the factor loadings greater than 0.6 after Varimax rotation.   Finally, the overall mean response magnitudes of the 44 face neurons were compared between direct and averted gaze directions in each head orientation.

RESULTS

General

In almost all recording sessions, the monkeys performed the G-DNMS task more than 98% correctly.   A total of 1091 single neurons were recorded from the amygdala of the four hemispheres in two monkeys (Table 1).   Of these 1091 neurons, 55 responded to more than one facial stimulus (face-responsive neurons) during the sample phase.   Of these 55 face-responsive neurons, 36 were classified as face neurons that responded more strongly to the facial stimuli than to the simple figures.   In the comparison phase, of the 1091 neurons, 61 responded to

more than one facial stimulus (face-responsive neurons). Of these 61 face-responsive neurons, 44 were classified as face neurons. In the target phase, of the 1091 neurons, 62 responded to more than one facial stimulus (face-responsive neurons). Of these 62 face-responsive neurons, 47 were classified as face neurons. In the following analyses, the responses only in the comparison phase were analyzed in detail (see EXPERIMENTAL PROCEDURES).

The basic discharge rates in the control phase of 37 excitatory and seven inhibitory face neurons ranged from 0.24 to 38.23 spikes/s (8.17±1.69 spikes/s, mean±SEM) and from 1.34 to 24.67 spikes/s (8.86±3.37 spikes/s), respectively. Discharge rates of the face neurons for excitatory responses to the best facial stimulus ranged from 1.33 to 59.66 spikes/s (22.96±2.42 spikes/s). The discharge rates of the face neurons for the inhibitory responses to the best stimulus ranged from 0 to 16 spikes/s (6.46±2.40 spikes/s). Of these 44 face neurons, the activity of 18 (41%) was differential to gaze direction, while the activity of 26 (59%) was differential to head orientation based on the repeated measures 3-way ANOVA. Of the 44 face neurons, 22 were classified as identity-differential neurons based on the repeated measures 3-way ANOVA.

Of the 1091 neurons recorded, 28 responded during the delay period. Of these 28 neurons, activity of 10 was associated with specific stimuli; activities of the neurons increased in the delay period when the specific stimuli were presented. Activity of the 15 neurons increased during the delay period regardless of the visual stimuli. The remaining three neurons responded not only in the delay period but also during the comparison period regardless of the visual stimuli.

Gaze direction-differential and head orientation-differential neurons

Figure 2A illustrates a sample response of a gaze direction- and head orientation-differential neuron. This neuron responded to all four profile faces regardless of the models, but more strongly to those with directed gaze (Fig. 2Aa).

However, the same neuron did not respond to the frontal faces of the both models (Fig. 2Ab).   The response magnitudes of this neuron to each head orientation with direct and averted gaze directions are shown in Fig. 3A.   The response magnitudes of this neuron to the profiles were larger than those to the frontal faces and in the faces with averted gaze than in the faces with directed.   The results of the repeated measures 3-way ANOVA indicated that there were significant main effects of gaze direction [$F(1, 53)=5.02$, $p<0.05$] and head orientation [$F(1, 53)=50.95$, $p<0.001$], but no significant main effect of model [$F(1, 53)=2.56$, $p>0.05$].   These results indicated that this neuron was gaze direction-differential and head orientation-differential.

Gaze direction-differential and head orientation-nondifferential neurons

Sample histograms of a gaze direction-differential and head orientation-nondifferential neuron are shown in Fig. 2B.   Among the four profile faces, this neuron strongly responded to the model A face with a direct gaze (Fig. 2Ba).   On the other hand, the same neuron responded to all three frontal faces of the model A regardless of gaze direction (Fig. 2Bb).   The neuron did not respond to the same facial stimuli of the other face model at all.   Figure 3B shows comparison of the response magnitudes of the same neuron between the frontal and profile head orientations in each gaze direction.   In the model A faces with direct gaze, the response magnitudes of this neuron to the profile were stronger than those in the frontal head orientation.   In the averted gaze direction, the magnitudes of responses to the frontal face of model A were stronger than those to the profile face of the same model.   The repeated measures 3-way ANOVA on the response magnitudes indicated that there was a significant main effect of gaze direction [$F(1, 41)=15.76$, $p<0.0005$], but no significant main effect of head orientation [$F(1, 41)=3.37$, $p>0.05$].   These results indicated that this neuron was gaze direction-differential and head orientation-nondifferential.   Furthermore, the results of the repeated measures 3-way ANOVA indicated that there were a

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significant main effect of model [$F(1, 41)=66.35$, p<0.0005], indicating that this neuron was identity-differential.

Gaze direction-nondifferential and head orientation-nondifferential neurons

Figure 2C shows sample results of a gaze direction-nondifferential and head orientation-nondifferential neuron.   This neuron responded to the all four profile faces, but more strongly to the faces with averted gazes (Fig. 2Ca).   Furthermore, the same neuron responded less to the frontal faces with gaze averted to the right of the monkey (Fig. 2Cb).   The response magnitudes of the neuron to each head orientation with directed and averted gaze directions are shown in Fig. 3C.   The results of the repeated measures 3-way ANOVA indicated significant main effects of model [$F(1, 41)=5.36$, $p<0.05$], but no significant main effects of head orientation [$F(1, 41)=2.85$, $p>0.05$] and gaze direction [$F(1, 41)=0.25$, $p>0.05$]. These results indicated that this neuron was gaze direction-nondifferential, head orientation-nondifferential and identity-differential.

Interaction-sensitive neurons

Of 44 face neurons, 17 displayed significant interactions between gaze direction and head orientation (i.e., interaction-sensitive neurons), 27 displayed no significant interactions between same factors (i.e., interaction-insensitive neurons). Fig. 4 shows examples of the mean response magnitudes of the two interaction-sensitive neurons to the profile and frontal faces with direct and averted gazes.   Of the 17 interaction-sensitive neurons, nine responded only to one of the four combinations of the two factors (profile/frontal faces vs. directed/averted gazes) (Fig. 4A; responsive to the profile faces with direct gaze), two was unresponsive only to one of the four combinations (Fig. 4B; unresponsive to the profile faces with averted gaze).   The remaining six interaction-sensitive neurons showed an intermediate characteristic of the above the two neuronal types.   Of nine responsive neurons to one combination, five and two responded to the profile

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faces with directed gazes and the frontal faces with directed gazes, respectively. The remaining two neurons responded only to the frontal faces with averted gazes and the profile faces with averted gazes, respectively. Of the two neurons unresponsive to one combination, 1 and 1 were unresponsive to the profile faces with directed gaze and the profile faces with averted gaze, respectively.

Group analysis of the face neurons: modulation by gaze direction and head orientation

The group analyses indicated that both gaze direction and head orientation are important determinants of the activity of amygdalar face neurons. First, the ratio of the neurons differentially responsive to head direction was significantly larger in the gaze direction-differential neurons (83.3%, 15/18) than in the gaze-nondifferential neurons (42.3%, 11/26) (chi-squared test, $p<0.05$). This indicated that the activity of the amygdalar face neurons was significantly modulated by both gaze direction and head orientation.

Second, the ratio of gaze direction- and head orientation-differential neurons in the interaction-sensitive neurons (11/17) was significantly larger than those of the gaze direction-differential and head orientation-nondifferential neurons (1/17), the gaze direction-nondifferential and head orientation-differential neurons (2/17), and the gaze direction-nondifferential and head orientation-nondifferential neurons (3/17) (chi-squared test, $p<0.05$). These results indicated that the activity of the amygdalar face neurons was significantly modulated by interaction between gaze direction and head orientation.

Third, Table 2 shows the results of the factor analysis. Factor analysis using the scree method revealed that two factors were derived from these facial stimuli, and accounted for 82.39 % of variance of the data. Each factor was highly correlated with each of the two head orientations with factor loadings more than 0.6. The results of the factor analysis suggest that the head direction is the most globally coded feature by the amygdala neurons, that is, the factor loading

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structure can be explained pretty well by head direction only.

Group analysis of the face neurons: responsiveness to direct gazes

The amygdalar face neurons seemed to be more sensitive to the facial stimuli with the direct gazes than those with the averted gazes.   Fig. 5 shows the overall mean response magnitudes of the 17 interaction-sensitive neurons (Fig. 5A), and the 27 interaction-insensitive neurons (Fig. 5B) to the directed and averted gazes in the frontal, profile, and all four faces.   In the 17 interaction-sensitive neurons, there were no significant differences in the overall mean response magnitudes between the direct and averted gazes for the profile faces (Fig. 5Aa; paired t-test, *n.s.*).   The same results were true for the frontal faces (Fig. 5Ab; paired t-test, *n.s.*), and the all faces (both profile and frontal faces) (Fig. 5Ac; paired t-test, *n.s.*). In the 27 interaction-insensitive neurons, the overall mean response magnitude to the profile faces with direct gazes was larger than that to the profile faces with averted gazes (Fig. 5Ba; paired t-test, $p<0.05$).   In the frontal faces, there were no significant differences in the overall mean response magnitudes between the direct and averted gazes (Fig. 5Bb; paired t-test, *n.s.*).   The mean magnitude to the all facial stimuli with direct gazes was significantly larger than those to the all facial stimuli with averted gazes (Fig. 5Cc; paired t-test, $p<0.05$).

Then, we analyzed responsiveness of the all 44 face neurons at once instead of dividing into the separate groups.   The overall mean response magnitude of the 44 face neurons to the profile faces with direct gazes was significantly larger than that to the profile faces with averted gazes (Fig. 6A; paired t-test, $p<0.05$).   In the frontal faces, there was a similar tendency between direct and averted gazes (Fig. 6B; paired t-test, $p<0.10$).   When responses to the both frontal and profile faces were included in the analysis, the mean magnitude of the 44 face neurons to the four facial stimuli with direct gazes was significantly larger than that to the four facial stimuli with averted gazes (Fig. 6Ca; paired t-test, $p<0.01$).   These results indicated that activity of the amygdalar face neurons was more sensitive to direct

gazes. Furthermore, when the neural data were limited between stimulus onset and 100 msec after stimulus onset, the same results were true for the all faces (Fig. 6Cb; paired *t*-test, $p<0.05$). However, when the neural data were limited between 100 and 300 msec after stimulus onset, there were no significant differences in overall mean response magnitudes between the direct and averted faces for the all faces (Fig. 6Cc; paired *t*-test, *n.s.*). These results suggest that neural activity of the amygdalar face neurons was more sensitive to the direct gaze in early latency less than 100 msec.

Location of neuronal types

We recorded the neuronal activity from various subnuclei of the amygdala, which included the lateral (AL), basolateral (ABl), corticomedial (CM), and basomedial (ABm) nuclei of the amygdala. Histological data indicated that all face-responsive neurons were located within the amygdala. Distributions of the various type face neurons were illustrated in Fig. 7. Although the gaze direction- and head orientation-differential neurons were distributed in all the nuclei in the amygdala, these neurons were predominantly located in the AL nucleus (filled circles in Fig. 7).

DISCUSSION

General response characteristics of the monkey amygdalar neurons

The present study identified a set of the amygdalar neurons that responded to human faces, and activities of some neurons were selective to the face models. These neurons were mainly located in the lateral and basolateral nuclei of the amygdala, which receive massive afferent inputs from the temporal cortex (Amaral et al., 1992; Aggleton and Saunders, 2000) where various types of face neurons were reported (Perrett et al., 1985, 1992; Rolls, 2000; Eifuku et al., 2004). Consistent with the present study, recent neurophysiological studies have also

reported amygdalar neurons in the basolateral and lateral nuclei that responded differentially to human and monkey faces (Nakamura et al., 1992; Kuraoka and Nakamura, 2006; Gothard et al., 2007). However, relatively fewer neurons responded to the facial stimuli in the present study than those in the previous studies (Kuraoka and Nakamura, 2006; Gothard et al., 2007). These previous studies emphasized that responses to faces were selective to facial identity and emotional expressions. In the present study, we used only two facial identities with neutral expression since we focused on neuronal responses to gaze directions and head orientations. Therefore, it is likely that the unresponsive neurons in the present study might respond to facial stimuli of other persons with various facial expressions. We are currently analyzing the monkey amygdalar neurons using such an expanded stimulus set, and the preliminary results indicated that more amygdalar neurons responded to such facial stimuli (unpublished data).

Some amygdalar neurons responded during the delay period in the present study. Consistent with the present study, Nakamura et al. (1992) using the similar task to the G-DNMS reported that the amygdalar neurons displayed changes in neural activity during the delay period.

It is noted that the monkeys were trained with the G-DNMS that required them to detect shift of gaze direction of the facial stimuli. Therefore, the differential amygdalar neuronal responses to the facial stimuli with the different gazes might be ascribed to training of the task. That is, these responses might be task-contingent responses. However, the previous available data suggest that this is unlikely. It is reported that amygdalar neurons responded to a directed gaze of a conspecific in a non-task condition (Brothers and Ring, 1993). A recent neuroimaging study, in which the monkey just passively looked at various facial expressions with head and eyes either averted from or directed to the monkey, also reported that activity of the amygdala increased in response to those faces (Hoffman et al., 2007). Furthermore, amygdalar neurons, which responded to various objects in a task condition, also responded to the same objects when the

experimenter directly presented the objects to the monkeys in clinical tests (Nishijo et al., unpublished data; Nishijo et al., 1993). These results suggest that activity of the amygdalar neurons is task (or situation)-independent (Nishijo et al., 1993), and amygdalar neurons would respond to facial stimuli with different gaze directions in a non-task context. Further studies in which amygdalar neurons are recorded from freely behaving monkeys are required to prove this possibility.

Comparison between human and non-human primates in facial recognition

The mechanisms in the monkey brain involved in facial recognition have been found to parallel those of humans (Rolls, 2000). Human and non-human primate faces have similar spatial configuration (Carmel and Bentin, 2002) and some human facial expressions have morphological analogues in monkeys (Preuschoft, 1992). Also, macaques and humans are known to employ similar strategies when gazing upon pictures of faces - directing it more to the eye region (Nahm et al., 1997). In contrast, Parr et al. (1999) found inversion effect in rhesus monkeys looking at conspecific faces but not in the case of human faces. This suggests discrepancies in perception mechanisms of human facial stimuli. Nevertheless, Guo et al. (2003) showed similar oculomotor strategies when macaques scanned both human and monkey faces in upright, inverted and scrambled pictures. These results suggest that macaques can effectively scan human faces.

The purpose of the present study was to analyze responsiveness of the monkey amygdalar face neurons to different gaze directions. The actual morphology of the eyes differs between monkey and human (Emery, 2000). That is, the major part of the visible eye in the monkey is the dark iris, while human have a large extent of white sclera (Kobayashi and Kohshima, 2001; Perrett and Mistlin, 1990). Since perception of gaze direction depends on the position of the irises (e.g., Todorovic, 2006), it is supposed that monkeys more easily discriminate gaze directions of human faces than those of monkey faces. Furthermore,

monkeys are more sensitive to the direct gazes than to averted gazes of human photos (Sato and Nakamura, 2001). Thus, we used the stimulus set consisting of the human faces.

Neural responsiveness to gaze direction and head orientation

Previous studies have reported that monkey amygdalar neurons respond to facial stimuli (Leonard et al., 1985; Nakamura et al., 1992; Gothard et al., 2007). Consistent with these studies, the present study identified face neurons in the monkey amygdala. Furthermore, the present study demonstrated that the monkey amygdalar face neurons were sensitive to both gaze direction and head orientation.

It is reported that mutual gaze (gazing at seen direct gaze of other individuals) was more frequently observed between infants and mothers than between infants and other individuals in rhesus monkeys, which is very similar to human mother-infant communication (Ferrari et al., 2009). Macaque monkeys could also follow the seen gaze direction and head orientation of other individuals including humans, as humans do (see a review by Emery, 2000). Both gaze direction and head orientation are sufficient indicators of attention direction (and therefore interest) of other individuals (Emery, 2000). Furthermore, consistent with the present results, macaque cortical neurons in the superior temporal sulcus (STS) well discriminated gaze and head directions (Jellema & Perrett, 2005). These results suggest that both gaze direction and head orientation are important social cues in macaque monkeys as well as humans.

It is noted that the monkeys were trained to attend and discriminate the gaze direction in the task without reference to head orientation and identity of the models in the present study. However, most amygdalar neurons were sensitive to head orientation and identity in addition to gaze direction. Consistent with the present results, previous psychological studies reported that perception of gaze directions was modulated by head orientation in the similar conditions (Hietanen, 1999; Langton, 2000; Langton et al., 2004). These results suggest that basic

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social signs such as gaze direction and head orientation are automatically (implicitly) and quickly processed (Frith and Frith, 1999; Jellema and Perrett, 2005). Furthermore, previous noninvasive studies suggest that the amygdala is involved in implicit processing of various facial information (Engell et al., 2007; Demos et al., 2008). These results further suggest a role of the amygdala in implicit social cognition.

Previous studies have reported that neurons that responded selectively to gaze and head direction have been identified in the monkey STS (Perrett et al., 1985; De Souza et al., 2005), which has reciprocal connections with the amygdala (Aggleton et al., 1980; Turner et al., 1980). Perrett et al. (1985) found three types of neuronal responses to four kinds of faces: frontal or profile faces with direct or averted gaze. The first type of STS neurons was one comparable to the gaze direction-differential and head orientation-nondifferential neurons in the present study. The second type of the STS neurons responded only to one of the four faces. The third type of STS neurons was unresponsive only to one of the four conditions. The present study identified all three types of the face neurons in the amygdala (two of these three types are shown in Fig. 4). The similarity of neuronal responses to facial stimuli in the STS and the amygdala suggests that reciprocal connections between the amygdala and STS form a fundamental neural network for cognition of gaze direction and head orientation. Consistent with this idea, human imaging studies reported that the amygdala and STS were co-active in response to facial stimuli (Corden et al., 2006; Ashwin et al., 2007).

Neural responsiveness to direct gaze

In the present study, response magnitudes to the direct gazes were significantly stronger than those to the averted gazes when the data for the all faces and those for the profile faces were analyzed. High sensitivity of the face neurons to gaze direction is consistent with human psychological studies, in which a patient S.M. with bilateral amygdalar lesions could not direct her gaze (i.e.,

attention) to the eye regions of facial photos (Adolphs et al., 2005) and during conversations with real people (Spezio et al., 2007). Furthermore, the human amygdala was more activated by a profile face with a direct gaze compared with an averted gaze (Kawashima et al., 1999; Corden et al., 2006), consistent with the present study. In addition, activity of the human amygdala increased when the subjects actively monitored gaze stimuli in a direct gaze-detection task (Hooker et al., 2003). These findings suggest that the amygdala might function as a component of an eye-direction detector, as existence of such system has been suggested by Baron-Cohen (1995) and Baron-Cohen et al. (1999). Instead, these amygdalar neurons might encode the arousal value associated with the direct / averted gaze (Hoffman et al., 2007). These results are also consistent with a monkey behavioral study showing that monkeys are more sensitive to the direct gaze of human photos than to averted gazes (Sato and Nakamura, 2001), suggesting that monkeys pay more attention to humans whose attention is directed to them through the same neural networks including the amygdala as those in humans. It is noted that differential responsiveness to direct gazes were evident in the early latencies less than 100 msec. This suggests that the amygdala receives this kind of information by way of direct and fast subcortical visual route (Johnson, 2005).

However, when the data for the frontal faces were analyzed, there was a tendency that response magnitudes to direct gazes were stronger than those to averted gazes, but this difference was statistically insignificant (Fig. 6B). For monkeys, attention directed to them is a more important social signal than that directed elsewhere, since it is essential for them to communicate with other individuals whose attention is directed to them for appropriate social interaction (Gómez, 2004). The previous behavioral studies reported that monkeys and humans displayed behavioral sensitivity to both head and gaze directions (Perrett and Mistlin, 1990). Furthermore, recent studies reported that great apes followed attentional directions of a human experimenter based mainly on the human's head

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orientation, although eye direction played some roles as well (Tomasello et al., 2007). These findings suggest that frontal faces in which head orientation was directed to the subjects are important social signals regardless of gaze direction. Therefore, absence of significant difference in mean response magnitudes between the direct and averted gazes for the frontal faces might be attributed to social significance of the frontal faces regardless of gaze direction.

Neural correlates of gaze perception based on the interaction between gaze direction and head orientation

The results of the ANOVAs in individual neurons revealed that some face neurons displayed interactions between gaze direction and head orientation. However, the factor analysis indicated that most of the variance of cell responses was due to head orientation; the factor(s) related to gaze direction were not extracted from the present population data. This suggests that modulation of amygdalar neural activity by head orientation was relatively independent of the other factors (gaze direction and/or identity), while modulation by gaze direction was more dependent on the other factors (head orientation and/or identity). Consistently, as discussed in the above section, there were no significant differences in mean response magnitudes between the direct and averted gazes for the frontal faces, while there were significant differences for the profile faces.

Significant interaction between gaze direction and head orientation observed in the present study is consistent with previous human psychological studies, in which perception of gaze direction was modulated by head orientation (Hietanen, 1999; Langton, 2000; Langton et al., 2004). These psychological studies further suggest that information about gaze direction and head orientation is processed by separate systems in parallel since head orientation and gaze direction are mutually influential, and that both kinds of information interact with each other. Furthermore, this interaction could occur early in the face information processing since this interaction was unaffected by various transformations of original facial

images such as inversion manipulation (Langton, 2000; Wilson et al., 2000; Langton et al., 2004). Furthermore, we analyzed the response latencies to the facial stimuli, which were defined as the time when neuronal activity increased or decreased $\geq$ 2.0 SD of the baseline activity. Response latencies depended on both neurons and facial stimuli, and a total of 265 cases were analyzed. Of these, 105 cases showed short response latencies less than 100 msec in the amygdala, while neurons in the inferotemporal cortex, from which the amygdala receives cortical inputs, fire 100-140 msec after stimulus presentation (Rolls et al., 1977). These findings suggest that the amygdala, which receives direct and fast subcortical visual inputs (Johnson, 2005), might be one of the brain areas where information about gaze direction and head orientation might interact before reaching the cortical areas.

Amygdalar role in social cognition

Neuropsychological studies reported that detection of direct gaze was impaired in adult autistic subjects (Howard et al., 2000), and that orienting to the eye regions of other people was disturbed in autistic children (Leekam and Ramsden, 2006). These autistic people display neuroanatomical abnormalities in the amygdala (Schumann and Amaral, 2006), and functional connectivity between the amygdala and other cortical areas is decreased in these people (Welchew et al., 2005). Imaging studies reported abnormal activity in the amygdala in autistic subjects: hypoactivation of the amygdala when they were making mentalistic inferences from regions of another person's eyes (Baron-Cohen et al., 1999), and amygdalar hyperactivation in response to seen eyes (Dalton et al., 2005). Furthermore, amygdalar volume was negatively correlated to some autistic symptoms, while it was positively correlated to social cognition ability in normal subjects (Dziobek et al., 2006), and reflexive attentional shift induced by gaze cues was disturbed in patients with the unilateral anterior medial temporal regions including the amygdala (Okada et al., 2008). These results suggest that the

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human amygdala is critical in processing information from the eye region of other individuals, and that this process is disturbed in autistic people with abnormal amygdalar activity. The present results provide an important clue in elucidating the role of the amygdala in social cognition and development of social cognition in human infants.

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Fig. 1.   Task paradigm of a delayed non-matching-to-sample task based on gaze direction (G-DNMS).

A: facial stimuli used in the G-DNMS.   Stimulus set consisted of 10 faces (four profile faces and six frontal faces).   B: simple geometric patterns.   C: stimulus sequence in the G-DNMS, in which stimuli were sequentially presented with delay.

Fig. 2.   Raster displays of neuronal activity, averaged peri-event histograms and response magnitudes to various facial stimuli of the three different face neurons (A, B, and C).

A: a gaze direction-differential and head orientation-differential neuron. This neuron responded to all four profile faces, regardless of the model, but more strongly to those with directed gaze (a).   However, the same neuron did not respond to the frontal faces of either model (b).   B: a gaze direction-differential and head orientation-nondifferential neuron.   This neuron strongly responded to the profile face of the model A with gaze directed toward the monkey (a). Furthermore, the neuron strongly responded to all three frontal faces of the model A regardless of gaze direction (b).   C: a gaze direction-nondifferential and head orientation-nondifferential neuron.   This neuron responded to all four profile faces, but more strongly to faces with averted gaze regardless of the model (a). Furthermore, the same neuron responded less to both frontal faces with gaze averted to the right of the monkey (b).   Horizontal bars under the histograms indicate the stimulus presentation period (500 msec).   A vertical line in each of the raster displays and histograms indicates the stimulus-onset point.   Calibration at the right bottom of the figure: number of spikes per trial in each bin.   Bin width: 50 msec.

Fig. 3.   Comparison of response magnitudes to various facial stimuli of the same neurons shown in Fig. 2A, B, and C.

A-C: the gaze direction-differential and head orientation-differential (A), gaze direction-differential and head orientation-nondifferential (B), and gaze direction-nondifferential and head orientation-nondifferential (C) neurons shown in Fig. 2A, B, and C, respectively.   Black and white bars indicate the response magnitudes to the frontal and profile faces, respectively.   Each p-value represents a significant level for the main effects of gaze direction and head direction in repeated measures ANOVA.

Fig. 4.   Mean response magnitudes of the two different interaction-sensitive neurons to the profile and frontal faces with direct and averted gazes.

A: This neuron strongly responded only to the profile faces with gaze directed toward the monkey.   On the other hand, the same neuron did not respond to the frontal faces with averted gaze at all.   B: This neuron responded to both the profile and frontal faces except the profile faces with averted gazes.   Gray and white bars indicate the mean response magnitudes ±SEM to direct and averted gazes, respectively.   *, significant difference from other stimuli (Bonferroni's methods, $p<0.05$).

Fig. 5.   Comparison of mean response magnitudes to the direct and averted gazes of the profile (a), frontal (b) and all faces (c) in the interaction-sensitive (A), and the interaction-insensitive neurons (B) in the amygdala.

Gray and white bars indicate the overall mean response magnitudes ±SEM to direct and averted gazes, respectively.   Each p-value represents a significant level by paired t-test.

Fig. 6.   Comparison of mean response magnitudes to direct and averted gazes in
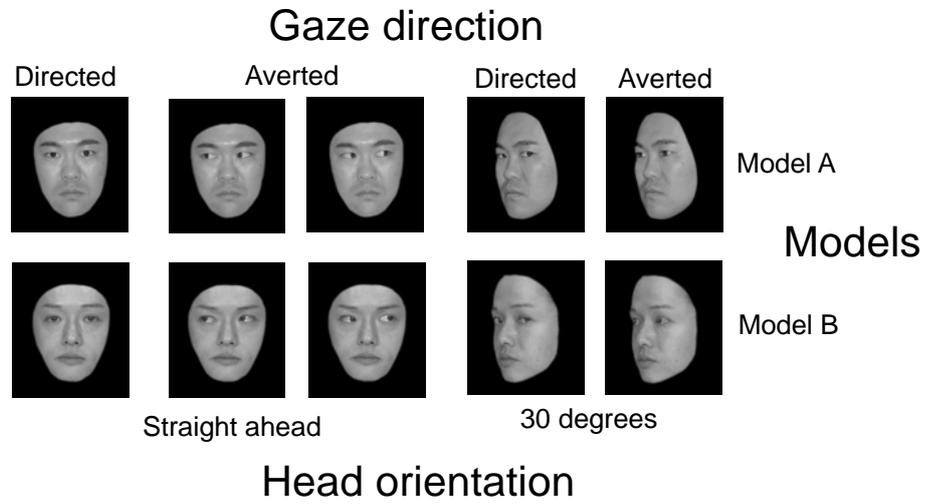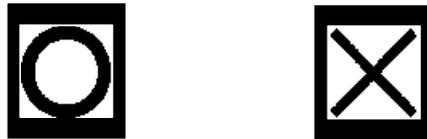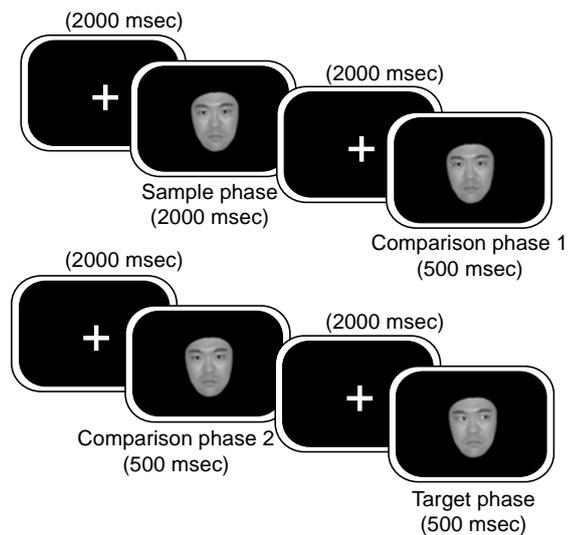
profile face (A), frontal face (B) and all faces (C) of all face neurons in the amygdala.   In (C), the neural data were analyzed in different periods between 0 and 500 msec after stimulus onset (a), between 0 and 100 msec after stimulus onset (b) and between 100 and 300 msec after stimulus onset (c).

Gray and white bars indicate the overall mean response magnitudes ±SEM to direct and averted gazes, respectively.   Each p-value represents a significant level by paired t-test.

Fig. 7.   Recording sites of the 61 face-responsive neurons.

Filled circles, gaze direction-differential and head orientation-differential neurons; left half-filled circles, gaze direction-differential and head orientation-nondifferential neurons; right half-filled circles, gaze direction-nondifferential and head orientation-differential neurons; open circles, gaze direction-nondifferential and head orientation-nondifferential neurons; open squares, neurons that responded to both the facial stimuli and geometric patterns. CM, corticomedial group of the amygdala; AL, lateral nucleus; ABl, basolateral nucleus; ABm, basomedial nucleus; Hip, hippocampus.
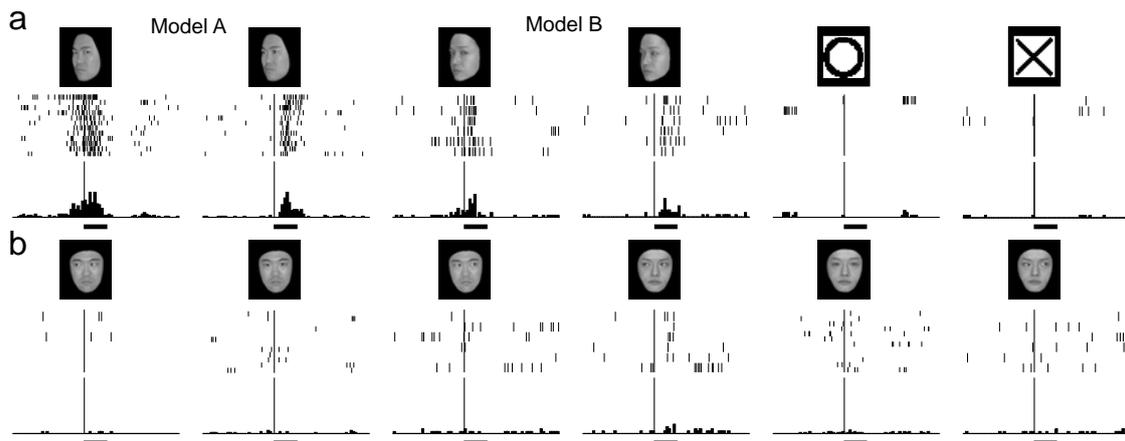
A. Facial stimuli

Gaze direction

Directed　　　　Averted　　　　　　Directed　　Averted

Model A

Models

Model B

Straight ahead　　　　　　30 degrees

Head orientation

B. Geometric patterns

C. G-DNMS task

(2000 msec)

+

(2000 msec)

Sample phase
(2000 msec)

+

Comparison phase 1
(500 msec)

(2000 msec)

+

(2000 msec)

Comparison phase 2
(500 msec)

+

Target phase
(500 msec)

Figure 1

A. Gaze direction-differential and head orientation-differential

B. Gaze direction-differential and head orientation-nondifferential

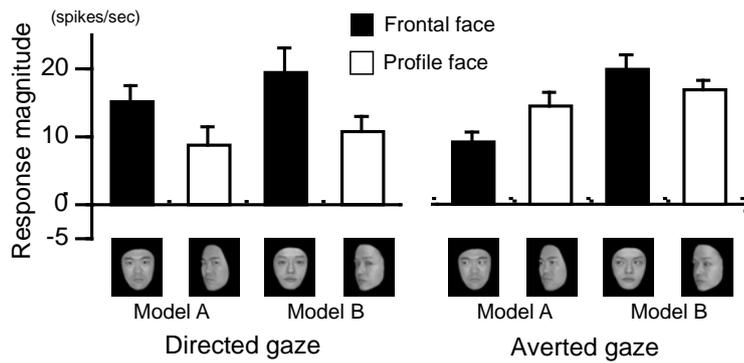# C. Gaze direction-nondifferential and head orientation-nondifferential

1 spikes
1 sec
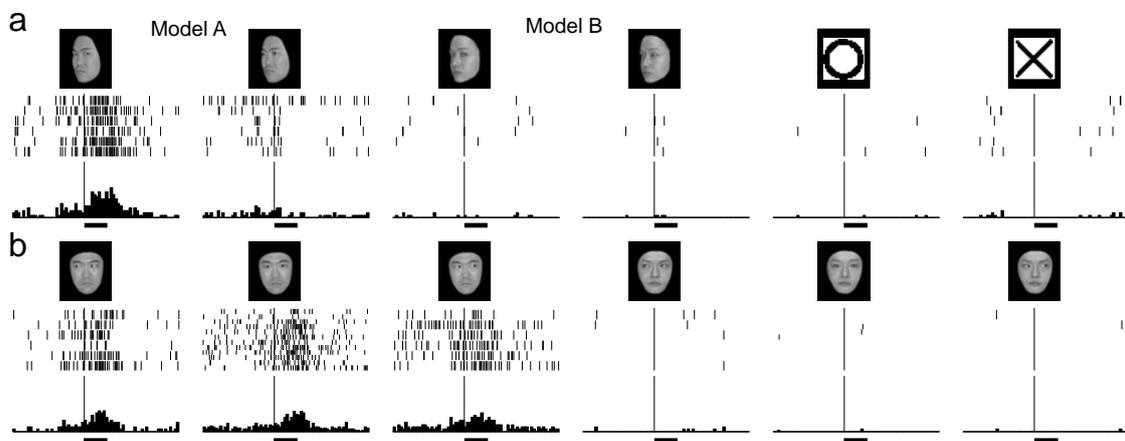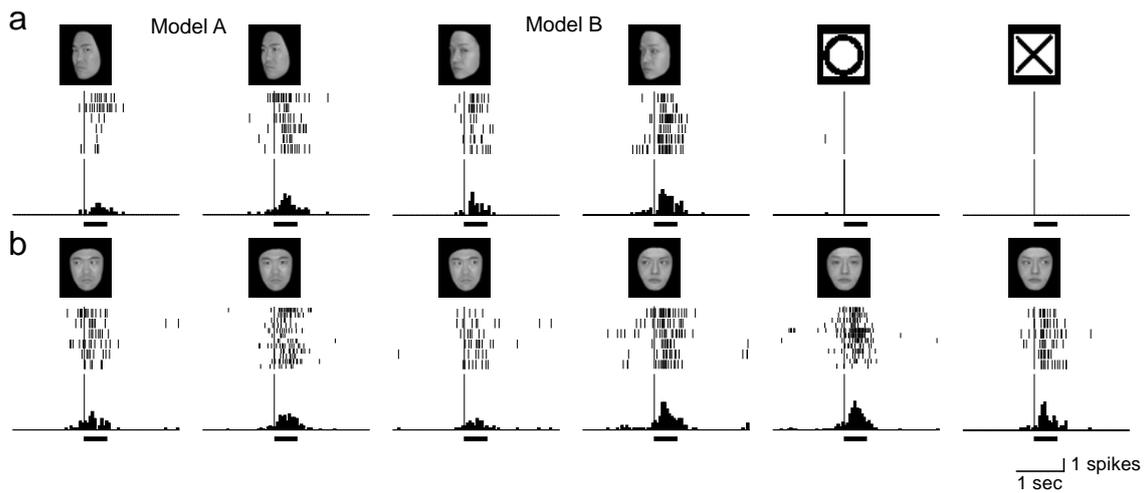
Figure 2

A. Gaze direction-differential and head orientation-differential
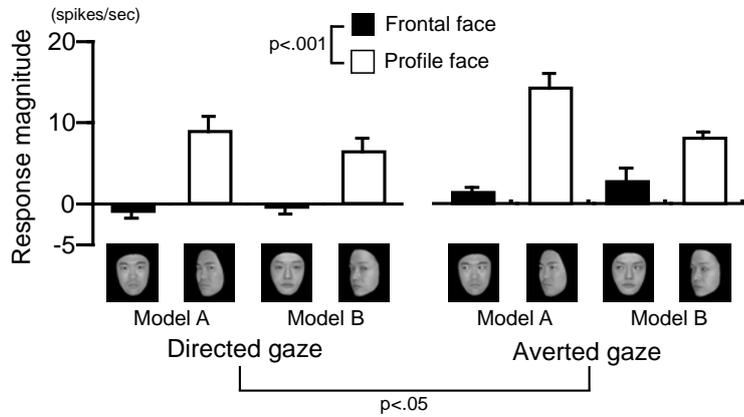
B. Gaze direction-differential and head orientation-nondifferential
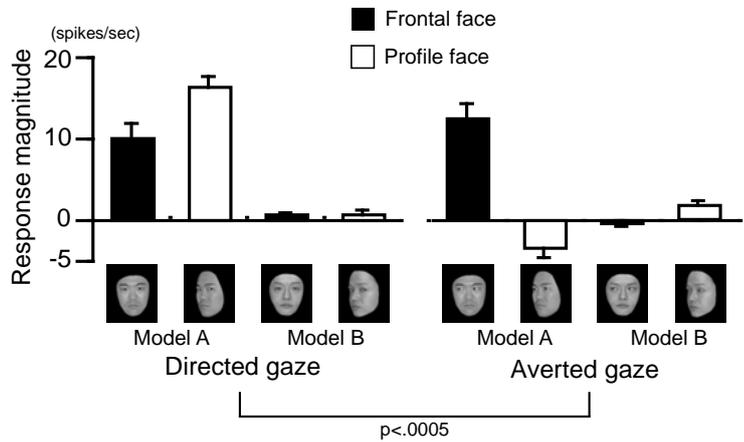
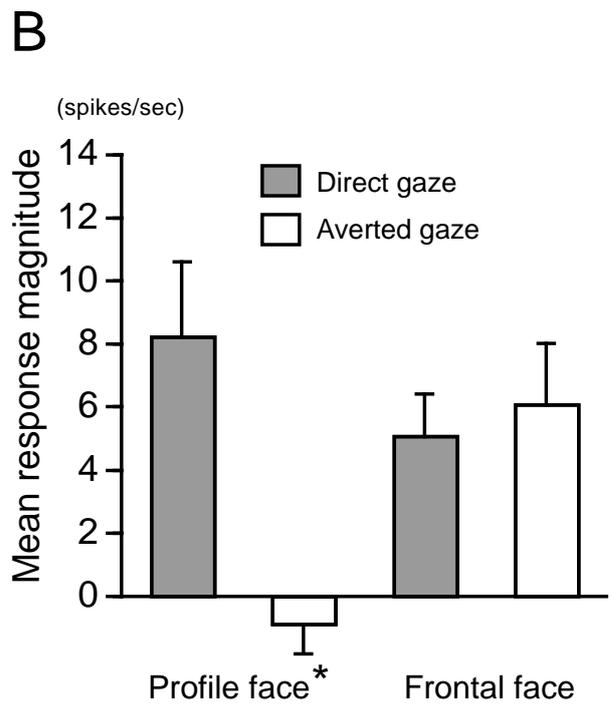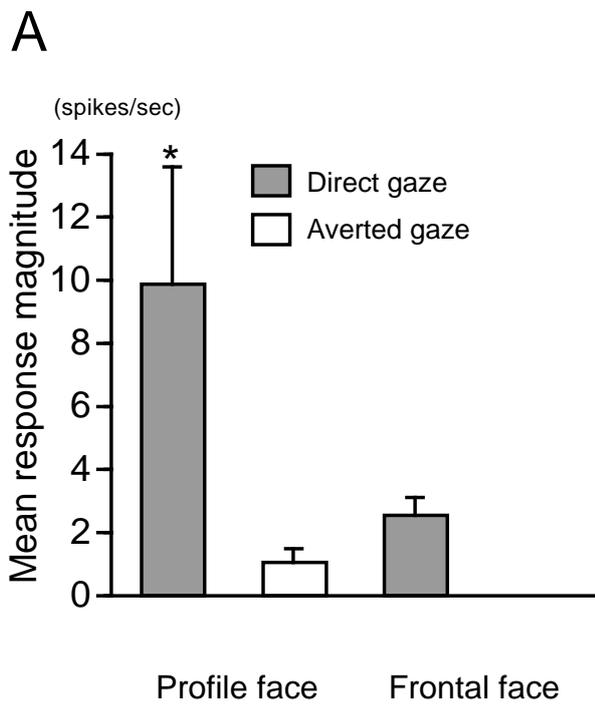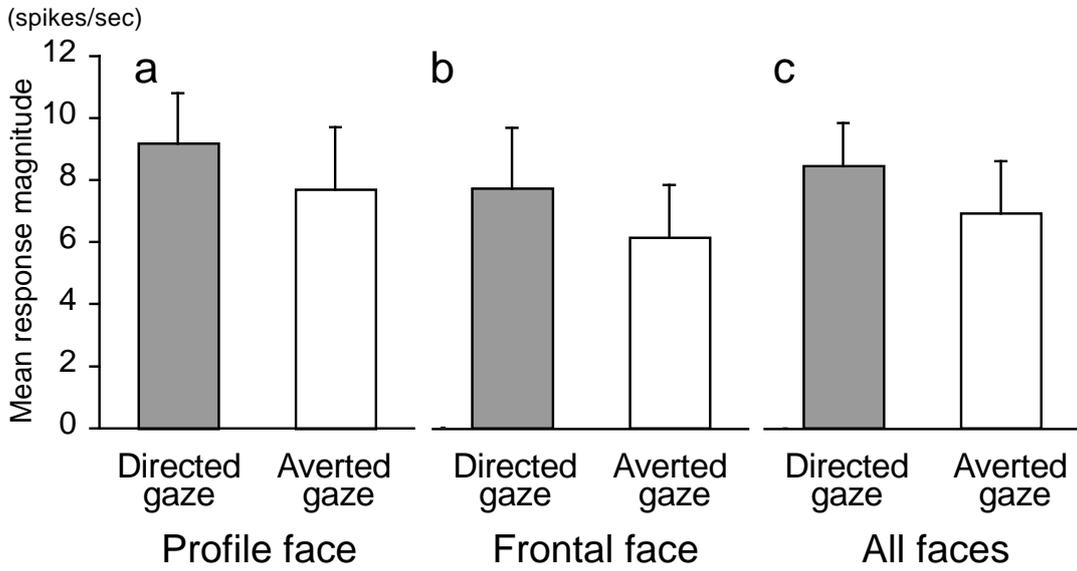C. Gaze direction-nondifferential and head orientation-nondifferential

Figure 3

Figure 4

A. Interaction-sensitive neurons

(spikes/sec)

a — Profile face

Mean response magnitude

Directed gaze	Averted gaze

b — Frontal face

Directed gaze	Averted gaze

c — All faces

Directed gaze	Averted gaze

B Interaction-insensitive neurons

(spikes/sec)

a — Profile face

p<.05

Mean response magnitude

Directed gaze	Averted gaze

b — Frontal face

Directed gaze	Averted gaze

c — All faces

p<.05

Directed gaze	Averted gaze

Figure 5

41

A

(spikes/sec)

p<.05

Mean response magnitude

Direct gaze | Averted gaze

Profile face

B

p<.10

Direct gaze | Averted gaze

Frontal face

Ca.

(spikes/sec)

p<.01

Mean response magnitude

Direct gaze | Averted gaze

All faces

b. <100msec

p<.05

Direct gaze | Averted gaze

All faces

c. 100-300msec

n.s.

Direct gaze | Averted gaze

All faces

Figure 6

CM
ABm
AL
ABl
AP21

CM
ABm
AL
ABl
AP20

CM
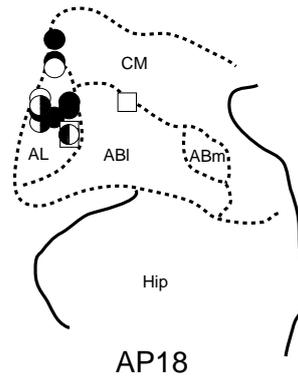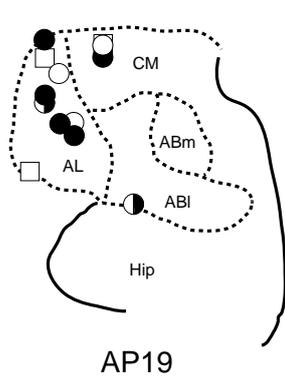ABm
AL
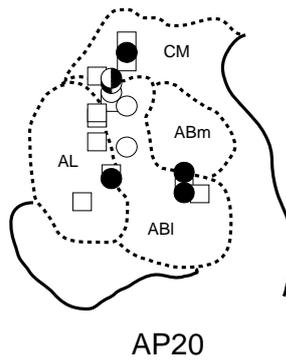ABl
Hip
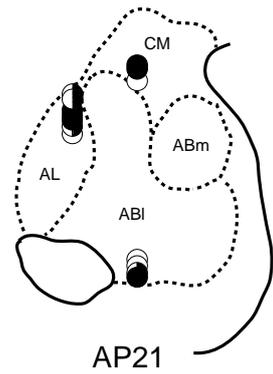AP19

CM
AL
ABl
ABm
Hip
AP18

Figure 7

Table 1. Summary of the face and face-responsive neurons in each recorded hemisphere.

Recorded hemisphere	First monkey		Second monkey		Total
	Right	Left	Right	Left	
No. of neurons recorded	218	598	209	66	1091
No. of face-responsive neurons	31	20	4	6	61
No. of face neurons	21	17	2	4	44
Ratio of face-responsive neurons (%)	14.22	3.34	1.91	9.09	5.59
Ratio of face neurons (%)	9.63	2.84	0.96	6.06	4.03

Table 2. Factor loading matrix for 44 face neurons resulted from orthogonal rotation (Varimax).

Facial stimuli	Factor 1 (Frontal face)	Factor 2 (Profile face)	Communality
	0.87861	0.36636	0.90618
	0.86435	0.37621	0.88864
	0.81274	0.41233	0.83056
	0.79413	0.46529	0.84714
	0.33099	**0.92420**	0.96369
	0.32499	**0.71705**	0.61977
	0.47936	**0.71553**	0.74176
	0.54655	**0.70283**	0.79269
Eigenvalues	3.55393	3.03652	6.59045
% of Variance explained	44.44	37.95	82.39

Note that factor loadings more than 0.6 are listed in bold-faced type.