Neural basis of saccade target selection in frontal eye field during visual search

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CONSPICUOUS visual features commonly attract gaze[1,2], but how the brain selects targets for eye movements is not known. We investigated target selection in rhesus monkeys performing a visual search task[3] by recording neurons in the frontal eye field, an area known to be responsible for generating purposive eye movements[4,5]. Neurons with combined visual- and eye movement-related activity were analysed. We found that the initial visual responses to search stimulus arrays were the same whether the target or a distractor was in the response field. We also found that the neural activity evolved to specify target location before the execution of eye movements, ultimately peaking when the target was in the response field and being suppressed when the target was beside but not distant from the response field. These results demonstrate a possible mechanism by which a desired target is fixated and inappropriate eye movements are prevented.

Neurons were monitored in two rhesus monkeys in the rostral bank of the arcuate sulcus where low-intensity microstimulation elicited saccadic eye movements; this property functionally defines the frontal eye field (FEF)[6]. Neurons that were active both in response to visual stimuli and preceding saccadic eye movements were analysed because they were active during target identification and response selection. The activity of neurons was compared during a simple detection task and during a more natural search task (Fig. 1). The activity of one neuron is shown in Fig. 2. In detection trials this cell was active only when the target fell in its response field. In search trials this neuron initially responded to either the target or a distractor in its response field, but the activation changed during the latent period before the movement. If the stimulus in the response field was the target, the discharge rate peaked. If the target was distant from the response field, activation evoked by the distractor in the response field continued through the saccade. If the target appeared near but not centred in the response field, the neuron discharged an abbreviated burst and was then inactive until the saccade.

To quantify the activity associated with visual responses and saccade initiation, discharge rate was measured during a 100-ms interval following stimulus presentation and for 100 ms preceding saccade initiation (Fig. 3). During detection trials neural activity varied with target direction (Fig. 3*a*, *b*). Differential discharge rates were evident as soon as the cell responded to the target stimulus and continued until the saccade was initiated (Fig. 3*a*). During search trials the spatial and temporal pattern of activation was markedly different. Either the target or a distractor in the response field elicited equivalent activation; thus, for 100 ms following the appearance of the search array, target location could not be identified on the basis of neural activity (Fig. 3*c*, *d*). Such a lack of significant variation of the initial visual response to the search array was evident in 25 of 28 neurons recorded in two monkeys (ANOVA, $P > 0.05$). But within 100 ms of the saccade, the pattern of neural activity had changed to vary systematically with target location. The presaccadic activation of 27 of the 28 neurons varied significantly with target direction during search trials (ANOVA, $P < 0.05$). Moreover, the pattern of variation of saccade-related activation in search trials was different from that observed in detection trials due to the suppression of the activation elicited by the distractor in the response field when the target was near the response field. This suppression was observed in 22 of the 27 neurons with significant presaccadic variation. The five neurons in which suppression was not observed had unusual response fields that were either unusually large or located in the ipsilateral hemifield. Suppression strength was measured as the ratio of the activation when the target was beside the response field to the activation when the target was in the hemifield opposite the response field, after subtracting baseline activity from both; cells with no suppression had a ratio of 1.0. The mean ± s.e.m. suppression ratio was 0.66 ± 0.04 (minimum = 0.08).

Visual guidance of eye movements requires information about what is where in the visual field. After each fixation, the target of the next fixation can be selected only as such information becomes available. During visual search, the FEF visual-movement neurons we recorded exhibited a transition from an early nonspecific response to a pattern of activation that specified the target for the upcoming saccade. A similar evolution of neural activity has been observed in inferior temporal cortex during visual search[7], which may influence FEF neurons. Likewise, the growth of activity of presaccadic movement neurons in the superior colliculus reflects the presence of a target or distractor in the movement field[8,9]. The FEF heavily innervates the superior colliculus[10], so the activation of colliculus neurons may reflect the response selection we observed in the

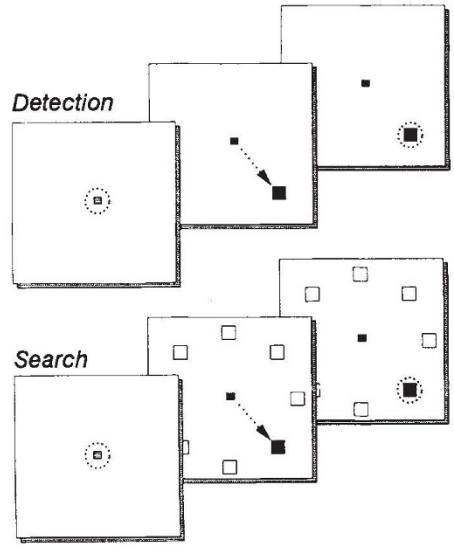

FIG. 1 Detection (top) and search (bottom) trial displays. The dotted circle indicates the required focus of gaze in each interval, and the dotted arrow indicates the saccadic eye movement. Each trial began with presentation of a coloured spot on a video monitor (front panels). After fixation of this spot for 300–500 ms, a target appeared at 1 of 8 locations of equal eccentricity (middle panels). A change of colour of the fixation spot permitted monkeys to shift gaze; for all of the data reported in this paper the colour change of the fixation spot was simultaneous with presentation of the stimuli. Juice reward was given for fixating the target with a single saccadic eye movement with a latency less than 800 ms (rear panels). Baseline neural activity was measured during the fixation period before the target and distractor stimuli were presented. On detection trials the target was presented alone to map the spatial extent of response fields and to identify the type of neuron being recorded. A number of different populations of neurons have been identified in FEF[4,5]. The focus of this study was visual-movement neurons that began to discharge a consistent interval after stimulus presentation and continued firing until the saccade was generated. On search trials the target was presented with 7 distractors all of the same eccentricity; the stimuli were positioned at the optimum eccentricity for each neuron. Stimuli were scaled in size for eccentricity to yield equivalent performance. During search trials the target was distinguished from distractors by colour (red/green or magenta/cyan) or spatial frequency (low/high). Detection and search trials were run in separate blocks. Standard methods for collecting single-neuron activity and eye position were used[16].

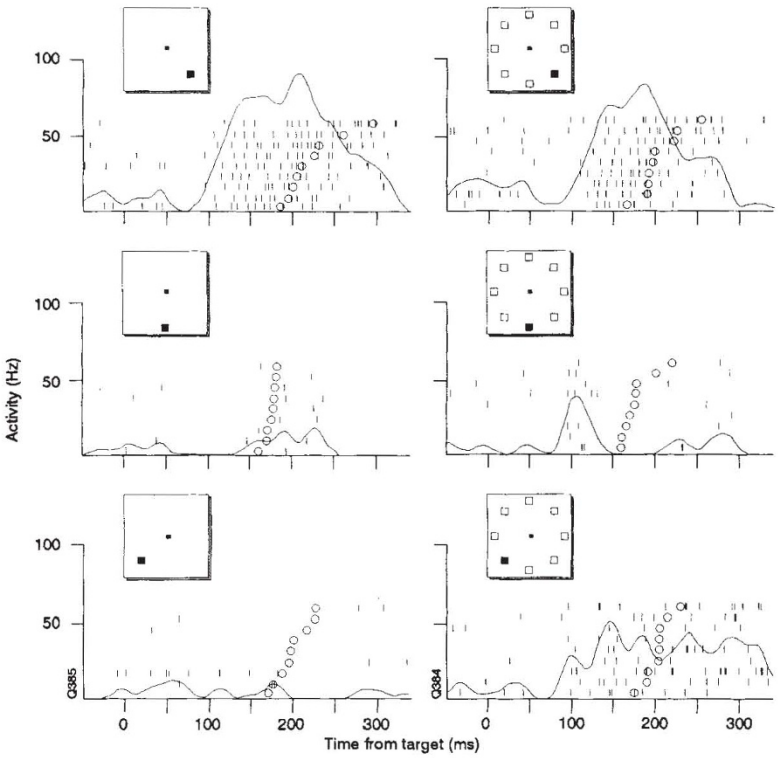

FIG. 2 Neural activity is shown during detection (left) and search (right) trials with the illustrated stimulus configurations. The target was a green 1° square presented either alone or with 7 red 1° square distractors at 10° eccentricity. In the raster displays vertical tickmarks represent times of neuronal discharges, one row per trial. Saccade initiation is indicated by a circle. The rasters are aligned on target presentation and sorted according to saccade latency. Superimposed on the raster is the average spike density function obtained by convolving each spike train with a gaussian filter (σ = 10 ms). The neuron responded before saccades to a lone target only when it fell in the lower right quadrant (upper left panel); the neuron began to fire with a consistent latency of ~90 ms after the target appeared and continued firing until the saccade was executed. Activity remained at baseline when the target appeared at other locations (middle and lower left panels). During search trials a stimulus appeared in the cell's response field on every trial. When the target was in the response field (top right panel), the activation was similar to that observed in the detection trials with the target at the same location (top left panel). When the target was distant from the response field (bottom right panel), the neuron responded to the distractor in the response field and continued discharging at a reduced rate until the saccade. But when the target fell beside the response field (middle right panel), the neuron discharged only briefly, becoming inactive before the saccade.

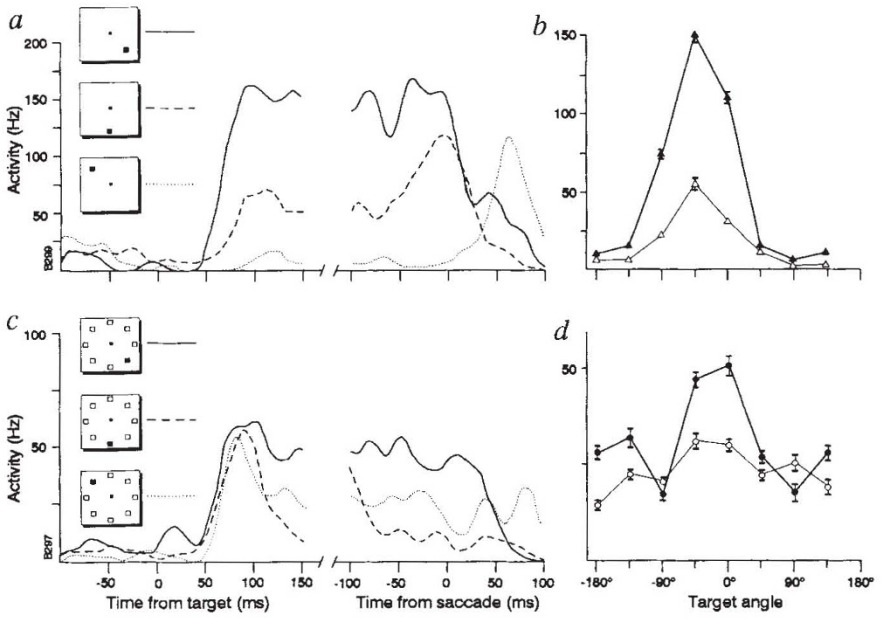

FIG. 3 Evolution of target selection in another FEF neuron. The target was an achromatic 0.33° square, 3 cycle deg^{-1} checkerboard presented alone or with 7 0.33° square, 4.5 cycle deg^{-1} distractors at 4° eccentricity. Average spike density functions are shown for detection trials (*a*) and search trials (*c*) aligned on the time of target presentation (left) or saccade initiation (right). Different line types show the activation associated with the three illustrated stimulus configurations. During detection trials both the visual and the saccade-related activity decreased as the target was presented further from the neuron's response field which was located in the lower right quadrant. During search trials an initial visually evoked response occurred in all trials, and the magnitude of this early activation did not vary with search stimulus configuration. But in the interval before the saccade was initiated the activation of the neuron changed according to the location of the target relative to the response field, being maximal when the target was in the response field (solid line), and suppressed when the target was beside (dashed line) but not distant from (dotted line) the response field. Average discharge rate ± s.e.m. for 100 ms following stimulus presentation (open symbols) and average discharge rate ± s.e.m. for 100 ms before saccade (closed symbols) is plotted as a function of target direction for detection (*b*) and search (*d*) trials. 0° is right, and 90° is up. During detection trials the variation of the visually evoked response with target angle was significant (ANOVA $F(7,65) = 16.4$, $P < 0.001$) as was the variation of the saccade-related activation ($F(7,65) = 86.0$, $P < 0.001$). The pattern of variation with target direction resembled a gaussian curve. During search trials, the neuron responded to the stimulus in its response field on every trial, and the initial response to the stimulus array did not vary significantly with target direction ($F(7,130) = 2.0$, $P > 0.05$). In contrast, the presaccadic activation varied significantly with target location ($F(7,130) = 8.08$, $P < 0.001$). Moreover, the pattern of variation of saccade-related activity with target direction in search trials was markedly different from that in detection trials, resembling a difference-of-gaussian or Mexican hat curve because of the suppression when the target was beside the response field.

FEF. Visual–movement cells, however, probably do not project directly to the superior colliculus or brainstem[11,12] and thus may represent an intermediate processing stage.

Before saccades executed during visual search, FEF visual–movement neuron activity became suppressed if a salient target was near its response field. Perhaps such suppression reduces the probability of an eye movement to a distracting stimulus within a neuron's response field. The central facilitation and surrounding suppression of visual–movement neuron activity resembles the centre-surround receptive field organization of neurons in the early visual system[13]. By analogy to these systems, we surmise that the suppression observed in the FEF arises through lateral inhibition. In fact, models of visual search use topographic representations of the locations of conspicuous features in the image derived from lateral inhibition within feature maps[14,15]. Our findings provide neurophysiological evidence for a mechanism by which conspicuous stimuli in complex scenes may attract gaze. □

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Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack

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EXPRESSION of viral genes in transgenic plants is a very effective tool for attenuating plant viral infection[1–3]. Nevertheless, the lack of generality and risk issues related to the expression of viral genes in plants[4] might limit the exploitation of this strategy. Expression in plants of antibodies against essential viral proteins could provide an alternative approach to engineer viral resistance. Recently, expression of complete[5–7] or engineered[7–9] antibodies has been successfully achieved in plants. The engineered single-chain Fv antibody scFv (refs 10, 11) is particularly suitable for expression in plants because of its small size and the lack of assembly requirements. Here we present evidence that constitutive expression in transgenic plants of a scFv antibody, directed against the plant icosahedral tombusvirus artichoke mottled crinkle virus, causes reduction of infection incidence and delay in symptom development.

A panel of monoclonal antibodies was raised against the artichoke mottled crinkle virus (AMCV) virion using standard methods[12], and the binding properties of individual antibodies were analysed by enzyme-linked immunosorbent assay (ELISA). One of these, the F8 antibody (belonging to the IgG2b subclass), had the highest affinity for AMCV coat protein in both dissociated and polymerized forms. In addition, epitope mapping experiments showed that the F8 antibody recognizes a highly conserved site on the coat protein that is involved in divalent-cation regulated swelling of tombusvirus[13]. For this study, existing data on the crystal structure[14] of the type-member of the tombus group, the tomato bushy stunt virus (TBSV), and on the nucleotide sequence of AMCV coat protein[15] were fundamental.

The hybridoma cell line expressing the F8 antibody was selected to isolate full-length complementary DNA clones of the heavy and light immunoglobulin chains. The variable domains (V_H and V_L) were amplified by polymerase chain reaction (PCR) using 'universal primers' and inserted into vectors for *Escherichia coli* expression of scFv antibody, in which the two variable domains are connected by a linker peptide (Fig. 1).

Bacterial periplasmic extracts were tested for correct expression of the engineered antibody (scFv[F8]) by western blotting (not shown) using the anti-Tag 9E10 antibody (see legend to Fig. 1). The binding activity of the bacterially produced scFv[F8] antibody against AMCV particles was assayed by ELISA. No binding was detected to four other purified proteins (bovine serum albumin, lysozyme, keyhole-limpet haemocyanin, concanavalin A) or to unrelated (cucumber mosaic virus, CMV) and related (TBSV) icosahedral plant viruses (not shown). Thus, scFv[F8] retains the specificity of the parental antibody. Furthermore, a saturation curve radioimmunoassay was used to determine the affinity range of both scFv[F8] and parent antibody. In these assays the amount of bound antibody on antigen adsorbed on solid phase was detected using the recombinant fusion protein LG (ref. 16) as a second radiolabelled reagent. The affinity constant was evaluated as the reciprocal of the antibody concentration giving 50% saturation of binding. Although avidity effects cannot be excluded, the affinity of the scFv was 100-fold lower ($10^7\ M^{-1}$) than that of the original antibody ($10^9\ M^{-1}$).

To determine whether the engineered antibody was effective in neutralizing the virus in the plant, the scFv[F8] construct was cloned into a plant expression vector to yield the pBG-scFv[F8]-BIN plasmid (Fig. 1). This encodes a cytoplasmic version of the scFv[F8] protein, in which the leader sequence for secretion has been deleted. Transgenic *Nicotiana benthamiana* plants, symptomatic hosts for AMCV, were regenerated after *Agrobacterium*-mediated transformation, essentially as described[17]. Northern blot analysis revealed that several (40% of the analysed plants) kanamycin-resistant plants accumulated varying levels of scFv[F8] messenger RNA of the predicted size (Fig. 2*a*). This was confirmed at the protein level by western blotting (maximum expression being 0.1% of total soluble leaf proteins) (Fig. 2*b*); ELISA provided evidence for binding specificity of the plant-derived scFv[F8] antibody (not shown). Comparison of data from ELISA and western blots demonstrated that all of the expressed antibody fragment in transgenic plants is active.

To verify the effects of the scFv[F8] antibody transgene on

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