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Activity of prefrontal neurons during location and color delayed matching tasks

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MANY cells in prefrontal cortex show enhanced activity prior to movement onset in delayed or memory-guided saccade tasks. This activity is a possible neural correlate of spatial attention and working memory. The goal of this study was to determine whether delay activity is evoked when non-spatial cues such as color are used to guide saccades. Monkeys were trained on a saccade target selection task in which they were cued for either the location or color of the rewarded target. When the location of the target was specified explicitly, many cells showed visual responses and delay activity that were spatially selective. Color selective visual responses or delay activity were both rare and weak. However, for many cells, spatially selective delay activity could be evoked when color was used to specify the location of the target. These results indicate that color is capable of eliciting spatially selective activity from cells that have no overt color selectivity. *NeuroReport* 10:1315–1322 © 1999 Lippincott Williams & Wilkins.

Key words: Color; Prefrontal cortex; Macaque; Space

Introduction

One of the most widely held notions about the organization of primate visual cortex is that there are parallel functional streams for spatial information on the one hand and object attributes such as shape and color on the other [1,2]. These streams are believed to correspond respectively to visual cortical areas of the parietal and temporal lobes. The idea that there is functional segregation in posterior visual areas has led to the hypothesis, which has been rapidly gaining support, that information about spatial location and object identity is re-integrated in prefrontal cortex by the anatomical and functional convergence of the dorsal and ventral streams. This is an appealing idea because prefrontal cortex has been implicated in voluntary movement control (reviewed in [3]) and many such behaviors depend on both spatial and feature-based sensory representations. For example, searching for a familiar face in a crowd might depend on the ability to use a representation of the person's face to compute spatially coded motor commands for eye movements. It is therefore worthwhile to work out the details of how cues such as shape and color interact with spatial representations that may be used to guide movement.

Prefrontal cortex appears to play a key role in delayed response tasks that require subjects to attend to and remember a spatial location or object attribute [4–8]. Several previous studies have looked at the integration of shape and location cues in prefrontal cortex [9–14], and also at the integration of color and location [15–19]. These studies vary with respect to several particulars, including the region of prefrontal cortex investigated (areas 8, 12, 45, 46, or some combination), the type of cue used (color or shape), and the type of behavioral response required (hand movement or eye movement). The present study used electrical microstimulation to locate the small saccade region of the frontal eye fields (FEF; areas 45 and 8c), where there is anatomical evidence for convergence of inputs from parietal and temporal visual areas [20,21]. Recordings were taken from this area as well as the adjacent cortex on the anterior bank of the arcuate sulcus (area 8a). The behavioral task was a delayed saccade to a target cued either by location or color. In addition, we have looked specifically at the issue of overt color selectivity, and we have also analyzed activity both during the delay and pre-saccade intervals of the task. Some previous studies are similar to the current study, but there are still important differences. Fuster and colleagues [17–19] have looked at activity in area 46 using color cues and a manual response task. Several studies have used shape as a cue in a variety of prefrontal areas, including FEF [9–14]. Schall and colleagues [15,16] have looked at presaccadic activity in FEF using color as a cue in a visual search task. The unique contribution of this study is to show that spatially selective delay activity can be evoked by color cues in FEF neurons that lack overt color selectivity, and that this same population of neurons may be involved in saccade target selection based on either spatial or color cues.

Materials and Methods

General: Experiments were conducted on two juvenile male rhesus monkeys (*Macaca mulatta*). Our methods were approved by the Institutional Animal Care and Use Committees at Columbia University and the New York State Psychiatric Institute. Monkeys were trained to move voluntarily from their home cage to a primate chair. A method modified from Wurtz [22] was used to train each monkey to attend a stationary target. Sterile surgery was then performed under general anesthesia (isoflurane 1–3%) to implant a coil of wire on one eye [23] and to secure a post to the skull for head restraint. For all subsequent training and experiments, the monkey's head was secured to the primate chair and a set of field coils was lowered over the chair so that we could use a magnetic search coil to monitor horizontal and vertical eye position.

Behavioral tasks: Monkeys were trained to make voluntary saccades to targets presented on a color CRT monitor. Trials were initiated by requiring the monkey to look at a central fixation target. After a short interval, an instructional cue was presented, and after another delay interval, one or two peripheral targets appeared. At the same time, the central fixation light was turned off and the monkey was required to make a saccade to the cued target. Delays were typically 1000 ms in duration, but occasionally shorter. The monkey's performance was monitored by tracking his eye position relative to a ±3° fixation window centered around the target. Correct performance of the task was rewarded with drops of fruit juice or water.

The response fields of prefrontal neurons were mapped using either a computer mouse-controlled bar or a mapping task in which on individual trials a single stimulus (white square, 1°, 15 cd/m^2) was presented at one of 16 locations centered on the fixation point at the center of the screen. These 16 locations comprised eight directions spaced 45° apart and two eccentricities, generally 6 and 12° but sometimes scaled to accommodate neurons that preferred larger or smaller eccentricities. In addition, the mapping task included 'go' and 'no-go' trials in which the animal either made a saccade to the peripheral stimulus or maintained fixation, respectively. The behavioral condition was signaled by the color of the initial fixation target at the beginning of each trial; white for go trials and yellow for no-go. For the three delayed saccade tasks, one target was always placed squarely inside the response field and the other target was placed at an equal eccentricity 180° away. The range of target eccentricities was 6–15° (mean 10.1°), although it should be noted that due to the limited resolution of the mapping procedure, there is no guarantee that the targets were always placed at the optimal eccentricity for a given neuron.

Eye movement recording: Eye position was monitored using a monocular scleral search coil system (CNC Engineering). Separate horizontal and vertical eye position signals were fed through an analog differentiator (lowpass, −3 dB at 25 Hz) to yield horizontal and vertical eye velocity. The eye position and eye velocity signals were then digitally sampled by computer at 1 kHz/channel and stored on disk for further analysis. We used an automatic algorithm to detect the first saccade that occurred during a 400 ms interval after the go signal (fixation target offset). The algorithm used an acceleration criterion of 500 deg/s^2 to detect the beginning and end of each saccade. Eye acceleration was computed by off-line differentiation of eye velocity.

Visual stimulation: Visual stimuli were generated and controlled by a CRS VSG2/3F video framebuffer with an on-board microprocessor (Texas Instruments TMS 34020). The output from the video board was displayed on a calibrated 27 inch (Mitsubishi) color monitor with a 60 Hz noninterlaced refresh rate. The monitor was placed at a viewing distance of 30 inches so that the display area subtended roughly 40° horizontally by 30° vertically. The spatial resolution of the display was 1280 pixels by 1024 lines, and the depth was 8 bits/pixel. Saccade targets were small (1.0°) colored squares presented on a uniform gray background. The target luminance was 15.0 cd/m^2, while the background was 5.0 cd/m^2. The framebuffer was programmed to send out digital pulses (frame sync) for timing purposes at the beginning of each frame in which a stimulus was turned on. These pulses were sampled by the computer and stored along with the eye movement data.

Neuronal recording and stimulation: Stainless steel recording chambers were implanted at stereotaxic coordinates 15–18 L, 20–25 A, following previous studies of frontal eye fields [24]. Neuronal activity was recorded using tungsten or platinum–iridium microelectrodes (impedance 0.5–2.0 MΩ). The electrode signal was amplified, filtered and passed through a time-amplitude window discriminator to separate action potentials from background noise. The time of occurrence of each action potential was recorded with a resolution of 0.01 ms. Electrical microstimulation was used to determine whether selected recording sites were located within the FEF [25,26]. Trains of biphasic pulses (70 ms; 0.2 ms/phase, 200 or 350 Hz) were delivered while monkeys fixated a central target, which was turned off for 500 ms as the electrical stimulus was delivered. Pulse amplitude was varied between 0 and 100 μA to ascertain the threshold for electrically evoked saccades. At 11 of 30 sites, saccades were evoked with currents $< 50\,\mu$A, and the average saccade amplitude was 2.5° (range 1.2–4.9°). Most recording sites were within 2–3 mm of the site of an evoked saccade and no recording site was > 5 mm anterior. These results place the majority of recording sites within the small saccade region of the FEF (area 45 and the ventral portion of area 8c) and the adjacent cortex on the anterior bank of the arcuate sulcus (area 8a; [21]).

Results

We trained two monkeys to perform variants of a standard oculomotor delayed response task. In these tasks, an instructive cue was presented briefly, followed by a short time delay. After the delay, the monkey executed a saccadic eye movement to one of two possible targets. The monkey was rewarded if his choice of target matched some aspect of the cue presented before the delay. In the location cue task, the monkey was informed of the exact location of the rewarded target by a small white square that was flashed for 300 ms at the location where that target would appear after the delay interval. Figure 1 (left column) shows the activity of a prefrontal neuron during this task. The cell had a strong transient visual response to the appearance of the cue in its receptive field, followed by sustained activity during the delay interval. The delay activity was significantly stronger when the cue was presented inside the receptive field (Fig. 1a, mean firing rate 17.2 spikes/s) compared with outside (Fig. 1b, mean firing rate 5.6 spikes/s). The difference (inside–outside) is shown in Fig. 1c.

We found that 56 of 103 (54%) prefrontal neurons

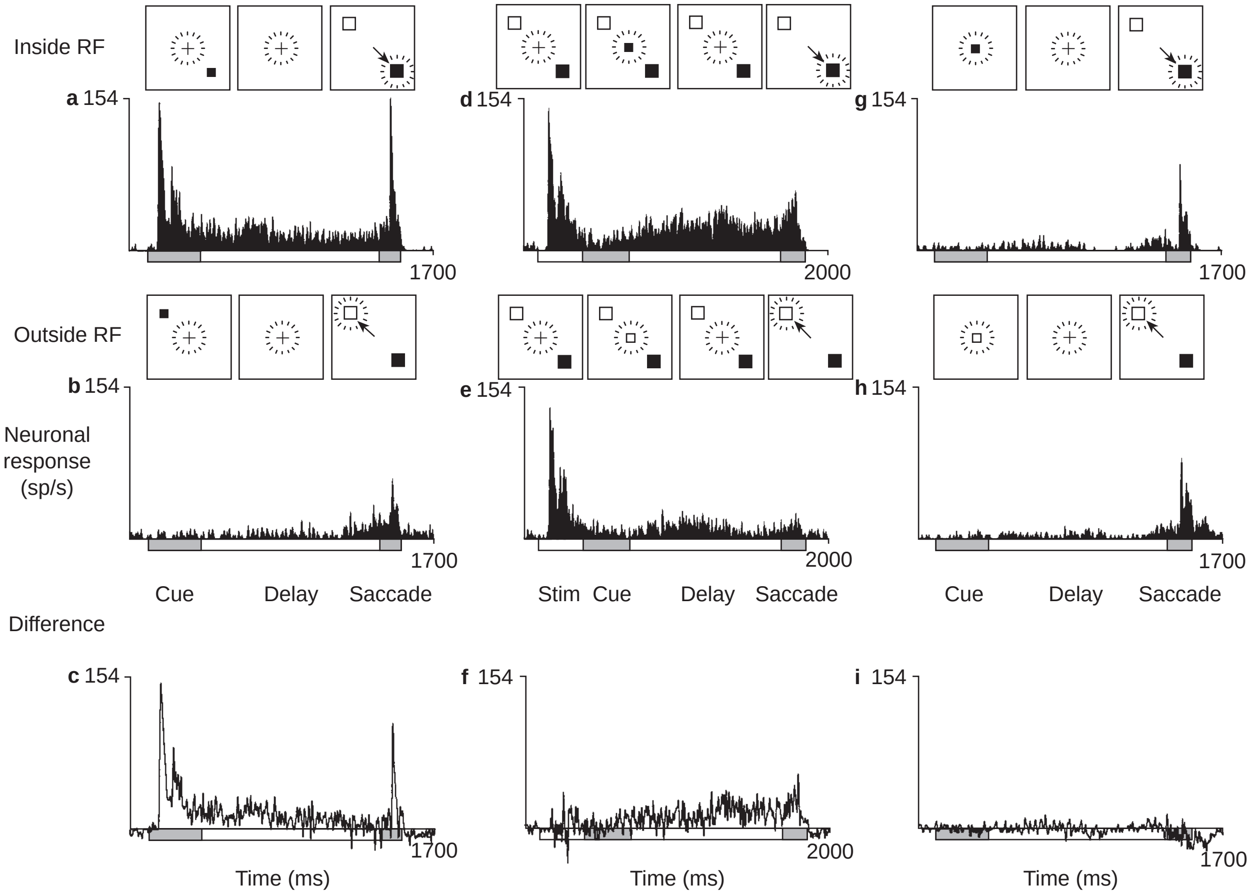
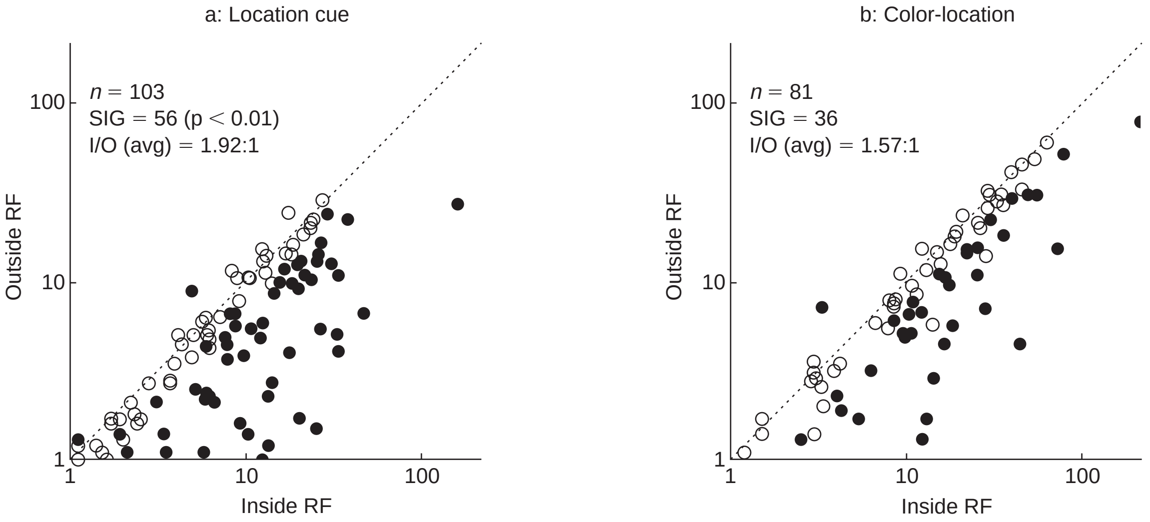


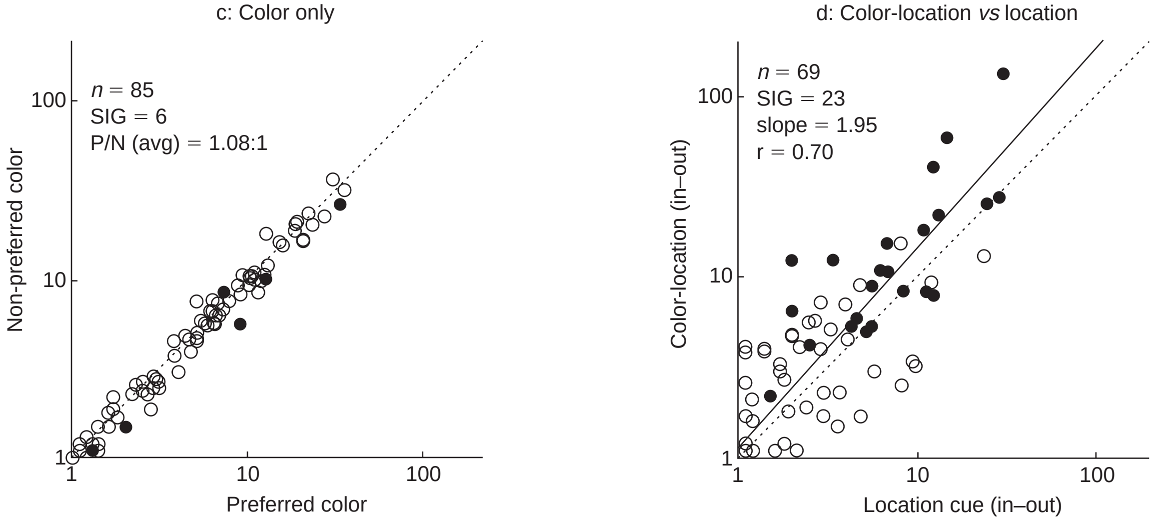
FIG. 1. Activity of a single prefrontal neuron during three delayed response tasks. All three tasks ended with the monkey making a saccadic eye movement to one of two possible targets. (**a**,**b**,**c**) In the location cue task, a peripheral stimulus was presented during the cue interval to signal the location of the rewarded target. The boxes above each histogram are representations of the visual display the monkey was viewing. The (+) is the initial fixation target, the small black square is the cue, and the larger black and white squares are the targets. The spoked ring indicates the desired eye position and the arrows indicate the direction of the saccade. Neither of these were shown to the monkey. (**d**,**e**,**f**) Activity during the color-location task. The target array was presented at the beginning of the trial and the color of the rewarded target was provided by a central cue 300 ms after the onset of the target array. (**g**,**h**,**i**) Activity for the color only task. The three tasks were run concurrently with their trials randomly interleaved. Histograms were smoothed using a Gaussian filter with s.d. = 4 ms. The neuron's RF was in the lower right quadrant and the stimulus eccentricity was 6°.

had delay activity that was significantly stronger (unpaired *t*-test, $p < 0.01$) when the cue was presented inside the neuron's response field compared with when the cue was presented outside the response field (Fig. 2a). Averaged over all 103 cells, the delay activity was almost twice as strong when the cue was inside the RF as when it was outside (average inside:outside ratio = 1.92:1).

In the first color cue task, two peripheral targets were presented at the start of each trial and the monkey was cued for the color of the rewarded target. The color and location of the rewarded target were independently randomized across trials so that there was no long term-association between a particular color and location. Nevertheless, on each trial, the monkey could use color to infer the correct location by inspection of the target array. We refer to this as the color-location task. Activity of a typical neuron is shown in Fig. 1 (middle column). The average firing rate during the delay interval on inside RF (Fig. 1d) trials was 24.3 spikes/s, while that on outside RF (Fig. 1e) trials was 10.4 spikes/s. For the population of 81 neurons, 36 (44%) showed stronger delay activity when the rewarded target was inside the response field of the neuron than when the rewarded target was outside the RF (unpaired *t*-test, $p < 0.01$). Averaged over all 81 cells, activity during the delay was roughly 60%


a: Location cue

b: Color-location


c: Color only

d: Color-location *vs* location

FIG. 2. Average firing rate (spikes/s) during the entire delay interval for all three tasks. Each data point represents a single neuron. All plots are in log–log coordinates. n is the total number of cells in each condition, SIG is the number showing significant spatial selectivity, I/0 and P/N are the average response ratios inside:outside or preferred:null. (**a**) Delay activity during the explicit location cue task. Delay activity evoked when the cue was inside the response field is plotted against activity when the cue was outside the RF. Filled circles are cells that showed a significant difference (unpaired *t*-test, $p < 0.01$). The average ratio inside/outside was 1.92:1. (**b**) Delay activity during the color-location cue task. (**c**) Delay activity during the color only task. The preferred color was determined by the sensory response to a single red or green target presented inside the RF. (**d**) Comparison of the raw difference in firing rate (inside RF−outside RF) for the explicit and implicit cue tasks. Filled symbols represent cells that had significant spatially selective delay activity on both tasks. The solid line is the linear regression of y *vs* x (slope = 1.95, r = 0.7).

stronger when the cue indicated the target inside the RF than when the target was outside (Fig. 2b; average inside:outside ratio = 1.57:1).

Color alone was not effective in activating these cells. While nearly all cells gave vigorous sensory responses when colored targets were presented inside the RF, few cells showed any preference for red or green. Color-selective activity was tested in a variant of the color location task in which the color cue appeared before the delay but the target array did not appear until after the delay (Fig. 1, right column). Hence, during the delay interval, the monkey knew the color of the correct target, but he did not know where it would appear. One would not expect to find spatially selective delay activity with this task and in fact only one cell out of 85 gave a significant result (unpaired *t*-test, $p < 0.01$) when delay activity was sorted by target location. Six cells showed significant color-selective delay activity, but the difference between the preferred and non-preferred colors was rather small (average preferred:non-preferred ratio = 1.08:1, Fig. 2c). It should be noted that each cell's preferred color was assigned on the basis of its sensory response to a single red or green target presented within the RF (single target trials were randomly interleaved with two-target trials). As overt color selectivity was virtually absent, the classification of cells according to color preference was essentially random. Hence, there is no reason to expect that the color preference exhibited during the delay would correspond to the sensory preference, and this is confirmed by the fact that the points in Fig. 2c appear to be randomly distributed around the 45° line.

Sixty-nine neurons were tested with both the color location and location tasks, and 23 (33%) showed significant spatially selective delay activity for both tasks. Figure 2d compares the raw difference in average delay activity (inside−outside) for the two tasks. Overall, there was a fairly strong correlation in the strength of delay activity evoked by the two tasks ($r = 0.7$). However, many cells were significantly activated by one task but not the other (13/69 by the location task alone, and 6/69 by the color location task alone).

For most cells, delay activity evoked by the location cue task was constant over the time course of the delay interval, but delay activity evoked by the color location task increased over time. To examine the time course of delay activity, we selected 18 cells that showed significant spatially selective delay activity during both tasks ($p < 0.01$) and that were tested with a delay of 1000 ms. The activity of these cells was averaged to construct composite time histograms (Fig. 3). For the location cue task (left column), activity was maintained at a constant level throughout the delay. For the color location task, there was an equipotent visual response before the cue. After the cue, the visual response slowly evolved to signal the location of the rewarded target. It took about 100–200 ms from cue offset for the differential activity to rise above baseline, consistent with behavioral observations on the time course of attentional shifts [27].

The design of these experiments allowed us to assess whether neural activity is movement-related or purely sensory. For any given target array, the monkey may make a movement either into or away from the cell's RF depending on which target has been cued. Over a series of trials, different movements are made while the visual stimulus (i.e. target array) remains constant. If the neuronal response is purely sensory, then it should also remain constant. But if the response varies with saccade direction, then it may be related to the attentional or motor aspects of saccade target selection [15,16]. We quantified movement-related activity for each cell by calculating the firing rate during a 50 ms interval before the onset of the saccade on each trial. For the location cue task, 44/103 (43%) cells showed significant spatially-selective pre-saccadic activity and the average strength of modulation was 34% (Fig. 4a). Interestingly, both the proportion of significant effects and the strength of effect were smaller than what was found for the delay activity on the same task (Fig. 3a), and there were more cases where activity was actually stronger preceding saccades made to the target outside the RF. For the color location task, the same proportion of cells (37/81, 46%) showed significant effects but the average effect was almost twice as strong as in the location task. For color alone, the results were about the same as the location task when activity was sorted by target location (Fig. 4c), but there was virtually no effect when activity was sorted by target color (Fig. 4d).

Discussion

Anatomical studies indicate that the small saccade region of the frontal eye field (sFEF) receives input from both parietal and temporal visual areas [20,21]. This raises the possibility that sFEF integrates information about both the identity and the location of attended objects and uses this information to select targets for saccadic eye movements. Our results confirm earlier reports that this area lacks overt feature selectivity [16,28]. Nevertheless, color can selectively activate neurons in this area when it specifies the location of a saccade target. Work by Schall and colleagues [16] has also emphasized that FEF neurons lack patent feature selectivity, but

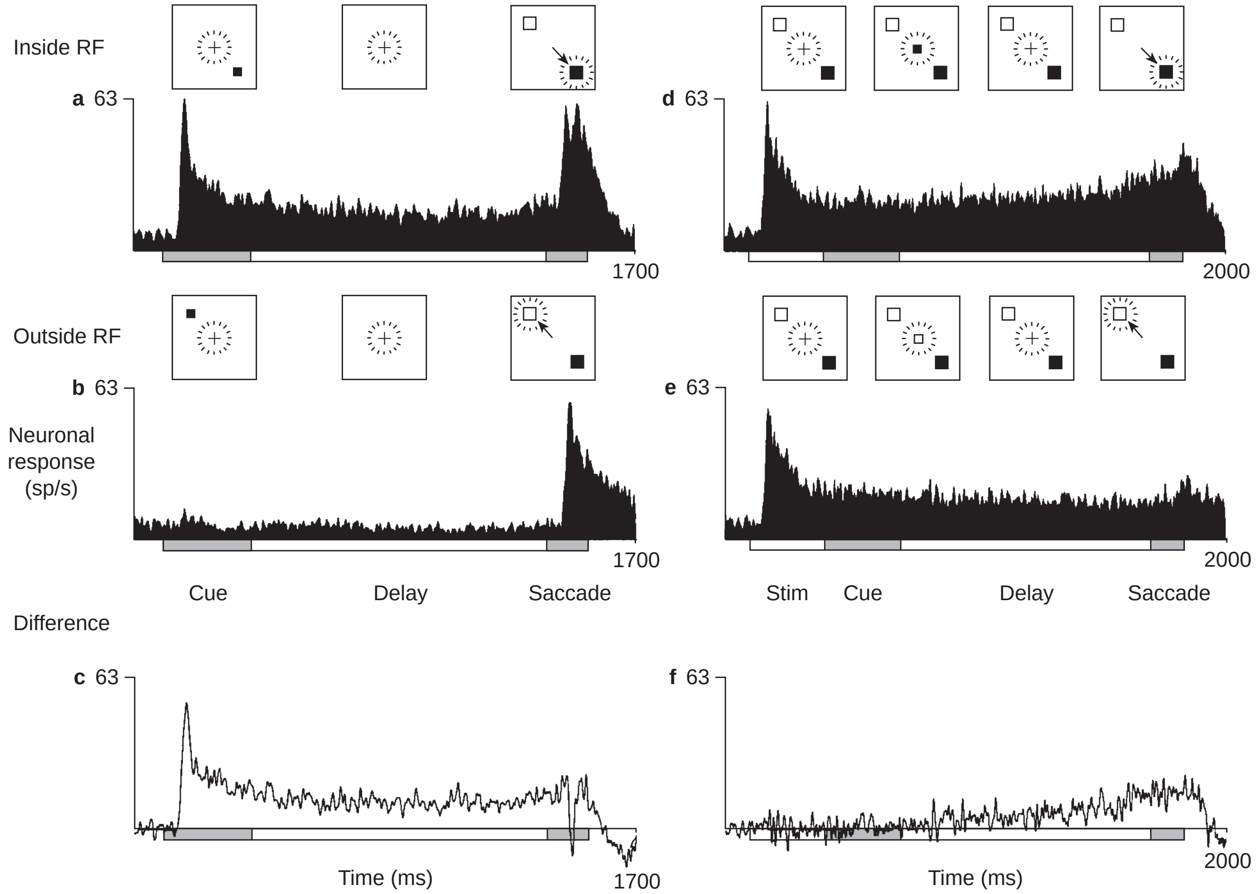


FIG. 3. Average activity of 18 cells with statistically significant spatial selectivity during the delay interval. (**a**–**c**) location cue. (**d**–**f**) Color location cue. The average histograms for all cells were added together and normalized by the number of cells. The activity of each individual cell was not normalized before being added to the total.

respond selectively when form or color is used as a cue in parallel visual search tasks. In their experiments, the pre-saccadic activity of the neurons was selective for the location of the most salient visual target. Our experiments extend these results by showing that spatial selectivity is present during the delay interval, well in advance of the movement. There are some reports of a small proportion of overtly color selective cells in dorsolateral prefrontal cortex (area 46), but the degree of selectivity was not reported [17–19].

Work from several laboratories [9,10,14] has provided evidence for a convergence of location and shape information in prefrontal cortex. In some of these studies, prefrontal neurons showed overt shape selectivity apart from their spatial selectivity. If feature selectivity in prefrontal cortex reflects the organization of posterior visual areas, then it is possible that there are significant differences in the way that color and shape are represented in prefrontal cortex. Shape is represented explicitly in both temporal [29,30] and parietal visual areas [31], but available evidence suggests that color is not strongly represented in parietal cortex [32].

It is not clear whether the cells that are modulated during both the location and color location tasks represent a site where spatial and color information are integrated or whether these cells are merely downstream from the site of integration. It is possible that the activity we have observed reflects the output of a decision process that selects the location of the saccade target and then relays this decision to FEF. In this case, the delay activity might be interpreted as a motor signal that is tied to the production of the saccade. We think this is unlikely simply because such a scenario would lead to the expectation that cells show the same pattern of delay activity regardless of the task. In fact, both the strength and time course of spatial delay activity were task dependent, suggesting that location and color cues act through different mechanisms.

When pre-saccadic activity was analyzed, it was found to be substantially task dependent. This is not surprising when one considers that most cells had strong visual responses to stimuli presented inside the response field regardless of the direction of the saccade, although the response was generally stronger when the saccade was directed inside the RF.

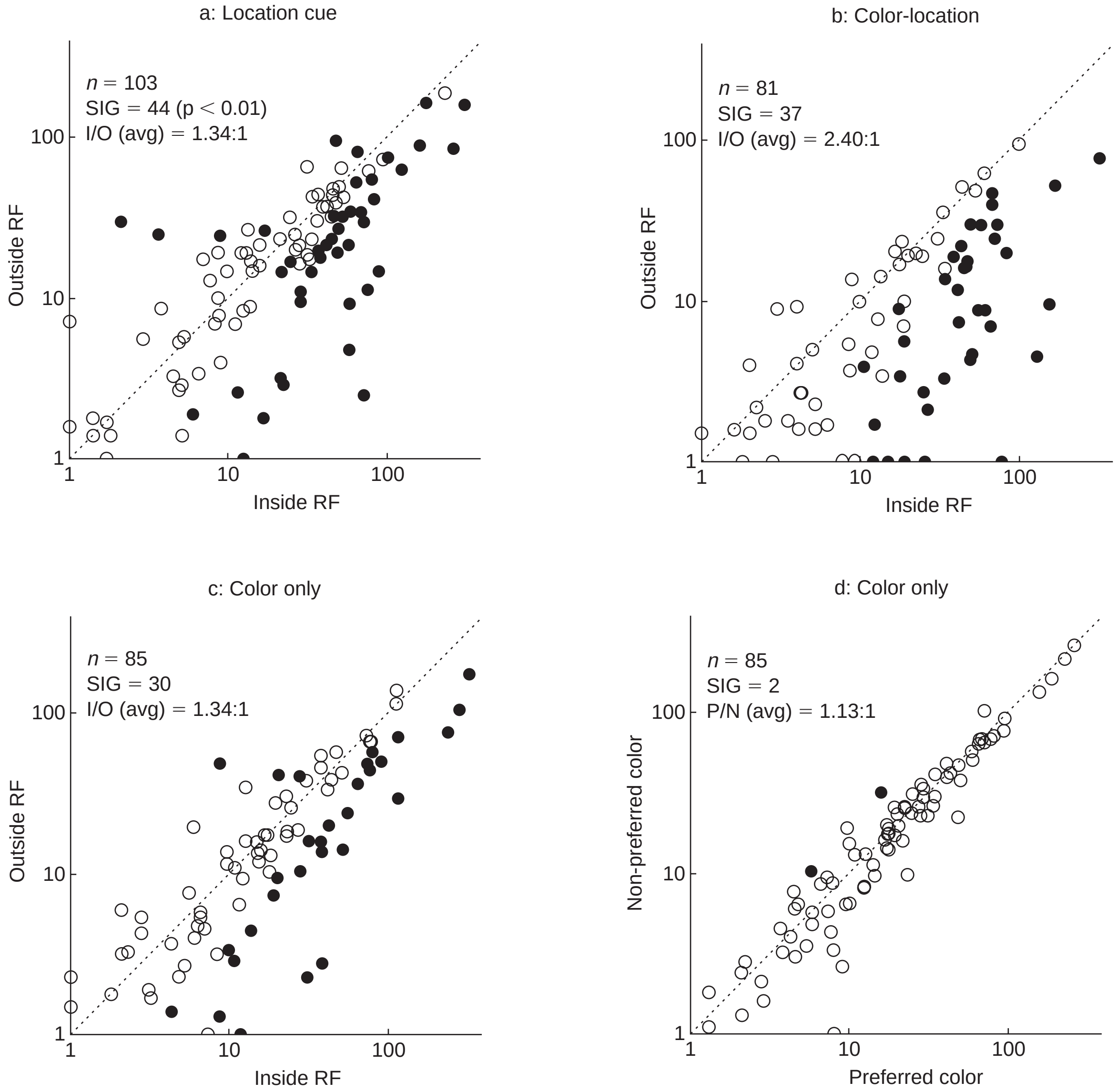


FIG. 4. Average firing rate during pre-saccadic interval for three tasks. Conversions are the same as for Fig. 2. (**a**) location task. (**b**) Color location task. (**c**) Color only task sorted by target location. (**d**) Color-only sorted by target color.

When a target appears suddenly in the RF just prior to the saccade, as in the location and color only tasks, a visual response leaks through even when the saccade is directed elsewhere. In the color location task there is no abrupt stimulus onset near the time of the movement and this may be why the presaccadic activity is stronger. Visual transients (both onsets and offsets) are powerful stimuli for capturing attention and others have noted that they can dominate neuronal responses in parietal cortex [33]. The same appears to be true of FEF neurons. The task-dependence of pre-saccadic activity can thus be explained by the predominantly visual nature of the neurons in our sample and may reflect an attentional modulation of this visual response.

Although we have emphasized the aspect of these experiments that deals with the integration of color and location cues, it seems likely that there are differences in the tasks that go beyond physical differences in the manner of cue presentation. For example, the location cue task may selectively tap into a transient mechanism for pre-attentive orienting [34]. In the color location task, attention may initially be divided between the two peripheral targets, but then gradually shift to the location of the target that matches the color of the cue [35]. Hence, these tasks may activate different attentional mechanisms or activate the same mechanism in different ways. Differences in the way that spatial attention is deployed in the two tasks may account for the different time courses of delay activity shown in Fig. 3.

Conclusion

Spatially selective activity can be evoked in FEF neurons when a color cue is used to indicate target location, even in cells that have no overt color selectivity. Furthermore, many cells in this area show spatially selective activity during the presaccadic interval, regardless of whether the target is specified by location or color. This region may be part of a neural substrate that links feature-based visual representations with eye movements.

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