Impact of Experience on the Representation of Object-Centered Space in the Macaque Supplementary Eye Field

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ABSTRACT

Many neurons in the macaque supplementary eye field (SEF) exhibit object-centered spatial selectivity, firing at different rates when the monkey plans a saccade to the left or right end of a horizontal bar. Is this property natural to the supplementary eye field or is it a product of specialized training in the laboratory? To answer this question, we monitored the activity of single SEF neurons in two monkeys before and after training to select eye-movement targets by an object-centered rule. During stage 1, the monkeys performed a color delayed-match-to-sample (DMS) task in which a red or green central cue dictated an eye movement to the matching end of a horizontal bar. Many neurons at this stage exhibited object-centered spatial selectivity. During stage 2, the monkeys performed a color-conditional object-centered task in which a green or red central cue instructed an eye movement to the left or right end of a gray bar. More neurons exhibited object-centered spatial selectivity during this stage than during stage 1. During stage 3, the monkeys again performed the color DMS task. The fraction of neurons exhibiting object-centered spatial selectivity remained at a level comparable to that observed during stage 2 and above that observed during stage 1. Thus object-centered spatial selectivity was present before training on an object-centered rule, was enhanced as a product of object-centered training, and outlasted active use of an object-centered rule. We conclude that neural representations of object-centered space, naturally present in the primate brain, can be sharpened by training.
INTRODUCTION

The SEF, an area on the dorsomedial shoulder of the frontal lobe, has traditionally been regarded as serving oculomotor functions. This view is supported by the fact that intracortical microstimulation of the SEF elicits saccadic eye movements (Fujii et al. 1995; Lee and Tehovnik 1995; Mann et al. 1988; Martinez-Trujillo et al. 2003a,b, 2004; Missal and Heinen 2001, 2004; Mitz and Godsalk 1989; Russo and Bruce 1993; Schall 1991a; Schlag and Schlag-Rey 1985, 1987a, b; Tehovnik and Lee 1993; Tehovnik et al. 1994, 1998, 1999; Tehovnik and Slocum 2000; Tehovnik and Sommer 1996, 1997; Tian and Lynch 1995) and by the fact that SEF neurons are active during the planning and execution of saccades (Bon and Lucchetti 1991, 1992; Chen and Wise 1995a, b, 1996, 1997; Coe et al. 2002; Fujii et al. 2002; Hanes et al. 1995; Lee and Tehovnik 1995; Mushiake et al. 1996; Olson and Gettner 1995, 1999, 2002; Olson et al. 2000; Olson and Tremblay 2000; Russo and Bruce 1996, 2000; Schall 1991a,b; Schlag and Schlag-Rey 1985, 1987b; Schlag et al. 1992; Schlag-Rey et al. 1997; Tremblay et al. 2002).

SEF neurons represent saccade direction with respect to either a retina-centered or a head-centered reference frame. Electrical stimulation at some sites elicits saccades of fixed direction regardless of initial gaze angle, a result suggestive of retina-centered coding (Martinez-Trujillo et al. 2003a, 2004; Russo and Bruce 1993; Schlag and Schlag-Rey 1987a,b). Electrical stimulation at other sites elicits saccades to a fixed end-point, as expected from head-centered coding (Bon and Lucchetti 1992; Lee and Tehovnik 1995; Mann et al. 1988; Mitz and Godsalk 1989; Schall 1991a; Schlag and Schlag-Rey 1985, 1987a,b; Tehovnik and Lee 1993; Tehovnik et al. 1994, 1998, 1999; Tehovnik and Slocum 2000; Tehovnik and Sommer 1996, 1997). Some neurons are selective for saccades in a fixed direction regardless of starting point, as expected from retina-centered coding (Russo and Bruce 1996), while others are selective for saccades that bring the gaze to a given angle, as expected from head-centered coding (Mann et al. 1988). Recent studies, including those involving head-unrestrained animals, have shown that the nature of the egocentric reference frame may vary regionally within the SEF (Martinez-Trujillo et al. 2004; Park et al. 2006).
The contribution of the SEF to saccadic control must occur at a comparatively high level, for neuronal activity reflects many factors other than saccade direction. Factors known to influence neuronal activity in the SEF include the nature of the rule underlying selection of the target (Olson et al. 2000), the presence of conflict (Amador et al. 2004; Nakamura et al. 2004; Schlag-Rey et al. 1997), the occurrence of an error (Stuphorn et al. 2000), the anticipation of reward (Amador et al. 2000; Stuphorn et al. 2000), the learning and retention of arbitrary associations between visual stimuli and saccades (Chen and Wise 1995a, b, 1996, 1997; Mann et al. 1988), the ordinal position of a saccade in a planned sequence (Isoda and Tanji 2002, 2003; Lu et al. 2002) and execution of a hand movement (Fujii et al. 2002; Mushiake et al. 1996).

One of the most striking observations in support of the view that the SEF serves high-order functions is the finding, obtained in monkeys trained to select saccade targets by an object-centered rule, that around half of SEF neurons are selective for the object-centered location of the target, firing differentially before saccades to the right or left end of a horizontal bar even when the direction of the saccade is held constant (Olson 2003; Olson and Gettner 1995, 1999; Olson and Tremblay 2000; Tremblay et al. 2002). Object-centered signals in the SEF represent an abstract form of spatial information – the location of the target relative to the object – independent of concrete factors such as the nature of the cue conveying the instruction (Olson and Gettner 1999) or the physical properties of the object (Olson and Tremblay 2000). Although we have adopted the convention of referring to neurons as exhibiting “object-centered” selectivity if they are sensitive to the location of the target on the bar and “saccade direction” selectivity if they are sensitive to the physical direction of the saccade, we do not mean to rule out the interpretation that object-centered neurons represent the direction of the planned saccade as defined relative to an object-centered frame. Recently, there have been indications that neurons in other brain areas, including the superior colliculus (Horwitz et al. 2004b) and posterior parietal cortex (Chafee et al. 2005), also encode the object-centered location of a target selected by the monkey for action or attention.

Is object-centered spatial selectivity a natural property of neurons in brain areas where it has been observed or is it a result of training? The answer to this question has not been evident from the results of studies documenting the phenomenon up to this time.
because all such studies were carried out in monkeys trained to select targets on the basis of their object-centered location. To resolve this issue, we recorded from SEF neurons before and after training monkeys on the use of an object-centered rule. The results indicate that object-centered spatial selectivity is present in the SEF before monkeys have learned to select targets on the basis of an object-centered rule but is enhanced by training on the use of such a rule.

**METHODS**

**Subjects**

Two adult male rhesus monkeys were used (Macaca mulatta; laboratory designations Bi and Ro hereafter referred to as M1 and M2). At the beginning of the series of experiments reported here, M1 was ten years old and had been trained to perform a memory guided saccade task. M2 was seven years old and had been trained to maintain fixation on static targets. Neither monkey had any prior training on making object-centered spatial judgments. Experimental procedures were approved by the Carnegie Mellon University Animal Care and Use Committee and were in compliance with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

**Preparatory Surgery**

At the outset of the training period, each monkey underwent sterile surgery under general anesthesia maintained with isoflurane inhalation. The top of the skull was exposed, bone screws were inserted around the perimeter of the exposed area, a continuous cap of rapidly hardening acrylic was laid down so as to cover the skull and embed the heads of the screws, a head-restraint bar was embedded in the cap, and scleral search coils were implanted on the eyes, with the leads directed subcutaneously to plugs on the acrylic cap (Robinson 1963). Following initial training, a 2-cm-diameter disk of acrylic and skull, centered on the midline of the brain approximately 5mm rostral to the genu of the arcuate sulcus, was removed and a cylindrical recording chamber was
cemented into the hole with its base just above the exposed dural membrane.

**Behavioral Control and Data Collection**

All aspects of behavioral procedure, including presentation of stimuli, monitoring of eye movements, and delivery of reward, were under the control of a computer running Cortex software (http://www.cortex.salk.edu). Eye position was monitored by means of a scleral search coil system (Riverbend Instruments, Inc., Birmingham, AL). The X and Y coordinates of eye position were stored at 10 ms intervals with approximately 0.1 deg resolution. Stimuli generated by an active matrix LCD projector were rear-projected on a frontoparallel screen 25.4 cm (M1) and 24.5 (M2) cm from the monkey's eyes. Reward in the form of 0.1 cc of juice was delivered through a spigot under control of a solenoid valve upon successful completion of each trial.

**Tasks**

The monkeys were first trained to perform the *color DMS task* and later trained to perform the *color-conditional object-centered task* (Fig. 1). The tasks were identical with respect to the timing of events and the geometry of the stimuli. They differed only with respect (1) to the coloring of the target bar (red on one end and green on the other in the color DMS task vs. uniformly gray in the object-centered task) and (2) the rule by which the monkey selected one end of the bar as the target for a saccade (the end of the same color as the cue in the color DMS task vs. the end associated with the color of the cue – left for green and right for red – in the color-conditional object-centered task). Each trial began with presentation of a central fixation spot and attainment of fixation in an approximately 5° x 5° fixation window. Then an instructional cue was presented at the fovea. After a further delay, a target display consisting of two dots connected by a horizontal bar appeared in the upper visual field. After another delay, the fixation spot was turned off. At this time, the monkey was required to make a saccade to the target (the left or right end of a horizontal bar), landing within a 5° x 5° window centered on it and maintaining gaze on it for an interval that varied randomly across trials in the range 0-300 ms. Upon termination of this interval, the display was extinguished and juice reward was delivered. The twelve conditions in the color DMS task (Fig. 1B) were
interleaved randomly subject to the constraint that one trial conforming to each condition had to be completed successfully before the beginning of the next block of twelve trials. The six conditions in the object-centered task (Fig. 1C) were interleaved subject to the constraint that one trial conforming to each condition had to be completed successfully before the beginning of the next block of six trials.

**Stimuli**

*Geometry.* The displays (Fig. 1) were identical for the two monkeys but the distance from the eyes to the monitor was less for M2 by a factor of 0.96. In analyzing saccade trajectories, we scaled up data from M2 by a factor of 1.04 so as to achieve register with data from M1. The values given here are for M1. The fixation spot was a 0.43° x 0.43° white square presented at the center of the screen. The foveal color cue was a 1.7° x 1.7° gray, green, or red square. The bar display consisted two 1.3° x 1.3° squares centered on the ends of a horizontal gray bar 7.1° long and 0.28° thick. The bar display was centered at one of three locations at an elevation of 11.4°. One location was centered above fixation. The other two points were offset to the right or left by half a bar length. This design allowed analyzing neuronal activity accompanying a saccade to the same dot (at the near left or near right location in Fig. 1B and C) as a function of whether it occupied the left or right end of a bar.

*Luminance and hue.* The fixation point had a luminance of 83 cd/m² and CIE x and y chromaticity coefficients of 0.28 and 0.32. The red cue and target dot had a luminance of 33 cd/m² and CIE x and y chromaticity coefficients of 0.33 and 0.17. The green cue and target dot had a luminance of 67 cd/m² and CIE x and y chromaticity coefficients of 0.25 and 0.66. The gray bar and target dot had a luminance of 57 cd/m² and CIE x and y chromaticity coefficients of 0.27 and 0.31.

**Recording Sites**

The location of the recording sites relative to gross morphological landmarks was assessed by analysis of structural MR images. Scanning was carried out in a Brüker 4.7 T magnet in which the anesthetized monkey was supported by an MR-compatible stereotaxic device. Fiducial marks made visible by means of a contrast agent included
the centers of the ear bars and selected locations inside the recording chamber. Frontoparallel and parasagittal slices of 2 mm thickness were collected over the entire extent of the cerebral hemisphere. To determine the location of recording sites relative to functional divisions of cortex, we mapped out regions under each chamber from which oculomotor responses could be elicited at low threshold (≤ 50 µA) by electrical microstimulation (1.65 ms biphasic pulses delivered through the recording microelectrode at a frequency of 300 Hz in trains 200 ms long). Recording sites during all three stages of data collection overlapped within a bilateral region of the frontal lobe identified as the SEF on the basis of electrical microstimulation mapping (saccades could be elicited at currents of less than 50 µA) and on the basis of its location as established through the analysis of MR images (2-8 mm rostral to the genu of the arcuate sulcus and within a few mm of the interhemispheric cleft).

**Single-Neuron Recording**

At the beginning of each day's session, a varnish-coated tungsten microelectrode with an initial impedance of several megohms at 1 KHz (Frederick Haer & Co., Bowdoinham, ME) was advanced vertically through the dura into the immediately underlying cortex using a hydraulic microdrive (Narashige, Tokyo, Japan). The electrode could be placed reproducibly at points forming a square grid with 1 mm spacing (Crist et al. 1988). Single neurons were isolated using both online and offline template-matching and principal components analysis sorting (Plexon Inc, Dallas, TX). In this study, we deviated from standard practice in previous studies of object-centered selectivity in the SEF (Olson 2003; Olson and Gettner 1995, 1999; Olson and Tremblay 2000; Tremblay et al. 2002) by recording from all isolated neurons regardless of whether they appeared to exhibit task-related activity on preliminary testing. This approach was designed to ensure uniform sampling throughout all stages of the experiment. Data collection continued until 16 trials had been completed under each condition (96 trials in the object-centered task or 192 trials in the color DMS task) unless the neuron was lost. In the event of the neuron’s being lost, the data were retained and included in the database for subsequent analysis if at least eight trials had been completed successfully under each condition. The
average number of trials per condition, across all recorded neurons, was 14.1 during stage 1, 15.1 during stage 2 and 11.3 during stage 3.

**Statistical Analysis of Neuronal Activity**

To characterize the dependence of each neuron’s firing rate on conditions varying across trials in the task, we carried out independent ANOVAs on data from two epochs: a pre-bar-onset epoch (foveal cue onset + 100 ms to bar onset + 100 ms) and a post-bar-onset epoch (bar onset + 100 ms to fixation point offset + 100 ms). The offset of 100 ms was introduced to take into account the approximate latency of visual responses in the SEF (Pouget et al. 2005). There were three fully orthogonal factors: saccade direction (near left or near right in Fig. 1B and C), the object-centered location of the target (on the left or right end of the bar) and (in the case of the color DMS task) the color of the target (red or green). In all ANOVAs, the criterion for significance was taken as \( p < 0.05 \) unless otherwise stated. In comparing, across training stages and monkeys, the counts of neurons exhibiting significant effects in the ANOVAs, we used a \( \chi^2 \) test. For example, if (in case 1) 30 neurons exhibited a significant effect and 70 did not, and (in case 2) 60 neurons exhibited a significant effect and 240 did not, then we would compute \( \chi^2 \) on the basis of the observed values (30, 70, 60, 240) and the predicted values (22.5, 77.5, 67.5, 232.5), and would stipulate one degree of freedom in assessing the significance of the outcome. The predicted values in this example reflect the null hypothesis that both samples were drawn from the same parent population in which 22.5% (90/400) neurons exhibited a significant effect. In any comparison involving, as this one did, a single degree of freedom, we incorporated a Yates correction.

**Regression of Neuronal Activity on Saccade Metrics**

In order to determine whether firing rate was correlated with object-centered location independently of any effect arising from subtle variations in saccades between bar-left and bar-right trials, we performed a multivariate regression analysis, fitting three models to data collected from each neuron during trials in which the target (the right or left end of a bar) appeared at a given screen location:
1) \( Y = \beta_0 + \beta_1 \text{Obj} + \beta_2 \text{Lat} + \beta_3 \text{Vel} + \beta_4 \text{Amp} + \beta_5 \text{Xpos} + \beta_6 \text{Ypos} \)

2) \( Y = \beta_0 + \beta_1 \text{Lat} + \beta_2 \text{Vel} + \beta_3 \text{Amp} + \beta_4 \text{Xpos} + \beta_5 \text{Ypos} \)

3) \( Y = \beta_0 + \beta_1 \text{Obj} \)

where \( Y \) = firing rate measured during the post-bar-onset period (target onset +100 ms to saccade initiation +100 ms), \( \text{Obj} \) = object-centered location (0 or 1 for bar-left or bar-right), \( \text{Lat} \) = latency (from fixation spot offset), \( \text{Vel} \) = peak velocity, \( \text{Amp} \) = amplitude, and \( \text{Xpos} \) and \( \text{Ypos} \) = final x and y landing positions respectively. Having fitted the parameters of each model to a neuron’s data, we determined, using an F-test, whether the full model (1), when compared to each of the reduced models (2, 3) accounted for significantly more of the variance in the data than could be explained by its larger number of degrees of freedom. If model 1 provided a significant improvement over model 2, we concluded that neuronal activity depended significantly on object-centered location independently of any tendency for saccade parameters to co-vary with object-centered location. If model 1 provided a significant improvement over model 3, we concluded, by similar reasoning, that neuronal activity depended significantly on variations in the saccade. We computed F as:

\[
F_{(DF_{\text{reduced}} - DF_{\text{full}})} = \frac{\left( SS_{\text{reduced}} - SS_{\text{full}} \right)}{DF_{\text{reduced}} - DF_{\text{full}}} \left( \frac{SS_{\text{full}}}{DF_{\text{full}}} \right)
\]

where, for both the full model and the reduced model, \( SS \) was the residual sum of squares obtained upon fitting the model to the data and \( DF \) was the number of degrees of freedom on which \( SS \) was based (\( n - p - 1 \) where \( n \) was the number of trials and \( p \) was the number of free parameters in the model).

The properties of the saccade executed on each trial were measured by the following series of steps. First, the direction of gaze was determined for each 10 ms bin during a 500 ms epoch beginning with offset of the fixation spot. Then the instant of maximal velocity was identified by finding the pair of adjacent 10 ms bins \((B_m\text{ and }B_{m+1})\) for which the displacement of the eye in degrees of visual angle \((\Delta E_m)\) was maximal. The
maximal velocity, in degrees of visual angle per second, was given by $100*\Delta E_m$. The start of the saccade was identified by moving backward in time until encountering a pair of bins, $B_s$ and $B_{s+1}$, for which $\Delta E_s < \Delta E_m/4$. Saccadic reaction time was taken as the interval between offset of the fixation spot and the beginning of bin $B_{s+1}$. The finish of the saccade was identified by moving forward in time until encountering a pair of bins, $B_f$ and $B_{f+1}$, for which $\Delta E_f < \Delta E_m/4$. Saccade amplitude was taken as the distance in degrees of visual angle between eye positions recorded at $B_s$ and $B_{f+1}$. The final position of the eye was estimated on the basis of $B_{f+7}$ so as to allow time for asymptotic deceleration without exceeding the minimal reaction time for any corrective saccade.

RESULTS

Stage 1: Color DMS Task

TRAINING. Two monkeys (M1 and M2) were trained to perform an oculomotor color delayed match to sample (DMS) task. A red or green foveal cue presented early in each trial instructed the monkey to make a saccade to the red or green end of a horizontal bar presented later in the trial (Fig. 1A and B). The chromatic match appeared with equal frequency on the bar’s left and right ends. Thus the monkey had to select the target on the basis of its color without regard to its object-centered location. Training through a series of steps culminating in mastery of the color DMS task occupied 11 and 9 months in M1 and M2 respectively.

BEHAVIOR. In assessing behavioral performance during neuronal data collection sessions, we confined our attention to a set of trial conditions across which the direction of the saccade and the object-centered location of the target were fully counterbalanced (conditions in the “near left” and “near right” columns of Fig. 1 B and C).

The percent correct score for each session was computed as the number of trials on which the monkey made a saccade to the correct end of the bar expressed as a percentage of all trials on which he responded with any saccade to offset of the fixation spot. The average percent correct score across all neuronal data collection sessions was 95% and
90% in M1 and M2 respectively. Error trials were excluded from all further stages of analysis.

The reaction time for each session was computed as the average interval between offset of the fixation spot and initiation of the saccade on correct trials. The average reaction time across all neuronal data collection sessions was 97 and 188 ms in M1 and M2 respectively. The low values in M1 indicate that on many trials the monkey had committed to execution of the saccade before offset of the fixation spot. The monkeys were not counted wrong so long as the gaze remained within the central window until extinction of the fixation spot.

The landing points of saccades to targets at the four possible locations (far left, near left, near right and far right) formed four tight clusters (Fig. 2A and B). However, on quantitative analysis, we found that there were subtle differences between bar-left and bar-right trials. Differences with respect to the vertical axis were small and inconsistent. Differences with respect to the horizontal axis conformed to a systematic pattern whereby the saccade deviated slightly from the end of the bar toward its center, as reported previously (Olson and Tremblay 2000). Thus, across conditions in which the target dot on the left end of the bar and the target dot on the right end of the bar occupied the same screen location, the gaze consistently landed slightly farther to the right when the dot on the bar’s left end was the target than when the dot on the bar’s right end was the target. The mean offset between the two landing points was 0.42 degrees of visual angle (0.56° in M1 and 0.27° in M2). We consider below whether this small deviation could have had an impact on neuronal activity.

NEURONAL ACTIVITY. The first stage of neuronal data collection immediately followed the training period, occupying 3 months in M1 and 5 months in M2. So as to ensure unbiased sampling, we selected every well isolated neuron for study without reference to whether it showed obvious task-related activity. The recording sites are shown in Fig. 3C and D. We collected data from a total of 259 neurons (99 in M1 and 160 in M2). Among these neurons, some exhibited clear signs of object-centered spatial selectivity. The most dramatic example is shown in Fig. 4A. Following the appearance of the target bar, this
neuron fired more strongly when the target was on the bar’s left end than when it was on the right regardless of where the bar was on the screen.

To determine how common this form of selectivity was, we carried out an ANOVA on data from each neuron based on eight conditions in which the target was at the near left or near right location on the screen (Fig. 1B). Across these conditions, object-centered location was fully counterbalanced against the two other factors that might have influenced neuronal activity: saccade direction and color. The full results of the ANOVA are presented in Table 1.

During Delay 1, which intervened between presentation of the colored sample and onset of the target bar (Fig. 1A, panel 3), the monkey knew neither the object-centered location of the yet-to-appear target nor the direction of the required saccade. Accordingly, we expected to observe neither object-centered selectivity nor selectivity for saccade direction. Indeed, significant main effects of object-centered location and saccade direction were no more common than expected by chance ($\chi^2$ test, p > 0.05). During the same epoch, however, the monkey was required to hold in working memory the color of the sample. Accordingly, neurons might reasonably be expected to exhibit color selectivity. Indeed, the rate of incidence of main effects of color (24/259 = 9% of neurons) significantly exceeded the frequency expected by chance in each monkey ($\chi^2$ test, p = 0.0026). We conclude that selectivity for the color of the cue, although not common, was genuinely present.

During Delay 2, which intervened between the onset of the target bar and permission to execute a saccade (Fig. 1A, panel 4), both the saccade direction and the object-centered location of the target were known. There was a significant main effect of saccade direction during this period in 85/259 = 33% of neurons. There was a significant main effect of object-centered location in 69/259 = 27% of neurons. Critically, the rate of object-centered selectivity exceeded the rate expected by chance at a high level of significance ($\chi^2$ test, p << 0.0001). Object-centered selectivity was not only statistically significant but also robust, as revealed by plots of population activity among all studied neurons (Fig. 4B and C) and among those in which a main effect of object-centered location achieved significance (Fig. 4D and E). Results from the two monkeys were not identical. Saccade-direction effects were most frequent in M1 (Fig. 7A), whereas object-
centered effects were most frequent in M2 (Fig. 7B). This difference was highly significant ($\chi^2$ test, $p << 0.0001$). It is important therefore to note that the frequency with which neurons exhibited object-centered selectivity exceeded the number expected by chance at a very highly significant level in each monkey independently ($\chi^2$ test, $p << 0.0001$). We conclude that neurons in the SEF were sensitive to the object-centered location of a saccade target even in monkeys never trained to select targets by an object-centered rule.

Did neurons that were selective for object-centered location form a group separate from those that were selective for saccade direction? To answer this question, we compared the number of cases in which a neuron exhibited both forms of selectivity to the number expected on the basis of the assumption that the two traits were distributed independently. We found that neurons with both forms of selectivity were actually more common than expected from independent distribution of the two traits ($\chi^2$ test, $p < 0.00039$). The relatively high rate of incidence of neurons with dual selectivity is evident upon inspection of Fig. 7A and B (gray bars).

The use of categorical measures based on classifying neurons according to whether or not they exhibited a statistically significant effect may have masked a subtle tendency for neurons to group by functional category. To circumvent this problem, we carried out an additional step of analysis based on continuous measures. We plotted, across all neurons, an index of the strength of the object-centered signal (the fraction of cross-condition firing rate variance accounted for by the object-centered location of the target) against an index of the strength of the saccade-direction signal (the fraction of cross-condition firing-rate variance accounted for by saccade direction). The distribution of neurons with respect to the relative strengths of the two signals appeared to be continuous and balanced (Fig. 8A). The fraction in which the object-centered signal was stronger was not significantly different from the fraction in which the saccade-direction signal was stronger.

Could neuronal activity apparently dependent on the object-centered location of the target actually have been dependent on small differences between saccades made to the left and right ends of bars? In a previous study of monkeys trained to select saccade targets by an object-centered rule, we ruled out this possibility by demonstrating that
neuronal firing rate continued to depend on the object-centered location of the target even after the influence of variations of saccade direction had been factored out (Tremblay et al. 2002). We took a similar approach here, carrying out a multivariate regression analysis to assess whether neuronal activity depended on the object-centered location of the target as distinct from the properties of the saccade (latency, velocity, amplitude and landing position) (see Methods: Regression of Neuronal Activity on Saccade Metrics). The analysis was carried out for each of two target locations (near left and near right in Fig. 1B) in each of 69 neurons exhibiting a significant main effect of object-centered location in the ANOVA. Thus 2 x 69 = 138 cases were analyzed. There was a significant dependence on object-centered location, even with the influence of saccade metrics factored out, in 62/138 = 45% of cases. The absence of a significant effect in other cases was due to a loss of power attendant on analyzing subsets of the full data set. In only 15% of cases was firing significantly correlated with the parameters of the saccade when the influence of object-centered location was factored out. We conclude that object-centered spatial selectivity was genuine rather than an indirect manifestation of sensitivity to subtle differences between saccades occurring on bar-left and bar-right trials.

**Stage 2: Object-Centered Task**

**TRAINING.** During the second stage of training, we induced transfer from the color DMS task to the color-conditional object-centered task (Olson and Gettner 1999) by limiting the color DMS task to conditions in which the green dot was on the left end of the target bar and the red dot on the right end and gradually desaturating the red and green of the target dots (but not of the foveal cue) until they were a uniform gray. Training proceeded swiftly (occupying 7 and 3 days in M1 and M2 respectively).

**BEHAVIOR.** During the subsequent neuronal data collection period, each monkey performed proficiently, making saccades to the bar’s left (or right) end when the cue was green (or red), regardless of the bar’s location on the screen. The average percent correct score was 89% in each monkey. The average reaction time was 89 and 86 ms in M1 and
M2 respectively. From the short duration of the reaction times, it is evident that on many trials the monkeys committed to execution of the saccade before offset of the fixation spot. The saccadic landing point varied slightly but systematically according to whether the target at a given location on the screen was the left or right end of a bar (Fig. 2C and D). The mean horizontal displacement between the bar-left and bar-right landing points (resulting from a tendency for the saccade to deviate toward the center of the bar) was 0.42° in M1 and 0.43° in M2. In all of these respects, behavior was roughly comparable during stage 2 to that observed during stage 1.

**Neuronal activity.** During the immediately ensuing period of neuronal data collection, which occupied two months in each monkey, we recorded from 217 neurons (118 in M1 and 99 in M2). The recording sites are shown in Fig. 3E and F. Among these neurons, some exhibited obvious object-centered spatial selectivity. The most dramatic instance is shown in Fig. 5A. On trials in which the monkey had been instructed to make a saccade to the bar’s left end, this neuron began firing before onset of the target bar and continued to fire strongly until execution of the saccade. It hardly fired on trials in which the instruction was to go to the bar’s right end.

To assess the frequency with which neurons exhibited object-centered and saccade-direction selectivity, we analyzed data from each neuron using a series of ANOVAs. The procedure was identical to that employed during stage 1 with the exception that the color of the cue was not included as an independent factor because color and object-centered location were indissociable (each color cued a particular object-centered location). The full results are summarized in Table 2.

During Delay 1, which intervened between presentation of the colored sample and onset of the target bar (Fig. 1A, panel 3), 47/217 = 22% of neurons exhibited a significant main effect of color or object-centered location (the two forms of selectivity were not dissociable because each color cued a particular object-centered location). This percentage is significantly greater (χ² test, p = 0.00026) than the percentage (9%) exhibiting color selectivity in the same epoch during stage 1 (neurons could exhibit only color selectivity during Delay 1 in the color DMS task because during this epoch the object-centered location of the upcoming target was not yet known). The increase from
9% selective for color at stage 1 to 22% selective for color or object-centered location at stage 2 presumably reflects the addition to a small population of genuinely color-selective neurons of a second population of neurons selective for the object-centered location associated with the color. Significant main effects of saccade direction were no more common than expected by chance ($\chi^2$ test, $p > 0.05$) in harmony with the fact that the bar was not yet visible and the saccade direction therefore could not be known.

During Delay 2, which intervened between the onset of the target bar and permission to execute a saccade (Fig. 1A, panel 4), there was a significant main effect of object-centered location in $99/217 = 46\%$ of neurons. The object-centered signals were robust, as revealed by plots of population activity among all studied neurons (Fig. 5B and C) and among those in which a main effect of object-centered location achieved significance (Fig. 5D and E). The percentage of neurons exhibiting object-centered spatial selectivity at this stage was significantly greater than the percentage (27%) doing so during stage 1 ($\chi^2$ test, $p < 0.0001$). Even with the data subdivided by monkey, this effect achieved significance ($\chi^2$ test, $p = 0.0003$ in M1 and $p = 0.02$ in M2). In contrast, the proportion of neurons exhibiting saccade-direction selectivity ($50/217 = 23\%$) was significantly less than the percentage (33%) observed during stage 1 ($\chi^2$ test, $p = 0.024$).

The enhancement of object-centered selectivity was evident not only within the recorded population as a whole but also within the subset of spatially selective neurons. Among these neurons, the incidence of object-centered selectivity was greater during stage 2 (Fig. 7C and D) than during stage 1 (Fig. 7A and B). The shift between stage 1 and stage 2 in the categorical distribution of spatially selective neurons (selective for object-centered location, selective for saccade direction or selective for both) was significant in data from the two monkeys combined ($\chi^2$ test, $p < 0.0001$) and remained significant with consideration restricted to each individual monkey ($\chi^2$ test, $p = 0.00032$ in M1; $p = 0.037$ in M2). The distributions were statistically indistinguishable between the two monkeys at stage 2 ($p = 0.52$) as if training on the object-centered task had brought them to a common asymptotic level.

Finally, the enhancement of object-centered selectivity was evident in continuous measures applied to all neurons without regard to whether selectivity was significant (Fig. 8A and B). Neurons in which object-centered location accounted for more firing
rate variance than saccade direction (represented by points above the identity line) were significantly more numerous during stage 2 than during stage 1 ($\chi^2$ test, $p = 0.00021$). We conclude that training the monkeys to select targets by an object-centered rule produced an enhancement of selectivity for the object-centered location of the target as compared to selectivity for the direction of the saccade.

To be sure that object-centered spatial selectivity was genuine and not a secondary consequence of neuronal sensitivity to differences in saccade metrics between bar-left and bar-right trials, we conducted a multiple regression analysis identical to that employed during stage 1. The analysis was carried out for each of two target locations (near left and near right in Fig. 1B) in each of 99 neurons exhibiting a significant main effect of object-centered location in the ANOVA. Thus $2 \times 99 = 198$ cases were analyzed. In $88/198 = 44\%$ of cases, the firing rate remained dependent on object-centered location with the influence of saccade metrics factored out whereas the converse was true in only $14/198 = 7\%$ of cases. We conclude that object-centered selectivity was genuine.

The enhancement of object-centered spatial selectivity might have occurred rapidly, as a result of the monkeys’ mastering the object-centered task, or slowly, as a result of repeated practice on it. Mastery of the task was achieved in approximately a week. The subsequent period of data collection, during which the monkeys received continuous practice on the task, occupied approximately two months. We carried out a trend analysis to determine whether there had been a tendency for object-centered selectivity to increase over the course of the latter period. For each recording session, we computed a population index of object-centered spatial selectivity during the post-bar-onset epoch: the average across all neurons with object-centered spatial selectivity of $\text{Abs}((R-L)/(R+L))$, where $R$ and $L$ were the mean firing rates on bar-right and bar-left trials. We arranged the resulting values in sequence by session number. Then we fitted a line to them. If there had been a consistent tendency for the value to increase across sessions, then the slope of the line would have been positive. The slope was actually slightly negative in each monkey (M1 slope: $Y = -0.0026X$; M2 slope: $Y = -0.0015X$), although the effect did not attain significance. We conclude that the increase in object-centered
spatial selectivity was present as soon as the task had been mastered and did not increase slowly with practice.

**Stage 3: Color DMS Task after Object-Centered Training**

The enhancement of object-centered spatial selectivity observed during stage 2 might have depended on the monkeys’ actually performing the object-centered task at the time of neuronal data collection or, alternatively, once established, the enhancement might have been independent of task context. To distinguish between these possibilities, we collected data during a third period in which the monkeys again performed the color DMS task. The conditions were identical to those present during stage 1 with the sole exception that the monkeys performed object-centered sessions between color DMS sessions so as to keep fresh the effects of object-centered training.

**BEHAVIOR.** The mean percent correct across all neuronal data-collection sessions was 90% and 89% in M1 and M2 respectively. The mean reaction times were 156 and 200 ms. There was a systematic tendency for the saccade to deviate slightly toward the center of the bar. This resulted in a mean horizontal offset between bar-left and bar-right trials of 0.54° in M1 and 0.23° in M2 (Fig. 2E and F). These results are roughly comparable to ones obtained during performance of the color DMS task during stage 1.

**NEURONAL ACTIVITY.** We collected data from a total of 127 neurons (84 in M1 and 43 in M2). The recording sites are shown in Fig. 3G and H. Among these neurons, some exhibited obvious object-centered spatial selectivity. The most dramatic instance is shown in Fig. 6A. When the target occupied the bar’s left end, this neuron fired throughout the post-bar-onset delay period. It fired much less strongly when the target occupied the bar’s right end.

To assess the frequency of object-centered selectivity and selectivity for saccade direction, we analyzed each neuron’s data using a series of ANOVAs. The procedure was identical to that employed on data from stage 1. The full results are summarized in Table 3.
During Delay 1, which intervened between presentation of the colored sample and onset of the target bar (Fig. 1A, panel 3), 16/127 = 13% of neurons exhibited a significant main effect of color. This percentage was greater than that observed during stage 1 (9%) but the difference did not achieve significance ($\chi^2$ test, p > 0.05). The increase in the incidence of color selectivity may have resulted from activation of object-centered neurons by the color associated with their preferred direction. We present evidence favoring this interpretation below. Significant main effects of object-centered location and saccade direction were no more common than expected by chance ($\chi^2$ test, p > 0.05) in harmony with the fact that the bar was not yet visible, which meant that neither factor was known.

During Delay 2, which intervened between the onset of the target bar and permission to execute a saccade (Fig. 1A, panel 4), there was a significant main effect of object-centered location in 44/127 = 35% of neurons. The object-centered signals were robust, as revealed by plots of population activity among all studied neurons (Fig. 6B and C) and among those in which a main effect of object-centered location achieved significance (Fig. 6D and E). The percentage exhibiting a significant main effect of object-centered location (35%) was greater than during stage 1 (27%) and less than during stage 2 (46%) but neither difference achieved significance ($\chi^2$ test, p > 0.05). The percentage of neurons exhibiting saccade-direction selectivity (25/127 = 20%) was less than that observed at stage 1 (33%) and the effect did achieve significance ($\chi^2$ test, p = 0.01).

The enhancement of object-centered selectivity during stage 3 as compared to stage 1 was evident within the subset of spatially selective neurons. Among these neurons, the incidence of object-centered selectivity was greater during stage 3 (Fig. 7E and F) than during stage 1 (Fig. 7A and B). The difference between stages 3 and 1 in the categorical distribution of neurons (selective for object-centered location, selective for saccade direction or selective for both) was statistically significant ($\chi^2$ test, p = 0.002). In contrast, the difference between stages 3 and 2 was not significant ($\chi^2$ test, p > 0.05).

Finally, an enhancement of object-centered selectivity during stage 3 as compared to stage 1 was evident at the level of continuous measures applied to all neurons without regard to whether selectivity was significant (Fig. 8A vs. C). The fraction of object-centered neurons in which object-centered location accounted for more firing-rate
variance than saccade direction was greater during stage 3 (Fig. 8C) than during stage 1 (Fig. 8A) and the effect was statistically significant ($\chi^2$ test, $p = 0.0034$). In contrast, the difference between results obtained during stages 3 and 2 was not significant ($\chi^2$ test, $p > 0.05$). We conclude that training the monkeys to select targets by an object-centered rule produced an enhancement of object-centered selectivity that persisted even during color DMS performance.

To be sure that object-centered spatial selectivity was genuine and not a secondary consequence of neuronal sensitivity to differences in saccade metrics between bar-left and bar-right trials, we carried out a multiple regression analysis identical to that conducted during stage 1. The analysis was carried out for each of two target locations (near left and near right in Fig. 1B) in each of 44 neurons exhibiting a significant main effect of object-centered location in the ANOVA. Thus $2 \times 44 = 88$ cases were analyzed. In $47/88 = 53\%$ of cases, the firing rate remained dependent on object-centered location with the influence of saccade metrics factored out whereas the converse was true in only $7/88 = 8\%$ of cases. We conclude that object-centered selectivity was genuine.

Object-centered selectivity might have been strong early in each Color DMS session and then have declined as the monkey became used to selecting targets without recourse to use of an object-centered rule. To assess this possibility, we carried out a trend analysis on data from neurons with object-centered spatial selectivity. First, for each neuron, we subdivided the data collection session into successive blocks, each consisting of 12 successful trials. Then, for each block from the first to the last, we computed the average across all neurons of an index of object-centered selectivity: $\text{Abs}((R - L)/(R + L))$, where $R$ and $L$ were firing rates on bar-left and bar-right trials. Then we fitted a line to points representing the index as a function of block number. If there had been a consistent tendency for the value to decrease across sessions, then the slope of the line would have been negative. The slope was actually insignificantly positive. We conclude that the enhancement of object-centered spatial selectivity induced by training monkeys to perform the object-centered task persisted throughout the color DMS session.

As a check on the surprising conclusion that the effects of training on the object-centered task carried over into the color DMS task, we carried out a further analysis. To master the object-centered task required learning to associate two colors (green and red)
with two object-centered locations (left and right). These color-location associations, although irrelevant to performance of the color DMS task, might nevertheless have persisted in it, affecting behavior or neuronal activity. To determine whether the learned associations influenced behavior, we compared the monkeys’ performance on trials in which the colored targets were at locations congruent with their learned associations (green dot on the left and red dot on the right) and trials in which the targets had the opposite arrangement. Under incongruent conditions, the percent correct score was significantly lower in M1 (89% vs. 91%, Wilcoxon signed rank test, \( p = 0.02 \)) and the reaction time was significantly longer in M2 (203 ms vs. 196 ms, paired t-test, \( p = 0.02 \)). Thus color-location associations acquired during stage 2 affected behavior, with incongruence impeding correct performance in both monkeys. To determine whether the learned associations influenced neuronal activity, we constructed population histograms representing the activity of all neurons with significant object-centered selectivity. Two effects, absent among neurons studied at stage 1 before object-centered training (Fig. 9A), were present among neurons studied during stage 3, after object-centered training (Fig. 9B). First, during the epoch before the bar appeared, neurons fired more strongly when the cue was of the color associated with their preferred object-centered location. This effect was significant (sign test, \( p = 0.0013 \)). Second, following appearance of the bar, neurons fired more strongly when the color of the cue and the location of the target were incongruent, thus exhibiting conflict-related enhancement as described previously (Amador et al. 2004; Nakamura et al. 2004; Olson and Gettner 2002; Stuphorn et al. 2000; Tremblay et al. 2002). This effect was also significant (sign test, \( p < 0.0001 \)). The fact that color-location associations learned in the context of the object-centered task affected behavior and neuronal activity during color DMS performance adds credence to the notion that the enhancement of object-centered spatial selectivity induced by learning the object-centered task carried over into the color DMS task.

**DISCUSSION**

The first key finding of this study is that object-centered spatial selectivity was present in the SEF prior to training on object-centered tasks. This finding is novel. All
previous studies of object-centered spatial selectivity were carried out in monkeys already trained to perform object-centered tasks (Chafee et al. 2005; Horwitz et al. 2004b; Olson 2003; Olson and Gettner 1995, 1999; Olson and Tremblay 2000; Tremblay et al. 2002). It might be proposed that object-centered spatial selectivity – rather than being naturally present in the SEF – was induced by training on the color DMS task. We cannot firmly reject this interpretation on the basis of the present results. The central requirement of this task – that targets be selected on the basis of a color that could appear on either end of the target bar – is not likely to have induced SEF neurons to represent the object-centered locations of the targets. However, a secondary requirement – that monkeys select as target a part embedded in an object – might have had such an effect. The counter-argument to this proposal is that the behavior of looking at a significant feature embedded in an object is natural to monkeys. For example, when examining a face, they tend to look at the eyes and mouth (Guo et al. 2003). Thus natural experience received prior to training in the laboratory would presumably already have induced object-centered spatial selectivity in the SEF. On the issue of whether the trait is in fact induced by natural experience or, alternatively, is innate, we can of course offer no conclusion.

It is possible that SEF neurons exhibit object-centered selectivity under a more narrow range of conditions before training than after it. What if we had replaced the display actually used in this experiment – a pair of dots connected by a horizontal bar – with a display in which the cues favoring perceptual grouping into a single object were less prominent – for example, a pair of dots unconnected by a bar? We know that SEF neurons in monkeys trained on the bar task exhibit object-centered spatial selectivity this condition (Olson and Tremblay 2000). Prior to training this might not be so. To explore this and related issues will require further experiments.

The second key finding of this study is that training monkeys to select targets by an object-centered rule enhanced the strength of the object-centered representation in the SEF. This is not the first demonstration of training-induced effects in the SEF. Several prior studies have shown that SEF neurons active in conjunction with planning a saccade in a given direction become sensitive to color or pattern cues eliciting that saccade (Chen and Wise 1995a,b, 1996; Olson and Gettner 2002; Olson et al. 2000). For example, a
neuron active in conjunction with planning rightward saccades will begin to fire not only in response to a target flashed in the right visual field but also in response to a foveal digitized image instructing the monkey to make a rightward saccade (Olson et al. 2000). Similar effects of training have been observed in other oculomotor areas (Bichot et al. 1996; Grunewald et al. 1999; Horwitz et al. 2004a; Lauwereyns et al. 2001; Toth and Assad 2002). The present study is, however, the first to have shown that training monkeys to attend to locations as defined with respect to a particular reference frame induces neurons to represent locations relative to that frame.

Although object-centered spatial selectivity was the main focus of this study, we made an incidental observation that saccade-direction selectivity actually decreased as a result of training on the object-centered task. This is surprising because even the object-centered task required monkeys to make saccades in particular directions. Moreover, throughout the period of study, the monkeys undoubtedly made many more saccades under natural circumstances in the colony than under artificial circumstances in the laboratory. This finding cannot be written off to our having used an insensitive test for saccade-direction selectivity. It is true that the angular deviation between saccades to the near left and near right targets was only 35°. However, many SEF neurons do fire at significantly different rates in conjunction with saccades to locations this closely spaced (Olson 2003; Olson and Gettner 1995, 1999; Olson and Tremblay 2000; Tremblay et al. 2002). Furthermore, the sensitivity of the test was the same at all training stages, so that it allowed for fair comparison between stages. It is possible that any enhancement of object-centered spatial selectivity necessarily involves a reduction of saccade-direction selectivity. If, prior to training, a neuron already utilized its full dynamic range for representing saccade direction, firing maximally for saccades in one direction and minimally for saccades in the opposite direction, then, for a signal representing object-centered location to be added to the saccade-direction signal without saturation at the floor or ceiling, it would be necessary that the gain of the saccade-direction signal be reduced.

What could account for the fact that the enhancement of object-centered selectivity was full blown at the outset of recording, after only 3-7 days of training? The most straightforward answer to this question is that the enhancement depended on no more
than the monkey's acquiring the task set. The object-centered task requires attending to the location of the target as defined relative to the object, a mental act in which two things and their relation are held in mind at once. Monkeys may be naturally capable of this form of attention (although deploying it infrequently) and SEF neurons may naturally signal the object-centered location of the target when they engage in it. According to this line of argument the enhancement of object-centered spatial selectivity induced by training was simply the result of requiring the monkey to deploy attention to the object and the target simultaneously.

How, then, are we to account for the fact that the incidence of object-centered selectivity remained elevated in the context of the color DMS task during stage 3? The most straightforward answer is that the monkeys continued to allocate attention during stage 3 in a manner that had become habitual during stage 2, with the habit maintained by object-centered refresher sessions interleaved with color DMS sessions during stage 3. This is in harmony with other published observations. First, it is known, in monkeys performing fully interleaved color DMS and object-centered trials, that SEF neurons encode the object-centered location of the target on color DMS trials nearly as strongly as on object-centered trials (Tremblay et al. 2002). Second, we observed, in the same monkeys during the same sessions, that there was a carryover of color-location associations from the object-centered task (where they were relevant) to the color DMS task (where they were not). Third, there is abundant behavioral evidence that cognitive habits formed in the context of one task carry over into other tasks. For example, Stroop interference in humans arises because a learned cognitive skill (reading) is exercised habitually in the context of a task to which it is irrelevant (color naming) (MacLeod 1991).

ACKNOWLEDGMENTS

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Table 1. Color DMS task during Stage 1: Counts of neurons exhibiting selectivity for color, for object-centered location and for saccade direction

Results of ANOVAs carried out on data from 259 neurons during stage 1 of the study with firing rate as the dependent variable and with the color of the cue and target (red, green), the object-centered location of the target (bar-left or bar-right) and the direction of the saccade (near left, near right) as three factors. Independent analyses were carried out on data from the pre-bar-onset epoch (cue onset + 100 ms to bar onset + 100 ms) and the post-bar-onset epoch (bar onset + 100 ms to fixation point offset + 100 ms). “Color”: significant main effect of color. R>G and G>R: Stronger firing for red than for green and vice versa. “Object-centered”: significant main effect of object-centered location. “Saccade direction”: significant main effect of saccade direction. C>I: stronger firing when the target was at the end of the bar contralateral to the recording hemisphere (in the case of object-centered selectivity) or in the visual field contralateral to the recording hemisphere (in the case of saccade-direction selectivity). I>C: the opposite pattern. In this and subsequent tables, directionally selective neurons are subdivided according to preferred direction (ipsilateral or contralateral to the recording hemisphere) as a point of information. As no interesting trends were noted with respect to preferred direction, we do not dwell on this distinction in the text.
<table>
<thead>
<tr>
<th>Monkey</th>
<th>Object-Centered</th>
<th>Saccade Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&gt;I</td>
<td>I&gt;C</td>
</tr>
<tr>
<td><strong>Delay 1: Pre-Bar-Onset Epoch</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 (n = 118)</td>
<td>13 (11%)</td>
<td>13 (11%)</td>
</tr>
<tr>
<td>M2 (n = 99)</td>
<td>16 (16%)</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Total (n = 217)</td>
<td>29 (13%)</td>
<td>18 (8%)</td>
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<tr>
<td><strong>Delay 2: Post-Bar-Onset Epoch</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 (n = 118)</td>
<td>30 (25%)</td>
<td>24 (20%)</td>
</tr>
<tr>
<td>M2 (n = 99)</td>
<td>26 (26%)</td>
<td>19 (19%)</td>
</tr>
<tr>
<td>Total (n = 217)</td>
<td>56 (26%)</td>
<td>43 (20%)</td>
</tr>
</tbody>
</table>

Table 2. Object-centered task during Stage 2: Counts of neurons exhibiting selectivity for object-centered location and for saccade direction

Results of ANOVAs carried out on data from 217 neurons during stage 2 of the study. Conventions as in Table 1.
<table>
<thead>
<tr>
<th>Monkey</th>
<th>Color</th>
<th>Object-Centered</th>
<th>Saccade Direction</th>
</tr>
</thead>
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<tr>
<td></td>
<td>R&gt;G</td>
<td>G&gt;R</td>
<td>C&gt;I</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Delay 1: Pre-Bar-Onset Epoch</td>
<td>M1 (N=84)</td>
<td>7 (8%)</td>
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<td></td>
<td>M2 (N=43)</td>
<td>4 (9%)</td>
<td>3 (7%)</td>
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<td></td>
<td>Total (N=127)</td>
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<td>5 (4%)</td>
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<tr>
<td>Delay 2: Post-Bar-Onset Epoch</td>
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<td>3 (4%)</td>
<td>2 (2%)</td>
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<tr>
<td></td>
<td>M2 (N=43)</td>
<td>2 (5%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td></td>
<td>Total (N=127)</td>
<td>5 (4%)</td>
<td>9 (7%)</td>
</tr>
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</table>

Table 3. Color DMS task during Stage 3: Counts of neurons exhibiting selectivity for color, for object-centered location and for saccade direction

Results of ANOVAs carried out on data from 127 neurons during stage 3 of the study.

Conventions as in Table 1.
FIGURE LEGENDS

Fig. 1. The monkeys performed two tasks: the color DMS task and the color-conditional object-centered task. A: The timing of events was the same in both tasks: a central chromatic cue presented early in each trial instructed the monkey which end of a horizontal bar to select as saccade target at the trial’s end. B: Display and saccade geometry under twelve conditions in the color DMS task. In this task, the monkey had to make a saccade to the end of the bar that matched the central cue in color (green or red). C: Display and saccade geometry under six conditions in the color-conditional object-centered task. In this task, the monkey had to make a saccade to the end of the bar (left or right) associated with the color of the central cue (green or red).

Fig. 2. Saccadic landing positions. Means and standard deviations of saccadic end-points for each monkey in each task. A and B: Color DMS task during stage 1. C and D: Color-conditional object-centered task during stage 2. E and F: Color DMS task during stage 3. The large circles are centered on the locations of the targets at near left and near right locations (Fig. 1B and C). Each of these locations could be occupied by either the left end of a bar (bar left) or the right end of a bar (bar right). The landing points on bar right trials (squares) were displaced slightly to the left of the landing points on bar left trials (circles), indicating a tendency for the eye to deviate from the bar’s end toward its center. Scale is in degrees of visual angle relative to the initial fixation point.

Fig. 3. Recording sites were within a region, straddling the interhemispheric midline 2-8 mm rostral to the genu of the arcuate sulcus, from which eye movements could be elicited by microstimulation at low levels of current ($\leq 50 \mu A$). They thus met criteria for assignment to the SEF as established in classic studies (Russo and Bruce 1993, 2000; Schlag and Schlag-Rey 1985, 1987a, b). A and B: Parasagittal MR images through the medial face of the right hemisphere in M1 and M2. The approximately vertical line above the brain marks AP and ML zero in recording-grid coordinates. The center of the grid in each monkey was located approximately 5 mm rostral to the genu of the arcuate sulcus and over the interhemispheric cleft. The white rectangle superimposed on the gray
matter demarcates the region in which recording was carried out. C and D: Location relative to the center of the chamber of neurons recorded in the context of the color DMS task during stage 1. The area of the outer (open) bubble indicates the number of recorded neurons and the area of inner (filled) bubble indicates the number exhibiting significant object-centered spatial selectivity. Gray shading demarcates the region within which electrical microstimulation elicited eye movements. E and F: Same for the color-conditional object-centered task during stage 2. G and H: Same for the color DMS task during stage 3.

Fig. 4. SEF neurons exhibited object-centered spatial selectivity even during experimental stage 1, when monkeys were performing the color DMS task and had not yet been trained to select targets on the basis of their object-centered location. A: Data from a neuron exhibiting object-centered spatial selectivity. Panels in the same column represent conditions in which the target was at the same location on the screen but occupied different ends of the bar. The neuron fired more strongly during bar-left than during bar-right trials. Data have been collapsed across target color (red or green) because the neuron was not selective for color. B and C: Population histograms representing mean firing rate as a function of time during the trial for all neurons recorded in M1 (B) and M2 (C). The curves represent the firing rate when the target was at the neuron’s preferred end of the bar (solid curve) or at the opposite end (broken curve). The difference between them (indicated by the width of the black ribbon spanning the post-delay period on which statistical analysis was based) corresponds to the strength of the object-centered signal. Data are aligned on the moment of onset of the target bar. Cue and saccade times are indicated as a range because the interval between each of these events and bar onset varied randomly from trial to trial. D and E: Equivalent displays for neurons exhibiting significant object-centered spatial selectivity in M1 (D) and M2 (E). The inset pie-chart indicates the fraction of the recorded population represented by these neurons.
Fig. 5. SEF neurons exhibited enhanced object-centered spatial selectivity during experimental stage 2, when monkeys were performing the object-centered task. Conventions as in Fig. 4.

Fig. 6. SEF neurons continued to exhibit enhanced object-centered spatial selectivity during experimental stage 3, when monkeys were again performing the color DMS task. Conventions as in Fig. 4.

Fig. 7. Among spatially selective neurons, the fraction exhibiting selectivity for the object-centered location of the target as opposed to selectivity for saccade direction was greater during experimental stages 2 and 3 than during experimental stage 1. A and B: Experimental stage 1: the monkeys were performing the color DMS task and had not yet been trained on the object-centered task. C and D: Experimental stage 2: the monkeys were performing the color-conditional object-centered task. E and F: Experimental stage 3: the monkeys were again performing the color DMS task. Bar height indicates the percentage of all spatially selective neurons exhibiting a given type of selectivity. Black: object-centered location of target. White: direction of saccade. Gray: both object-centered location of target and direction of saccade. Monkey 1: A, C, and E. Monkey 2: B, D, and F.

Fig. 8. The strength of object-centered signals relative to saccade-direction signals was greater during experimental stages 2 and 3 than during experimental stage 1. In each panel, the fraction of cross-condition firing rate variance explained by object-centered location is plotted against the fraction explained by saccade direction. Points above the identity line represent neurons in which object-centered location exerted a stronger influence than saccade direction. A: Data from 259 neurons recorded during performance of the color DMS task in stage 1. Two neurons, in which the object-centered and saccade-direction indices were identical, were excluded from the inset counts. B: Data from 217 neurons recorded during performance of the color-conditional object-centered task in stage 2. Eight neurons, in which the object-centered and saccade-direction indices were identical, were excluded from the inset counts. C: Data from 127
neurons recorded during performance of the color DMS task in stage 3. Two neurons, in
which the object-centered and saccade-direction indices were identical, were excluded
from the inset counts. All points in each plot fall beneath the line $x + y = 1$ because the
analysis was based solely on conditions requiring saccades to the near-left or near-right
target (the same conditions on which the ANOVA was based). Across these conditions,
object-centered location and saccade direction varied orthogonally. None of the variance
accounted for by one factor could be accounted for by the other. Consequently the two
fractions could not sum to a value greater than one.

Fig. 9. Neuronal activity during performance of the color DMS task at stage 3 was
influenced by color-location associations learned during performance of the object-
centered task at stage 2. A: Population histograms representing the activity of all 69
neurons that exhibited significant object-centered selectivity in the color DMS task
during stage 1. B: Population histograms representing the activity of all 44 neurons that
exhibited significant object-centered selectivity in the color DMS task during stage 3.
During both stage 1 (A) and stage 3 (B), firing was stronger when the target was at the
preferred object-centered location (thick curves are higher than thin curves). Two other
effects were present only during stage 3. First, neuronal activity before onset of the bar
was stronger when the cue was of the color associated with the neuron’s preferred
object-centered location than when it was of the other color (blue curves are higher than
red curves). Second, neuronal activity after onset of the bar was stronger when the color
and location of the target were incongruent than when they were congruent (the broken
thick curve is above the continuous thick curve and the broken thin curve is above the
continuous thin curve).
Fig. 1. The monkeys performed two tasks: the color DMS task and the color-conditional object-centered task. A: The timing of events was the same in both tasks: a central chromatic cue presented early in each trial instructed the monkey which end of a horizontal bar to select as saccade target at the trial's end. B: Display and saccade geometry under twelve conditions in the color DMS task. In this task, the monkey had to make a saccade to the end of the bar that matched the central cue in color (green or red). C: Display and saccade geometry under six conditions in the color-conditional object-centered task. In this task, the monkey had to make a saccade to the end of the bar (left or right) associated with the color of the central cue (green or red).
Fig. 2. Saccadic landing positions. Means and standard deviations of saccadic end-points for each monkey in each task. A and B: Color DMS task during stage 1. C and D: Color-conditional object-centered task during stage 2. E and F: Color DMS task during stage 3. The large circles are centered on the locations of the targets at near left and near right locations (Fig. 1B and C). Each of these locations could be occupied by either the left end of a bar (bar left) or the right end of a bar (bar right). The landing points on bar right trials (squares) were displaced slightly to the left of the landing points on bar left trials (circles), indicating a tendency for the eye to deviate from the bar's end toward its center. Scale is in degrees of visual angle relative to the initial fixation point.
Fig. 3. Recording sites were within a region, straddling the interhemispheric midline 2-8 mm rostral to the genu of the arcuate sulcus, from which eye movements could be elicited by microstimulation at low levels of current (< 50 μA). They thus met criteria for assignment to the SEF as established in classic studies (Russo and Bruce 1993, 2000; Schlag and Schlag-Rey 1985, 1987a, b). A and B: Parasagittal MR images through the medial face of the right hemisphere in M1 and M2. The approximately vertical line above the brain marks AP and ML zero in recording-grid coordinates. The center of the grid in each monkey was located approximately 5 mm rostral to the genu of the arcuate sulcus and over the interhemispheric cleft. The white rectangle superimposed on the gray matter demarcates the region in which recording was carried out. C and D: Location relative to the center of the chamber of neurons recorded in the context of the color DMS task during stage 1. The area of the outer (open) bubble indicates the number of recorded neurons and the area of inner (filled) bubble indicates the number exhibiting significant object-centered spatial selectivity. Gray shading demarcates the region within which electrical microstimulation elicited eye movements. E and F: Same for the color-conditional object-centered task during stage 2. G and H: Same for the color DMS task during stage 3.
Fig. 4. SEF neurons exhibited object-centered spatial selectivity even during experimental stage 1, when monkeys were performing the color DMS task and had not yet been trained to select targets on the basis of their object-centered location. A: Data from a neuron exhibiting object-centered spatial selectivity. Panels in the same column represent conditions in which the target was at the same location on the screen but occupied different ends of the bar. The neuron fired more strongly during bar-left than during bar-right trials. Data have been collapsed across target color (red or green) because the neuron was not selective for color. B and C: Population histograms representing mean firing rate as a function of time during the trial for all neurons recorded in M1 (B) and M2 (C). The curves represent the firing rate when the target was at the neuron’s preferred end of the bar (solid curve) or at the opposite end (broken curve). The difference between them (indicated by the width of the black ribbon spanning the post-delay period...
on which statistical analysis was based) corresponds to the strength of the object-centered signal. Data are aligned on the moment of onset of the target bar. Cue and saccade times are indicated as a range because the interval between each of these events and bar onset varied randomly from trial to trial. D and E: Equivalent displays for neurons exhibiting significant object-centered spatial selectivity in M1 (D) and M2 (E). The inset pie-chart indicates the fraction of the recorded population represented by these neurons.
Fig. 5. SEF neurons exhibited enhanced object-centered spatial selectivity during experimental stage 2, when monkeys were performing the object-centered task. Conventions as in Fig. 4.
Fig. 6. SEF neurons continued to exhibit enhanced object-centered spatial selectivity during experimental stage 3, when monkeys were again performing the color DMS task. Conventions as in Fig. 4.
Fig. 7. Among spatially selective neurons, the fraction exhibiting selectivity for the object-centered location of the target as opposed to selectivity for saccade direction was greater during experimental stages 2 and 3 than during experimental stage 1. A and B: Experimental stage 1: the monkeys were performing the color DMS task and had not yet been trained on the object-centered task. C and D: Experimental stage 2: the monkeys were performing the color-conditional object-centered task. E and F: Experimental stage 3: the monkeys were again performing the color DMS task. Bar height indicates the percentage of all spatially selective neurons exhibiting a given type of selectivity. Black: object-centered location of target. White: direction of saccade. Gray: both object-centered location of target and direction of saccade. Monkey 1: A, C, and E. Monkey 2: B, D, and F.
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Fig. 9. Neuronal activity during performance of the color DMS task at stage 3 was influenced by color-location associations learned during performance of the object-centered task at stage 2. A: Population histograms representing the activity of all 69 neurons that exhibited significant object-centered selectivity in the color DMS task during stage 1. B: Population histograms representing the activity of all 44 neurons that exhibited significant object-centered selectivity in the color DMS task during stage 3. During both stage 1 (A) and stage 3 (B), firing was stronger when the target was at the preferred object-centered location (thick curves are higher than thin curves). Two other effects were present only during stage 3. First, neuronal activity before onset of the bar was stronger when the cue was of the color associated with the neuron's preferred object-centered location than when it was of the other color (blue curves are higher than red curves). Second, neuronal activity after onset of the bar was stronger when the color and location of the target were incongruent than when they were congruent (the broken thick curve is above the continuous thick curve and the broken thin curve is above the continuous thin curve).