Neurons in the human amygdala encode face identity, but not gaze direction

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The amygdala is important for face processing, and direction of eye gaze is one of the most socially salient facial signals. Recording from over 200 neurons in the amygdala of neurosurgical patients, we found robust encoding of the identity of neutral-expression faces, but not of their direction of gaze. Processing of gaze direction may rely on a predominantly cortical network rather than the amygdala.

Direction of gaze is one of the most potent social signals in primates¹ and is processed by a specialized network of brain regions². Although considerable work has elucidated key cortical components of this network, notably sectors of parietal cortex and the posterior superior temporal sulcus (pSTS)², subcortical contributions remain debated. In particular, there is good evidence both for and against a role for the amygdala. Some studies³, but not others⁴, have found impaired gaze perception following human amygdala lesions. Some neuroimaging studies found greater amygdala activation to direct rather than to averted gaze⁵, others observed greater activation to averted rather than direct gaze⁶,⁷, as well as interactions with emotional expression⁷, and some found greater activation just to the anticipation of direct gaze rather than to direct gaze per se⁸. Taken together, a sizeable literature addresses the role of the amygdala in processing eye gaze from faces, but there is no clear consensus.

The issue is also of clinical interest, as impairments in processing the social meaning of eye gaze are well documented in autism, and this impairment has been linked to amygdala dysfunction in neuroimaging studies⁹,¹⁰. Perhaps most relevantly, direct neuronal recordings in the human amygdala have found reliable responses to the eye region of faces, whereas these responses are markedly reduced in people with autism¹¹.

To directly address the amygdala’s role in processing eye gaze, we recorded from a total of 904 single and multi-units (225 single units, 679 multi-units) in the medial temporal lobe of 14 neurosurgical patients (223 units from the basolateral amygdala, 343 from hippocampus, 150 from entorhinal cortex, and 188 from parahippocampal cortex; Online Methods, Supplementary Tables 1 and 2, and Supplementary Fig. 1). Given the possibly complex interactions between facial expressions of emotion and gaze⁷,¹², we focused on only three dimensions of neutral-expression faces: their identity (five different actors with similar low-level visual image properties; Supplementary Table 3), their direction of gaze with head direction frontal (eight directions in 45-degree steps) and their head direction (with congruent eye gaze, eight directions) (Fig. 1 and Supplementary Fig. 2).

Consistent with prior recordings from the human amygdala¹¹,¹³, we found reliable responses to one or more persons featured in the stimulus viewing task (Fig. 1 and Supplementary Figs. 3 and 4). Two-way ANOVA of response firing rates yielded a significant main effect for person identity in 14% of amygdala units (P < 10⁻⁶, one-sided binomial test, Online Methods), but a main effect for gaze direction in only 6% (P = 0.23). Thus, we found no evidence for gaze selectivity (above what would be expected by chance). Moreover, the cells selective for person identity (31 cells; Supplementary Fig. 5) were almost entirely non-overlapping with the cells selective for gaze direction (14 cells, only three cells overlapped), and the proportion of gaze-selective cells was significantly smaller than the proportion that were identity selective (P = 0.007, Fisher’s exact test).

A separate comparison experiment carried out in 13 of the same patients revealed that the overall selectivity of amygdala neurons to faces, as compared with non-face stimuli, was typical, making it unlikely that we were recording from unusual locations or an unusual group of patients. As this comparison experiment was carried out with different stimuli and on a different day, it is not possible to link it to exactly the same neurons as in our main study, but it suggests sparse responses across a range of stimulus categories in the amygdala that are entirely consistent with prior findings¹³ (response probabilities and response magnitudes were equivalent for faces and non-faces; Online Methods and Supplementary Fig. 6).

Main effects for either person identity or gaze direction in our primary experiment were not observed above chance level in the other three MTL regions, except in parahippocampal cortex, where 11% of units (P = 0.001) also showed a main effect of identity. We confirmed that neuronal responses to direct and averted gaze did not differ above chance levels with direct post hoc tests (two-sided Wilcoxon rank sum tests comparing all direct gaze trials to all averted gaze trials, followed by one-sided binomial tests, P > 0.05 for all regions). These findings remained valid when we used a coarser distinction of gaze directions (left/middle/right or up/middle/down) or when we restricted our analysis to just single units (Online Methods).

To test whether an influence of person identity or gaze direction is present at the population level, we analyzed how images are segregated by response patterns using a categorization technique, representational similarity analysis¹⁴. Representational similarity matrices displaying the similarity in response between all pairs of the 45 stimuli (gaze and head directions pooled together) revealed a specific response pattern in the amygdala to person identity, which was reflected by similar responses to a given person that differed from responses to all other persons (P < 10⁻⁵, permutation test; Fig. 2). No comparable effect was found for gaze direction in any of the four MTL regions (P > 0.05). A significant influence of person identity was also found in the hippocampus (P = 0.001) and parahippocampal cortex (P < 10⁻⁴), but not in entorhinal cortex (P = 0.08). When comparing all effect

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sizes of person identity versus gaze direction yielded by the two-way ANOVA mentioned above at the population level, we confirmed significantly stronger effect sizes (ω²) for person identity than for gaze direction for the amygdala (P = 0.0001) and parahippocampal cortex (P = 0.008), but not for hippocampus and entorhinal cortex (P > 0.05; Online Methods). Thus, although we certainly do not rule out some degree of gaze encoding in the human amygdala, it was not detectable above chance levels, and it had a significantly weaker effect than identity encoding.

One shortcoming of our stimuli may have been their artificial nature: it is possible that amygdala neurons would respond to eye gaze, but only to dynamic, more naturalistic and more socially engaging stimuli rather than to static images. To address this possibility, we also conducted in the same 14 patients a ‘live encounter’ task. In this task, one of the experimenters (F.M. or J.N.) physically sat in front of the patient (Supplementary Fig. 7). Subjects were instructed to look at the experimenter for approximately 2 min while the experimenter randomly switched between looking directly at the subject (direct gaze), looking down (averted gaze) and closing his eyes (approximately every 2 s; all transitions were time-stamped and neuronal responses were analyzed with respect to them). This procedure was performed twice, resulting in 40 trials for each of the three conditions.

As with the first task, post hoc tests between the different gaze conditions failed to find any significant responses modulated by gaze in this live encounter experiment (all P > 0.05, one-sided binomial test; Fig. 1). In a second version of the live encounter task, recorded during four experimental sessions in two additional patients (Online Methods), the experimenter kept his gaze fixed on the subject while the subject switched between the three conditions. Of the 76 additional amygdala units recorded with this version, there was again no significant modulation by condition (all P > 0.05).

To our surprise, comparison of mean response activity during the static picture viewing versus live encounter task across units revealed a significantly higher activity during the static picture viewing (P = 0.013 in the amygdala, P > 0.2 for all other brain regions, two-sided Wilcoxon signed-rank test), a finding that was unexpected given the more arousing, socially engaging and ecologically valid nature of the live encounter task. Taken together, our findings are consistent with prior work showing that neurons in the human amygdala process the identity of faces, but we found no evidence for a role in processing gaze direction, either from eye gaze or head direction, even with a live person as the stimulus. These findings are surprising for at least two reasons. First, they seem discrepant with studies in monkeys, which have reported neuronal amygdala responses to gaze direction15,16; however, the proportion of such neurons may be very low (~5% in ref. 16) and the stimuli differed in several details from ours. Second, there is a body of data from neuroimaging that documents amygdala responses to gaze direction in both monkeys17 and humans5–8, although the conclusions of these varied studies, as noted earlier, do not cohere very well.

It is also important to note that a recent study in monkeys found that amygdala neurons respond to direct gaze, but only when the monkey is fixating onto the eyes of the stimuli18. In our experiment, subjects were required to attend to the eyes of the stimuli by the task that they had to perform, and task performance levels and observation by the experimenter both suggest that they fixated the eyes of the stimuli (95% task accuracy, Online Methods). As well, the additional four sessions of our live encounter task required subjects to fixate onto, or away from, the experimenter’s direct (and live) gaze; even here, to our surprise, we found no evidence for amygdala responses. It may be that human and monkey amygdala neurons respond differently to a context-specific’s gaze, for reasons that could range from species differences in basic perceptual processing to differences in the social meaning of the stimuli (the humans, unlike the monkeys, always know that they are in an experiment, even with the live encounter condition).

Another plausible explanation for our failure to find gaze-responsive neurons may be that we did not sample those regions of the amygdala in which such neurons might be concentrated: in the monkey, blood oxygen level–dependent (BOLD)–functional magnetic resonance imaging (fMRI) responses to gaze were found primarily in the central nucleus and bed nucleus of the stria terminalis17, but not in...
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Figure 2  Human amygdala neurons encode stimulus identity rather than gaze direction. Representational similarity analysis revealed the similarity of the population response from all units in a given region to every pair of stimuli, grouped by person identity across gaze directions (left column) and by gaze direction across persons (right column). Higher r values along the main diagonal of the matrices, indicating higher similarity within groups than between groups, were found for the amygdala for face identity, but not for gaze directions.

more basal or lateral parts of the amygdala, from which we recorded (Supplementary Fig. 1). A further possibility is that neurons in the amygdala may not spike in response to gaze, even though they receive cortical inputs that carry such information—inputs whose gaze-sensitivity might instead be reflected in BOLD responses, field potential changes19 or magnetoencephalography responses. It was also surprising that we did not find any response to gaze even with a real person as the stimulus; indeed, we found that, in general, this stimulus resulted in weaker responses than did our static images. Explanations here may include the possibility of habituation or top-down modulation of the amygdala as a result of context effects. Equally surprising was the fact that amygdala neurons were not even modulated by whether the experimenter had open or closed eyes: fMRI studies have found amygdala responses to eyes20, as have some single-unit studies11. One factor that may contribute to these differences is that these other studies both used emotional faces (happy or fear), whereas ours only showed neutral faces.

In conclusion, our findings argue that the role of the human amygdala in processing information about eye gaze should be reconsidered. The human amygdala’s strong selectivity for information from the eye region of faces may be instead used for a host of other kinds of processing, including recognition of identity and emotional expression11,13, whereas gaze processing may draw predominantly on a cortical network2. Given that neurons in the monkey amygdala do appear to respond to the eye region of faces18, our findings suggest that the specific ability for further fine discrimination of gaze direction may require cortical processing. Although speculative, this account would be consistent both with the more detailed resolution of visual representations in cortex and with the uniquely human evolution of the white sclera of the eyes to signal gaze direction, a cue unavailable to monkeys1.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

F.M. and R.A. designed the study. F.M. and O.T. implemented the experimental procedure. V.A.C. and F.M. carried out the neurosurgical procedures. F.M. and J.N. collected the electrophysiological data. F.M. analyzed the electrophysiological data. C.M.Q., J.N., C.E.E. and F.M. verified electrode locations. R.A. and F.M. wrote the paper. All of the authors discussed the results and commented on the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.
Subjects and recordings. 14 subjects (five male, 22–54 years old) undergoing treatment for pharmacologically intractable epilepsy were implanted with chronic depth electrodes to localize the epileptogenic focus for possible clinical resection. An additional two subjects (one male, 33 and 52 years old) were tested at a later date (on the second version of our live encounter task, see below). All studies conformed to the guidelines of the Medical Institutional Review Board at the University of Bonn. Informed written consent was obtained from each subject. Patients generally participated in a series of separate studies, consisting of typically 8–10 experiments spread out over the course of up to 2 weeks; recording sessions typically took from 1–6 h on any given day.

Recordings were obtained from a bundle of nine microwires (eight high-impedance recording electrodes, one low-impedance reference, AdTech) protruding from the end of each depth electrode. The differential signal from the microwires was amplified using a Neuralynx ATLAS system, filtered between 0.1 and 9,000 Hz, and sampled at 32 kHz. These recordings were stored digitally for further analysis. Spike detection, and sorting was performed after band-pass filtering the signals between 300 and 3,000 Hz as described previously21,22. The number of recording microwires per patient ranged from 32 to 96.

No statistical methods were used to predetermine sample sizes but our sample sizes are similar to those reported in previous publications23.

Microelectrode locations. For a detailed localization of amygdala recording sites, a post-implantation computer tomography was co-registered to a pre-implantation MRI scan. The fused image was then normalized to MNI space using FSL-32. The tips of the wire bundles were clearly visible (Supplementary Fig. 1a). Two independent raters confirmed that each of the 31 microwire bundle tips were located in the basolateral amygdala. Locations are shown in Supplementary Figure 1b and their coordinates are listed in Supplementary Table 2.

Experimental protocol. The main experimental protocol consisted of two parts. In the first part (static faces), subjects were presented with a random sequence of 210 photographs from five different persons whose gaze and head deviation varied in steps of 45°, thus resulting in eight different head directions and eight different gaze directions per person (Supplementary Fig. 2). In addition, five pictures of each person with direct gaze were added to the sequence which was shown twice in randomized order. Of the five persons featured in the stimulus set, one (F.M., person 5 in Supplementary Fig. 2) was familiar to all subjects, while the other four were unfamiliar to all subjects. Images were displayed on a computer monitor at a distance of approximately 50 cm from the subject, and covered a visual angle of approximately 20°. Each image was shown for 800 ms, followed by an inter-stimulus interval of 800 ms plus an additional random jitter between 0 and 500 ms. To keep subjects attentive, trials were presented in a one-back task with 20% of the images randomly repeated throughout the sequence. Subjects had to indicate a repetition of the exact same picture (that is, same person and same gaze/head direction) by pressing a key. False positive and negative responses were indicated by an acoustic signal. All patients were attentive to the task, with mean performance accuracy at 95% (range: 87–100%).

In the second part of the experimental protocol (live encounter), the experimenter (F.M. or J.N.) sat opposite the subject (Supplementary Fig. 7). Subjects were instructed to look at and attend to the experimenter for approximately 2 min. The experimenter randomly switched between looking directly at the subject (direct gaze), looking down (averted gaze), and closing his eyes approximately every 2 s. This procedure was performed twice, resulting in 40 trials for each of the three conditions. Each trial was time-stamped by the experimenter, who pushed one of three keys on a laptop keyboard synchronously with his switch in gaze. Each key corresponded to one of the three gaze conditions. The entire experimental session (static faces plus live encounter) lasted around 20 min and was always acquired and analyzed as one continuous recording. Some subjects performed two sessions on different days, which resulted in a total of 20 sessions from our 14 subjects.

A second version of the live encounter task, in which the experimenter (E.M.) kept his gaze fixed on the patient while the subject switched between the three conditions upon the experimenter’s command, was run with two additional patients (1 male, 33 and 52 years old) who did not participate in any of the other tasks. Two experimental sessions of this version were run with each of the two subjects, a total of 76 amygdala units (including 20 single units) were recorded.

An additional (third) comparison experiment was recorded in different sessions, on different days, from 13 of our 14 original subjects. This is described below. Since this experiment was done on a different day, it is not possible directly to link individual neurons to the same neurons from which we recorded in our two main experiments described above. Instead this comparison experiment serves as a general characterization of the response selectivity of amygdala neurons in our patients, comparing faces to non-face objects. The experiment was a screening task, to some degree customized for each patient (for example, pictures of each patient’s friends and family were used for that patient), that showed 100–150 images in a variety of different categories (faces, animals, landscapes, and objects).

Statistical analysis. For each of the 904 recorded single and multi-units, response activity during each of the 210 stimulus presentations was determined from the firing rate within the first 1000 ms after stimulus onset. For a more robust estimate of the response firing rates, we pooled identical head and gaze directions together, resulting in four trials per head/gaze direction and ten trials for direct gaze per identity. We then averaged across these groups, resulting in 45 mean firing rates that were fed into a one-way ANOVA with person identity and head/gaze direction as independent factors. After screening for cells with significant ANOVA effects at a level of alpha = 0.05, we used a one-sided binomial test to determine whether their number was significantly above chance level.

To also probe for weaker effects of gaze modulation, we compared firing rates for the 50 trials of direct gaze against the 160 trials of averted gaze or head using a two-sided Wilcoxon rank sum test and again verified significance with the one-sided binomial test. The same procedure was also carried out to compare direct versus averted gaze in the live encounter task, using again the first 1,000 ms of each trial to assess response activity (and each of these versus the eyes closed condition; none of these three pairwise contrasts for the live encounter task was statistically significant).

In an additional second-level analysis, we compared the mean response activity over all presented stimuli from the first task, to the mean response activity over all trials in the original version of the live encounter task, using a two-sided Wilcoxon signed-rank test across all units from a given region.

Representational similarity analysis. To analyze population response patterns to different stimuli in the amygdala on the basis of their representational similarity21,24,25, we generated representational similarity matrices for each region by determining for each pair of stimuli the similarity between the associated response patterns. Similarity was quantified as the linear correlation of activity across units in a given region. Response activity for each unit was z-score transformed based on the mean and s.d. across all trials. Similarity values displayed in the matrices were once grouped by person identity and once by gaze/head direction (Fig. 2). In both cases, similarity within groups was compared to similarity between groups by testing the appropriate similarity values (180 versus 810 for persons; 90 versus 900 for gaze/head directions) using a two-sided Wilcoxon rank sum test. Since the similarity values cannot be regarded as statistically independent due to their bivariate nature, the Wilcoxon test might yield spurious results. We therefore performed an additional permutation test by randomly shuffling the within-group and between-group labels 10,000 times and comparing the test statistics of the relabeled realizations to the original one, using once again a two-sided Wilcoxon rank sum test.

Control analyses: two-way ANOVA with collapsed gaze directions. Two-way ANOVA for five person identities and nine gaze directions yielded a significant main effect for person identities, but not for gaze direction. To rule out a lack of statistical power for the higher number of gaze directions compared to the number of persons, we collapsed across the three left, middle, and right gaze directions, respectively, and repeated the ANOVA. We obtained a main effect of persons in 11% of amygdala units (P < 10−3, one-sided binomial test), but a main effect for gaze direction in only 3% (P = 0.97). When collapsing across upper, middle, and lower gaze directions, we obtained a main effect of persons in 13% of amygdala units (P < 10−3), but a main effect for gaze direction in only 4% (P = 0.79).

Control analyses: two-way ANOVA for single units only. We repeated the original two-way ANOVA for five person identities and nine gaze directions for the subset of 225 single units included in our total set of 904 units.
Control analyses: direct comparison of effect sizes for two-way ANOVA. To directly compare effect sizes for person identity and gaze direction at the population level, we calculated omega squared ($\omega^2$) for each unit from the two-way ANOVA with five persons and nine gaze directions, and performed a paired $t$ test to test for significant differences. This procedure confirmed a significantly higher mean $\omega^2$ for person identity versus gaze directions in the amygdala (0.044 versus $-0.001$, $P = 0.0001$) and parahippocampal cortex (0.029 versus 0.004, $P = 0.0076$), but not in hippocampus (0.013 versus 0.004, $P = 0.073$) and entorhinal cortex (0.010 versus 0.006, $P = 0.546$).

Control analyses: left versus right amygdala analyses. 31 of 223 amygdala units from our main experiment showed a significant main effect of face identity in the two-way ANOVA. Of these, 9 of 97 (9.3%) were in the right amygdala, 22 of 126 (17.5%) in the left amygdala, which in this limited sample is not a sufficient lateralization to support any strong claims ($P = 0.12$, Fisher’s exact test). Of the 14 units that showed a main effect of gaze direction (though not significant above the expected type I error rate in the binomial test), 7 (7.2% of 97) were in the right and 7 (5.6% of 126) in the left amygdala ($P = 0.78$). Thus, no significant difference between the left and right amygdala was observed.

Control analyses: face selectivity of amygdala units. Since our stimulus material both in the picture task and in the live encounter task consists entirely of human faces, it is unclear how selectively the amygdala neurons reported in this study respond to faces. To shed light on this question, we analyzed another set of data, collected from these same patients, in which pictures from various stimulus categories (faces, animals, landscapes, and objects) were shown. These experiments were run as a screening procedure before other experiments not reported here, in all but one of the 14 patients originally included in this study. This procedure has been used in several publications by ourselves and other groups, described there in further detail. In brief, 100–150 stimuli were presented in randomized order six times each for 1 s. To determine whether an amygdala unit responded to one or more of the presented stimuli, we used the response criterion described in previous work. Briefly, we divided the 1,000 ms after stimulus onset into 19 overlapping 100-ms bins, and compared for each bin the spike rates for the six presentations of each stimulus to the baseline intervals of 500 ms before all the stimulus onsets in a session (approximately $125 \times 6$) by means of a two-sided Wilcoxon rank sum test, using the Simes procedure to correct for multiple comparisons and applying a conservative significance threshold of $P = 0.001$ to reduce false positive detections.

In total we recorded 384 amygdala units (including 124 single units) during 28 experimental sessions from 13 patients. It is important to note that, since these neurons were recorded on a different day than those we present in our main experiment, it is unknown to what extent this set of neurons corresponds to any of the neurons we present in our main experiment. Over all 28 sessions, we counted the number of instances when an amygdala neuron showed a significant response to a face image and divided this number by the total number of instances when a face image was presented to an amygdala neuron. The resulting response probability was based on the cumulative exposure of amygdala neurons to face images. For example, if 10% of amygdala neurons responded to 10% of all face stimuli in each session, the resulting response probability to faces would be 1%. The probability of responding to a non-face stimulus $RP_{nf}$ was calculated in the same manner. These two probabilities were compared using the chi-square test with Yates correction.

In addition, we calculated average response magnitudes for face and non-face stimuli. Of the total 384 amygdala units, 81 (including 28 single units) responded significantly to one or more of the presented pictures according to the response criterion described above. For each of these 384 units, we calculated the normalized response activity to each of the 100–150 presented stimuli as a $z$-score by subtracting the mean baseline activity (during the 500-ms interval before each stimulus presentation) across all stimulus presentations from the average response activity during the six presentations (1 s each) of a particular stimulus and dividing this difference by the s.d. of response activities across the 100–150 stimuli. We then averaged over the stimuli, separately for faces and non-faces, to obtain the mean response magnitudes to both stimulus classes for each neuron and statistically compared these using a two-sided Wilcoxon signed-rank test.

The response probability of amygdala units to face stimuli was 0.48%, the response probability for non-face stimuli was 0.53% (chi-square = 0.279; $P = 0.594$; Supplementary Fig. 6a). Restricting this analysis to single units only, these values were 0.39% and 0.47%, respectively (chi-square = 0.256; $P = 0.613$), thus corresponding well to previous findings from a different group of subjects. Mean response magnitude to faces versus non-faces was 0.19 versus 0.16 ($P = 0.377$) for all responsive units and 0.17 versus 0.15 ($P = 0.631$) for single units only (Supplementary Fig. 6b). Of the 81 responsive units, 46 (16 single units) responded exclusively to faces, 12 (7 single units) exclusively to non-faces, and 23 (5 single units) to both faces and non-faces. Scatter plots displaying response probabilities and response magnitudes to faces versus non-faces separately for each cell (Supplementary Fig. 6c,d) show that some, but not all, responsive units were able to discriminate between faces and non-faces.

A Supplementary Methods Checklist is available.

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