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Modulation of Presaccadic Activity in the Frontal Eye Field by the Superior Colliculus

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Berman RA, Joiner WM, Cavanaugh J, Wurtz RH. Modulation of presaccadic activity in the frontal eye field by the superior colliculus. *J Neurophysiol* 101: 2934–2942, 2009. First published March 25, 2009; doi:10.1152/jn.00053.2009. A cascade of neuronal signals precedes each saccadic eye movement to targets in the visual scene. In the cerebral cortex, this neuronal processing culminates in the frontal eye field (FEF), where neurons have bursts of activity before the saccade. This presaccadic activity is typically considered to drive downstream activity in the intermediate layers of the superior colliculus (SC), which receives direct projections from FEF. Consequently, the FEF activity is thought to be determined solely by earlier cortical processing and unaffected by activity in the SC. Recent evidence of an ascending path from the SC to FEF raises the possibility, however, that presaccadic activity in the FEF may also depend on input from the SC. Here we tested this possibility by recording from single FEF neurons during the reversible inactivation of SC. Our results indicate that presaccadic activity in the FEF does not require SC input: we never observed a significant reduction in FEF presaccadic activity when the SC was inactivated. Unexpectedly, in a third of experiments, SC inactivation elicited a significant increase in FEF presaccadic activity. The passive visual response of FEF neurons, in contrast, was virtually unaffected by inactivation of the SC. These findings show that presaccadic activity in the FEF does not originate in the SC but nevertheless may be influenced by modulatory signals ascending from the SC.

INTRODUCTION

Neuronal signals in cortex are usually thought to govern those in the brain stem. In the oculomotor system, for example, the generation of rapid, saccadic eye movements depends on activity in a pathway that extends from the cerebral cortex to the extraocular motor neurons (Wurtz and Goldberg 1989). Within this pathway, two structures have long been recognized as key: the cortical frontal eye field (FEF) and the superior colliculus (SC) (Schall 2002; Sparks and Hartwich-Young 1989). We typically consider communication between these two structures to be unidirectional; the FEF has well-established projections to the intermediate layers of the SC, so it is natural to think of saccade commands traveling downstream from the FEF to the SC (Helminski and Segraves 2003; Komatsu and Suzuki 1985; Schlag-Rey et al. 1992; Sommer and Wurtz 2000, 2001; Stanton et al. 1988a). Recent studies, however, have identified a pathway that ascends from SC to FEF via the mediodorsal thalamus (MD) (Lynch et al. 1994; Sommer and Wurtz 2002). This ascending pathway has been shown to transmit corollary discharge signals, which influence the spatial properties of visual receptive fields in the FEF around the time of saccades (Sommer and Wurtz 2004b, 2006). In those experiments, any presaccadic activity of the neurons was avoided by having monkeys make saccades away from the neuronal movement fields.

An untested hypothesis, therefore, is that this ascending path from SC also contributes to presaccadic activity in the FEF. This hypothesis may seem counterintuitive but could explain observed differences between the FEF and the lateral intraparietal area (LIP), another cortical structure that contributes to saccade planning and generation (Andersen 1989; Colby and Goldberg 1999). Neurons in both the FEF and LIP project to the intermediate layers of the SC, but these output neurons differ in the signals that they carry. FEF output neurons frequently have a burst of presaccadic activity in addition to visual and delay activity, and sometimes have only presaccadic activity (Segraves and Goldberg 1987; Sommer and Wurtz 2001). LIP output neurons, by contrast, have this presaccadic burst less frequently and never have only presaccadic activity (Pare and Wurtz 1997, 2001; Wurtz et al. 2001). The FEF neurons therefore resemble those in SC more closely than do LIP neurons. In keeping with this, the FEF appears to have more prominent input from SC than does LIP (Pare and Wurtz 2001), and the ascending path from SC to FEF is known to transmit the presaccadic burst (Sommer and Wurtz 2004a). Thus while the presaccadic activity in the FEF is traditionally presumed to emerge from earlier stages of cortical processing, we cannot yet reject the possibility that this presaccadic activity actually originates in the SC. In the present study, we tested the hypothesis that presaccadic activity in FEF requires input from SC. We examined the contribution of the SC to FEF activity by recording from FEF neurons while reversibly inactivating the SC. We asked whether SC inactivation reduces presaccadic activity; we also asked whether it modulates the passive visual response in FEF.

METHODS

In three adult male monkeys (*Macaca mulatta*) weighing from 8 to 11 kg, we implanted scleral search coils for measuring eye position, recording cylinders for accessing FEF and SC, and a post for immobilizing the head during experiments. We conducted 4 experiments in the first animal, 2 in the second, and 18 in the third (of these, our final analysis included 3, 2, and 13 experiments, respectively, and the modulatory neuronal effect we describe in the following text was observed in data from the first and third animal). All procedures were approved by the Institute Animal Care and Use Committee and complied with Public Health Service Policy on the humane care and use of laboratory animals.

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Outline of experimental steps

We began each experiment by determining the movement and visual receptive fields of neurons at a series of sites in SC and FEF. This preliminary mapping enabled us to inactivate portions of the SC with representations that overlapped those of the recorded FEF neurons. For each experiment, we first advanced an injection needle with an attached recording electrode ("injectrode") toward the SC. The injectrode targeted a region of the retinotopic map in SC. We then lowered a recording electrode into FEF and searched for neurons that had presaccadic activity (or in some experiments, visual responses) for locations that overlapped those represented in the targeted region of SC. When an FEF neuron was encountered, we characterized visual and presaccadic activity using delayed visually guided saccades. We then temporarily inactivated the SC with lidocaine, measuring decreases in saccade velocity to determine the efficacy of the inactivation. We recorded the FEF neuronal activity during three periods of the experiment, which were defined in off-line analysis: preinjection, deficit, and when possible, recovery.

Saccade task

The monkey's head was restrained, and it faced a tangent screen 57 cm in front of it. Fixation spots and visual stimuli were either red spots on a dark background, back-projected by a laser, or white spots on a gray background, back-projected by an LCD projector. Onset of the visual stimuli was determined from the time the laser spots were turned on or from the time a spot was flashed onto a photocell in synchrony with the appearance of the projector-generated stimulus. A computer running REX (Hays et al. 1982) controlled stimulus presentation, administration of reward, the recording of eye movements and single neuron activity, and the on-line display of results.

We used a delayed visually guided saccade task to characterize FEF activity during both the initial mapping and the inactivation. The monkey began each trial by fixating on a spot in the center of the screen for 250 ms. A target then appeared at one of five locations in the periphery. The center of these peripheral targets was chosen based on the visuomovement fields of the FEF neuron and by the trajectory of saccades evoked with microstimulation. The other four targets were placed a fixed distance around this center at cardinal locations (0, 90, 180, 270°) to estimate the extent of the field (see Fig. 1*A, top left inset*). For the data selected for analysis (see following text), average saccade amplitude was 17.3° (range: 10–25.5°) and average saccade direction was 17.2° (range: 0–56.3°). After the target appeared, the monkey had to maintain central fixation for an additional 500 ms. The disappearance of the fixation point was the monkey's cue to move the eyes to the target. Liquid reward was given if the monkey attained the target within 500 ms and maintained fixation there for an additional 250 ms.

Recording and microstimulation

One recording cylinder was implanted over the FEF for recording neuronal activity and one over the SC for both recording and subsequent inactivation. The FEF chamber was positioned approximately normal to the cranial surface. The SC chamber was tilted 38° backward from vertical so electrodes entering through this chamber would approach the SC approximately normal to the collicular surface. Both chambers were cemented in place with dental acrylic with additional acrylic to secure the eye coil wires and to attach to the titanium support screws.

After initial estimation using MR images, we located recording sites electrophysiologically. We recorded single neuron responses and microstimulated in FEF and SC with tungsten microelectrodes advanced by a stepper microdrive. Electrodes passed through guide tubes in a 1-mm-resolution grid in the recording cylinder (Crist et al. 1988). For the SC, the microelectrode was attached to an injection needle, described subsequently in METHODS. Neuronal responses were discriminated from background activity using a software-based waveform discriminator. To evoke saccades with microstimulation, we passed current for 70 ms using biphasic pulses (0.25 ms/phase at 350 Hz). We characterized visuomovement fields in FEF and SC by monitoring neuronal activity while the monkey made saccades to targets throughout the contralateral visual field. For the FEF, we targeted neurons in the anterior bank of the arcuate sulcus and verified their location with two criteria: saccade-related activity and the ability to evoke saccades with currents of $\leq$50 μA (Bruce and Goldberg 1985). For the SC, we identified entry into the structure by the robust visual responses encountered in the superficial layers. We differentiated the intermediate layers from the superficial layers by the emergence of presaccadic activity and the dramatic drop in the stimulation current required for evoking saccades.

In ~2/3 of the experiments, we used microstimulation of the SC to assess directly the connectivity of single neurons in the FEF to the SC. We stimulated through the SC recording electrode using single biphasic current pulses (0.15 ms/phase, negative-positive) and looked for evoked spikes in the FEF neuron under study. Orthodromic (synaptically driven) activation of the FEF neuron meant that it received input from the stimulated site in SC; antidromic activation (backfiring) of the FEF neuron meant that it sent output to the SC. Failure to obtain such activation from SC stimulation does not mean there is no connection, just that one could not be demonstrated, potentially due to a lack of precise alignment between the SC and FEF sites. Further details of the stimulation procedure are given in Sommer and Wurtz (1998).

SC injection procedure

Our injection targeted the intermediate layers of SC, which are the source of the known projection pathway from SC to FEF (Lynch et al. 1994; Sommer and Wurtz 2004a). We used lidocaine hydrochloride (2%) to inactivate the SC because it produces a relatively short-duration inactivation, making it possible to obtain data during a subsequent recovery period. The short duration (usually ~20 min), and rapid onset of the lidocaine inactivation also minimized time for spread in the SC. We reasoned that these advantages outweighed the disadvantage of potential inactivation of fibers of passage within the SC injection zone. Targeted injections were made through a 30-gauge needle injectrode; a tungsten microelectrode was cemented to its side and extended 500 μm beyond the end of the needle. This extension allowed us to identify potential SC sites before the injection needle reached the site. We advanced the injectrode until neuronal recording and/or microstimulation criteria indicated that the microelectrode was located in the SC intermediate layers. As in the FEF, these criteria were the presence of saccade-related activity, and the ability to evoke saccades with stimulation currents of $\leq$50 μA. To place the injection at the site of stimulation, we advanced the syringe 500 μm to account for the extension of the microelectrode 500 μm beyond the injection needle. We injected lidocaine either with manual pressure on the syringe plunger, or by computer control of a second microdrive attached to the plunger. Injections ranged from 0.25 to 4.0 μl lidocaine and were administered over the course of ~3 min. This wide range of injection volumes is due to variability in our early experiments, as we worked to establish the minimum volume required to elicit consistent behavioral effects during inactivation and refined the technical procedure. As the experiments progressed, volumes were less variable and averaged ~1.5 μl. We did not observe any relationship between the magnitude of neuronal effects and injection volume.

Analysis of saccades and neuronal activity during inactivation

We used the monkeys' saccades to a visual target as an assay of the inactivation of the SC. Our main measure was the decrease in eye

velocity, which can occur without altering saccade accuracy but is a clear indicator of neuronal changes in the SC (Hanes and Wurtz 2001). On the basis of the velocity measurement, the neuronal data were divided into three periods: a *preinjection period*, a *deficit period*, and a *recovery period*. The preinjection period consisted of the data collected before the injection of lidocaine. The deficit period began after the injection when the eye velocity decreased <80% of the mean velocity during the preinjection period. The deficit period ended when the eye velocity of two consecutive trials was greater than this decrease, i.e., exceeded 80% of the preinjection mean. The recovery period began when eye velocity reached 90% of that in the preinjection period. For some experiments, these three analysis periods were separated by two transition periods when eye velocities did not meet the requirements of either adjacent period. Data from the transition periods were not analyzed. We used a two-tailed Student's *t*-test to determine whether the eye velocity during the deficit period was significantly different from the preinjection period and, if available, whether the eye velocity during the recovery period was different from the preinjection period. These determinations were made for each of the five target locations individually.

For each experiment and each period therein, we assessed both visual and presaccadic activity in FEF. Visual activity was measured in a 100-ms window that began 50 ms after the stimulus appeared. Presaccadic activity was measured in a 50-ms window that began 50 ms before the start of the saccade. The saccade start was identified as the time that eye velocity and acceleration exceeded 100°/s and 5,000°/s^2, respectively. We only analyzed the injection results for FEF neurons that had significant visual or saccade-related activity during the preinjection period. Significant visual activity was recognized if activity in the visual window was ≥2 SD above activity measured in a prestimulus background epoch, a 200-ms window that began 160 ms before stimulus appearance. Significant presaccadic activity was recognized if activity in the saccade window was ≥2 SD above activity measured in a 100-ms window that began 300 ms before saccade onset.

Of the five target locations in each experiment, we chose only one location to evaluate neuronal changes during inactivation. The chosen location exhibited the strongest neuronal responses in the preinjection period and had a significant change in saccade velocity between preinjection and deficit periods. In other words, the location represented the best overlap between the FEF visuomovement field and the field affected by SC inactivation. Frequently, there were several locations for each experiment where we observed the FEF–SC overlap, but we do not think the inclusion of these multiple locations yields more information. First, the other locations would not be independent; they would all be for the same injection. Second, we thought that choosing the optimal site of overlap offered greater signal to noise than using an average that included suboptimal sites and introduced differences in saccade amplitude and direction. For the selected location, we used a Wilcoxon rank-sum test to determine whether FEF activity in the visual and/or presaccadic windows differed significantly between the preinjection and deficit periods of the experiment. For some experiments ($n = 10$), we were able to hold the FEF neuron long enough to collect data during a recovery period in which saccade velocities returned to ≥90% of the preinjection levels. When recovery data were available, we likewise tested for neuronal activity differences between the preinjection period and recovery period.

RESULTS

We conducted a total of 24 experiments in which we recorded from single FEF neurons during the inactivation of the intermediate SC. Of these, four were discarded during subsequent analysis due to an insufficient number of eligible saccade trials in the preinjection and deficit periods. We investigated the effect of inactivation on presaccadic (or visual) activity only for the experiments in which the FEF neuron had significant presaccadic (or visual) activity during the preinjection period. Consequently, an additional two experiments were discarded due to a lack of both visual and presaccadic activity. The remaining 18 experiments were included in analysis of the effect of SC inactivation on presaccadic activity ($n = 15$) or visual responses ($n = 8$) in FEF.

Decreased saccade velocities during SC inactivation

We first asked if the inactivation of SC was effective. We had two major requirements for the inactivation. First and foremost, we required that the inactivation produce a change in saccade behavior at target locations overlapping with the FEF visuomovement field. We measured changes in saccade velocity in order to map the SC "deficit field" (Hanes and Wurtz 2001). Figure 1*A* shows a monkey's saccade velocities for five targets that fell within the estimated FEF movement field, indicated by the dashed circle. We used the velocities to define three periods: preinjection, deficit, and recovery (red lines define the deficit period at each target location). For this experiment, saccade velocity was significantly lower during the deficit period than the preinjection period for all targets, indicating that the SC deficit field overlapped with the FEF visuomovement field. We note that the duration of the velocity decrease was not uniform across locations in this example; we observed similar variability in several other experiments and presume that it may reflect unevenness in the spread of lidocaine. The essential observation is that for each of the 18 experiments included here, we saw significant decreases in saccade velocity for at least two of the five target locations and always at a location where we also observed significant neuronal activity in FEF. The inactivations therefore successfully produced an overlap between the SC deficit field and the FEF visuomovement field.

Our second requirement was for the inactivation to be small enough that it did not prevent the monkeys from performing the saccade task. Eye traces from the same example experiment (Fig. 1*B*) show that we met this goal: the monkey was still able to make saccades to all targets during the deficit period despite small changes in trajectory. This was true for all reported experiments.

Thus these data demonstrate that the SC lidocaine injection was effective. Injection into a specific part of the SC saccade map caused a velocity change at a location encompassed by the FEF visuomovement field, but did not disrupt the monkey's ability to perform the saccade task.

Presaccadic activity in FEF is never reduced during SC inactivation but can be enhanced

Our central question was whether presaccadic activity in the FEF requires ascending signals from the intermediate layers of the SC. For each experiment, we followed activity at a single target location where initial FEF presaccadic activity was strongest and where the SC inactivation had affected saccade behavior (significant reduction of saccade velocities). Our expectation was that if FEF presaccadic activity originates in SC, then it should be reduced during SC inactivation.

We found that SC inactivation did not reduce presaccadic activity in the FEF. Figure 2*A* shows the averaged population

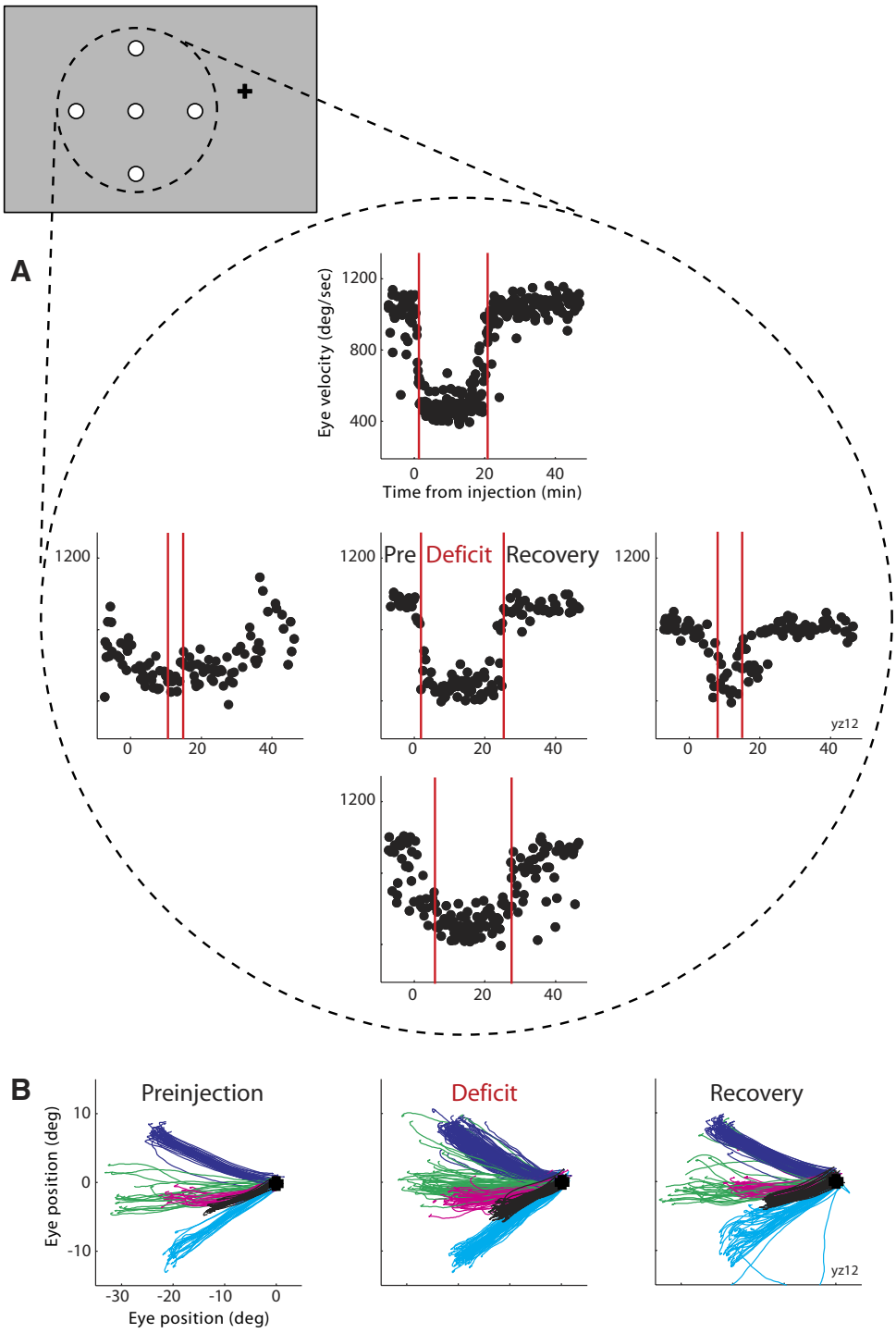

FIG. 1. Example of saccade changes during superior colliculus (SC) inactivation. *A*: saccade velocities show that the SC deficit field overlaps the frontal eye field (FEF) visuomovement field. *Top left inset*: the visuomovement field (dotted circle) of the FEF neuron in this experiment, which had presaccadic activity for saccades made from central fixation (crosshair) to each of 5 possible target locations (white circles). For this experiment, the center target was 5° below and 20° to the left of fixation. The other targets were 10° from the center target. The expanded view (large dotted circle) shows the peak eye velocity in degrees per second (*y* axis) for saccades to each target, plotted as a function of time (*x* axis). *Time zero* indicates the beginning of the lidocaine injection. Red lines indicate the beginning and end of the deficit period for each location (see METHODS). For this experiment, eye velocities were significantly lower during the deficit period than during the preinjection period at all target locations, and the center location was chosen for neuronal analysis due to its strongest presaccadic activity. *B*: eye movement trajectories from the same example experiment show that the monkey can still make saccades. Trajectories for all locations are shown for the three analysis periods: preinjection, deficit, and recovery. *x* and *y* axes represent the horizontal and vertical eye positions in degrees of visual angle.

activity of presaccadic neurons (spike density plots, $n = 15$), where activity is aligned on the beginning of the saccade. The population activity was not reduced during the deficit period (thick red line) but instead increased slightly compared with the preinjection period (thick blue line). The increase is also evident in the sample in Fig. 2*B*, which shows for each experiment the average activity during the presaccadic epoch, for the preinjection period (*x* axis) versus the deficit period (*y* axis). For this sample, presaccadic activity was larger overall during the deficit period than during the preinjection period (median 64.6 spikes/s during deficit, 56 spikes/s preinjection), but this difference was not statistically significant ($P = 0.19$, Wilcoxon signed-rank test).

We did not observe a significant reduction of presaccadic activity in any of the 15 SC inactivations. Instead, contrary to expectation, presaccadic activity *increased* significantly in a third of individual experiments (solid black dots in Fig. 2*B*, $P < 0.05$, Wilcoxon rank-sum test). An example of the increase is shown in Fig. 2*C*. In this example experiment, SC inactivation led to a significant reduction in saccade velocities at the selected location, and we were able to record from the FEF neuron not only during the deficit but also after velocities

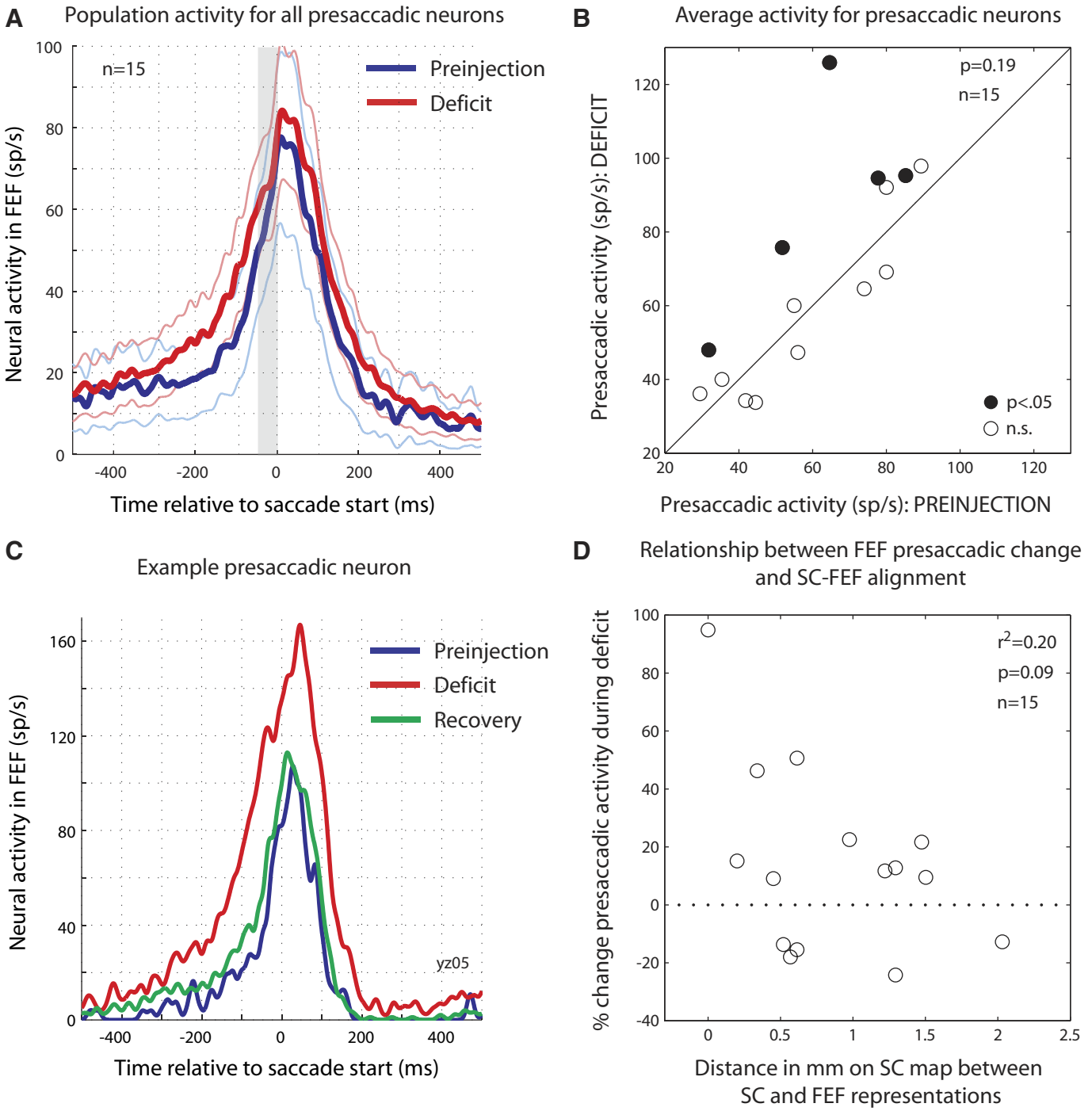

FIG. 2. Presaccadic activity in FEF during SC inactivation. *A*: population spike density plot for all FEF neurons with presaccadic activity ($n = 15$) shows that activity does not decrease during the deficit period (red line) compared with the preinjection period (blue line). FEF activity in spikes per second (y axis) is shown as a function of time (x axis), aligned on the beginning of the saccadic eye movement (*time 0*). Thin lines represent standard error. The shaded box indicates the presaccadic epoch used for computing average firing rates in *B*. *B*: each dot represents average presaccadic activity in a single experiment for the deficit period (y axis) plotted against the preinjection period (x axis). Solid dots indicate experiments with a significant difference between the 2 periods. *C*: presaccadic activity of an example neuron with a significant increase in activity during the deficit period compared with both preinjection and recovery periods. Conventions as in *A*. *D*: relationship between the inactivation-induced change in FEF activity and the alignment of spatial representations in FEF and SC. Each dot is from a single experiment. The x axis shows the distance in millimeters on the SC map between the SC injection site and the SC site that corresponds to the FEF representation. The y axis shows the percentage change in presaccadic activity during the deficit period compared with the preinjection period. The dashed horizontal line indicates no change in activity during deficit compared with preinjection.

recovered. The presaccadic activity for this neuron increased during SC inactivation (Fig. 2*C*, red line), and the increase between preinjection and deficit periods was significant ($P < 0.00001$). Presaccadic activity returned to preinjection levels during the recovery period (green line), and firing rates for these two periods were not significantly different ($P = 0.19$).

In summary, we can reject the hypothesis that the presaccadic bursts in FEF require inputs from SC. In the majority of

experiments, presaccadic activity was not altered by SC inactivation. Moreover, in the experiments where we did see significant modulation by SC inactivation, this modulation was never a reduction but always an increase in presaccadic FEF activity.

Understanding the increase in presaccadic activity

In light of the finding that a subset of experiments revealed a significant enhancement of presaccadic activity in FEF during SC inactivation, we wanted to know whether any factors distinguished these experiments from those in which FEF activity was unchanged. We considered three possibilities. First, we asked if the neurons with the presaccadic increase were uniquely characterized by direct connections with the SC (either input from or output to SC). In a subset of experiments ($n = 8$), we had microstimulated the SC to test for connectivity between the FEF neuron under study and the SC injection site (see METHODS). We found, however, that direct connections did not predict the presence or absence of modulation. For experiments with a significant presaccadic increase, we detected connectivity in two of the three cases tested (1 neuron received input from SC, the other projected to SC). Similarly, for experiments without modulation, we found connectivity in all five cases tested (2 received input from SC, 3 projected to SC). In other words, direct connections between the FEF neuron and the SC site were common and did not account for the pattern of modulation observed in FEF presaccadic activity.

Second, we asked if the change in presaccadic activity was due to differences in saccade metrics during the deficit period compared with the preinjection period. If the FEF were driving a different saccade after inactivation, its presaccadic activity might change accordingly. We addressed this possibility by conducting two further analyses, one on the entire population of presaccadic neurons and another on the subset of neurons that showed an increase in presaccadic activity. For the entire population, we computed for each experiment the percentage change between preinjection and deficit periods for presaccadic activity, saccade velocity, and saccade amplitude. We asked if the change in presaccadic activity was related to a change in either velocity or amplitude. It was not: changes in saccade metrics did not predict changes in firing rate (for velocity, $r^2 = 0.08$, $P = 0.30$; for amplitude, $r^2 = 0.04$, $P = 0.45$, linear regression). We conducted the next analysis on the five experiments where FEF presaccadic activity increased significantly after SC inactivation. If this increase was due to changes in saccade metrics, we reasoned that it should disappear if we analyzed trials from the preinjection and deficit periods with equivalent metrics. We focused on saccade amplitude as there were too few trials if we attempted to match velocities. We recalculated the median firing rates for a "matched" subset of deficit trials, for which the amplitudes were not significantly different from the preinjection period ($P > 0.05$, Wilcoxon rank-sum test). For two experiments, the number of deficit trials in this reanalysis decreased by 70–75% and consequently, the increase in presaccadic firing approached but no longer reached statistical significance. For the other three experiments, the increase remained significant. Moreover, for all five experiments, the "matched" subset had the same median firing rate obtained for the entire sample of deficit trials. In other words, even when saccade amplitudes did not differ significantly for deficit versus preinjection periods, we still observed the increased neuronal activity for deficit versus preinjection periods. These additional analyses show that the increase in FEF presaccadic activity is not readily attributed to altered saccade metrics after SC inactivation.

Third, we asked if the influence of SC inactivation on FEF activity was related to the spatial alignment between FEF and SC sites. Specifically, were changes in FEF activity stronger when the location represented by the FEF was more closely aligned with the representation at the site of inactivation in SC? We addressed this question by mapping the distance between FEF and SC movement fields in the coordinates of the SC map (Ottes et al. 1986). In this coordinate system, we can estimate the physical distance between the two field representations on the SC map and obtain a clearer understanding of how the SC inactivation might affect the saccade trajectories encoded by the FEF neuron. Figure 2*D* shows the relationship between the distance between SC and FEF sites (as represented on the SC map in mm; *x* axis) and the percentage change in FEF presaccadic activity during inactivation (*y* axis). The relationship was not statistically significant ($r^2 = 0.20$, $P = 0.09$, linear regression) although the data show a trend toward greater increases in presaccadic activity with smaller distances between the FEF and SC sites. Notably, we observed the largest increase in presaccadic activity for the single experiment in which the FEF and SC representations were precisely aligned (distance = 0). These data, while a limited sample, indicate that the alignment of FEF and SC sites may be an informative predictor of the effect of SC inactivation on presaccadic activity in FEF.

Visual responses are not systematically changed during SC inactivation

Earlier observations suggest that the ascending path from intermediate SC to FEF is unlikely to modulate the passive visual response in FEF (Sommer and Wurtz 2004a, 2006), but the effect of SC inactivation on visual activity in FEF had not yet been tested. We were able to investigate this in a subset of the FEF neurons that had visual activity ($n = 8$). The averaged population activity of these neurons (Fig. 3*A*) shows that the visual response was not significantly modulated during SC inactivation. When we measured the average activity in a visual epoch for each of the experiments, we found no significant difference between the preinjection and deficit periods in the sample of visual neurons (Fig. 3*B*; median: 30.9 spikes/s preinjection, 29.9 spikes/s during deficit; $P = 0.25$, Wilcoxon signed-rank test). Finally, we found that visual activity was significantly modulated in only two individual experiments, and this modulation was not systematic: activity increased in one case and decreased in the other (solid black dots in Fig. 3*B*, $P < 0.05$), Wilcoxon rank-sum test. We conclude that inactivation of SC does not strongly modulate the passive visual response in FEF.

DISCUSSION

SC drives neither presaccadic nor visual activity in FEF

Our central finding is that presaccadic activity in the FEF does not require input from the intermediate, saccade-related layers of the SC. Inactivation of SC failed to abolish or even significantly reduce the strength of presaccadic activity in the

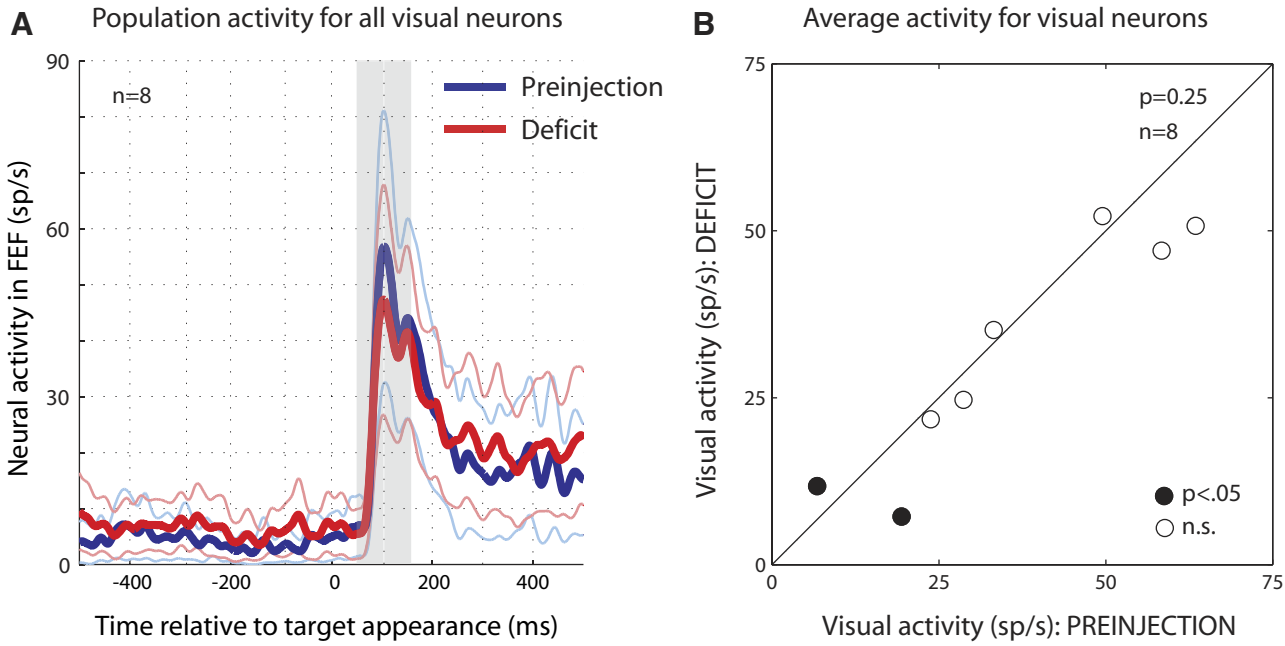

FIG. 3. Visual activity in FEF during SC inactivation. *A*: population spike density plot for all FEF neurons with a visual response ($n = 8$) shows that activity is not strongly modulated during the deficit period (red line) compared with the preinjection period (blue line). *B*: each dot represents average visual activity in a single experiment for the deficit period (y axis) plotted against the preinjection period (x axis). Conventions for each panel as in Fig. 2.

FEF. Accordingly, we must reject the hypothesis that the SC drives presaccadic activity in the FEF. At the outset, this hypothesis was appealing because it would account for the observations that FEF has more predominant presaccadic activity, as well as more predominant SC input, than does area LIP (Pare and Wurtz 1997, 2001; Sommer and Wurtz 2001; Wurtz et al. 2001). While logically attractive for differentiating FEF from LIP, this hypothesis receives no support from our experiments. The observed differences between FEF and LIP must emerge from other sources, presumably cortical.

We found that visual activity in the FEF, like presaccadic activity, is not driven by input from the SC. Visual responses in the FEF were effectively unchanged by SC inactivation. It is important to note that the visual activity under investigation here is the classic, passive visual response—the neuron's firing when a stimulus appears in the receptive field while the monkey fixates. This response is distinct from the visual processing previously examined during inactivation of the ascending SC-MD-FEF pathway (Sommer and Wurtz 2006). Sommer and Wurtz found that this pathway carries corollary discharge signals about impending saccades, which enable the FEF neuron to become responsive at the "future field" (the anticipated receptive field after the saccade was made). Inactivation of MD disrupted the shifting of FEF receptive fields from the current to the future locus: FEF neurons no longer exhibited the anticipatory response at the future field around the time of the eye movement. Importantly, however, MD inactivation did *not* change the classic, passive visual response in FEF. Our present results similarly show no change in the visual response during SC inactivation. Together these two findings indicate that the visual response in FEF is not driven from SC via the ascending pathway. These data therefore refute an earlier hypothesis generated in this laboratory (Sommer and Wurtz 1998). The earlier study examined the activity of FEF neurons that received input from SC and offered the testable hypothesis that the pathway from SC may provide visual input to FEF. Here we have tested this hypothesis directly and find no clear evidence that the intermediate SC contributes to the visual response in FEF. Further corroboration of this lack of SC visual input comes from a study of the signals represented at each stage of the ascending SC-MD-FEF pathway (Sommer and Wurtz 2004a). In that study, Sommer and Wurtz found that visual responses are more prominent in FEF than in either MD or SC and proposed that this likely reflects input from extrastriate visual areas to FEF. The conclusion is that the passive visual response in FEF is likely of cortical rather than collicular origin.

SC can modulate presaccadic activity in FEF

Our experiments demonstrate that although the intermediate SC does not drive activity in FEF, it can modulate presaccadic activity in the FEF. This modulation was not universal but was consistent in the third of experiments where we observed an effect of SC inactivation on presaccadic signals in FEF. To our surprise, the modulation was always an enhancement of FEF presaccadic activity during SC inactivation. What is the functional significance of this increase? One possibility is that it reflects a compensatory interaction between these two structures. It is well established that the combined ablation of FEF and SC eliminates the ability to generate saccades, but ablation of either structure alone causes only temporary saccade deficits (Schiller et al. 1980). Thus when one of these structures is compromised, the other can compensate. This compensation has also been evident in reversible inactivation studies. For example, monkeys can still make visually guided saccades during SC inactivation even when saccades cannot be evoked with electrical stimulation of the FEF (Hanes and Wurtz 2001). One interpretation of the present findings is that the increase in FEF activity serves to compensate for the disruption of the SC.

This interpretation is particularly compelling in light of our observation that the inactivation-induced increase was greatest for the experiment in which the FEF and SC representations were exactly aligned. In the context of that experiment, a simple explanation is that the presaccadic activity in the FEF neuron increased to counterbalance the impairment of the corresponding spatial representation in SC. The increase in FEF activity could have a compensatory effect via its direct projections to oculomotor regions of the pons (Huerta et al. 1986; Leichnetz et al. 1984; Segraves and Goldberg 1987; Stanton et al. 1988b).

Previous studies have shown that presaccadic activity in the FEF is related to eye-movement parameters (Dias and Bruce 1994; Everling and Munoz 2000). This relationship is important for the interpretation of the present findings. Inactivation of the SC leads to changes in the saccadic eye movements; indeed, for all experiments, changes in eye velocity were taken as an indicator that the inactivation was effective. By definition, the monkeys were making different saccades during the preinjection and deficit periods. One way of viewing the FEF activity, then, is that it could differ between preinjection and deficit periods because the activity is driving different saccades. We found, however, that differences in saccade velocity and amplitude did not account for the neuronal effects we observed. Even if we had uncovered an association, the fundamental conclusion would be unchanged. If the FEF knows that it is driving a different saccade, and therefore adjusts its activity, this too indicates that the FEF must be getting feedback from downstream structures.

Whatever the functional significance of the increases in presaccadic activity, they indicate that the FEF may receive modulatory input from the SC. It is useful, then, to consider the kind of communication between SC and FEF that could give rise to increased FEF activity during SC inactivation. One possibility is that the SC normally exerts a tonic inhibition on some portion of presaccadic FEF neurons, perhaps in a spatially specific manner; inactivation of the SC would release this inhibition and thus result in higher firing rates. Previous work has shown that the presaccadic burst is present at all three levels of the ascending path from SC to MD to FEF (Sommer and Wurtz 2004a). This finding indicates that these ascending connections are predominantly excitatory, which in turn predicts that the SC inactivation should have caused a *decrease* in presaccadic signals in FEF. To explain the observed increase, then, we need to invoke the presence of an inhibitory interneuron at the level of the FEF, which would exert tonic inhibition from the ascending path on at least some subset of FEF neurons. Preliminary evidence from analysis of action potential waveforms, in fact, indicates that about half of the FEF neurons that receive input from the SC-MD pathway are inhibitory interneurons (Shin and Sommer 2006, 2008). Of course, we do not know from our experiments that SC acts on FEF via the MD relay; other pathways may mediate the modulatory effects we observed. The general implication of this modulation is that subcortical changes, originating in the SC, may influence presaccadic activity upstream in the FEF.

Inactivation of the SC-FEF pathway

In both the present study and a previous study from this laboratory (Sommer and Wurtz 2006), we have examined how FEF activity is affected by inactivation of the ascending pathway from SC. As discussed in the preceding text, these studies differ in the kind of FEF activity under investigation: the previous study focused on the contribution of the ascending pathway to shifting receptive fields in FEF neurons. Presaccadic activity was not evaluated (indeed, the cells were selected on the basis of visual activity alone), and the passive visual response was evaluated only as a control. Another major difference between these two studies is obviously the site of inactivation. Here we have inactivated the source structure in the pathway, SC, whereas Sommer and Wurtz inactivated the relay, MD.

The choice to inactivate SC in the present study was motivated by four advantages. First, as a practical matter, it simplified the execution of an experiment that already demands simultaneous recording and inactivation, as well as the physiological identification of connected neurons in some cases. These technical challenges are considerably greater when one also requires identification of the spatially restricted relay zone in MD. Second, inactivation of SC allowed us to test (and ultimately extend) the previous finding that the ascending path through MD does not contribute to the passive visual response in FEF. Third, interpretation of the inactivation is in some respects more straightforward for SC than for MD. MD inactivation potentially affects not only the ascending input from SC but also the added input that emanates from cortical projections onto MD. SC inactivation does not entail this possible confound. Fourth, of greatest interest, inactivation of SC is conceptually relevant to earlier studies of the recovery of eye movements after impairment of either FEF or SC (Schiller et al. 1980). Inactivation of SC, like that of FEF, can significantly change the latency, velocity, and trajectory of visually guided saccades (Dias and Segraves 1999; Dias et al. 1995; Hanes and Wurtz 2001; Quaia et al. 1998; Sommer and Tehovnik 1997; Walton et al. 2008). Inactivation of MD has only a minimal effect on visually guided saccades (Sommer and Wurtz 2004b). We have postulated here that some of our results point toward a compensatory explanation: an increase in presaccadic activity in FEF could work to overcome the saccade impairment caused by SC inactivation. One interesting prediction of this interpretation is that inactivation of MD, which leaves saccade generation unimpaired, should not elicit any increase in presaccadic activity in FEF. Rather, the FEF modulation should be a specific compensation for the deleterious effect of SC inactivation on saccade generation.

As a final note, while this study concentrated on the saccade-related functions of the SC and FEF, recent research demonstrates that both structures also contribute to higher cognitive functions (Krauzlis et al. 2004; Moore et al. 2003; Schall 2002). In particular, other studies of SC inactivation indicate its role in the selection and representation of desired target locations (Hafed and Krauzlis 2008; McPeek 2008; McPeek and Keller 2004); inactivation or ablation of the FEF has also been shown to impair target selection (Keller et al. 2008; Schiller and Chou 1998; Schiller and Tehovnik 2003). These higher-order processes cannot be evaluated in the experimental design of the present study but represent an important avenue for understanding the reciprocal interplay between these two structures. Further research of this kind, which acknowledges both the oculomotor and cognitive functions of this pathway, will

help to elucidate the close interaction between the SC and FEF in guiding spatial behavior.

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