Separate Representations of Target and Timing Cue Locations in the Supplementary Eye Fields

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Campos M, Breznen B, Andersen RA. Separate representations of target and timing cue locations in the supplementary eye fields. *J Neurophysiol* 101: 448–459, 2009. First published November 12, 2008; doi:10.1152/jn.90704.2008. When different stimuli indicate *where* and *when* to make an eye movement, the brain areas involved in oculomotor control must selectively plan an eye movement to the stimulus that encodes the target position and also encode the information available from the timing cue. This could pose a challenge to the oculomotor system since the representation of the timing stimulus location in one brain area might be interpreted by downstream neurons as a competing motor plan. Evidence from diverse sources has suggested that the supplementary eye fields (SEF) play an important role in behavioral timing, so we recorded single-unit activity from SEF to characterize how target and timing cues are encoded in this region. Two monkeys performed a variant of the memory-guided saccade task, in which a timing stimulus was presented at a randomly chosen eccentric location. Many spatially tuned SEF neurons encoded only the location of the target and not the timing stimulus, whereas several other SEF neurons encoded the location of the timing stimulus and not the target. The SEF population therefore encoded the location of each stimulus with largely distinct neuronal subpopulations. For comparison, we recorded a small population of lateral intraparietal (LIP) neurons in the same task. We found that most LIP neurons that encoded the location of the target also encoded the location of the timing stimulus after its presentation, but selectively encoded the intended eye movement plan in advance of saccade initiation. These results suggest that SEF, by conditionally encoding the location of instructional stimuli depending on their meaning, can help identify which movement plan represented in other oculomotor structures, such as LIP, should be selected for the next eye movement.

INTRODUCTION

When different stimuli indicate *where* and *when* to make an eye movement, the brain areas involved in oculomotor control must selectively plan an eye movement to the stimulus that encodes the target position, but still encode the information available from the timing cue. This could pose a challenge to the oculomotor system since the representation of the timing stimulus location in one area might be interpreted by downstream neurons as a competing motor plan. Evidence from diverse sources has suggested that the supplementary eye fields (SEF) play an important role in behavioral timing. The general anatomical region in which the SEF resides—the medial frontal cortex—has been argued to be the source of the Bereitschaftspotential, or readiness potential, an electrical signature that immediately precedes self-generated movements (Ball et al. 1999; Kornhuber and Deecke 1965). The supplementary

motor area (SMA) has been shown to be activated during the internal generation of precisely timed movements at an instructed interval (Macar et al. 2004; Rao et al. 1997) and, in a survey of the neural mechanisms of interval timing, the SMA was the brain area found to be most consistently activated in neuroimaging studies (Buhusi and Meck 2005). Attention to the temporal duration of a stimulus without regard to motor planning has been shown in a functional magnetic resonance imaging (fMRI) study to selectively increase activity in a corticostriatal network that included the pre-SMA (Coull et al. 2004), which is adjacent and just medial to the SEF. Within the SEF, a subset of neurons exhibited preparatory set activity in an instructed saccade task that terminated at a time that corresponded to the beginning of activation of saccade burst neurons, suggesting that the preparatory set activity in SEF might actively regulate the timing of saccade initiation by removing inhibition (Hanes et al. 1995). The role of the SEF in the ordering of multiple movements in sequence has also been established in detail (Isoda and Tanji 2002, 2003). Based on these diverse findings, we hypothesized that the SEF might play a special role within the oculomotor network in representing timing information available from explicit timing cues.

When there are two instructional cues available to the monkey and each stimulus represents a different meaning based on the rules of the task the neurons involved in executing the task must represent these task-dependent meanings. SEF might play a critical role in this process. Damage to the lateral frontal cortex results in severe impairments in the ability to associate actions with arbitrary visual stimuli (Petrides 2007) and area 6 of Walker, in particular, is critically important when a monkey needs to select between distinct movements based on learned conditional relations with instructional cues (Petrides 1987). Area 6 also extends medially to include the SEF (Schlag and Schlag-Rey 1987) and neurophysiological evidence supports the view that SEF has the ability to conditionally associate specific eye movements with visual cues, since SEF neurons have been shown to increase activity while acquiring conditional oculomotor associations (Chen and Wise 1996). In the context of learned sequential eye movements, SEF neurons are capable of ignoring distractor stimuli and exclusively representing the location of a saccade target, which can be used to identify which stimulus is the appropriate target at each stage in the learned sequence (Lu et al. 2002). In this study we investigated how SEF represents multiple stimuli when both are relevant and meaningful to the task: one stimulus containing spatial information and the other timing information.

Parietal cortex is also critical in tasks featuring two instructional stimuli, given its role in conditional motor response tasks (Halsband and Passingham 1982). Since muscimol inactivation of the lateral intraparietal area (LIP) disrupts the selection of the correct target from an array of stimuli (Wardak et al. 2002), and human patients with bilateral parietal lesions have trouble filtering out distractors and selecting appropriate targets (Friedman-Hill et al. 2003), LIP is among the areas that appear to be participants in target selection processes. Anatomically, SEF and LIP are reciprocally connected cortical structures in the oculomotor network and both are connected to several other areas such as temporal and lateral prefrontal cortices (Andersen 1995; Huerta and Kaas 1990; Lynch and Tian 2006). LIP sits at the interface between sensory and motor cortices (Andersen and Buneo 2002), whereas SEF is in the frontal cortex. It has been argued that LIP represents a salience map (Bisley and Goldberg 2003), movement plans (Snyder et al. 1997), and default movement plans (Andersen and Buneo 2002; Cui and Andersen 2007; Snyder et al. 1997) similar to the default plans for reach found in dorsal premotor cortex (Cisek and Kalaska 2005). In the current experiments we will examine whether LIP neurons distinguish between the meaning of stimuli in terms of timing and target cues.

In this study we characterize the encoding of SEF neurons while monkeys performed a memory-guided saccade task with and without an asynchronously presented timing cue.

The timing cue was spatially irrelevant but was predictive of the timing of both the target presentation and the go signal for the saccade. We characterized individual neurons in SEF with regard to the encoding of the locations of these two stimuli. For comparison, we also recorded a small population of LIP neurons in the same task. The results presented here suggest mechanisms by which cortical oculomotor areas work together to perform an instructed eye movement when different stimuli are used to indicate *when* and *where* to generate a saccade.

METHODS

Experiments were performed with two behaving, male rhesus monkeys (*Macaca mulatta*). Each was chronically fitted with a stainless steel head post for head immobilization and two recording chambers over small craniotomies for electrode insertions. Experimental procedures were in accordance with the California Institute of Technology Institutional Animal Care and Use Committee.

Behavioral tasks

Two eye movement tasks were used: a memory-guided saccade task and a timing-cue task. In both tasks the monkey was instructed to perform a saccade from a central fixation point to one of 43 targets placed at regular intervals to cover the entire visual field out to 15° of visual angle in every direction from central fixation (Fig. 1, *top right*).
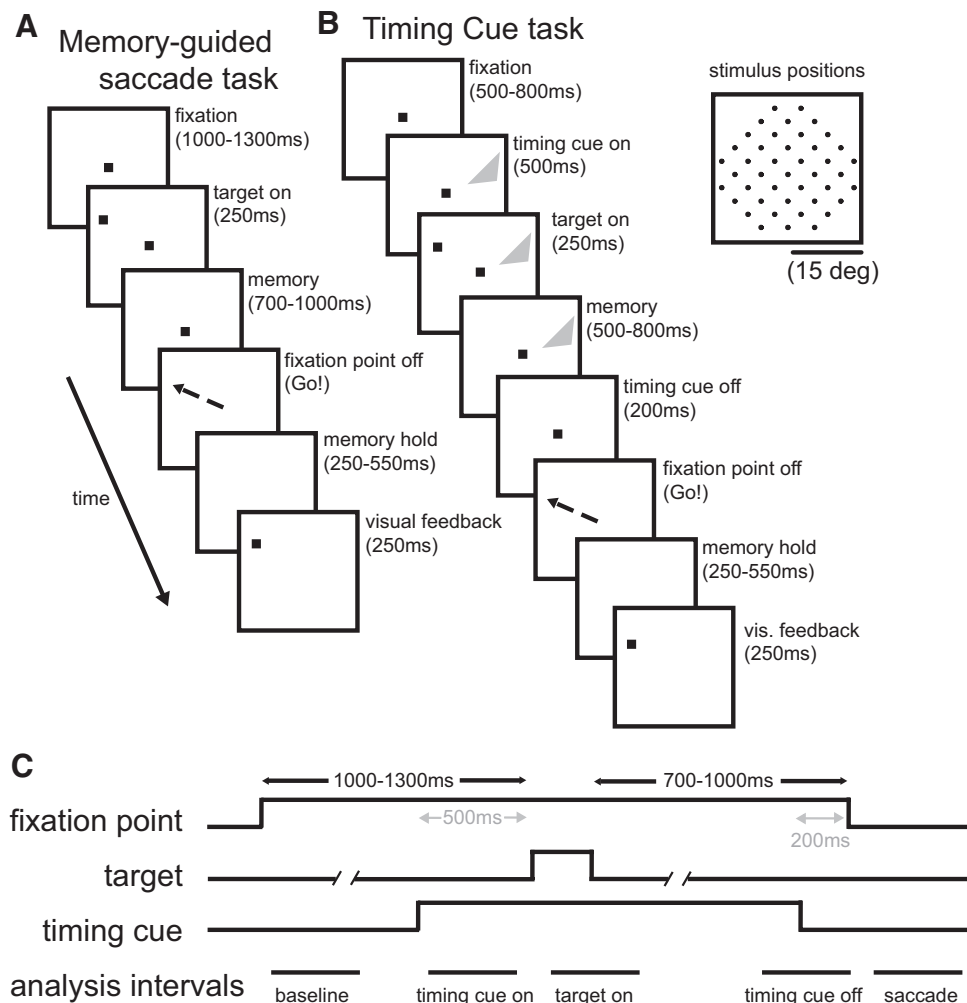

FIG. 1. Time course of oculomotor tasks. The temporal progression of each task is shown in successive panels from top left to bottom right. In the memory-guided saccade task (*A*), the monkey is required to acquire a central fixation point at the start of the trial. After a variable delay (1,000–1,300 ms), a cue is briefly flashed (250 ms) at one of 43 targets in the periphery (stimulus positions shown in box at *right*). Following a memory interval, the fixation point is extinguished and the monkey is required to saccade to the remembered target location and fixate there. After 250–550 ms, the target reappears and then, following an additional 250-ms fixation, the animal is rewarded with a drop of juice. In the timing-cue task (*B*), the monkey begins the trial by acquiring a central fixation point. After a variable delay (500–800 ms), a timing cue appears at one of the same 43 peripheral locations that are used as possible targets. The target appears 500 ms later and then the trial proceeds as in the memory-saccade task. The timing cue remains illuminated until 200 ms before the fixation point is extinguished. *C*: time course of stimulus presentations and intervals used in data analysis.

Trials from each task were interleaved, although timing-cue task trials were performed eight times more frequently, as explained in the following text.

In the memory-guided saccade task (Fig. 1A), monkeys were required to fixate a central fixation spot for a variable interval (1,000–1,300 ms), maintain central fixation while a peripheral target was briefly flashed, wait for a variable interval (700–1,000 ms) until the central fixation point extinguished, and then saccade to the remembered location. In the timing-cue task (Fig. 1B), the monkeys performed a memory-guided saccade task as described earlier, but a timing cue (green isosceles triangle or semicircle, base length 8° of visual angle) was presented peripherally while the monkey fixated at the start of the trial (after a variable interval 500–800 ms after the start of fixation). After a fixed interval following the timing-cue presentation (500 ms), the target (small white dot, diameter 1° of visual angle) was briefly flashed. The timing cue remained visible while the target was flashed and remained until 200 ms before the *fixation point off* event, which was the "go signal." At the go signal, the monkeys were required to saccade to the remembered location of the target. Note, as highlighted in Fig. 1C, that in the timing-cue task the *target on* and *fixation point off* events followed fixed intervals (*timing cue on*, 500 ms; *timing cue off*, 200 ms) and the occurrence of each of these events was therefore predictable. In the memory-guided saccade task, in contrast, these events followed variable intervals (*fixation*, 1,000–1,300 ms; *memory*, 700–1,000 ms) and the occurrence of these events was thus not predictable.

The timing-cue and target locations were chosen randomly with replacement from the possible 43 target locations. The orientation of the timing cue (±45, ±135°) and its identity (triangle or semicircle) were also chosen randomly with replacement at the start of each trial. Timing-cue and memory-guided saccade task trials were interleaved and the timing-cue trails were performed eight times more frequently than memory trials so that a sufficient number of trials would be recorded to compare different combinations of trials in which the target and timing cues appeared either in the same or opposite quadrants. We were particularly interested, for example, in trials in which both the target and the timing cue were presented in the same quadrant as the preferred direction of the neuron. A typical recording session included 400–1,000 correct trials depending on the isolation quality as monitored by the experimenter. Therefore in each recording session ≥8 trials were performed to each target in the timing-cue task and at least one trial was performed to each target in the memory-guided saccade task.

Recording procedure

Neurons were accessed with between three and six independently controlled Thomas Recording electrodes (Thomas Recording, Giessen, Germany), from one or two head-mounted micromanipulators. The electrodes were advanced with a Thomas microdrive system through a blunt stainless steel guide tube pressed against the dura for SEF recordings or a sharp stainless steel guidetube puncturing the dura and driven down 1 mm for LIP recordings. Neurons were generally found 1–3 mm beneath the exterior of the dura for SEF recordings and 5–9 mm beneath the level of the dura for LIP recordings. Both areas were identified based on a combination of anatomical localization using an MRI scanned after the chamber placement surgery and observation of saccade-related activity at each recording location. We also performed low-threshold microstimulation to evoke saccades with monkey M to confirm the location of SEF. The recording locations in frontal cortex and the results of microstimulation for monkey M are illustrated in Fig. 2. Images are presented according to MRI conventions, in which the left hemisphere is shown on the *right*.

Waveforms were amplified and isolated on-line with a commercial hardware and software package, and then isolation quality was later verified off-line (Plexon). Cell activity was monitored with custom-built on-line data visualization software written in Matlab.

Data analysis

Cell spiking activity was analyzed in four intervals defined with respect to events in the timing-cue task trials (see analysis intervals in Fig. 1C). The first two analysis intervals were chosen to include any initial visual responses to each instructional stimulus. The *timing cue on* interval was defined as the 350-ms interval starting 50 ms after the presentation of the timing cue. The *target on* interval was defined as the 350-ms interval starting 50 ms after the presentation of the target. The third interval included the time at the end of the memory period in which the timing stimulus was still present on the tangent screen, although the target was not, and continued to include any phasic response to the offset of the timing cue. This *timing cue off* interval was defined as the 350-ms interval starting 150 ms before the timing cue turned off and lasting until the go signal (fixation point off). The *saccade* interval was defined as the 350-ms interval starting 50 ms after the saccade go signal and was meant to include any activity related to either the go signal or the saccade execution itself. An additional baseline interval was defined as the 350-ms interval starting 50 ms after the fixation point appearance at the start of the trial. At this point in time the monkey was actively fixating and not planning or executing any eye movements.
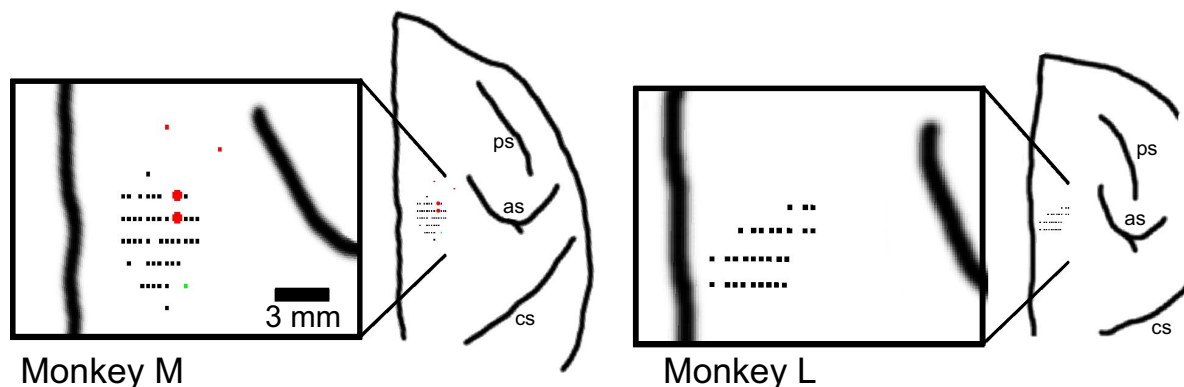

FIG. 2. Recording locations in frontal cortex. Electrode track locations (black dots) are plotted in relation to nearby sulci (principal sulcus [ps]; arcuate sulcus [as]; central sulcus [cs]) that were traced from postoperative magnetic resonance (MR) images (see METHODS). Large red dots near the center of the cluster of recording locations in Monkey M indicate the locations where saccades were reliably elicited with low current stimulation (30–50 μA). The smaller red dots at the top of the cluster are locations where eye movements were elicited, but only with higher currents (250 μA). The green dot at the *bottom right* of the cluster is a location where stimulation (250 μA) elicited a movement of the right arm. Left hemispheres are shown plotted on the *right*, according to MRI convention.

In the timing-cue trials there were two visual stimuli presented (timing cue and target) to the monkey asynchronously. To determine how individual neurons could be spatially tuned to each of these stimuli independently, trials were selected in which the target or timing cue appeared inside the response field or in an opposite location.

The direction of the response field was calculated using population vectors. We first identified the time interval that exhibited the strongest tuning for either stimulus by calculating the population vector of the firing activity in all intervals and taking the population vector with the maximum length as the period of strongest tuning. Two population vectors were calculated in each interval by multiplying the firing rates observed in the interval by the position of either the timing cue or the target and then dividing by the sum of the firing rates. The preferred direction was then defined as the direction of the largest of these eight population vectors. In the first interval, the *timing cue on* interval, the target had not yet been presented and, in this special case, the population vector was calculated according to where the cue would appear later in the trial. As expected, this never yielded the longest population vector for any of the spatially tuned neurons in our data set. After the preferred direction was identified in this way, all locations within the same quadrant (±45°) as the preferred direction were then defined to be *in* the response field of the neuron. All locations in the opposite quadrant were likewise defined to be *away* from the response field of the neuron.

This procedure to identify the response field is illustrated in the *top panels* of Fig. 4A. The *left panel* is an intensity plot of the mean firing rate activity from the *timing cue on* interval associated with timing-cue presentations at each of the 43 stimulus locations. In the *right panel* is an intensity plot of the mean firing rate activity from the *target on* interval associated with the target presentations at each of the 43 stimulus locations (the timing cue was also present in these trials, but at a randomly chosen location in each trial, and thus did not systematically affect the dependence of firing rate on target location). This particular SEF neuron exhibited spatial tuning only in the *target on* interval and was not modulated in the *timing cue on* interval. Population vectors were calculated in all four analysis intervals and with respect to both timing-cue and target locations. In this case the population vector from the *right panel* was also the longest of all eight population vector calculations and was therefore used as the preferred direction of this neuron. The response field was then defined with respect to this preferred direction in all time intervals and for both stimuli. The red dots superimposed on the firing rate intensity plots indicate locations *in* the response field quadrant and the yellow dots (target positions in the opposite quadrant) are the *away* locations.

To assess the significance of spatial tuning for either the timing cue or the target, average neural firing activity was compared using ANOVA ($P < 0.01$, with Bonferroni correction for multiple comparisons) for trials in four stimulus conditions: the combination of trials in which the timing cue appeared in the response field versus away and the target appeared within the response field or away. A neuron exhibited spatial tuning for a given stimulus if there was a significant difference between the firing rates observed in trials in which that stimulus was in the response field versus away, whereas the other stimulus was in a fixed location (either in or away). These stimuli configurations are illustrated on the *left side* of the rastergrams in Figs. 4–6. If a neuron exhibited an increased or decreased firing rate relative to the baseline interval (ANOVA, $P < 0.01$, with Bonferroni correction for multiple comparisons) but did not pass the criteria for spatial tuning, then it was classified as having a nonspatially tuned response. Examples of nonspatially tuned neurons are shown in Fig. 6.

Some neurons, especially in LIP, exhibited spatially tuned responses to both stimuli that were significantly different from baseline. We additionally observed that these responses were often significantly different from each other. Although it may be interesting to characterize the relative magnitude of the response to each stimulus, our experimental methods did not allow for a quantitative comparison between the stimuli responses for three reasons. First, the responses to the target may have been combined with the existing response to the timing cue since the timing cue was still visible during the target presentation. We could therefore only compare responses across trials from the two variants of the task—with and without the timing cue—but this approach also did not work well because we recorded fewer trials in the memory-guided saccade trials and, in many recordings, the response field was not adequately sampled. Second, the information available in the timing cue was not necessary for the successful completion of the task and therefore we could not be sure whether the monkey considered that information on a single-trial basis, thus introducing another source of variability to the neural response. Third, the number of neurons in our LIP sample was too small to justify concrete statements about whether the initial responses to both stimuli were indeed different in magnitude generally across the population and in which direction. Therefore for the purpose of this report we characterized only the initial responses to the target and timing cue as different from baseline.

RESULTS

Neurons were recorded from the SEF and LIP of two monkeys during the performance of interleaved memory-guided saccade task and timing-cue task trials (Fig. 1). All neurons encountered in these anatomical regions that could be reliably isolated were recorded. There were 57 task-related neurons found in SEF (monkey M: 38; monkey L: 19) from a sample of 187 recorded SEF neurons (monkey M: 87; monkey L: 100). There were 43 task-related neurons found in LIP (monkey M: 13; monkey L: 30) from a sample of 189 recorded LIP neurons (monkey M: 103; monkey L: 86).

The SEF recording locations are illustrated in Fig. 2 for both monkeys. Microstimulation was performed on monkey M to confirm the location of SEF. The large red dots near the center of the cluster of recording locations indicate the locations where saccades were reliably elicited with low current stimulation (30–50 μA). These results show that at least most of our recording locations were in SEF, according to the criterion that SEF is a region in which saccades are reliably elicited by low current stimulation. The recording locations of monkey L were similarly situated relative to the nearby anatomical landmarks.

Behavioral analysis verifies that subjects used the timing cue

Since the "go signal" (fixation point off) always followed the timing cue disappearance after a fixed duration (200 ms; see METHODS), we wanted to know whether the monkeys were using the timing information available from the timing cue. To test this possibility we analyzed the reaction times (time from the go signal to the target acquisition) in both trial conditions. We found that both monkeys exhibited shorter mean reaction times in trials in which the timing cue was presented. For monkey L, the mean reaction time in the regular memory-guided saccade trials was 224 ms (SE = 1.43 ms) and in the timing-cue trials was shortened by 44 ms to 180 ms (SE = 1.43 ms). We found the same trend for monkey M, for whom the mean reaction time in the regular memory-guided saccade trials was 204 ms (SE = 0.99 ms) and in the timing-cue trials was shortened by 41 ms to 163 ms (SE = 0.56 ms). As can be further seen in the distributions of reaction times in Fig. 3, there were many more short-latency (<150 ms) saccades in the trials in which the timing cue was presented. Based on this reaction time evidence we concluded that the monkeys were making use of

Reaction Times (Monkey L)
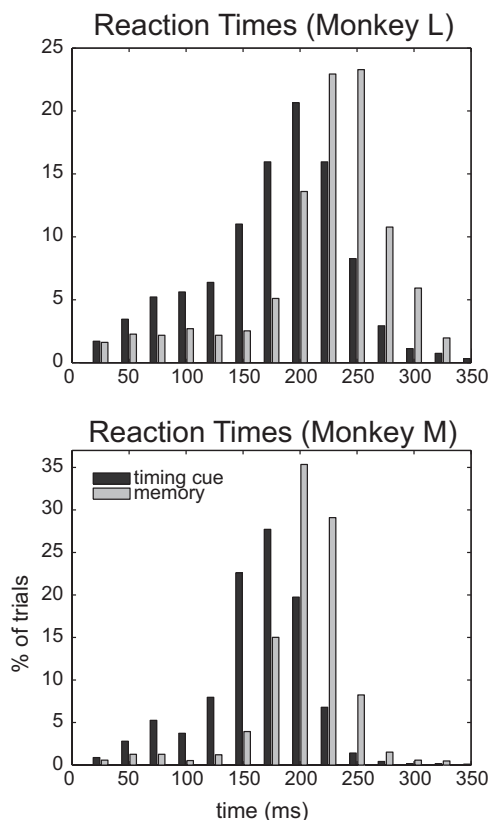

Reaction Times (Monkey M)

FIG. 3. Reaction times in both trial types. Reaction time distributions are shown for each monkey. Trials in which the timing-cue stimulus was presented (timing-cue task trials) are shown in black. Trials in which the timing-cue stimulus was not presented (memory-guided saccade task) are shown in gray.

the timing cue for its timing information. Therefore whereas the location, identity, and orientation of the timing cue were all irrelevant for saccade preparation by design, the timing cue was in fact informative with regard to indicating *when* the instructed saccade could be made.

Spatially tuned responses to the target and timing-cue stimuli

Several SEF neurons encoded the location of only one of the instructional stimuli. Figure 4 shows two typical examples of spatially tuned neurons from SEF that illustrate the general finding that most SEF neurons responded to one of the two stimuli. The first example neuron exhibits strong spatial tuning after the presentation of the target. This neuron's firing activity was unchanged by the presentation of the timing cue, regardless of where it was presented, and strongly activated by target presentations in the right hemifield (black, red, and magenta rastergrams). At the *top* of Fig. 4*A* are two spatial intensity plots that show the average firing rates associated with timing cue (*left*) or target (*right*) presentations at each of the 43 stimuli locations. The high intensity at the right side of the plot on the *right* shows that the neuron responded in a spatially tuned manner for the target stimulus presentation. The roughly even distribution of intensity in the plot on the *left* shows that the neuron did not encode the spatial location of the timing cue.

We ran a series of statistical tests (ANOVA, $P < 0.01$ with Bonferroni correction for multiple comparisons) to quantify the

tendency of a given neuron to encode the location of one or both stimuli. Each test evaluated whether individual neurons were encoding the location of either the timing cue or the target, or both, by comparing firing rates in cases in which these stimuli were presented either in the response field or at an opposite location (see METHODS). The first four rastergrams in the *middle* of Fig. 4*A* show responses of the example neuron for the combinations of trials in which the target and timing cue were either in the response field or in an opposite location. The *bottom two rows* show neural responses when the target was in or away from the response field in the memory-guided saccade task trials (in which the timing cue was not presented at all). At the *bottom* of Fig. 4*A* are the mean firing rates for these six conditions with corresponding SE error bars calculated in 50-ms intervals. As can be seen, the two conditions in which the timing cue was presented inside the response field (black and green lines) show no change in activity during the *timing cue on* interval. In the *target on* interval, in contrast, the conditions in which the target was presented inside the response field (red, magenta, and black lines) were significantly elevated compared with trials in which the target was presented away (green, blue, and cyan).

The second example SEF neuron shown in Fig. 4*B* responded to the timing cue only. This neuron exhibited a transient increase in activity following the presentation of the timing cue in a large portion of the visual field, with strongest modulations observed when the timing cue was presented up and slightly to the right (black and green). Timing-cue presentations in the direction opposite to the preferred direction of this neuron (red and blue), which was less activated compared with trials in which the timing cue was presented inside the response field (black and green), still showed an increase in firing activity relative to memory trials in which no timing cue was presented at all (magenta and cyan), indicating that the response field was very large. During the presentation of the target this neuron was essentially unmodulated. These two SEF example neurons thus responded to visual stimuli in particular locations, but only when the stimuli were members of its preferred category. In the population of task-related SEF neurons we found that there were more neurons tuned to the target during the *target on* interval (26/57, 46%) than were found to be tuned for the timing cue during the *timing cue on* interval (10/57, 18%), with only three neurons tuned for both stimuli (see Fig. 7 and additional details in *Population results* in the following text).

We recorded a small population of task-related LIP neurons ($n = 43$) for comparison with the SEF population. The spatial representations differed in qualitatively significant ways between the two areas. Whereas spatially tuned SEF neurons usually responded to only one of the stimuli, spatially tuned LIP neurons would usually respond to both the target and the timing cue. The example neuron shown in Fig. 5 illustrates the general finding that LIP neurons responded with similar intensity to the timing cue and the target. During the presentation of the timing cue, this neuron was vigorously activated if it appeared in the *lower left*. Similarly, during the presentation of the target, the neuron was again vigorously activated when the target appeared in the same area. This example LIP neuron thus responded to visual stimuli in particular locations irrespective of the category to which the stimulus belonged. Some LIP neurons exhibited initial responses to both stimuli that were significantly different *from each other*, but the experimental
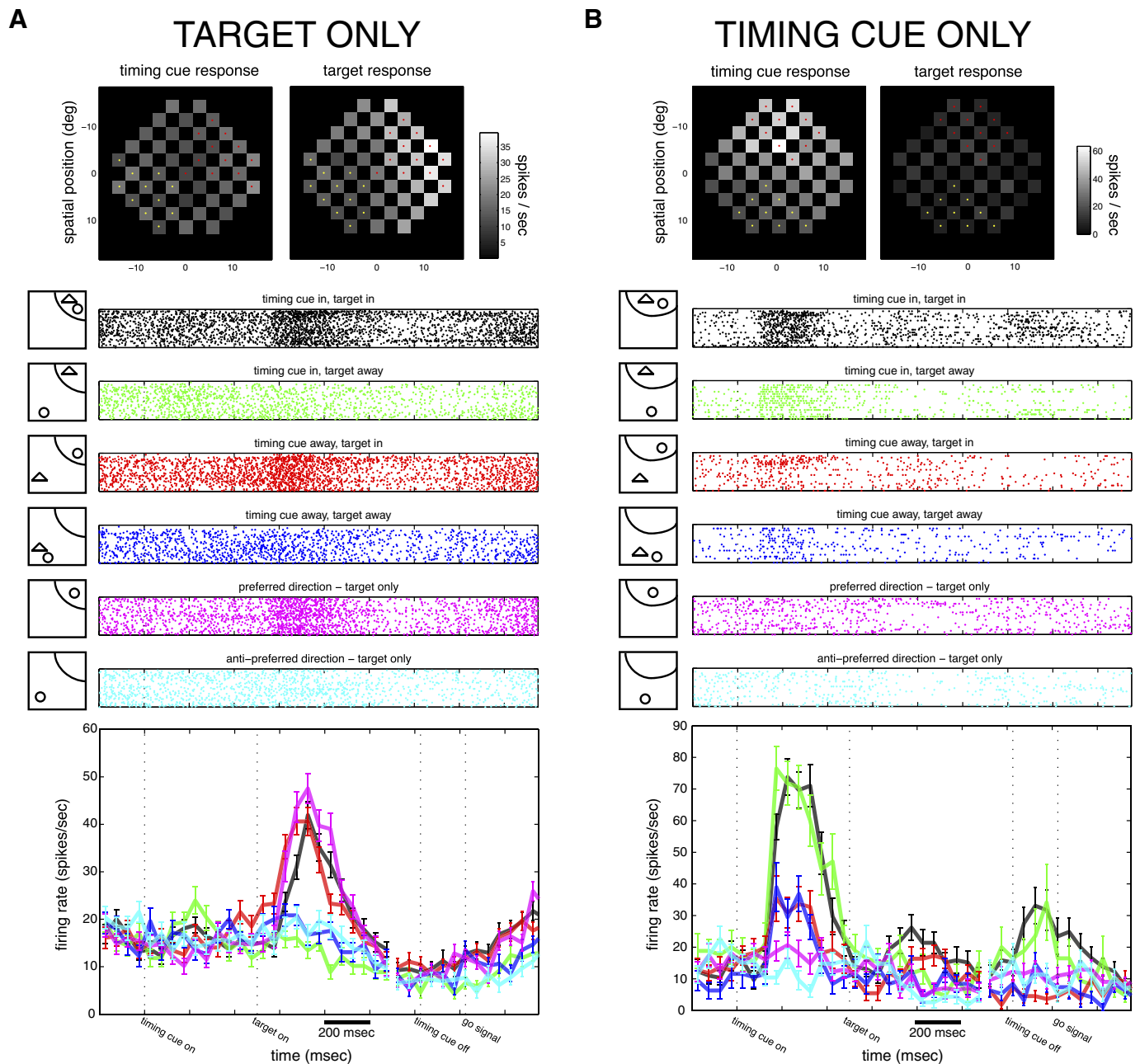
A TARGET ONLY

B TIMING CUE ONLY

FIG. 4. Example spatially tuned supplementary eye field (SEF) responses to target and timing-cue stimuli. *A*: example neuron that responds only to the target and ignores the timing-cue stimulus. *Top row left*: firing rate intensity plot showing mean firing rates associated with timing-cue presentations at the 43 stimulus positions (350-ms interval starting 50 ms after target presentation). *Top row right*: firing rate intensity plot showing mean firing rates associated with target and timing cue presentations (350-ms interval starting 50 ms after target presentation). *Middle*: spike trains for 6 different stimulus configurations. Stimulus configurations are shown schematically on the *left*, with the curved line indicating the response field, the triangle representing the timing cue, and the circle representing the target. Event times are labeled at *bottom*. *Bottom*: mean spike firing rates in time with SE for the 6 stimulus configurations (color coded to match histograms in *middle*). *B*: example SEF neuron that responds only to the timing-cue presentation, with a smaller, but significant response when the timing cue is extinguished.

methods did not allow for a quantitative comparison between these initial responses, so we report only that the responses were present or absent (see METHODS). In the population of task-related LIP neurons we found similar numbers of neurons to be tuned to the timing cue during the *timing cue on* interval (19/43, 44%) and tuned for the target during the *target on* interval (17/43, 40%), with the majority of these neurons (14) tuned for both stimuli (see Fig. 7 and additional details in *Population results* in the following text).

Since the LIP neurons tended to respond to both instructional stimuli on their initial presentation, it is interesting to consider what happens to these responses during the course of planning the eye movement to the target. The LIP neuron shown in Fig. 5 exhibited rich firing rate dynamics during the time intervals that followed the target presentation, which reflect the population of LIP neurons. Midway through the memory period the two stimuli conditions featuring the timing cue in the response field were highest (black and green), with
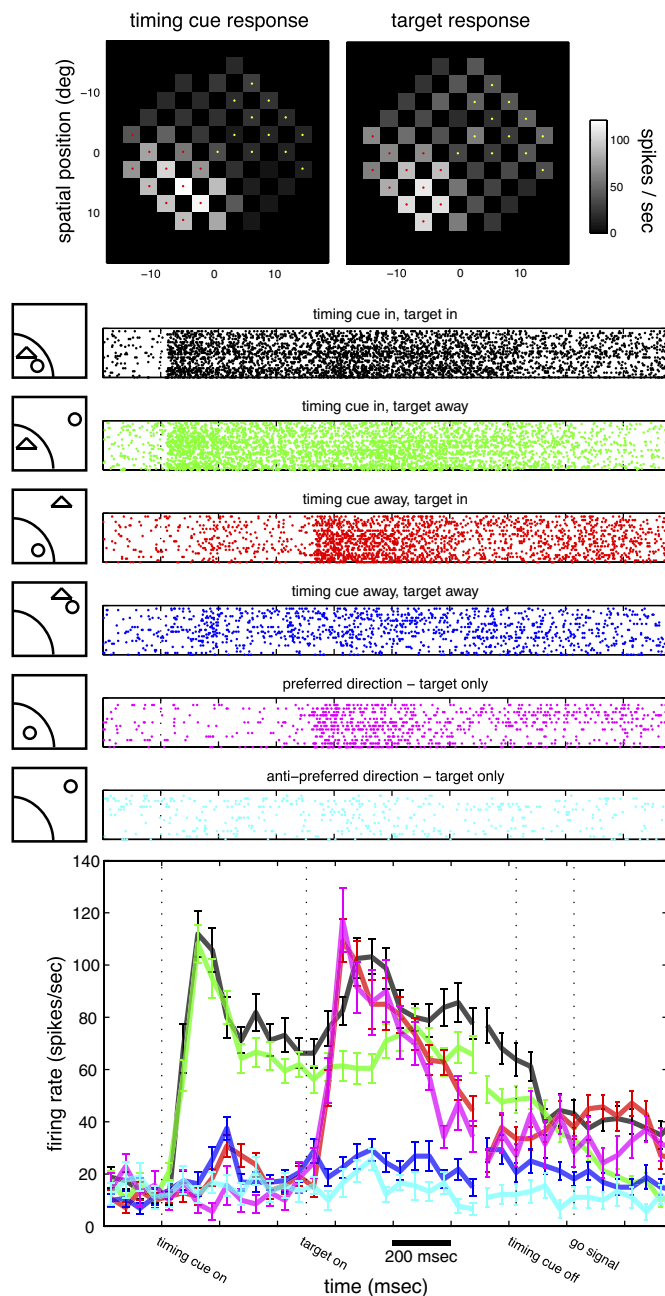
LIP - TARGET & TIMING CUE

FIG. 5. Example spatially tuned lateral intraparietal (LIP) response to target and timing-cue stimuli. Example LIP neuron that responded similarly to the target and timing-cue stimuli. Same conventions as Fig. 4.

the firing rate slightly lower when only the target had been in the response field (red and magenta). At this point in the trial the target had already extinguished and the timing cue remained present on the screen. After the timing cue extinguished, the firing rates for trials in which only the timing cue was in the response field (green) dropped, whereas at the same time the firing rates for timing-cue trials in which only the target was presented in the response field (red) began to rise. The trials in which both stimuli had been in the response field (black) remained elevated during this transition interval, perhaps representing the sum on these decreasing and increasing

signals. Finally, during the saccade, the trials in which the target was presented in the response field (red and black) were statistically indistinguishable and the trials in which the target was presented opposite the response field (green and blue) were equally low. During the saccade, the previous location of the timing stimulus thus had no influence on the firing rate of this example neuron. These dynamics illustrate how this single neuron represented locations of the two instructional stimuli at slightly different strengths throughout the entire trial and then represented the location of the target exclusively during saccade execution. It is important to note that the visual conditions are different for the two cues. The target stimulus is only briefly flashed followed by a "memory" period in which it is not present, whereas the timing cue is continuously present until just before the go signal to make the eye movement. Thus visual input may contribute substantially to the activity related to the timing stimulus.

Nonspatially tuned responses in SEF

Many SEF neurons exhibited nonspatial responses at various times in the trial. The two neurons shown in Fig. 6 were activated in nonoverlapping intervals in the task. The first was active from the beginning of the trial until just after the target extinguished, although there was a brief and slight suppression when the timing cue was presented. The second neuron was activated in the interval that began around the time that the target stimulus disappeared and continued until just after the go signal. For these neurons the directions of the response fields were calculated in the same way as described earlier (see METHODS), but since these neurons were not spatially tuned, the resulting response field definitions were essentially arbitrary. As can be seen in these figures, the firing rates were not modulated based on stimuli configuration, but rather on the interval within the trial. These nonspatial responses may explain the ability of other SEF neurons to selectively represent either the target or timing-cue stimuli (see DISCUSSION).

Population results

The mean firing rates averaged over all task-related neurons (exhibiting spatially tuned or nonspatially tuned modulations in at least one interval) are shown in the *top panels* of Fig. 7, separately for each of the six stimuli conditions (color coded to match previous rastergrams), and two anatomical areas: SEF (*left*) and LIP (*right*). The numbers of individual cells found to exhibit tuning for either stimulus or to be nonspatially responsive (see METHODS) are shown in the *bottom panels* of Fig. 7.

There are three notable features of the mean firing rate plots. First, in the SEF population activity, the initial response to the target is higher than the initial response to the timing cue, whereas in LIP the mean population responses are similar for both stimuli presentations. As can be seen in the cell counts in the *bottom panels*, this difference in mean population activity for the two stimuli can be mainly attributed to the different numbers of neurons responding to each stimulus. In SEF there were about 2.5-fold as many neurons encoding the location of the target as the timing cue, whereas in LIP these numbers were nearly equal. When considering the neurons that encoded the location of only one stimulus, there were significantly more SEF neurons encoding the location of the target than the timing
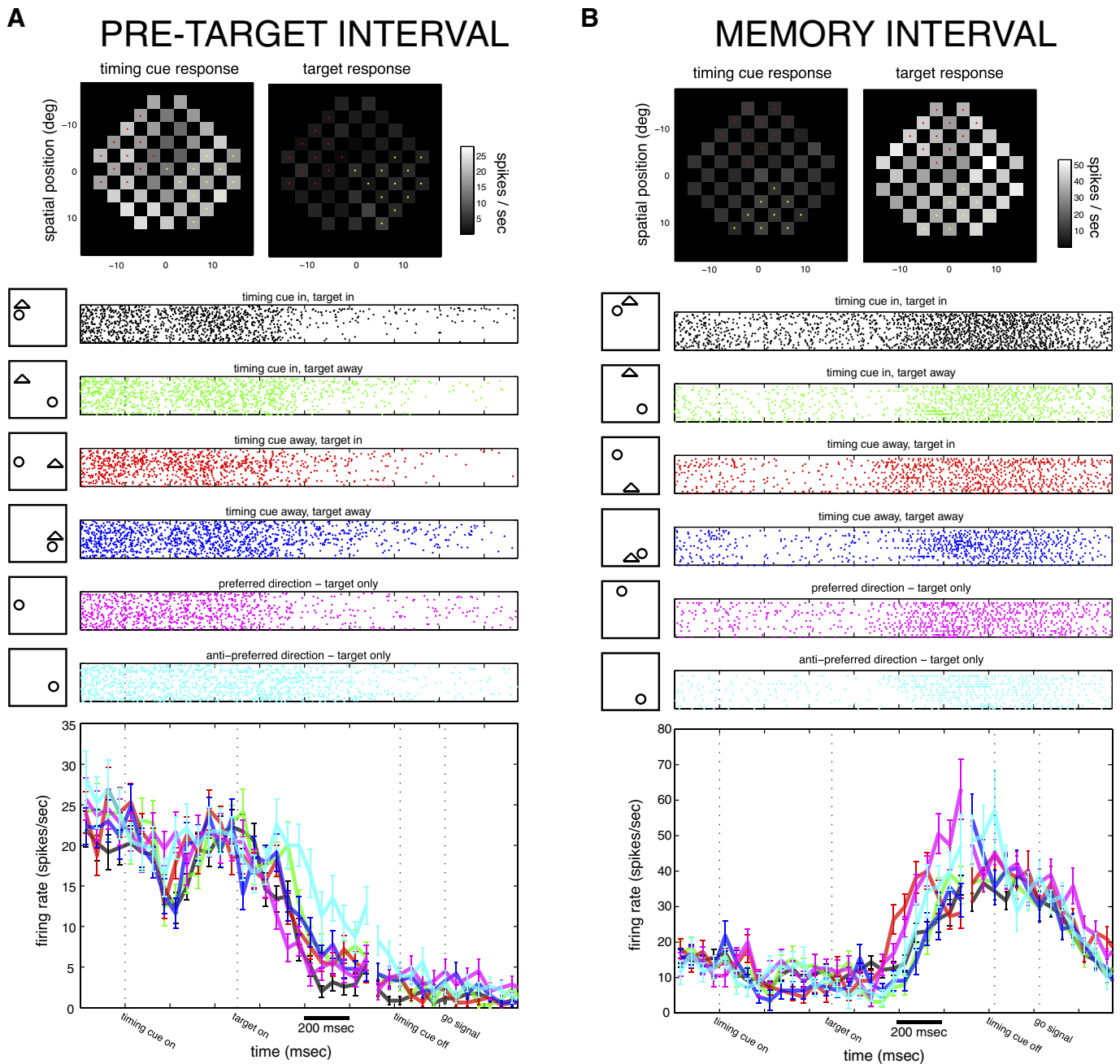
A PRE-TARGET INTERVAL

B MEMORY INTERVAL

FIG. 6. Example nonspatially tuned SEF neurons. *A*: example SEF neuron with elevated firing activity extending from the start of the trial to just after the target presentation (target on). *B*: example SEF neuron with elevated activity in the interval extending approximately from the time the target is extinguished (250 ms after target on) until the fixation point was extinguished (go signal), with a steeper initial rise of activity in the memory-guided saccade trials (cyan and magenta). See text for additional description.

cue (target:timing cue = 23:7, χ^2, $P < 0.01$). The LIP population had a statistically equal number of neurons encoding each stimulus independently (target:timing cue, 5:3, χ^2, $P > 0.5$), although one must be cautious when interpreting statistical measures on such a small sample. Some of the neurons were significantly activated following the presentation of both stimuli, although at different levels. However, this differential modulation was observed in a small number of neurons.

Second, the mean firing activity when the timing cue was in the response field and the target was away (green lines in

top panels) was strikingly different between the two populations in the intervals following the saccade target presentation. In the SEF population this green line was essentially equal to the blue and cyan lines, which represent the conditions in which no stimuli were presented in the response field. In the LIP population the green line was significantly elevated with respect to these stimuli away conditions (blue and cyan) and partially overlapping with the conditions in which the target was presented in the response field (black, red, and magenta). This indicates that the LIP population maintained the representation of the
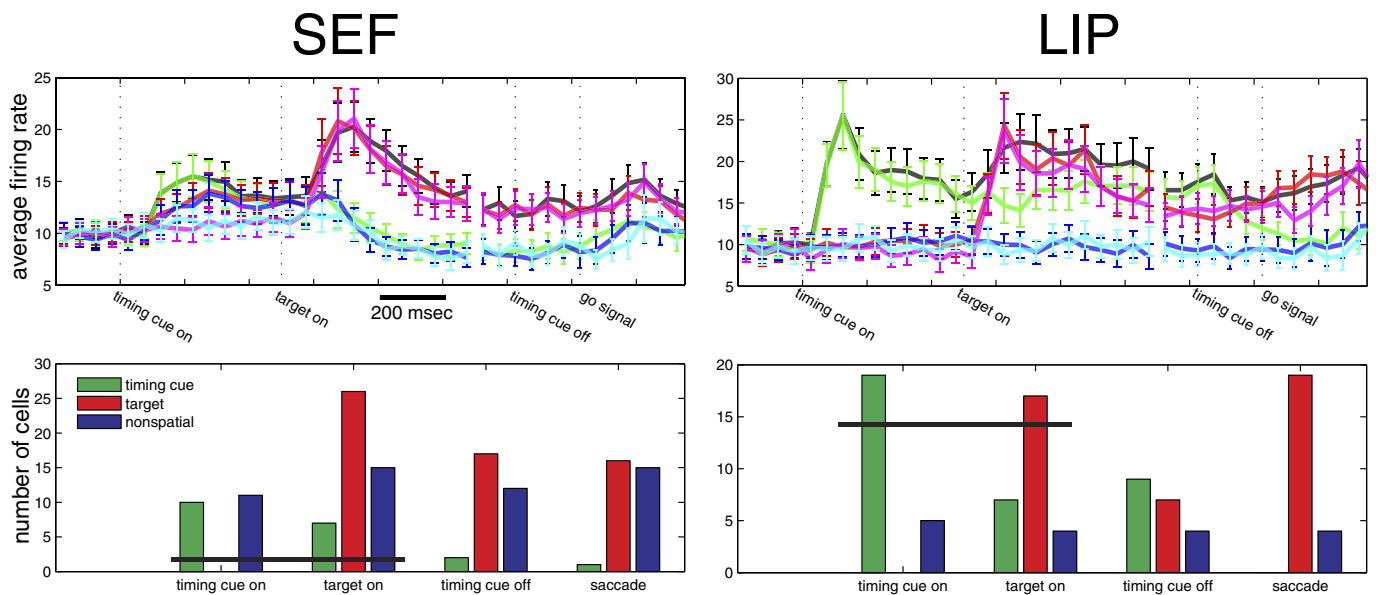
FIG. 7. Population results. *Top*: mean firing rates for all task-related neurons in the SEF (*left*) and LIP (*right*) populations. Error bars are SE. Colors correspond to the stimuli configurations shown in Figs. 4–6. *Bottom*: cell counts of neurons satisfying the criteria for spatial tuning for the timing cue (green) or target (red), or for nonspatial tuning (blue), at each of 4 intervals in the task (see METHODS).

location of the timing cue in the period following the target presentation, whereas the SEF population did not.

Third, the blue and cyan lines are essentially flat in the LIP population plot, indicating the LIP neurons were generally not modulated if no stimulus was presented within the response field. The blue and cyan lines in the SEF population plot, however, showed a substantial modulation over the course of the trial. This was due in part to neurons with large response fields, as shown in Fig. 4*B*, and in part to a substantial number of neurons that exhibited untuned but significant modulations at different points in the trial (Fig. 6).

In the *bottom panels* of Fig. 7 are shown the summary counts of all of the neurons in our database tuned for the timing cue or target location or nonspatially modulated at different intervals in the task. Timing-cue tuning was tested in four intervals: *timing cue on*, *target on*, *timing cue off*, and *saccade*. Target tuning was assessed during the latter three of these intervals (see METHODS for interval definitions). In the SEF population, there were more neurons found to be tuned to the target during the *target on* interval (26/57, 46%) than were found to be tuned for the timing cue during the *timing cue on* interval (10/57, 18%), with only three neurons tuned for both stimuli (shown as a black horizontal line). During the *timing cue off* period there were many more SEF neurons tuned to the location of the target (17, 30%) than to the timing cue (2, 4%). Similar numbers of spatially tuned LIP neurons were found to be tuned to the timing cue during the *timing cue on* interval (19/43, 44%) and tuned for the target during the *target on* interval (17/43, 40%), with the vast majority of these neurons (14, shown as a black horizontal line) tuned for both stimuli. During the *timing cue off* interval there were similar numbers of LIP neurons tuned to the location of the timing cue and the target. In monkey M there were more neurons tuned for the target compared with the timing cue (3:1) during the *timing cue off* interval; in monkey L, however, there were fewer neurons tuned for the target compared with the timing cue (4:8). This inconsistency could be attributable to the small sample size. At

the time of the saccade all of the spatially tuned LIP neurons were tuned for the target location only.

To assess nonspatial modulations we compared firing rates in the same four intervals used in the spatial tuning analysis (*timing cue on*, *target on*, *timing cue off*, and *saccade*) with baseline firing rates (see METHODS). Several neurons were found to exhibit significant modulations but did not pass the tests for spatial tuning described earlier and therefore exhibited nonspatially tuned responses. The numbers of these are shown as blue bars at the *bottom* of Fig. 7. There was a higher percentage of nonspatially tuned neurons in SEF than in LIP.

The ratios of nonspatially tuned to spatially tuned neurons during the *timing cue on* interval—when spatial tuning was assessed with respect only to the timing cue location—were strikingly different between the two populations. In SEF there were 11 nonspatially tuned neurons compared with 10 spatially tuned neurons, meaning that a little more than half of the neurons in SEF that were modulated at that time were not encoding the location of the timing cue. In LIP there were four nonspatially tuned neurons compared with 18 spatially tuned neurons at that time, meaning that 82% of the neurons in LIP that were modulated were encoding the location of the timing cue. Therefore both populations responded to the appearance of the timing cue, but in LIP this response was usually spatially specific, whereas in SEF this response was either spatially specific or not with equal probability. That is, whereas most responding LIP neurons indicated where the timing cue was located, about half of the responding SEF neurons simply indicated that it had appeared.

DISCUSSION

In this report we have shown that spatially tuned SEF neurons usually encoded the location of only one stimulus in a task in which different stimuli indicated *where* and *when* to make an eye movement. This finding implies that the locations of these two instructional stimuli were represented in separate

subpopulations of SEF neurons, with each subpopulation generating a map of visual space to unambiguously represent the location of one stimulus. It has been shown previously that response fields in SEF are conditional on the meaning of the stimuli appearing inside them (Chen and Wise 1996; Chen et al. 2001; Lu et al. 2002), similar to responses in ventrolateral prefrontal neurons that appear to integrate task rules (Sakagami and Pan 2007). In this study we have gone further to show that SEF separately encodes the location of two stimuli when both are relevant to saccade behavior.

The SEF neurons may have responded to the timing cue because it was a meaningful stimulus in an oculomotor task and not specifically because it carried timing information. Previously, SEF neurons have been shown to respond to stimuli that carry information relevant to the guidance of oculomotor behavior that are not themselves saccade targets. SEF neurons respond in the antisaccade task, in which the location of the spatial cue instructs the monkey to generate a saccade in the opposite direction (Amador et al. 2004; Schlag-Rey et al. 1997). SEF neurons have also been shown to respond to object-centered cues that indicate a portion of an object to which the monkey should generate a saccade without specifying the exact saccade metrics (Olson and Gettner 1995). In the context of a sequential saccade task, SEF neurons encoded target direction depending on the numerical position of saccades (rank order) in an instructed sequence, suggesting that the SEF contributes to the temporal ordering of multiple saccades (Isoda and Tanji 2002, 2003; Lu et al. 2002). The sequential saccade results imply that for a potential saccade target at a given location, distinct subsets of SEF neurons encoded the location of that target depending on whether it was the first, second, and so forth target in an instructed sequence. These results thus overlap with ours, since they imply that the SEF uses distinct subpopulations of neurons to encode the location of different types of visual cues (first vs. second saccade, target vs. timing cue, etc.). We suspect that the SEF might encode the locations of all task-related cues in distinct populations of neurons.

Although the meaning of the stimuli is important—and may be more important than that meaning being related to behavioral timing—there are several reasons to believe that the SEF is especially concerned with behavioral timing. As reviewed in the INTRODUCTION, the general anatomical region in which the SEF resides has been implicated as the source of the Bereitschaftspotential (Ball et al. 1999; Kornhuber and Deecke 1965) and is activated during the internal generation of precisely timed movements (Buhusi and Meck 2005; Macar et al. 2004; Rao et al. 1997) or attention to the temporal duration of a stimulus (Coull et al. 2004). Preparatory set activity in SEF might actively regulate the timing of saccade initiation by removing inhibition (Hanes et al. 1995). There is clearly an abundance of evidence suggesting that the SEF would be involved in representing instructional stimuli related to behavioral timing in particular.

Our results relate especially well to a previous microstimulation study in which stimulation was applied at one of two different points within a delayed saccade task—either 500 ms after fixation onset (and before the presentation of the saccade target stimulus) or 50 or 100 ms before the go signal triggering the saccade. This study found that the saccades evoked by the stimulation during fixation tended to be either fixed vector or goal-directed, in accordance with previous stimulation studies.

However, when intracortical microstimulation (ICMS) was delivered to the SEF in the period after the cue signal was already presented, and before the go signal triggering the saccade (the dimming of the fixation point), the saccades evoked by the ICMS were directed toward the target instructed by the cue, usually with a shortened reaction time. The authors speculated that these two types of evoked saccades suggest two distinct function roles by which SEF contributed to oculomotor behavior. The former shows that SEF contributes to the selection of the saccade goal and the latter suggests a role of the SEF in inducing the initiation of the saccade goals that have already been selected (Fujii et al. 1995). These functional roles parallel our main result—that SEF encodes saccade targets and timing cues. Although one must be careful when interpreting microstimulation results, it seems possible that the stimulation applied just prior to the go signal in that study may have played a role similar to that of the offset of the timing-cue stimulus in our study.

In contrast to the finding that individual SEF neurons responded to either the location of the target or the timing cue, LIP neurons usually represented the locations of both stimuli, indicating that the ensemble of LIP neurons encoded both stimuli together in one map. The response fields of LIP neurons did not appear to be similarly conditional on stimulus meaning other than as potential saccade targets or as cues to which the monkey should attend. In previous studies, LIP neurons have been shown to be *modulated* by the meaning of stimuli, for example, by simultaneously representing an informative stimulus feature such as color or category (Assad 2003; Freedman and Assad 2006). In this study, we have also observed unequal responses to the two stimuli in LIP but we did not report on these response differences for a few reasons (see METHODS). The modulation by stimulus category observed in LIP for directional stimuli was quite large and reminiscent of the SEF responses that we are reporting here to be conditional on the meaning of the stimulus, but there were important differences. Most notably, in the initial visual sample period the LIP neurons responded to presentations of the nonpreferred stimulus category as well, albeit with a comparatively lower firing rate. We have found that, in contrast, spatially tuned neurons in SEF did not respond at all to the presentation of the nonpreferred stimulus. Furthermore, because the same study found that neurons in area MT, a lower-level cortical area that provides input to LIP about directional stimuli, did not contain category-encoding responses (Freedman and Assad 2006), we suspect that the category information did not come from the bottom-up, but instead reflected information transmitted from top-down inputs. Similarly, the initial response to a distractor in LIP can be attenuated if it is predictable (Ipata et al. 2006), reflecting the effect of top-down influences on the response to sudden-onset stimuli (Jonides and Yantis 1988). The results presented here suggest that the separate representations of different instructional stimuli found in SEF could be the source of some of these top-down influences.

Many SEF neurons responded to the timing-cue presentation irrespective of its location, such as the example neurons shown in Fig. 6. These nonspatially tuned neurons could provide the mechanism whereby other SEF neurons selectively encode the location of only one stimulus. For example, the neuron shown in Fig. 6*A*, active from the beginning of the trial until just after the presentation of the target, could inhibit other neurons

during the presentation of the timing cue, effectively gating the flow of information into a neuron such as the one shown in Fig. 4*A*, which selectively encodes the cue location. A similar mechanism was posited by Hanes and colleagues (1995) to account for the observation that preparatory set activity terminated at the same time that saccade-related activity began in distinct populations of SEF neurons. These authors noted that the precise temporal relationship suggested that the preparatory set activity might actively regulate the timing of saccade initiation by removing inhibition from saccade-related neurons and, furthermore, that similar mechanisms might guide saccade selection, as suggested by the results presented here. Our results do not imply, however, that SEF activity precisely triggers oculomotor behavior. Schall and colleagues (2002) claimed that FEF is intimately involved in the triggering of eye movements by virtue of neural activation reaching a threshold at a time that is related to reaction times on a trial-by-trial basis. SEF neurons did not exhibit this same property and thus were supposed not to be directly involved in saccade timing. In our report, we claim only that the timing cue is important for predicting the signal to execute the saccade. The detailed mechanics and precise timing of saccade initiation are a different matter not directly addressed in this study.

The neural activity tuned for the timing-cue location during the *timing cue off* interval indicates that LIP maintains representations of the locations of both target and timing-cue stimuli until just before the eye movement plan to the target is executed. This sustained representation could reflect a default plan to the timing cue, which is canceled later in the trial because of the constraints imposed in the experiment. LIP has been shown to represent default eye movement plans (Andersen and Buneo 2002; Snyder et al. 1997), including the period in trials in which the animals have not yet chosen whether to look or reach to a target cue (Cui and Andersen 2007). The representation of the timing cue in LIP during this interval could also reflect attentional processes—that the monkey was required to pay attention to the timing-cue stimulus. This representation could in turn serve to enhance the representation of that stimulus in lower-level areas (Saalmann et al. 2007), to more reliably perceive the timing information it contained. The design of our current study does not distinguish between these possibilities. The sustained representation of the timing cue could reflect that the monkey is paying attention to this stimulus because it is relevant to the task or it represents a default plan to saccade to the timing-cue stimulus, or some combination of these effects.

It has been shown previously that multiple stimuli can be represented simultaneously in LIP (Bisley and Goldberg 2003). However, in our study the timing-cue stimulus appears *before* the saccade target and persists after the saccade target disappears. The response to the timing cue in the memory period was therefore not a response to a suddenly appearing visual stimulus. It would be informative to test whether LIP neurons will continue to represent a persistent cue stimulus that is presented prior to the target as in this study, if it were not informative for saccade timing or any other aspect of saccade planning. An earlier study of the representation of a distractor in LIP featured a briefly flashed visual stimulus at the end of the memory period, such that it extinguished at a fixed 100-ms interval prior to the saccade go signal (Powell and Goldberg 2000). In light of the findings reported here, we would propose

to retest LIP responsivity when the distractor does not carry such reliable timing information to see whether the LIP responses will be as vigorous.

These data from LIP and SEF offer insights into the mechanisms of saccade planning in the distributed cortical oculomotor network. We found that most LIP neurons responded to both the timing-cue and target stimuli in a spatially tuned manner. Some SEF neurons were spatially tuned and others were not and the spatially tuned SEF neurons usually responded to one or the other cue. The spatial representations in the two areas suggest that LIP is involved in identifying the locations of all stimuli relevant to saccade planning, whereas SEF selectively maintains a representation of each stimulus separately, perhaps so that it can influence the target-selection process occurring in LIP and other areas. Furthermore, the spatial representation of timing-cue information in SEF—with individual neurons having spatially circumscribed receptive fields—may account for the recently observed human behavioral evidence that visual events are timed by neural mechanisms that are spatially selective (Burr et al. 2007).

Our results show how task-relevant stimuli—particularly with respect to movement planning and behavioral timing—are encoded in separate populations of frontal neurons. These separate representations might be matched with the combined representation of potential target locations in parietal cortex, in the process of selecting the appropriate target. This might prove to be a general mechanism for identifying various kinds of information that are relevant for the planning and execution of movements.

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