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Report

Neurons in the Monkey Amygdala Detect Eye Contact during Naturalistic Social Interactions

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Summary

Primates explore the visual world through eye-movement sequences. Saccades bring details of interest into the fovea, while fixations stabilize the image [1]. During natural vision, social primates direct their gaze at the eyes of others to communicate their own emotions and intentions and to gather information about the mental states of others [2]. Direct gaze is an integral part of facial expressions that signals cooperation or conflict over resources and social status [3–6]. Despite the great importance of making and breaking eye contact in the behavioral repertoire of primates, little is known about the neural substrates that support these behaviors. Here we show that the monkey amygdala contains neurons that respond selectively to fixations on the eyes of others and to eye contact. These "eye cells" share several features with the canonical, visually responsive neurons in the monkey amygdala; however, they respond to the eyes only when they fall within the fovea of the viewer, either as a result of a deliberate saccade or as eyes move into the fovea of the viewer during a fixation intended to explore a different feature. The presence of eyes in peripheral vision fails to activate the eye cells. These findings link the primate amygdala to eye movements involved in the exploration and selection of details in visual scenes that contain socially and emotionally salient features.

Results

We recorded neuronal activity from the amygdalae of three monkeys while they viewed videos of natural behaviors displayed by unfamiliar conspecifics (henceforth, "movie monkeys"). Two of the three subjects also viewed a representative static frame extracted from each video. We identified the segments of time when the viewer monkeys fixated on various facial features of the movie monkeys (Figure 1) and confirmed previous reports on the primacy of eyes as targets of viewing interest (e.g., even though the eyes occupied only 2.6% of the video frames, monkeys Q, Z, and G spent 39.1%, 26.8%, and 17.2% of the time fixating on them, respectively; they spent significantly less time fixating on the mouth: Chi-square test comparing the percent of time that the eyes or mouth were fixated, $p < 0.00001$) [7–11]. We and others have previously shown that videos promote interactive looking behaviors, e.g., eye contact, gaze following, gaze avoidance, and the reciprocation of facial expressions [12–17], as they better approximate natural interactions [18–20]. Indeed, videos captured the viewer's attention for longer periods of time (paired t test comparing time spent fixating the eyes of videos and the eyes of static images: Z, $t_{2234} = 14.08$, $p < 0.00001$; G, $t_{2791} = 7.99$, $p < 0.00001$). When scaled for the total time spent looking at videos and static images, however, the viewer monkeys fixated on the eyes of both stimuli in equal proportion (Chi-square test comparing amount of time that monkeys Z and G spend fixating the eyes of videos and images: Z, $\chi^2_{df=1} = 1.489$, $p = 0.222$; G, $\chi^2_{df=1} = 0.001$, $p = 0.974$).

Neurons in the Amygdala Respond to Fixations on the Eyes

Of 318 well-isolated neurons, 38 neurons (12%) significantly changed their firing rate when the subjects fixated on the eyes of the movie monkeys (Wilcoxon rank-sum test, $p < 0.05$; Q, 8/104 cells, 7.7%; Z, 27/171 cells, 15.8%; G, 3/42 cells, 7.1%) (Figures 2A–2D; Movies S1 and S2 available online). These "eye-fixation cells" did not respond (or responded with a reduced firing rate) when subjects fixated on other facial features, e.g., the mouth (Wilcoxon rank-sum test, $p < 0.05$; Supplemental Experimental Procedures). The response patterns registered during fixation on the eyes were (1) tonic excitation spanning the entire duration of fixations on the eyes (Figure 2B), (2) phasic excitation with an average duration of 120 ± 42 ms (Figure 2C), and (3) phasic inhibition (Figure 2D). The same analysis applied to fixations on other facial features (e.g., the mouth) failed to identify cells that were selective for any other targets. We found, however, 14 cells that responded to all fixations independent of the target (Figure 2E). These "nonselective-fixation cells" were the only other type of fixation-related neuron identified. The average activity of all 318 recorded cells indicates that the population responded more strongly during fixations on the eyes than during fixations on other features (Figure S1B, bottom).

The response latency of the eye-fixation cells varied between 80 and 140 ms with a mean latency of 118 ± 29 ms, which is shorter than the response latency of canonical visually driven cells in the amygdala (mean response latency to the presentation of visual stimuli: 157 ± 58 ms; paired t test, $t_{56} = 3.3299$, $p = 0.0015$; previously reported latencies of visually responsive neurons in the amygdala exceed 100 ms, e.g., [21–24]) (Figure S1A). The eye-fixation cells were not topographically clustered (Figure S2; histology and recording site reconstruction) (4/45, 9%, in the centromedial and 34/273, 12%, in the basolateral nuclei; Yates chi-square: $\chi^2_{df=1} = 0.189$, $p = 0.664$).

A Subpopulation of Eye-Fixation Cells Respond to Eye Contact

Each video depicted social signals displayed toward and away from the viewer (direct and averted gaze of the movie monkey). Displays with direct gaze created opportunities for the viewer to establish eye contact with the movie monkey. We identified periods of eye contact by combining the scan path of the viewer with an ethogram that marked the gaze direction of the movie monkey.

A group of ten eye-fixation cells responded with significantly higher firing rates during eye contact than during fixations on eyes with averted gaze (Figure 3) (two-tailed bootstrap by

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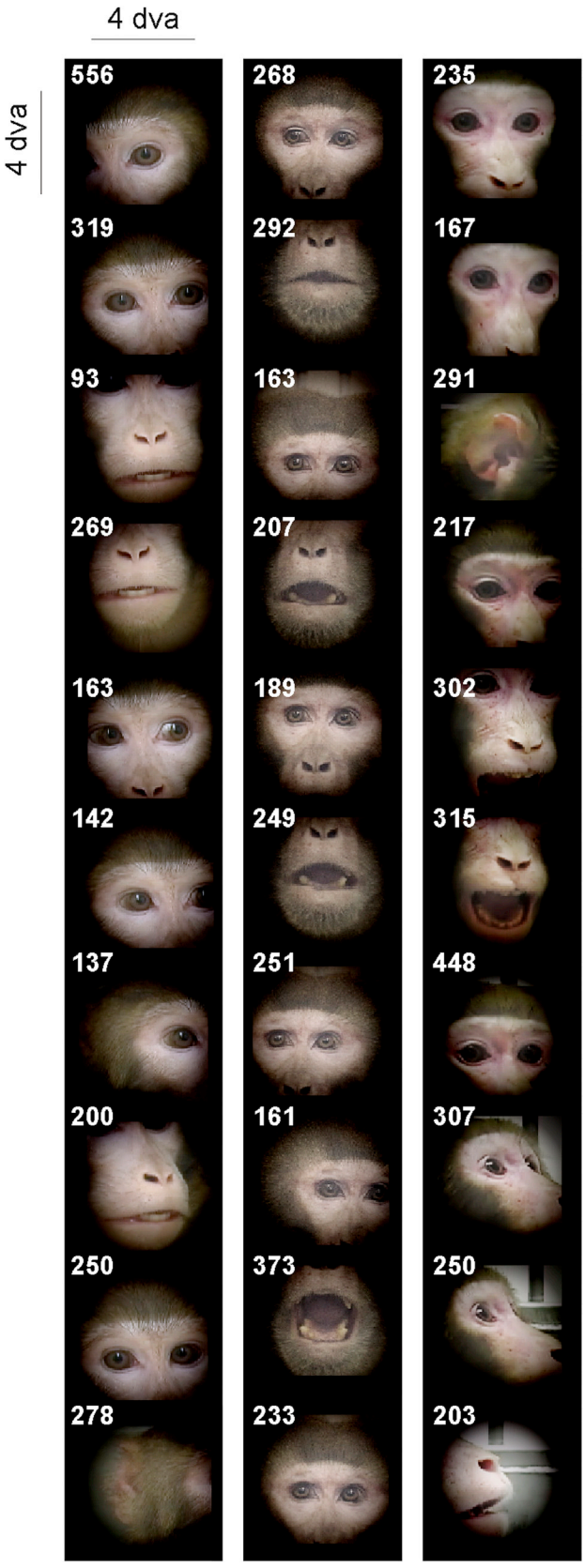

Figure 1. Fixations on Videos of Conspecifics

Each column depicts the first ten fixations made by a monkey as he viewed a video of a conspecific. The fixated region is depicted as a 4 × 4 degree of visual angle "bubble" extracted from the video. The number in the upper-left quadrant of each bubble indicates the duration (ms) of each fixation. The viewer monkeys fixated on the eyes of the movie monkeys more often than any other facial feature. See also Figures S1 and S2 and Movies S1 and S2.

shuffling movie monkey ethogram, $p < 0.05$; Supplemental Experimental Procedures). The high sensitivity to eye contact is illustrated by an increase of up to a 76 Hz firing rate during eye contact compared to a mean rate of 10 Hz during fixations on averted eyes for a neuron with a baseline firing rate of 5 Hz (Figure 3 and Movie S3). By contrast, two cells responded with significantly greater firing rates during fixations on eyes with averted gaze (mean rate during eye contact and during fixations on averted eyes: cell 1, 15.2 Hz, 7.7 Hz; cell 2, 12.0 Hz, 9.7 Hz).

To further characterize the cells that responded to fixations on the eyes and to eye contact, we determined (1) whether fixating on the eyes of a static image is sufficient to drive a neural response (2) whether the appearance of eyes in the center of gaze without the subject actively saccading is sufficient to activate a response, and (3) whether the eye-fixation cells are a special class of cells whether or they share features with other visually responsive neurons in the amygdala.

Fixating on the Eyes of Static Images Is Sufficient to Drive the Activity of Eye-Fixation Cells

Of the 38 eye-fixation cells, 14 were recorded in two monkeys that viewed the same movie monkeys in videos and video frames presented as static images. Fixating on the eyes of dynamic and static images induced similar changes in firing rate (Figure 4) (mean difference in rate, 0.63 ± 3.57 Hz, equivalent to a 7% ± 25% change; $t_{13} = -0.6598$, $p = 0.529$). Two eye-contact cells were also tested with static images. Both cells responded during fixations on eyes with direct gaze (the equivalent of eye contact) with elevated firing rates (mean rate during eye contact and during fixations on averted eyes: cell 1, 19.7 Hz, 28.3 Hz; cell 2, 7.1 Hz, 9.7 Hz). The temporal patterns of the spike trains, i.e., phasic versus tonic responses, were similar during eye fixations on dynamic and static stimuli. Figure 4 shows side-by-side eye-fixation cells that respond with excitatory phasic, excitatory tonic, and inhibitory responses to fixations on eyes in static and dynamic images. Although a more complete answer is expected to emerge from a larger population of eye-contact cells, these initial findings indicate that these cells differentiate direct and averted gaze independent of the dynamic/static properties of the stimulus.

Can Eye-Fixation Cells Be Activated in the Absence of Saccades?

Although eye-fixation cells were discovered by alignment of neural activity to saccades and fixations on videos, it is unclear whether the action of making a saccade to the eyes is necessary to elicit an eye-fixation response. Is the mere presence of eyes at the center of the visual field sufficient to elicit a response? To address this question, we recorded the activity of five eye-fixation cells in an experiment where the subject fixated on a cue that triggered the immediate presentation of a static image of a face. When the face appeared, its eyes fell either at the center of gaze (fovea) or at a distance greater than 4° of visual angle from center of gaze (Figure S3). All five eye-fixation cells responded similarly after saccades to the eyes and the appearance of eyes at the center of fixation (Figures S3B–S3D), indicating that saccades are not a sine qua non requirement for the activity of eye cells. Indeed, the eye-contact-selective cells increased their firing rate when the

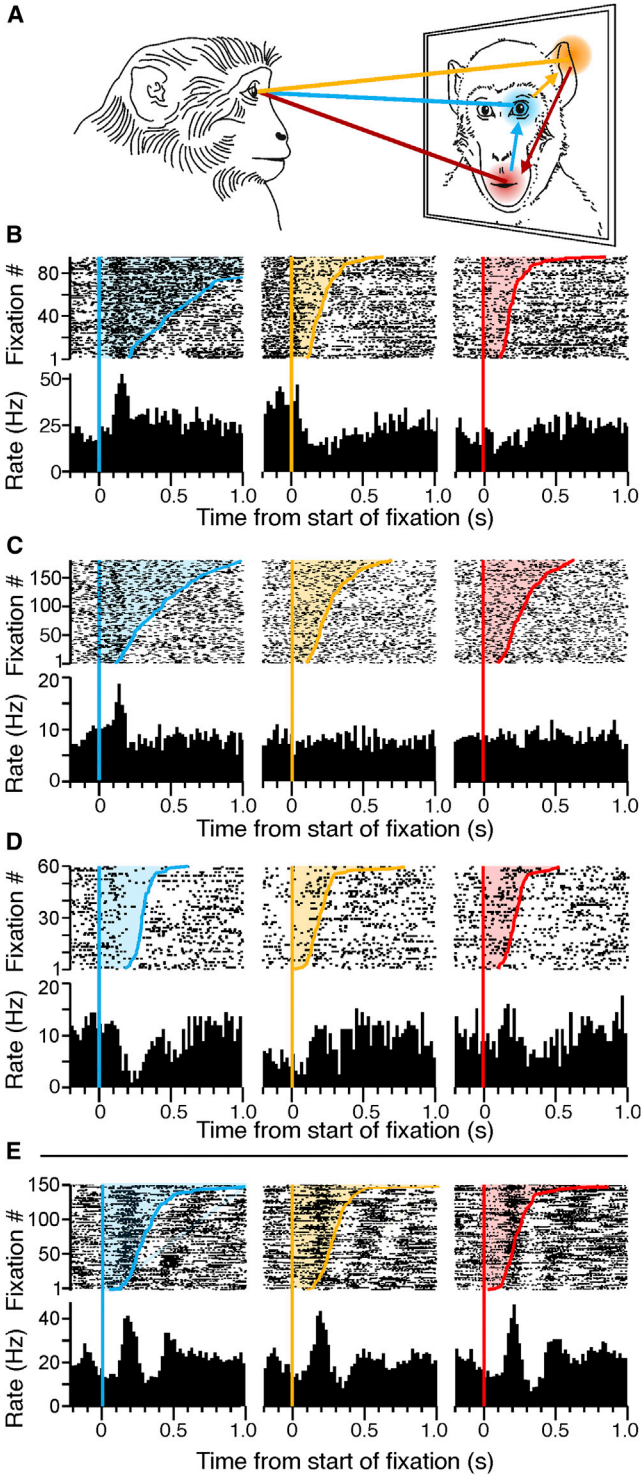

Figure 2. Fixating on the Eyes Activates Neurons in the Monkey Amygdala

(A) Fixations were classified in three categories (indicated by color-coded, shaded areas): fixations on the eyes that were preceded by fixations elsewhere (blue), fixations on another feature that followed fixations on the eyes (yellow), and fixations on other features that were preceded by fixations on other areas (red).

(B–E) Raster plots and perievent time histograms illustrating the activity of four neurons during each of the three types of fixations. Rasters are sorted by fixation duration. Fixations begin at 0 s and end at the curved line.

(B) The firing rate of this neuron increased for the entire duration that the viewer fixated on the eyes but was reduced when the viewer fixated on other features.

movie monkey changed its direction of gaze from averted to direct while the viewer fixated on the eyes (e.g., Figures 3B and 3D at 3.3 s and 3.5 s, respectively). This finding suggests that the term "eye-fixation cells" should be replaced by "eye-centered cells," or simply "eye cells." Further analysis indicated that eye cells responded with the same latency to the active (saccade) and passive (image) appearance of the eyes within the fovea (latency of neural response fixation after saccades to the eyes: 121+27 ms; latency of neural response to the appearance of eyes on the fovea: 133+74 ms; t test comparing difference in latencies, $t_3 = -0.574$, $p = 0.697$). These cells responded differentially to the eyes compared to the mouth for both the active and passive looking conditions (mean difference in firing rate to static images with central presentation on eyes compared to mouth: 15.5 ± 6.2 Hz, 46.2% ± 18.5%; paired t test, $t_4 = 5.5939$, $p = 0.005$). By comparison, three nonselective-fixation cells were tested in this paradigm, and they responded similarly to the appearance of any feature at the center of the gaze (Figure S3E; t test comparing mean firing rate, $t_2 = 1.512$, $p = 0.135$).

Eye Cells Are a Specialized Class of the Canonical Visually Responsive Neurons in the Amygdala

By definition, the eye cells are visually responsive neurons with spiking time-locked to the appearance of the eyes in the fovea. Many visually responsive neurons in the monkey amygdala respond to the onset or offset of visual stimuli (phasic image-on/image-off cells) or to the entire time an image is presented (tonic responses) [21–25]. We have previously shown that selectivity for the content of the images is expressed by changes in (1) the polarity of the response, i.e., inhibitory or excitatory, (2) the magnitude of the response, and (3) the temporal pattern of the response (e.g., bursting, phasic, or tonic changes in firing rate; see Figure 2 in [25]). The nonspecific fixation cells and the eye cells share these properties with the rest of visually responsive neurons in the amygdala. For example, the eye cell depicted in Figure S4A responded with a tonic increase in firing rate relative to interstimulus baseline. Superimposed on this tonic elevation of firing rate were further elevations in rate during fixations on the eyes. Furthermore, the firing rate during fixations on directed eyes (eye contact) was further elevated compared to fixations on averted eyes. Thus, the primary response of these cells signals the presence of videos, while the secondary and tertiary response signals fixations on eyes and eye contact, respectively, in a pattern of nested selectivity.

Re-examining our 318 recorded neurons in this light, 248 (78%) responded to the onset/offset of the presence of videos depicting conspecifics. All 52 identified fixation cells (38 eye-fixation cells and 14 nonselective-fixation cells) responded to the appearance of visual stimuli on the monitor *and* to the presence of eyes in the fovea (i.e., 52/248, 21%, of the visually responsive cells also responded to fixations on the eyes; Figure S4). Moreover, the pattern of their response (tonic versus phasic and inhibitory or excitatory) was preserved for images, fixations, and the content of fixations (Figures S4A–S4D).

It is critical to emphasize that the response of fixation cells to the onset of visual stimuli is independent of the monkey's

(C) This neuron showed a phasic increase in firing rate during fixation on the eyes but no change in firing rate when during fixation on other features.

(D) This neuron was inhibited when the viewer fixated on the eyes and was released from inhibition when the subject looked away from the eyes.

(E) This neuron reliably increased its firing rate after the onset of a fixation, regardless of what the viewer fixated on (bin size = 20 ms).

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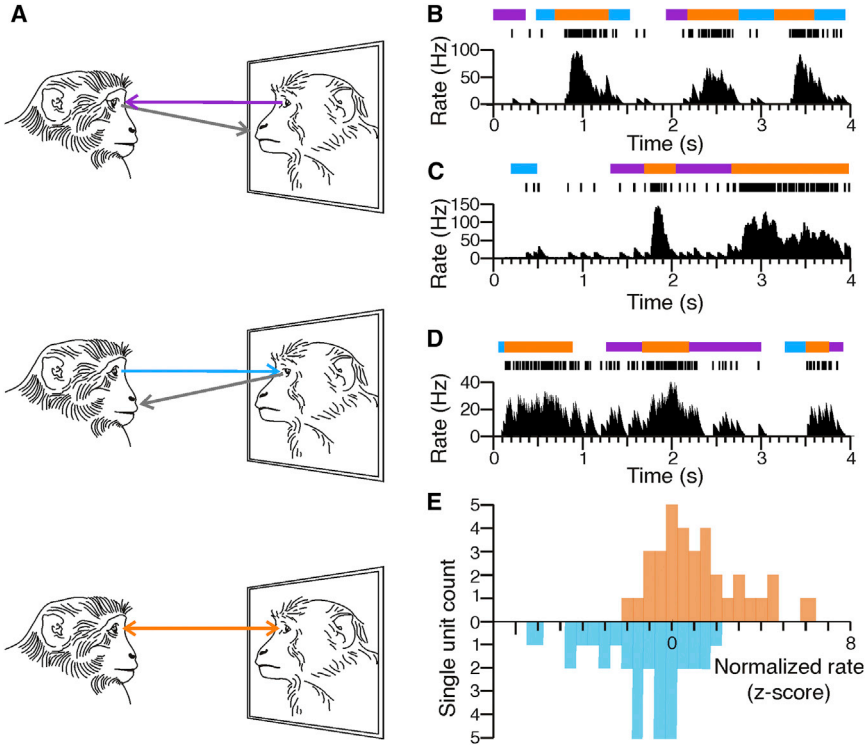

Figure 3. Eye-Contact Cells

(A) Categories of gaze interactions between the viewer and the movie monkeys. Top: the movie monkey gazes directly at the viewer, but the viewer does not fixate on his eyes (this scenario is depicted in the purple bars in B–D). Middle: the subject fixates on the movie monkey's eyes but the movie monkey's gaze is averted (blue bar in B–D). Bottom: eye contact is established between the two monkeys (orange bar in B–D).

(B–D) Spike train and mean firing rate of three eye-contact cells. Note that each cell increased its firing rate during periods of eye contact (orange) but exhibited little or no change in firing rate when the subject fixated on the eyes of monkeys with averted gaze (blue).

(E) Mean normalized firing rates of all 34 eye cells during periods of eye contact (orange) and during fixations on eyes with averted gaze (blue). On average, the population of eye cells has a greater firing rate during fixations on eyes with direct gaze. The overlapping regions of the two histograms represent those eye cells that fire with comparable rates during fixations on eyes with direct and averted gaze. Firing rate was normalized (Z score) to the mean and SD of the firing rate during fixations on the eyes.

See also Movie S3.

subsequent eye movements. In all instances, the neural response for stimulus onset preceded the neural response elicited by the first fixation that the monkeys made (latency of neural response after onset of video stimuli: 147 ± 56 ms; latency of first eye-movement on the visual stimuli: 251 ± 91 ms; rank-sum test, $z = 6.451$, $p < 0.0001$). Indeed, the neural response evoked by the visual stimulus is more strongly time locked to the appearance of the stimulus than to the first fixation on that stimulus (maximum response rate when aligned to onset of visual stimulus versus first fixation: 50.3 ± 43.59 Hz versus 34.8 ± 25.9 Hz; signed-rank test, $z = 5.655$, $p < 0.0001$).

Discussion

We identified eye cells in response to videos, a naturalistic, ethologically valid alternative to static images of facial expressions. The videos engaged the viewer monkeys in socially meaningful looking behaviors rarely observed in responses to static images [17]. It was assumed that the higher level of engagement of the viewer with the videos was the primary cause for the activation of the eye cells. The controls we report here, however, show that eye cells are active even when the viewer scans static faces and that active eye movements are not necessary for eye-cell activity. Why then did we miss the eye cells in the data recorded in the past decade in response to static images of facial expressions? Because the timing of fixations and saccades is inconsistent across trials and averaging the spike trains across trials eliminated the chance to observe these short-lived fixation-related changes of firing rates.

These findings confirm earlier observations that neurons in the amygdala show several levels of *nested selectivity*. Indeed, our 2007 report on the selectivity of amygdala neurons [24] shows that the vast majority of neurons in the amygdala are category selective, responding differentially to monkey faces, human faces, and objects. Face-selective cells show additional selectivity for individuals. These identity-selective cells further differentiate between the facial expressions of that particular individual (see Figure 5 in [24]). The eye cells follow the same scheme. At a primary level, well-illustrated by across-trial averages, these cells respond to the onset/offset of images or to the entire display of the visual stimulus (either videos or static images). At a secondary level, discrete but significant changes occur in relation to fixations. At a tertiary level, these discrete variations differentiate eye contact form fixations on averted eyes. There might be quaternary or even higher-order levels (such as eye contact during appeasing or affiliative interactions, or eye contact with a friend or foe), but the design of the current experiment precluded such analyses. The idea that, in the amygdala, the most socially salient stimuli elicit the highest firing rates holds true: at the population level, neurons that signal eye contact elicit the highest firing rates, similarly to threatening faces that elicited higher firing rates than neutral and appeasing expressions [24]. One consequence of the nested selectivity in the amygdala (that receives broad inputs from all sensory modalities and broadcasts to an equally large array of targets the outcome of the computation that the take place therein) is that the changes in firing rates, especially their timing to behavioral events, carry information about multiple dimensions of a stimulus and therefore may retain in a small population of neurons the diversity of its inputs.

The observation that neurons in the amygdala respond selectively to eyes that fall on the fovea and do not respond to the presence of eyes in peripheral vision raises the question of retinotopy or some form of spatial segregation of foveal and peripheral vision in the amygdala. Retinotopy is unlikely when considering the gradual expansion of visual receptive fields along the ventral visual pathway [26]; indeed, the receptive fields in areas that project to the amygdala, e.g., area TE

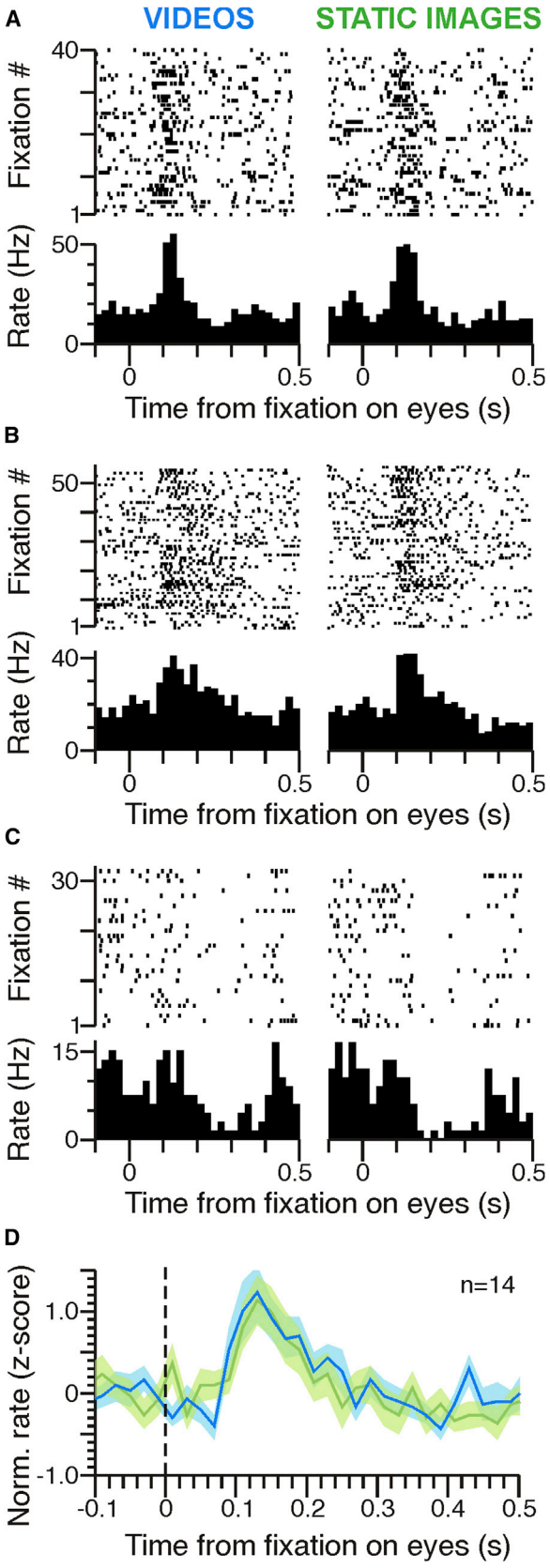

Figure 4. Fixating on the Eyes of Static Images Is Sufficient to Drive the Eye Cells

(A–C) Raster plots and perievent time histograms depicting the activity of three neurons during fixations on the eyes of monkeys shown in videos (left) and in static images (right). The neurons exhibited equivalent changes in response magnitude during all time bins spanning the fixation (rank-sum test, $p > 0.05$).

(A) An eye cell that responded with a phasic increase in firing rate during fixations on the eyes of monkeys depicted in videos and static images.

(B) An eye cell that responded with a broad phasic increase in activity during fixations on the eyes in videos and static images.

(C) A neuron that exhibited delayed inhibitory activity during fixations on the eyes in video and static images.

(D) Mean normalized firing rate (±SEM) of 14 eye cells during fixation on the eyes of monkeys depicted in videos (blue) and static images (green). Firing rate was normalized (Z score) to the mean and SD of the firing rate in a 100 ms window preceding fixation on the eyes.

See also Figures S3 and S4.

[27, 28], are large enough to encompass an entire hemifield [29–31]. Neurons in TE, however, exhibit heightened sensitivity for details that fall within the fovea [32–34]. It is unclear whether a mere change in sensitivity is sufficient to account the eye cells. The amygdala might receive information about the location of objects and events from alternative sources. The visual space in the parietal cortices seems to be a likely candidate; however, this possibility has not been experimentally explored. Recent reports on the spatial selectivity of neurons recorded from the amygdala [35] suggest that neurons therein carry spatial information about the location of reward, although the spatial scale might be too coarse for differentiating between foveal and peripheral presence of eyes.

Regardless of the neural mechanisms that gave rise to their properties, eye cells might play an important role in species-specific social behaviors in primates. These cells might represent an evolutionary specialization to support meaningful forms of social interaction mediated by gaze [2, 36]. Eye contact, its duration, and the way it is achieved or avoided are meaningful communicative signals. A confident, dominant monkey initiates eye contact by staring at the eyes of others and waiting for the targeted eyes to return direct gaze; submissive individuals might engage briefly in eye contact or may choose to avoid it altogether [3, 4, 37]. In humans, the majority of psychiatric and neurodevelopmental disorders show disruption in the use of eyes and eye contact during social interactions [38–42]. For example, patients on the autism spectrum typically fail to solicit and reciprocate eye contact [40, 43–45]. Further studies that block or enhance the activity of eye cells in the amygdala will complete our understanding of their potential role in natural and pathological social behaviors.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, three movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.08.063.

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Supplemental Information

Neurons in the Monkey Amygdala Detect Eye Contact during Naturalistic Social Interactions

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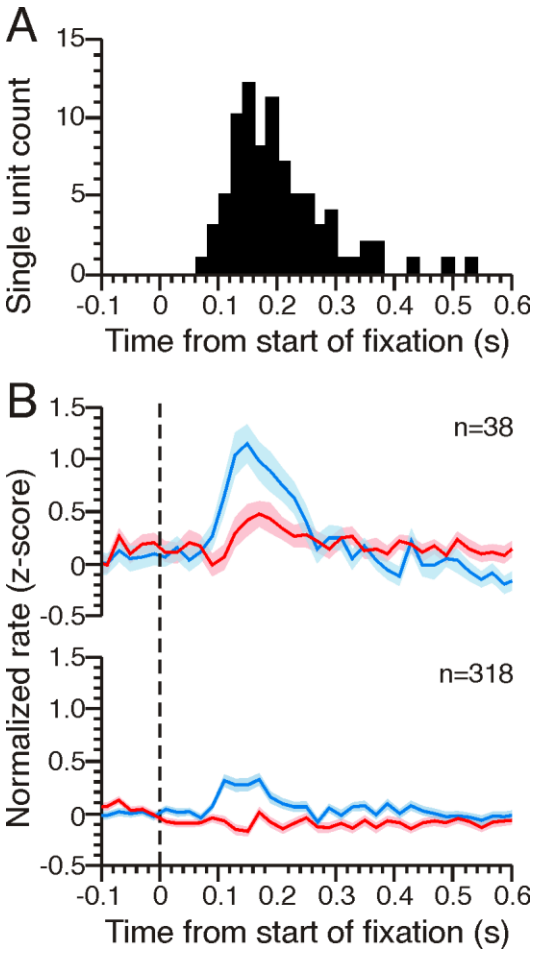

Figure S1, Related to Figure 1: *Population response of eye cells*. (A) Histogram depicting the time bins that each cell exhibited a significant difference in firing rate during fixations on eyes compared to fixations on other facial features. (B) Mean normalized firing rate (±SEM) of 38 eye cells (top panel). Population activity during eye-fixations is illustrated in blue; activity during fixations on other facial features is illustrated in red. Note that fixations on the eyes elicited increases in firing rate earlier than fixations on other features. (bottom panel) Mean normalized firing rate (±SEM) of all 318 cells recorded from the amygdala. In all four traces, the normalized z-score was computed using the average and standard deviation of the firing rate in the 100 ms window preceding fixation onset. (bin size=20ms).

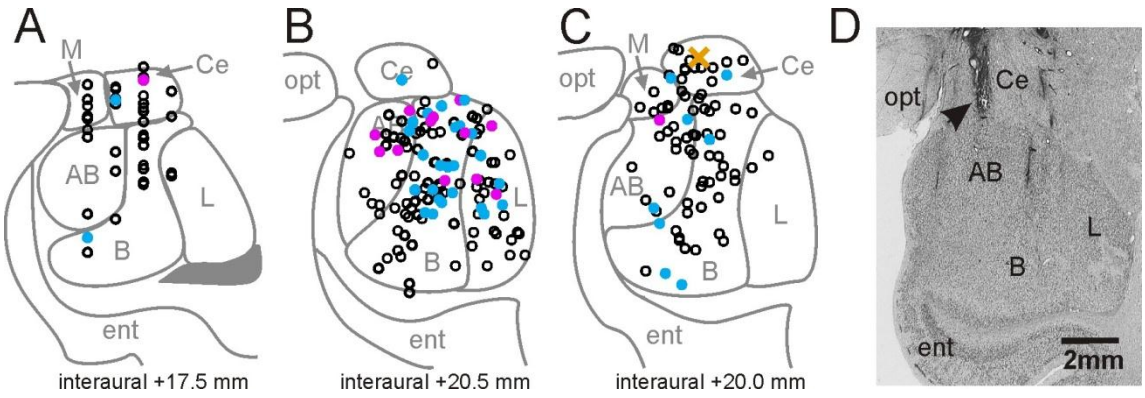

Figure S2, Related to Figure 1: *Histology and MRI-based reconstruction of recording sites*. **(A,B,C)** Sites of neurons recorded from the amygdalae of monkeys G, Z, and Q, respectively. Eye cells are indicated by blue circles. Nonselective-fixation cells are indicated by purple circles. The sites of all other cells recorded in the amygdala are illustrated by open circles. In monkey Q, the projected recording sites were confirmed by an electrolytic lesion placed at the site indicated by the orange X. **(D)** Nissl-stained coronal section of the amygdala of monkey Q, with the site of the electrolytic lesion indicated by the arrow. (Nuclei of the amygdala: AB=accessory basal, B=basal, Ce=central, L=lateral, M=medial; ent=entrorhinal cortex; opt=optic nerve).

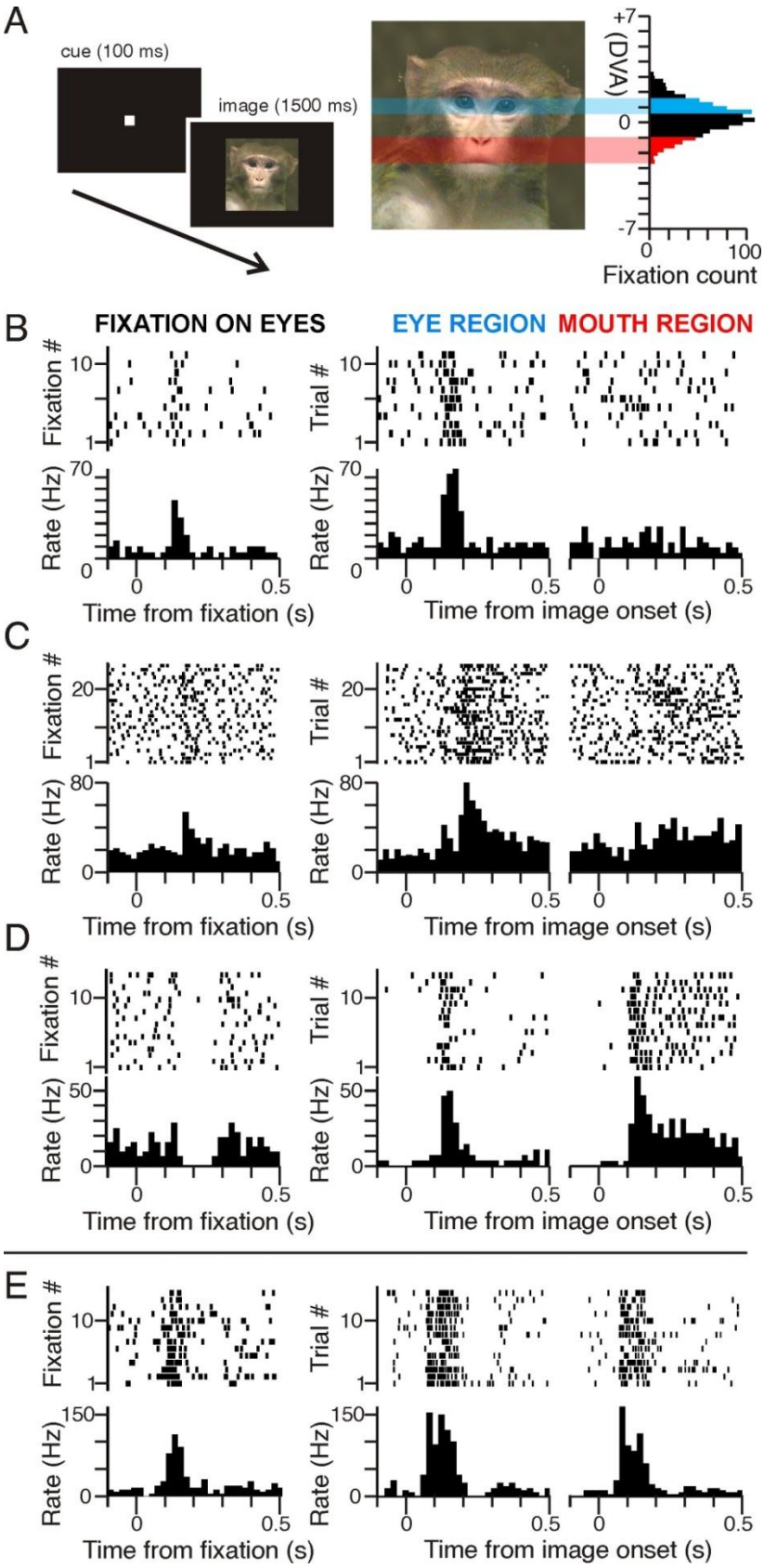

Figure S3, Related to Figure 4: *Eye cells respond independently of saccades.* **(A)** (left) Sequence of an experimental trial; the subjects fixated a cue (white square) to trigger the presentation of a static image of a face. (right) The histogram shows the vertical location of the fixations at image onset. The fixations are distributed around the center of the image. Some fixations, however appear at the level of the eyes (blue) and others around the mouth (red). **(B-D)** Raster plots and peri-event time histograms for three eye cells. The left panel depicts the response of each cell during fixations on the eyes of the movie monkeys. The middle and right panel show the response following the presentation of static faces with either the eyes (middle) or mouth (right) centered at location where the subject was fixating. **(B)** A cell that responded with a phasic increase in firing rate when the eyes were fixated in videos or when the eyes appeared at the center of gaze when the static image was first displayed. **(C)** A cell that responded with a phasic increase in firing rate when the eyes were fixated in videos and with a broader phasic increase in firing rate when the eyes were fixated at the onset of the display of the static image **(D)** A cell that was inhibited during fixations on the eyes during videos was also inhibited when the eyes of a static image fell on the center of the gaze. Note that at ~ 0.15 s after image onset there is a brief increase of firing rate that marks the appearance of the image but is independent of the fixation content. **(E)** A nonselective fixation cell that responds with a phasic increase in firing rate when either the eyes or the mouth appear at the center of gaze.

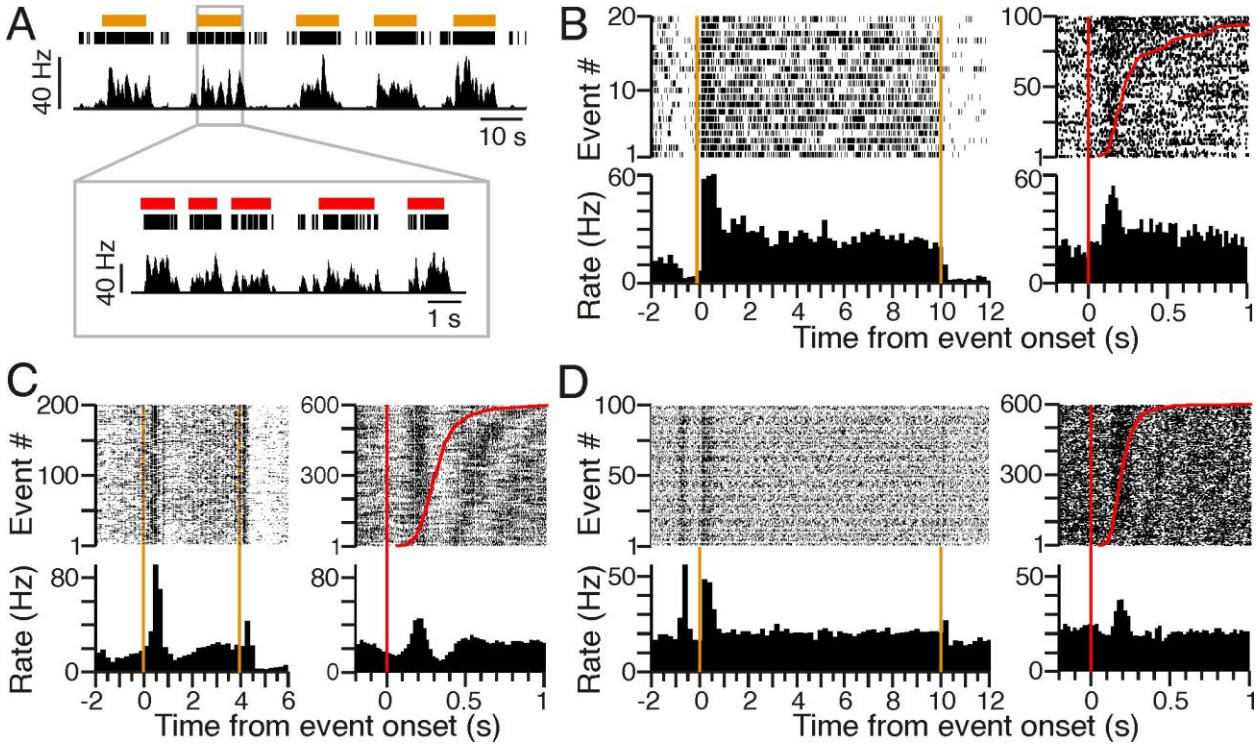

Figure S4, Related to Figure 4: *Nested selectivity of visually-responsive neurons in the monkey amygdala.* **(A)** The spike train and firing rate of cell that was tonically activated by the presentation of video stimuli (orange bars). The same cell shows, at a shorter time scale, significant changes in firing rate during fixations on the eyes (red bars). **(B)** Rasters and perievent time histogram for the neuron shown in panel A. (left) At the onset of the video, this cell exhibited a phasic increase in firing rate that was maintained above baseline levels for the entire duration of the stimulus. (right) The same cell showed secondarily a phasic increase firing rate during fixation on the eyes of a viewer monkey. The elevated firing rate was maintained until the viewer made a saccade away from the eyes (as indicated by the red curved line that marks the end of fixations on the eyes). **(C)** A cell that exhibited a phasic increase in firing rate at the onset and offset of a 4 s video; this cell showed similar increases in firing rate at the start and end of fixations. **(D)** A cell that responded to the onset of the video and of fixation on the eyes with a phasic increase in firing rate. (peri-stimulus bin size=200 ms; peri-fixation bin size =20 ms; instantaneous firing rate binned by convolution with 100 ms gaussian distribution).

Supplemental Experimental Procedures

Surgical procedures

All surgical procedures were carried out in compliance with NIH guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Arizona. A detailed description of the surgeries has been previously reported by Gothard et al., 2007 [S1]. Briefly, three adult male monkeys (*Macaca mulatta*) received a pre-surgical MRI scan to determine the location of the amygdala in stereotaxic coordinates. Each monkey was then implanted with (1) a recording chamber above the right amygdala and (2) titanium posts (Thomas Recording, Germany) for head immobilization during acute neurophysiological recordings. A craniotomy (~13 mm in diameter) was drilled in the center of each chamber. Between recordings the craniotomy was sealed with a silicone elastomer to maintain sterility and prevent scarring of the dura [S2]. MRI with contrast verified the orientation of the chamber relative to the amygdala.

Neural recordings

Single unit activity was recorded from monkeys Z and Q using a custom built 7-channel Eckhorn drive (Thomas Recording, Germany) that advanced 7 microelectrodes (quartz-glass insulated tungsten electrodes, 80-100 μm diameter, 1-2 MΩ impedance) into the right amygdala [S1]. In monkey G, a custom-built NAN drive (NAN Instruments, Israel) advanced into the brain a single reference electrode and 3 recording microelectrodes (quartz-glass insulated tungsten electrodes, 250 μm diameter, 1-2 MΩ impedance). The anatomical location of each electrode tip in the amygdala was calculated based on the post-surgical MRI, a method which has been validated histologically [S1]. Single unit activity was preamplified via a head stage with 20 gain (Thomas Recording, Germany; Neuralynx, Montana), and a headstage with unity gain for the NAN system; the signals were amplified and filtered (1,000 gain; 600-6,000 Hz filter, Lynx-8, Neuralynx, Bozeman, MT, USA), and sampled continuously at 40 kHz (Power 1401, Cambridge Electronic Design [CED], Cambridge, UK). The activity of a neuron was recorded if its firing rate exceeded 0.5 Hz and the signal to noise ratio of the trace exceeded 2:1. As the electrodes were lowered into the amygdala, the monkey was presented with images to (1) keep the monkey occupied and (2) engage the activity of visually-responsive neurons. Single-units were sorted off-line using the Spike 2 template-matching algorithm and principal component analysis (CED, Cambridge, UK).

Stimuli

Video stimuli. Monkeys viewed either 4 or 10 s video clips extracted from raw video footage of macaque (*Macaca mulatta*) behavior. To ensure the stimuli were as natural as possible, no editing was performed and all video footage was continuous in time. Each movie depicted either (1) a single monkey in a plexiglass cage, displaying various social signals and facial expressions, or (2) multiple monkeys engaged in group interactions in the field station of the California National Primate Research Center (for details see [S3]) and freely-ranging monkeys on the island of Cayo Santiago (courtesy of Dr. Lisa Parr, Emory University). In each 10 s video, the movie monkey looked toward or away from the video-camera, providing periods when the viewer monkey could perceive the stimulus as having direct or averted gaze. In total, 126 movies of single monkeys were shown (42 identities x 3 social behaviors). The social group videos depicted 2-7 monkeys engaged in a variety of behaviors, e.g., allogrooming, aggression,

eating, playing. As with the single monkeys, these videos contained segments where at least one of the monkeys looked directly at the video camera. In total, 165 movies of this type were shown. Examples of movie stimuli are provided in Movies S1, S2, and S3.

Static images. To compare the response of eye cells on videos and static images, single frames were extracted from the 4 s video stimuli and presented as single static images. To determine the response of eye cells in the absence of saccades, faces of 20 unfamiliar monkeys were used as image stimuli. The faces displayed neutral expressions and either direct or averted gaze. All faces were superimposed on scrambled backgrounds matched for color and contrast (for an example of the stimuli used see Figure 7 or Mosher et al., 2011 [S4])

Behavioral task and recording procedure

Data were recorded in 18 sessions from monkey Q, 37 sessions in monkey Z, and 16 sessions in monkey G. During each session, 22-100 different videos were presented in pseudorandom succession, 1-5 times each.

Monkeys were seated in a custom-built primate chair with their eyes located at 57 cm from an LCD monitor spanning 37 x 38 degrees of visual angle (dva) with a refresh rate of 60 Hz. The eye-position of the subject monkey was recorded within $\pm$1 dva resolution using an infrared eye-tracker with a sampling rate of 240 Hz (ISCAN Inc., Woburn, MA, USA). The presentation of visual stimuli was executed by Neurobehavioral Systems Presentation software (Albany, California, USA). Custom-made hardware was designed to interface with Presentation so that the display of each video frame could be recorded with 1 ms resolution. The eye-movements of the subject, the display of the stimuli, and the neural recordings were co-registered in time using a CED Power 1401 data acquisition system and Spike 2 software (CED, Cambridge, UK).

Stimuli were displayed in a trial structure. At the onset of a trial, the monkey was presented with a cue subtending 2 dva. Fixating the cue for 100 ms triggered the presentation of either a video or static image. During recording sessions that included both videos and static images, both types of stimuli had equal probability of being presented and were shown in pseudorandom succession. Video stimuli spanned 26 x 18 dva and consisted of 120 frames (4 s) or 300 frames (10 s) displayed at a temporal resolution of 30 frames per second. The corresponding static images also spanned 26 x 18 dva and were also displayed for 4 s. During stimulus presentation, the subjects were free to look anywhere on or off the monitor. At the termination of the stimulus, monkeys G and Z received 0.5-1 mL juice reward; monkey Q received no reward. Each stimulus was followed by a 4-12 s inter-movie-interval during which time the display monitor remained with a blank screen.

Note: To test the response of fixation cells in the absence of saccades (Figure 7), static images were presented at 14 x 14 dva for 1.5 seconds. The monkeys were allowed to freely view the images but were required to keep their gaze within the perimeter of the image at all times.

Histology

The reconstruction of intra-amygdala recording sites was based on histological and MRI analysis. Histological analysis was performed for monkey Q. After euthanasia, the brain was extracted from the skull and submerged in 4% phosphate-buffered formaldehyde (pH 7.2). The block containing the amygdala was sectioned in the coronal plane at 40 µm thickness and the sections through the amygdala were mounted on microscopic slides and stained with the Nissl method to determine the nuclear boundaries and the site of an electrolytic lesion made during his

final experiment (two sequential 100 μA direct current pulses, 10 s in duration). Monkey Z and G are currently involved in ongoing studies, thus precluding histological confirmation of the electrode tracks.

Data Analysis

Analyses were carried out using custom-designed programs in MATLAB R2009 (The MathWorks, Natick, MA, USA).

Classification of eye cells.

A cell was classified as an eye cell if its response met two criteria: (1) a significant change in firing rate time-locked to the onset of fixations, and (2) a significant difference in firing rate during fixations on the eyes compared to fixations on the mouth or other facial region (Wilcoxon ranksum test for both comparisons, $P<0.05$). If a cell met the first criterion but failed to meet the second it was classified as a non-selective fixation cell.

To identify at every time-point where the monkey was fixating, the eye-movements of the viewer monkey were co-registered with the presentation of each video frame. Eye-movements greater than 1 dva were detected automatically -- the onset of each fixation was identified as the point in time when the movement of the eye decelerated to less than 2 dva/s^2. The onset of each fixation was manually verified. Fixations on the eye and mouth region were classified based on regions-of-interest boundaries manually outlined by three experimenters.

Responses of eye cells to eye-contact

Eye-contact was defined as time periods when the viewer monkey fixated the eyes of the movie monkey and the movie monkey's gaze was directed at the viewer. To determine if a neuron responded to eye-contact we used a two-tailed bootstrapping analysis:

(1) We calculated the difference in firing rate between periods of eye-contact and periods when the viewer fixated eyes with averted gaze.
(2) We randomly shuffled the ethogram of the movie monkey's gaze direction 1,000 times, with replacement. Ethograms were shuffled by taking the 10 s ethogram of one video and randomly replacing it with the 10 s ethogram of another video that was presented within the same experimental recording. Shuffling only the ethogram preserves all aspects of the neural data (no shuffling spike times) and all aspects of the viewer's eye-movements (notably the epochs when the viewer fixated the eyes of the movie monkey).
(3) Based on these 1,000 shuffled data, we obtained a distribution of firing rates using the same calculation as in (1).
(4) We compared the empirical firing rate during true eye-contact with the distribution of firing rates obtained from the shuffled data. If the empirical firing rate for eye-contact was greater than 97.5% of the shuffled values then the neuron was classified as an eye-contact cell (two-tail bootstrap, $\alpha=0.05$).

The mean response of each cell during eye-contact and during fixations on eyes with averted gaze was normalized to the average firing rate of the neuron (z-score) and is summarized in Figure S1.

Response latencies of fixation cells.

Neural response latencies were determined by applying the cumulative sum procedure to peri-event time histograms with a binsize of 5 ms (Ellaway, 1978). The cumulative sum procedure involves (1) calculating the mean firing rate during a baseline time period, (2) subtracting the mean baseline firing rate from the value of each bin in the peri-event time histogram, and (3) adding together the values in the histogram that occur within consecutive time bins. The response latency was identified as the time bin at which the normalized cumulative sum measure (z-score) exceeded a value of 2.575 (the 99% confidence level of the z-score measure). For stimulus-evoked responses, the 200 ms period preceding stimulus onset was used as baseline; for fixation-related responses, the 200 ms period preceding the saccade was used.

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