Task-relevant Output Signals are Sent from Monkey Dorsolateral Prefrontal Cortex to the Superior Colliculus during a Visuospatial Working Memory Task

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Abstract

■ Visuospatial working memory is one of the most extensively investigated functions of the dorsolateral prefrontal cortex (DLPFC). Theories of prefrontal cortical function have suggested that this area exerts cognitive control by modulating the activity of structures to which it is connected. Here, we used the oculomotor system as a model in which to characterize the output signals sent from the DLPFC to a target structure during a classical spatial working memory task. We recorded the activity of identified DLPFC–superior colliculus (SC) projection neurons while monkeys performed a memory-guided saccade task in which they were required to generate saccades toward remembered stimulus locations. DLPFC neurons sent signals related to all aspects of the task to the SC, some of which were spatially tuned. These data provide the first direct evidence that the DLPFC sends task-relevant information to the SC during a spatial working memory task, and further support a role for the DLPFC in the direct modulation of other brain areas. ■

INTRODUCTION

The prefrontal cortex has been shown to be involved in a variety of cognitive functions. Studies investigating the response properties of single neurons in monkey prefrontal cortex have revealed a complex mixture of signals related to processes such as learning (Asaad, Rainer, & Miller, 1998), attention (Lebedev, Messinger, Kralik, & Wise, 2004; Everling, Tinsley, Gaffan, & Duncan, 2002; Rainer, Asaad, & Miller, 1998), decision-making (Kim & Shadlen, 1999), and flexible stimulus–response mapping (Johnston & Everling, 2006a; Everling & DeSouza, 2005). Of the many cognitive functions ascribed to the prefrontal cortex, working memory has perhaps been the most extensively investigated. It has been known for some time that prefrontal lesions produce profound impairments in monkeys performing delayed-response tasks (Jacobsen, 1936). A substantial body of literature has shown that prefrontal neurons display enhanced activity during delay periods intervening between the presentation of a stimulus and the required behavioral response (Miller, 1996; Fuster & Alexander, 1971; Kubota & Niki, 1971), a finding consistent with a role of the prefrontal cortex in short-term on-line maintenance of information.

One form of working memory that has been particularly well studied is visuospatial working memory. This function has typically been studied using memory-guided saccade tasks in which subjects are required to make saccades to the remembered locations of briefly presented targets. Evidence from lesion studies in humans (Pierrot-Deseilligny, Muri, Nyfeller, & Milea, 2005) and inactivation studies in primates (Sawaguchi & Iba, 2001), as well as single-neuron recordings, have suggested that the dorsolateral prefrontal cortex (DLPFC) plays a critical role in this function. Humans and monkeys with lesions of the DLPFC make errors in this task consistent with a specific disruption of spatial working memory. DLPFC neurons have been shown to exhibit spatially selective activity related to the stimulus, delay, and saccade in memory-guided saccade tasks (Funahashi, Bruce, & Goldman-Rakic, 1989, 1990, 1991; see Constantinidis & Wang, 2004; Goldman-Rakic, 1995, for review). The spatially selective delay-period activity of DLPFC neurons has been generally interpreted as mnemonic in nature, representing the active maintenance of spatial information required to guide the forthcoming saccade (Takeda & Funahashi, 2002; Constantinidis, Franowicz, & Goldman-Rakic, 2001). Delay activity is often absent or attenuated on error trials (Funahashi et al., 1989), suggesting that it plays a crucial role in the guidance of memory-based actions.

Models of prefrontal function have suggested that it participates in cognitive processes via a top–down control

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mechanism in which it acts to coordinate or modulate the activity of target structures to which it is connected (Miller & Cohen, 2001). In the case of memory-guided saccade tasks, such modulation could act to enhance or sustain the cue, delay, or saccade-related activity of other brain areas. The superior colliculus (SC) is a midbrain oculomotor structure that receives extensive projections from the DLPFC (Leichnetz, Spencer, Hardy, & Astruc, 1981; Goldman & Nauta, 1976), and has been shown to exhibit task-related responses, including delay-period activity, during memory-guided saccade tasks (Paré & Wurtz, 2001; Kojima, Matsumura, Togawa, & Hikosaka, 1996). Here, we used the oculomotor system as a model to investigate the signals sent by the output neurons of the DLPFC to a target structure during a mnemonic task. We employed antidromic activation to identify neurons sending a direct projection to the SC, and recorded the activity of these neurons while monkeys performed a memory-guided saccade task. Based on the results of studies investigating signals sent from cortical areas such as the FEF (Sommer & Wurtz, 2000) and the lateral intraparietal area (LIP; Paré & Wurtz, 2001) to the SC, and studies of the general population of DLPFC neurons during memory-guided saccades, we reasoned that cortico-tectal DLPFC neurons would carry a suite of signals related to most aspects of the task. We found that many cortico-tecal neurons exhibited spatially selective cue, delay, saccade, and postsaccadic activity. These data provide the first evidence that the DLPFC sends mnemonic signals directly to a target structure. Our findings are also consistent with the results of previous studies that have shown that the SC receives information regarding all aspects of memory-guided saccades from cortical oculomotor areas, suggesting that such nonselective output is a common functional principle of cortico-tectal projections.

METHODS

Two rhesus monkeys (*Macaca mulatta*) weighing 6.5 and 8 kg were subjects in the present experiment. Recording chambers were stereotactically implanted over both the right lateral DLPFC (A–P 31 mm, M–L 18 mm) and the SC (P 1.0 mm, D 5.0 mm, M–L, 0 mm) of both animals. The SC chamber was tilted 38° posterior from vertical. Details of the surgical procedures have been described previously (Everling & DeSouza, 2005). Postsurgical MRIs were performed on both animals to confirm the locations of the recording chambers and to allow reconstruction of recording locations. All experimental methods described were carried out in accordance with the guidelines of the Canadian Council on Animal Care policy on the care and use of experimental animals, and an ethics protocol approved by the Animal Users Subcommittee of the University of Western Ontario Council on Animal Care.

Behavioral Task

Monkeys performed a standard memory-guided saccade task (Figure 1A). Each trial began with the presentation of a small white fixation point (0.15°) at the center of the display screen. Monkeys were required to fixate this spot within a 0.5° × 0.5° window for 500 msec. Following this, a 0.15° visual stimulus was presented pseudorandomly with equal probability in one of eight cardinal directions (counterclockwise from right: 0°, 45°, 90°, 135°, 180°, 225°, 270°, or 315°) at a distance of 8° from fixation, for a duration of 100 msec. The radial direction and eccentricity of the stimuli were chosen to fall within the response fields of DLPFC neurons as demonstrated in previous studies (Miller, 1996; Funahashi et al., 1989, 1990, 1991; Boch & Goldberg, 1989). The animals were required to maintain central fixation during stimulus presentation. Following stimulus presentation, the animals were required to maintain fixation for a 1000 msec delay period during which the fixation point remained illuminated, and the stimulus was not visible. At the end of the delay period, the fixation spot disappeared, and the animals were required to make a saccade to the remembered stimulus location. To obtain a juice reward, the animals were required to generate a saccade toward the remembered stimulus location within 500 msec. Saccade endpoints were required to fall within a 5° × 5° window. If the saccade endpoint fell within this window, the target stimulus reappeared following a delay of 100 msec, and the juice reward was presented. Eye movements were recorded at 1000 Hz using a magnetic search coil technique (DNI, Newark, DE). Both monkeys had received extensive training in this task for previous experiments, and consequently, exhibited high levels of performance (Monkey R—98.2% correct, Monkey W—97.6%). Saccadic reaction times (SRTs) had a mean value of 172.2 msec (*SD* = 11.8).

Recording Technique

We recorded single cortico-tectal neurons from the right lateral prefrontal cortex. Arrays of two to six electrodes were driven individually within the DLPFC recording chambers using a computer-controlled multimicroelectrode drive (NAN, Plexon, Dallas, TX). Neurons were identified as sending a direct projection to the SC using antidromic activation. Arrays of three to four tungsten microelectrodes (impedance 0.5 MΩ, FHC, Bowdoinham, ME) were chronically implanted in the intermediate layers of SC ipsilateral to the DLPFC recording site. The intermediate layers were identified using single-neuron recordings and microstimulation (Everling & Munoz, 1999). One electrode was implanted at an eccentricity of <2°, and the others were implanted at eccentricities of 5° to 15° on the collicular motor map (Robinson, 1972). Electrodes were implanted for a

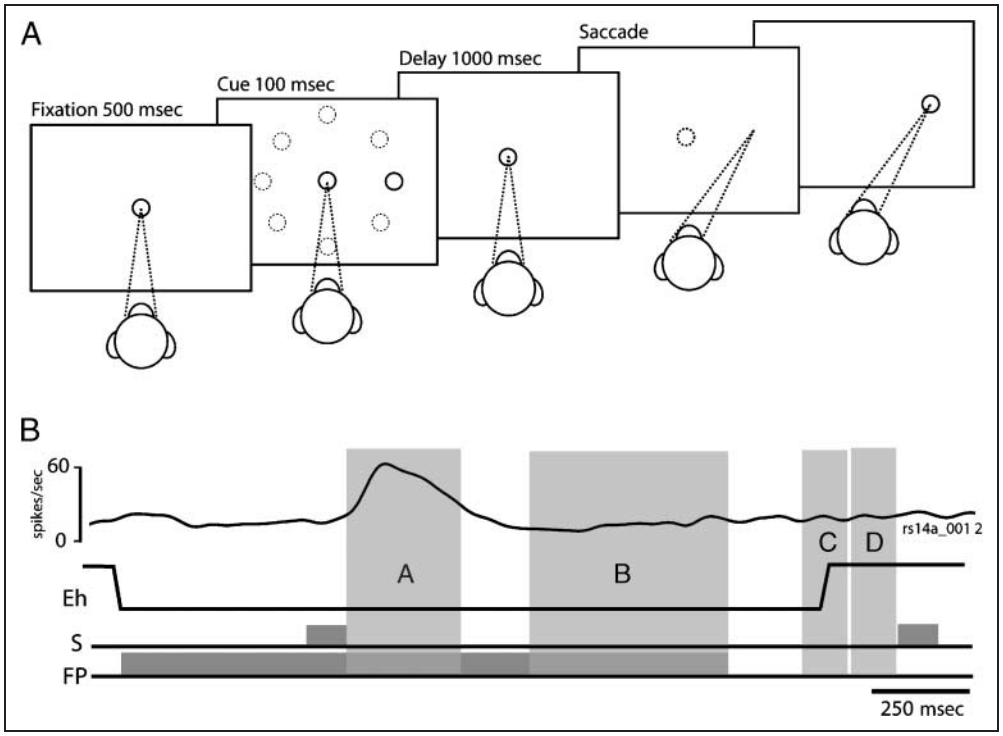

Figure 1. Experimental task and statistical analysis epochs. (A) Memory-guided saccade task. Each trial began with the presentation of a fixation point at the center of the screen which the monkey was required to fixate. A visual cue stimulus was briefly presented at one of eight locations. A delay period followed in which the animal was required to maintain fixation. At the end of the delay, the fixation point disappeared, signaling the monkey to make a saccade to the remembered location of the cue. (B) Statistical analysis epochs. We carried out statistical analyses in five epochs corresponding to key trial events. A = cue; B = delay; C = saccade; D = postsaccade. Line labeled FP depicts onset and offset of fixation, S cue stimulus, and Eh, horizontal eye position. At top, spike density function from a single neuron showing overlap of statistical windows with neural activity.

period of 4 to 6 weeks. These electrodes were tested periodically to ensure that eye movements could be elicited at low thresholds (10–40 μA), in order to verify that they had not shifted within the SC. In a given experimental session, we first isolated the activity of a single DLPFC neuron. The activity of the neuron was then monitored while single biphasic current pulses (0.3 msec) were delivered to the SC through one of the implanted electrodes and an indifferent electrode. The indifferent electrode was bathed in a medium of physiological saline (0.9% NaCl) inside the SC recording chamber, and was not inserted into the brain. Neurons were classified as antidromic if stimulation elicited action potentials meeting all of the following criteria: fixed threshold, fixed latency, and collision testing (Lipski, 1981) (see Figure 2B and C). Due to the relative scarcity of neurons that could be identified, and to increase the yield of identified neurons, we recorded only the activity of neurons meeting the above criteria. If a given neuron could not be identified, the neuron was abandoned, another neuron was isolated, and the antidromic technique was applied for this new neuron. The threshold for eliciting antidromic responses was defined as the current level that elicited an action potential approximately 50% of the time. Threshold varied between 100 and 1400 μA, with a mean value of 601 μA and a median of 600 μA. Thresholds for all antidromically identified neurons are presented in Figure 3A.

Data collection commenced once an antidromic neuron had been identified. Waveforms were digitized, stored, and sorted off-line using 2-D and 3-D principal components analysis (Plexon). To verify that none of our recording locations were in FEF, we subsequently delivered stimulation pulses at our recording sites. No eye movements were elicited by microstimulation at currents of up to 200 μA (100 msec, 300 Hz, 0.3-msec biphasic pulses).

Data Analysis

Trials were divided into five epochs within which we calculated mean spike counts for each neuron for statistical analysis (see Figure 1B). The ''cue'' epoch was 300 msec in length commencing 100 msec following presentation of the visual stimulus. This epoch began 100 msec after stimulus presentation to compensate for the latency of visual responses in prefrontal neurons. The ''delay'' epoch was 500 msec in length and consisted of the 500 msec immediately preceding offset of the fixation spot. The ''saccade'' epoch was 100 msec long and consisted of the 50 msec immediately preceding and following saccade onset. We also included a ''postsaccade'' epoch, which consisted of the 50 msec immediately following saccade end. Finally, we included a ''baseline'' epoch (not depicted in Figure 1B), which consisted of a 1500-msec period during

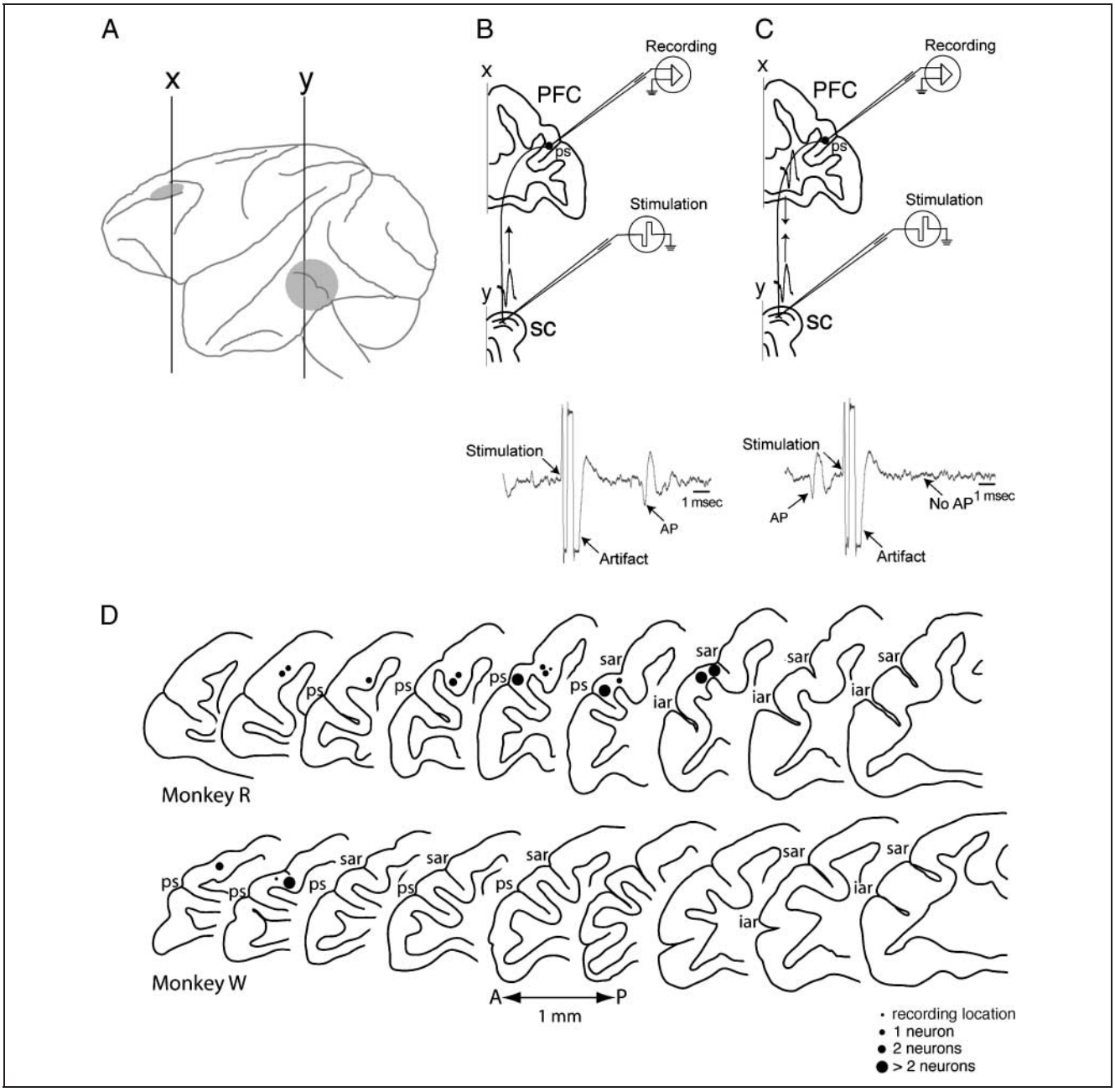

Figure 2. Schematic representation of antidromic identification technique and recording locations. (A) Lateral view of macaque brain showing locations of slice planes depicted in B and C; shaded area under x, PFC; under y, SC. (B) Top: Schematic representation of experimental method for antidromic activation. Bottom: Waveforms depicting activity recorded in the PFC showing artifact caused by stimulation and stimulation-evoked action potential (AP). (C) Top: Schematic representation of collision test. Bottom: Activation waveforms depicting PFC activity during collision test. Spontaneous action potential (AP) triggers stimulation pulse to the SC. Following collision, no action potential is observed in PFC neuron D, recording locations in monkeys R and W, reconstructed from MRI images and known depths of neuronal recordings. Slices are separated by 1 mm. Black dots represent recording locations, and numbers of neurons showing significant statistical effects are coded by dot size. iar = inferior arcuate sulcus; ps = principal sulcus; sar = superior arcuate sulcus.

the intertrial interval immediately preceding trial onset. We chose a long duration for this epoch to average out the effects of uncontrolled eye movements on neuronal firing during the intertrial interval. We also visually inspected this baseline activity for each neuron to ensure that it remained stable, and did not show wide variations that could be accounted for by activity related to such eye movements. We found no wide variations in any case.

Analyses of cue, delay, saccade, and postsaccadic activity were carried out using separate two-way ANOVA with the factors *epoch* and *direction*. The *epoch* factor was composed of the trial epoch of interest (i.e., cue) and the baseline epoch. The *direction* factor was composed

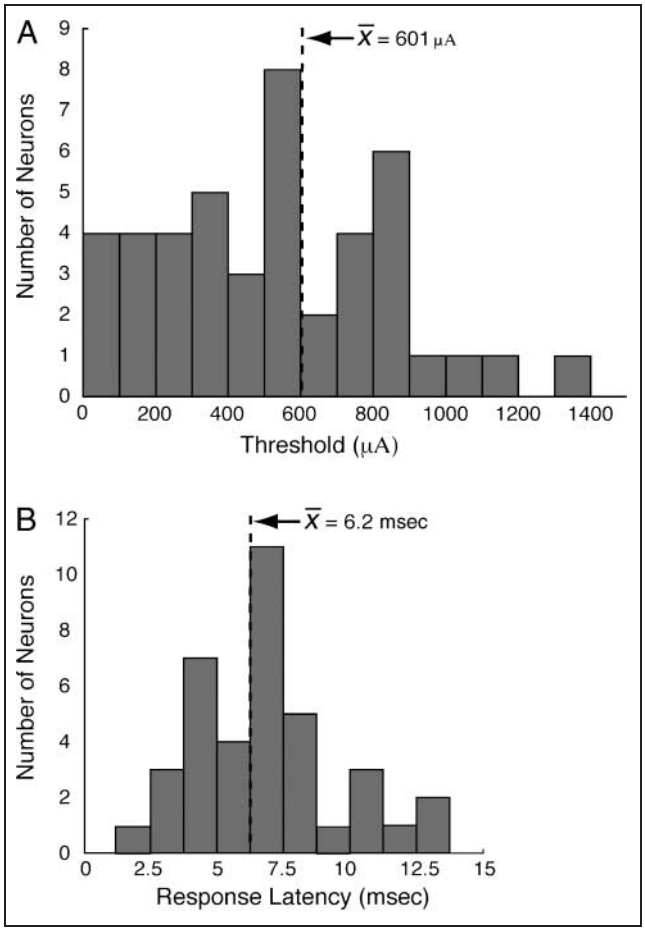

Figure 3. Histograms depicting distribution of numbers of neurons activated versus antidromic threshold (A), and antidromic latency (B).

of the eight stimulus locations presented during the task (0°, 45°, 90°, 135°, 180°, 225°, 270°, or 315°). Thus, for each neuron, four separate ANOVAs were calculated, one for each trial epoch of interest. All ANOVAs were evaluated at $p < .05$. These analyses were designed to be comparable with previous studies of mnemonic activity in DLPFC neurons (Funahashi et al., 1989, 1990, 1991), which classified neurons in a similar manner to that described here. A main effect of *epoch* indicated that a neuron showed a significant difference in activity between the epoch of interest and baseline activity. Such neurons were classified as being *responsive* during that epoch. A main effect of *direction* during a given epoch indicated that the activity of a neuron was modulated by the stimulus location. Such neurons were classified as *direction-selective*. An interaction between these factors indicated that a given neuron was both responsive and direction-selective. In some cases, the responsive and direction-selective classifications were mutually exclusive. That is, a neuron showing a significant effect of direction in a given epoch did not also show a significant effect of epoch (i.e., no overall effect relative to baseline), or an interaction between these factors. Statistically, this could occur if a neuron was strongly modulated for one direction, but the overall modulation across all directions relative to baseline was not sufficient to reach statistical significance. This occurred in a minority of neurons (15%). Most neurons showing an effect of direction were also responsive, or exhibited an Epoch × Direction interaction.

To further quantify direction-selective activity, we calculated two indices for each neuron showing a significant effect of direction or an Epoch × Direction interaction in the ANOVA described above. Because the activity of neurons could either increase or decrease during a given epoch relative to baseline, we calculated an excitation/inhibition (EI) index to determine whether the predominant response of each neuron was excitatory or inhibitory, and to quantify this response. To compute this index, we first determined the direction at which the neuron's response showed the greatest absolute difference from baseline. We then computed a contrast ratio comparing the baseline activity of the neuron with the activity in the relevant epoch using the following equation:

$$\mathrm{EI}_{\mathrm{index(epoch)}} = (\mathrm{epoch}(d) - \mathrm{base}(d))/(\mathrm{epoch}(d) + \mathrm{base}(d))$$

where base(d) represents the mean baseline activity at the best direction and epoch(d) represents the mean activity in the epoch of interest at the best direction. Values of this index could range from −1 to +1, with negative values indicating an inhibitory response relative to baseline, and positive values indicating an excitatory response.

To determine whether the responses of neurons exhibited laterality, we computed an ipsi/contra (IC) index. In all analyses, ipsilateral stimuli were defined as those appearing on the same side as the recorded hemisphere and contralateral stimuli were those appearing on the opposite hemisphere. Because we recorded from the right hemisphere in both animals, ipsilateral stimuli were always those appearing to the right of fixation, and contralateral stimuli were always those appearing to the left. To compute this index, we first calculated the absolute mean difference from baseline for the contralateral and ipsilateral stimulus directions. We then calculated the IC index as the contrast ratio using these values:

$$\mathrm{IC}_{\mathrm{index(epoch)}} = (\mathrm{Contra}_{(\mathrm{epoch})} - \mathrm{Ipsi}_{(\mathrm{epoch})})/(\mathrm{Contra}_{(\mathrm{epoch})} + \mathrm{Ipsi}_{(\mathrm{epoch})})$$

where Contra represents the absolute mean difference from baseline for contralateral stimuli, and Ipsi is the corresponding value for ipsilateral stimuli. Values of this

index could range from −1 to +1, with negative values indicating greater activity for ipsilateral stimuli and positive values indicating greater activity for contralateral stimuli.

To investigate the directional tuning properties of prefrontal projection neurons, we estimated tuning curves using a Gaussian function. This function was originally used to investigate visual receptive fields and movement fields of FEF neurons (Bruce & Goldberg, 1985), and subsequently, employed in studies investigating the tuning properties of prefrontal neurons during memory-guided saccades (Funahashi et al., 1989, 1990, 1991):

$$f(d) = B + R{*}e^{-1/2((-d-D)/T_d)^2}$$

where $f(d)$ represents the frequency of discharge, d represents stimulus direction, B represents baseline discharge rate, D represents the direction at the peak of the function (i.e., best direction), R represents response magnitude, and T_d is the index of tuning with respect to stimulus direction. This function was fit to individual neurons by fixing the value of B at the mean activity calculated during the baseline epoch for that neuron, and using least squares regression. This function was fit to all neurons showing a significant effect of direction or an Epoch × Direction interaction in the ANOVAs described above. Thus, separate tuning functions were calculated for cue, delay, saccade, and post-saccadic activity.

To investigate the relationship between neural activity and SRT, we computed Pearson product–moment correlations between SRT and neural activity for neurons showing a significant effect of direction or an Epoch × Direction interaction in the ANOVA on activity during the *saccade* epoch. Correlations were calculated for the best direction for each neuron, defined as the direction for which activity during the saccade epoch showed the maximum difference from baseline. Correlations were calculated using SRTs for the best direction and mean neural activity for the same direction calculated in a 200-msec window that included the last 100 msec of the delay epoch and the 100-msec immediately preceding saccade onset. This window was chosen because it included the time at which the animal was preparing to execute a saccade to the remembered stimulus location, and excluded any response to the visual reinforcement stimulus as the stimulus was not presented until the monkeys had correctly made a saccade to the remembered location. The statistical significance of correlations was assessed using t tests evaluated at $p < .05$.

All analyses described above were based on correct trials. Trials associated with incorrect responses, broken, incorrect, or inaccurate fixation, or failure to generate a saccade within 500 msec were excluded. We attempted to perform an analysis on error trials, however, because both animals were quite proficient at the experimental task, an insufficient number were available for statistical analysis.

RESULTS

Cortico-tectal Neurons

We report the activity of 46 neurons from the right DLPFC of two monkeys (35 monkey R, 11 monkey W) (Figure 2C) that were identified as sending a direct projection to the SC. Of these neurons, 41/46 (89.1%) showed a statistically significant difference in activity in at least one of the trial epochs we analyzed.

The latencies of the antidromic responses of the population of cortico-tectal neurons we recorded are presented in Figure 3B. To investigate the relationship between antidromic latency and response properties of cortico-tectal neurons, we divided the population of neurons into two groups on the basis of their median antidromic latency (6.0 msec). We found no difference in the proportion of neurons that were modulated in any epoch between these two groups (χ^2 test, $p = .22$). We carried out an identical analysis on the basis of antidromic thresholds, dividing neurons into groups with thresholds above and below the median threshold of 600 μA. As with latency, we found no differences in the proportion of neurons modulated in any epoch (χ^2 test, $p = .26$).

All neurons were antidromically activated by microstimulation delivered via an electrode implanted in the rostral SC. Only three neurons (6.5%) could also be activated through a caudal SC electrode. Although it is tempting to infer that this indicates that the DLPFC neurons we identified terminated selectively in the rostral SC, it seems more likely that this is a result of the anatomical fact that all fibers entering the SC do so via the rostral pole (Stanton, Goldberg, & Bruce, 1988). Thus, stimulation of this area may simply enhance the probability of antidromically stimulating a DLPFC neuron because of the higher density of incoming axons in this area relative to the caudal SC. As our main objective was to identify the types of signals sent from the DLPFC to the SC, we made no attempt to determine the explicit topography of DLPFC projections within the SC.

Because all neurons were antidromically activated by stimulation at a site close to the rostral pole of the SC, we calculated the effective spread of current to determine whether areas anterior to the SC could have been activated. Current spread was calculated using the equation $D = (I/K)^{0.5}$ (Tehovnik, 1996), where D represents the distance of current spread, I represents the stimulating current, and K represents the current/distance constant. Using a K value of 381 μA/mm^2 (Sommer & Wurtz, 1998), we calculated that current spread varied between 0.51 and 1.91 mm at the lowest and highest currents used (100–1400 μA). At the highest level, it is possible that the stimulating current encroached on areas rostral to the SC. However, most neurons were activated with currents

lower than this value. Ninety percent of the neurons reported here had thresholds of 850 μA or lower. The current spread at this level would be approximately 1.49 mm, which would extend a minimal distance outside of the SC. In any case, most of the current spread would be contained within the SC. It therefore seems most likely that any axons stimulated were within this area. We obtained similar results using the method of computing current spread described by Ranck (1975).

DLPFC Neurons Send Cue-related Signals Directly to the SC

To investigate whether DLPFC neurons sent information regarding the visual stimulus to the SC, we carried out a two-way ANOVA comparing baseline activity with activity during a 300-msec window that began 100 msec after stimulus presentation across the eight cardinal directions in which the stimulus was presented. We found that many neurons (24/46 = 52.2%) were significantly modulated during the cue epoch relative to baseline. We classified these neurons as being *responsive* during this task epoch. A number of neurons were also modulated by stimulus direction (11/46 = 23.9%), or showed a significant interaction between epoch and direction (12/46 = 26.1%). We classified both of these groups of neurons as being direction-selective as their activity was modulated by stimulus direction in both cases. Neurons showing only an effect of *epoch* were classified as having omnidirectional responses. We applied the same criteria for directional selectivity for the other trial epochs described below. In total, 15/46 = 32.6% showed either an effect of direction or an Epoch × Direction interaction, and were thus classified as exhibiting directional selectivity during the cue epoch. One such neuron is shown in Figure 4A. This neuron responded strongly to contralateral visual stimuli, with greatest activity for the upper contralateral quadrant. No such responses were observed for ipsilateral stimuli.

To further quantify direction-selective cue-period responses, we computed the mean cue-period activity for each stimulus direction for each neuron classified as direction-selective using the criteria described above. We then fit this mean activity with a Gaussian tuning function (see Methods). These parameters of these fits provide estimates of best direction for each neuron (D), as well as tuning width (T_d). Figure 4B presents the results of a Gaussian fit to the activity of the neuron shown in Figure 4A. This best direction for this neuron (D) was 171°, and the tuning width was 38°. Of the 15 neurons we classified as being direction-selective, we were able to fit 5 (33%) using this function. The remaining 10 neurons were poorly fit, and we were thus unable to

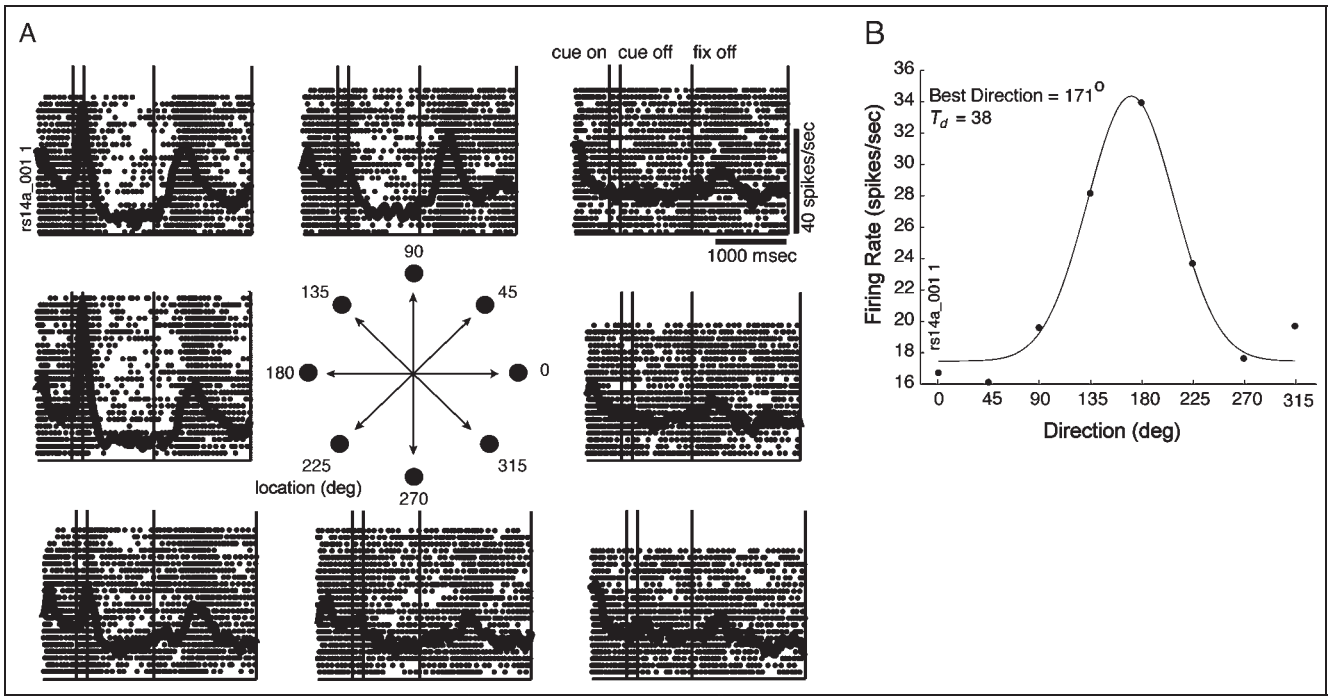

Figure 4. Single DLPFC cortico-tectal neuron showing significant effects during the cue epoch. (A) Single-neuron activity. Each panel depicts activity of the neuron for trials on which the cue stimulus was presented in the location depicted in the schematic at center. This neuron showed directionally selective activity during the cue epoch, with best responses for stimuli presented in the upper quadrant of the visual field contralateral to the recorded hemisphere (ANOVA, $p < .05$). Rasters and spike density functions are aligned on cue stimulus presentation. Spike density functions were constructed by convolving spike trains with a 30-msec Gaussian activation function. (B) Gaussian tuning function for cue epoch activity for neuron shown in A. Function was fit to the mean cue epoch activity for each direction (see Methods). This neuron was well tuned with best direction of 171° and a width of 38°.

obtain estimates of best directions and tuning widths using this method for these neurons. For the neurons that were fit, best directions ranged between 165° and 300°, and tuning widths ranged from 30° to 65°.

To further quantify the nature and magnitude of the responses exhibited by DLPFC–SC projection neurons during the cue period, we calculated an excitation/inhibition index for the best direction for each direction-selective neuron (see Methods). We found no significant differences in the numbers of neurons showing excitatory and inhibitory responses, or the numbers of neurons showing best responses for ipsilateral or contralateral stimuli (t test, $p > .05$). In order to investigate the relationship between the laterality of neurons responses and the sign (positive or negative, relative to baseline) of their best responses, we performed an analysis comparing the number of neurons exhibiting each possible combination of laterality and sign of response (i.e., decrease–contralateral, decrease–ipsilateral, increase–contralateral, increase–ipsilateral). We found no relationship between the nature of each neuron's response and its laterality (Fisher's Exact Test, $p = .40$), indicating that the cue-period activity of these neurons is not selectively excitatory or inhibitory for ipsilateral or contralateral stimulus locations.

To investigate the population responses of neurons showing significant modulations in activity during the cue epoch, we constructed average spike density functions for neurons classified as being responsive or direction-selective on the ANOVA described above. Because the responses of many of these neurons were significantly modulated by direction, and because the preferred directions of the population of neurons varied considerably, a simple averaging of responses across all stimulus directions would provide little information regarding the population activity of these neurons, as any activity differences would tend to be lost in averaging. To derive an estimate of population activity, we first determined the preferred direction for each neuron by finding the stimulus direction yielding the greatest mean activity during the cue epoch for each neuron. We also determined the nonpreferred direction for each neuron by finding the direction yielding the lowest mean activity during the cue epoch. This activity was then averaged across all neurons. Population activity for the preferred and nonpreferred directions during the cue epoch is presented in Figure 5A. For the preferred direction, activity increased in response to presentation of the visual stimulus, whereas activity was inhibited following stimulus presentation for the nonpreferred direction.

DLPFC Neurons Send Delay-related Signals Directly to the SC

To determine whether DLPFC neurons sent delay-related information to the SC, we carried out an identical set of analyses to those described for cue-related activity on the activity of each neuron during the 500 msec immediately preceding offset of the fixation spot. We found that many neurons were modulated during the delay epoch relative to baseline (23/46 = 50%). A total of 13 neurons (28.2%) were modulated by stimulus direction, and 9 neurons (19.6%) showed a significant Epoch × Direction interaction. Altogether, 16 neurons (34.8%) showed either a main effect of stimulus direction, or an Epoch × Direction interaction, and were thus classified as direction-selective. A neuron showing direction-selective delay-period activity is shown in Figure 6A. This neuron showed the greatest activity for stimuli presented in the upper left quadrant of the visual field. This neuron also exhibited cue-related responses for leftward stimuli. The Gaussian tuning curve fitted to the mean delay-period activity for each stimulus direction for this neuron is presented in Figure 6B. The best direction for this neuron was 121°, and the tuning width was 39°. We were able to fit 7 of the 16 neurons showing direction-selective activity using this function. Similar to the cue-related activity described above, tuning was extremely variable across neurons. Best directions could be in either hemifield, ranging from 0.44° to 325°, and tuning widths varied widely, ranging from 13° to 118°.

As with cue-period activity, we calculated both excitation/inhibition and ipsilateral/contralateral indices for the 16 neurons we classified as having direction-selective delay-period activity. Again, we found no significant differences between the number of neurons showing excitatory or inhibitory responses (t test, $p > .05$), or an ipsilateral or contralateral preference (t test, $p > .05$). We also found no clear relationship between the neuron's laterality and sign of response (Fisher's Exact Test, $p = .440$).

To investigate the population responses of neurons showing significant delay-period activity, we first determined the preferred and nonpreferred directions for each neuron showing a significant effect in the ANOVA of delay-period activity as described above for cue-period activity, and averaged these responses across all trials for those directions. This activity is depicted in Figure 5B. As can be seen in the figure, activity for the preferred direction had a tendency to slowly increase throughout the delay period, whereas activity for the nonpreferred direction was lower and remained stable throughout the delay period.

DLPFC Neurons Send Saccade-related Activity Directly to the SC

In addition to cue and delay-related activity, we found that a number of DLPFC–SC projection neurons carried saccade-related signals. We performed an ANOVA using the factors described above on the activity in a 100-msec window centered on saccade onset. As with cue and delay activity, we found that many neurons were modulated during the saccade epoch relative to baseline (18/46 = 39.1%). We found a total of 13 neurons (28.3%) with

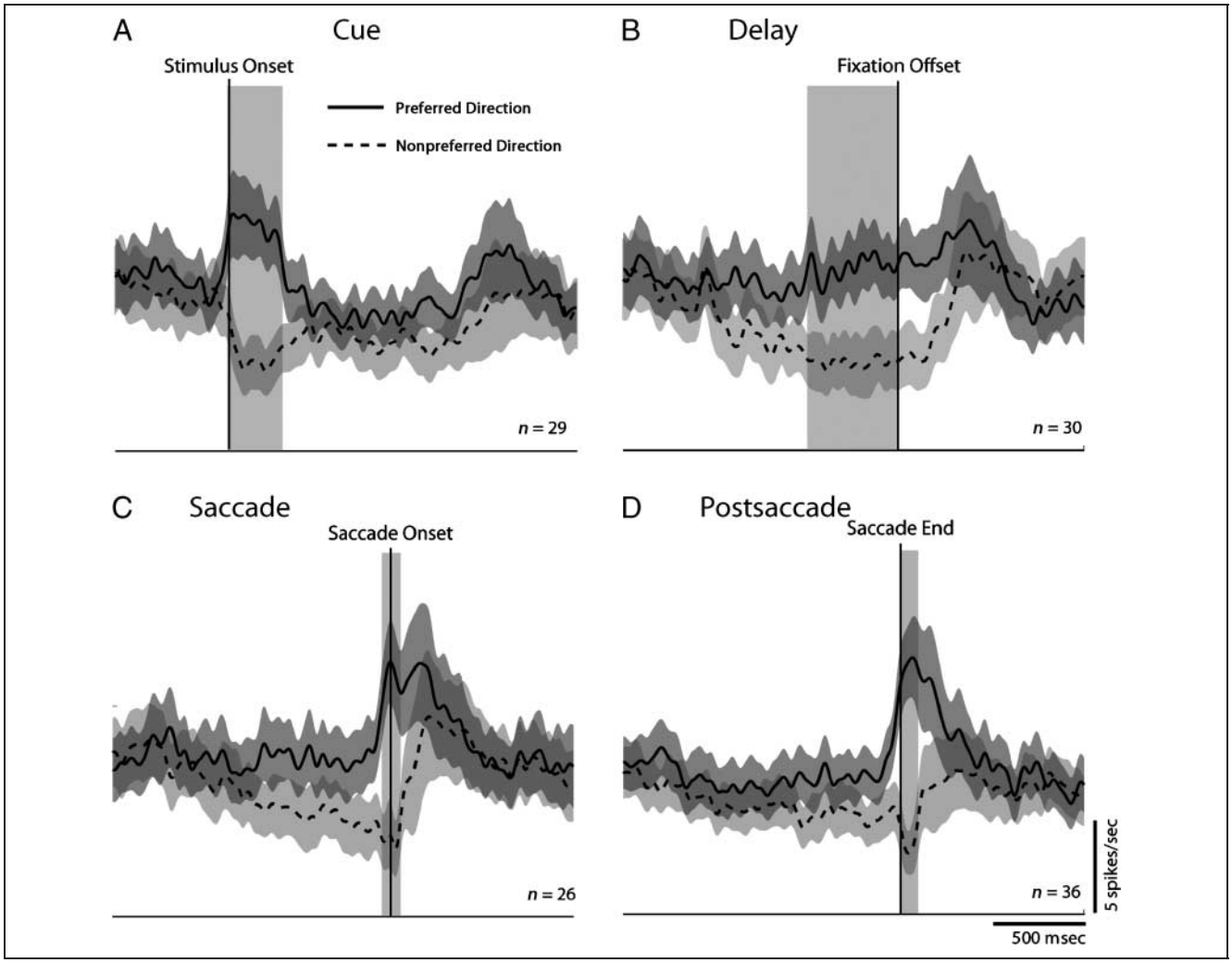

Figure 5. Spike density functions for the preferred and nonpreferred directions for the population of neurons showing significant effects on ANOVA for each epoch. Solid lines represent activity for the preferred direction, dashed lines, nonpreferred direction. Envelopes depict standard error of the mean (*SEM*). Shaded areas represent statistical windows used to test statistical significance of individual neurons in ANOVAs. (A) Activity during the cue epoch. Functions are aligned on cue stimulus onset. (B) Activity during the delay epoch. Functions are aligned on fixation offset. (C) Activity during the saccade epoch. Functions are aligned on saccade onset. (D) Activity during the postsaccade epoch. Functions are aligned on saccade end.

saccade-related activity that was modulated by stimulus direction, and 14 neurons (30.4%) that showed an Epoch × Direction interaction. Altogether, 18 neurons (39.1%) showed either a main effect of direction or an Epoch × Direction interaction, and were thus classified as direction-selective. One such neuron is depicted in Figure 7A. This neuron showed the greatest activity for saccades made to the upper right quadrant of the visual field, and little activity for other directions. The tuning curve for this neuron is shown in Figure 7B. The best direction for this neuron was 59°, and the neuron was broadly tuned, with a tuning width of 59°. Of the 18 neurons classified as direction-selective, 9 were well fit by the Gaussian function. As with direction-selective activity for the cue and delay epochs, the best directions of these neurons varied widely, ranging from 21° to 345°. Tuning widths were also highly variable, ranging from 21° to 95°.

Similar to cue and delay-related activity, we found no differences in the numbers of neurons with saccade-related activity that preferred the ipsilateral or contralateral fields (t test, $p > .05$), or with excitatory or inhibitory responses (t test, $p > .05$). Again, we found no clear relationship between these variables for this population of neurons (Fisher's exact test, $p = .386$).

As with the cue and delay epochs, we investigated responses for the preferred and nonpreferred directions for the population of neurons showing saccade-related modulations in activity. These data are depicted in Figure 5C. Population responses for the preferred direction showed a sharp increase in activity preceding saccade onset that peaked at saccade onset and declined thereafter. For the nonpreferred direction, activity showed a gradual decline during the saccade epoch.

To investigate the relationship between presaccadic activity and SRTs, we calculated correlations between

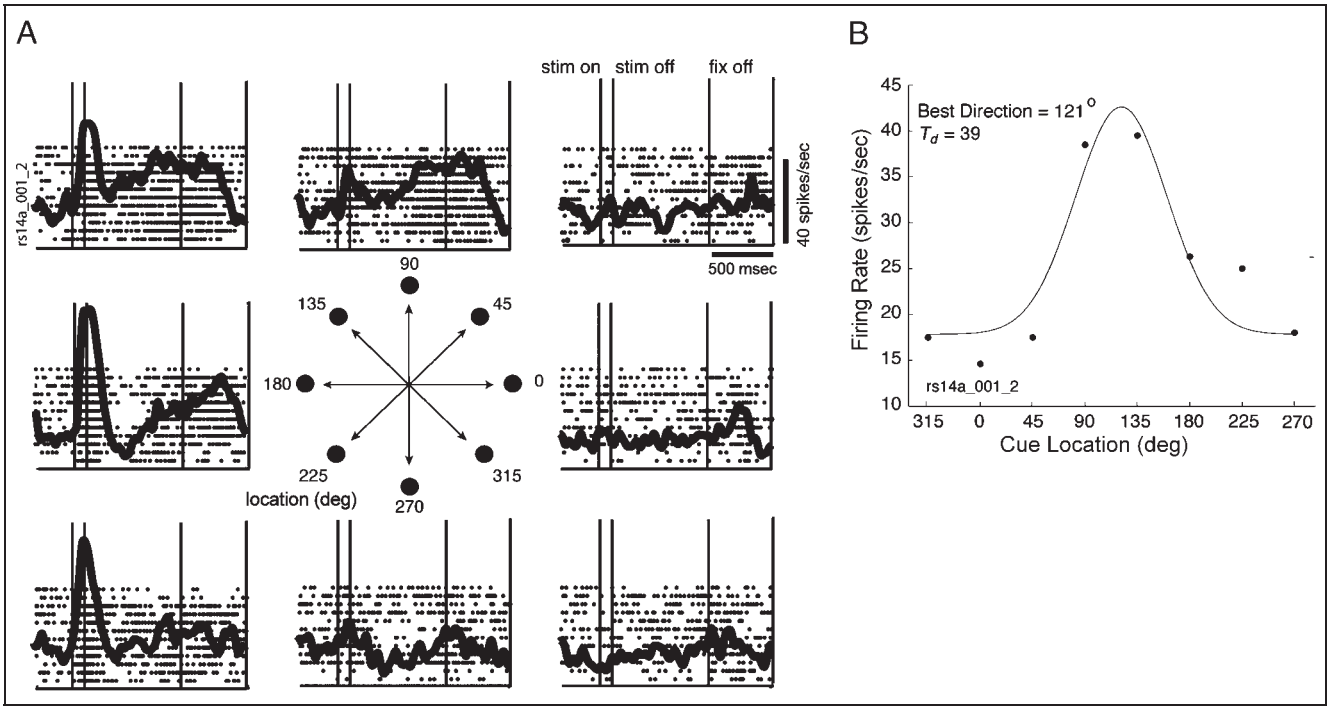

Figure 6. Single DLPFC cortico-tectal neuron showing significant effects during the delay epoch. (A) Single-neuron activity, plotted as in Figure 4. Rasters and spike density functions aligned on fixation offset (response). This neuron showed directionally selective activity during the delay epoch, with best responses for stimuli presented in the upper quadrant of the visual field contralateral to the recorded hemisphere (ANOVA, $p < .05$). (B) Gaussian tuning function for cue epoch activity for neuron shown in A. Function was fit to the mean delay epoch activity for each direction (see Methods). This neuron was well tuned with best direction of 121° and a width of 39°.

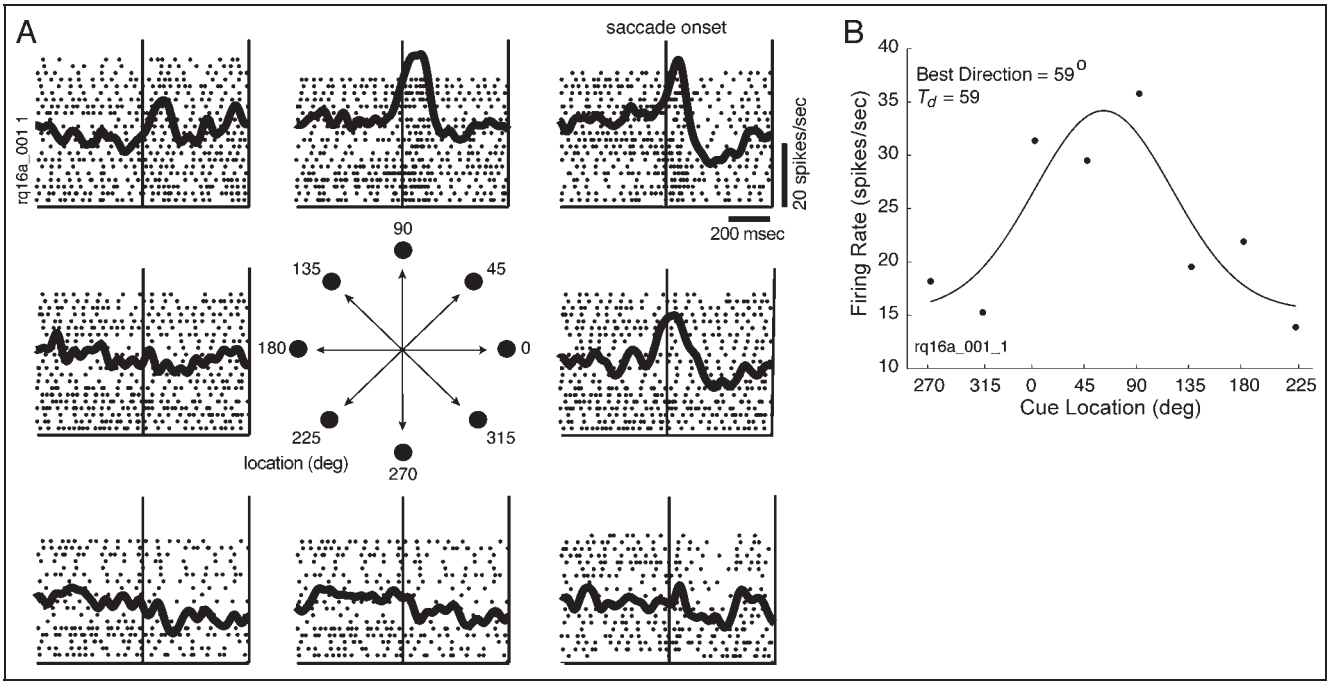

Figure 7. Single DLPFC cortico-tectal neuron showing significant effects during the saccade epoch. (A) Single-neuron activity, plotted as in Figures 4 and 6. Rasters and spike density functions aligned on saccade onset. This neuron showed directionally selective saccade-related activity, responding most strongly for saccades directed to the upper quadrant of the visual field ipsilateral to the recorded hemisphere (ANOVA, $p < .05$). (B) Gaussian tuning function for saccade epoch activity for neuron shown in A. Function was fit to the mean activity in the saccade epoch for each direction (see Methods). This neuron was well tuned with a best direction of 59° and a width of 59°.

the spike counts in a 200-msec window that included activity in the last 100 msec of the delay epoch and the 100 msec immediately following fixation offset and SRT for the best direction of each neuron showing a significant effect in the ANOVA described above (see Methods). SRT has been previously shown to be correlated with prestimulus neural activity in the SC (Everling, Dorris, Klein, Munoz., 1999; Dorris & Munoz, 1998; Dorris, Paré, & Munoz, 1997) and the FEF (Everling & Munoz, 1999; Dias & Bruce, 1994), as well as presaccadic activity of prefrontal neurons in oculomotor delayed response tasks (Watanabe, Igaki, & Funahashi, 2006). Based on these findings, we reasoned that DLPFC–SC projection neurons might carry a saccadic ''trigger'' signal. Correlation coefficients for each neuron with significant saccade-related activity are presented in Figure 8. Surprisingly, we found that presaccadic activity was significantly correlated with SRT for only 3 of 26 neurons. To verify this finding, we repeated the correlation analysis using analysis windows of up to 400 msec in duration, which included the 100 msec after fixation offset and increasing periods of the delay epoch. Similar results were obtained. This suggests that the presaccadic activity of this population of neurons is not directly related to saccade initiation.

DLPFC Neurons Send Postsaccadic Activity to the SC

In addition to cue, delay, and saccade-related activity, we found that many neurons carried postsaccadic signals to the SC. We found 25 neurons (54.3%) that showed a significant effect of epoch, and 19 neurons (41.3%) with a significant effect of direction. A total of 19 neurons (41.3%) showed a significant Epoch × Direction interaction. Of the neurons showing a significant effect on the ANOVA, we classified 24 (52.2%) as direction-selective. Figure 9A depicts a postsaccadic direction-selective neuron. This neuron showed a significant difference from baseline, and responded most strongly following saccades to the right (ipsilateral) hemifield. We fit the mean postsaccadic activity for each direction for neurons classified as direction-selective with a Gaussian function as described for the other trial epochs. We were able to fit 8 of 25 neurons (32%). The tuning function for the neuron shown in Figure 9A is presented in Figure 9B. The best direction for this neuron as derived from the tuning function was 345° and the tuning width was 25°. As with tuning parameters for the cue, delay, and saccade epochs, best directions varied widely, ranging from 59° to 345°. Tuning widths were also variable, ranging from 25° to 136°.

As with cue, delay, and saccade-related activity, we found no difference between the number of neurons with ipsilateral and contralateral responses (t test, $p > .05$) or excitatory and inhibitory responses (t test, $p > .05$). We also found no clear relationship between these variables (Fisher's exact test, $p = .274$). Thus, as in the cue, delay, and saccade epochs, ipsilateral and contralateral hemifields appear to be equally represented, and this population of neurons responds with both increases and decreases in activity relative to baseline.

Population responses for preferred and nonpreferred directions for neurons showing a significant effect on the ANOVA of postsaccadic activity are presented in Figure 5D. For the preferred direction, neurons showed a sharp increase in activity at saccade end. In contrast, for the nonpreferred directions, responses showed a sharp decrease.

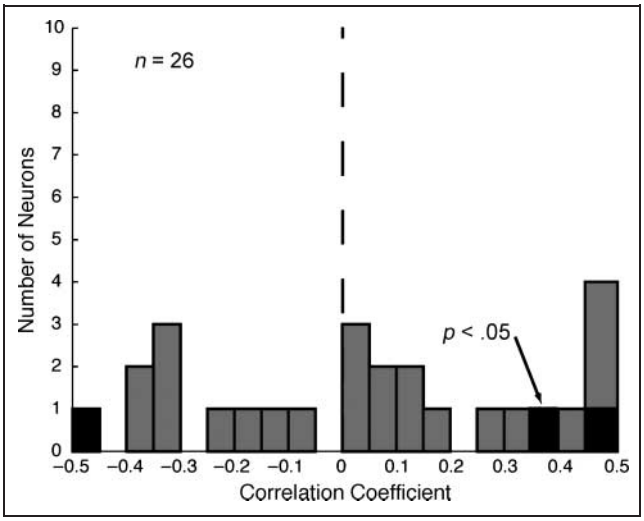

Figure 8. Histograms depicting correlations between neural activity and SRT for neurons showing a significant effect on ANOVA during the saccade epoch. Correlations were computed between mean activity for the best direction for each neuron in a 200-msec window that included the last 100 msec of the delay epoch, and the 100 msec immediately following fixation offset, and the SRT for the corresponding condition. Best direction was determined as that showing the greatest modulation in saccade-related activity compared to baseline. Neural activity was correlated with SRT for only 3 of 26 neurons (t test, evaluated at $p < .05$). Neurons showing a significant correlation are highlighted in black.

Individual DLPFC Neurons Send Varied Combinations of Signals to the SC

We found that most neurons were responsive in more than one analysis epoch. Of the 31 neurons showing a significant effect in at least one epoch, 25 (80.6%) showed a significant effect in more than one epoch. Thus, most DLPFC–SC projection neurons carried signals selective for combinations of cue, delay, saccade, and postsaccadic activity.

DISCUSSION

A key feature of models of DLPFC function is the assertion that it acts to modulate the activity of structures to which it is connected. Here we used the saccadic eye movement system in general, and visuospatial working

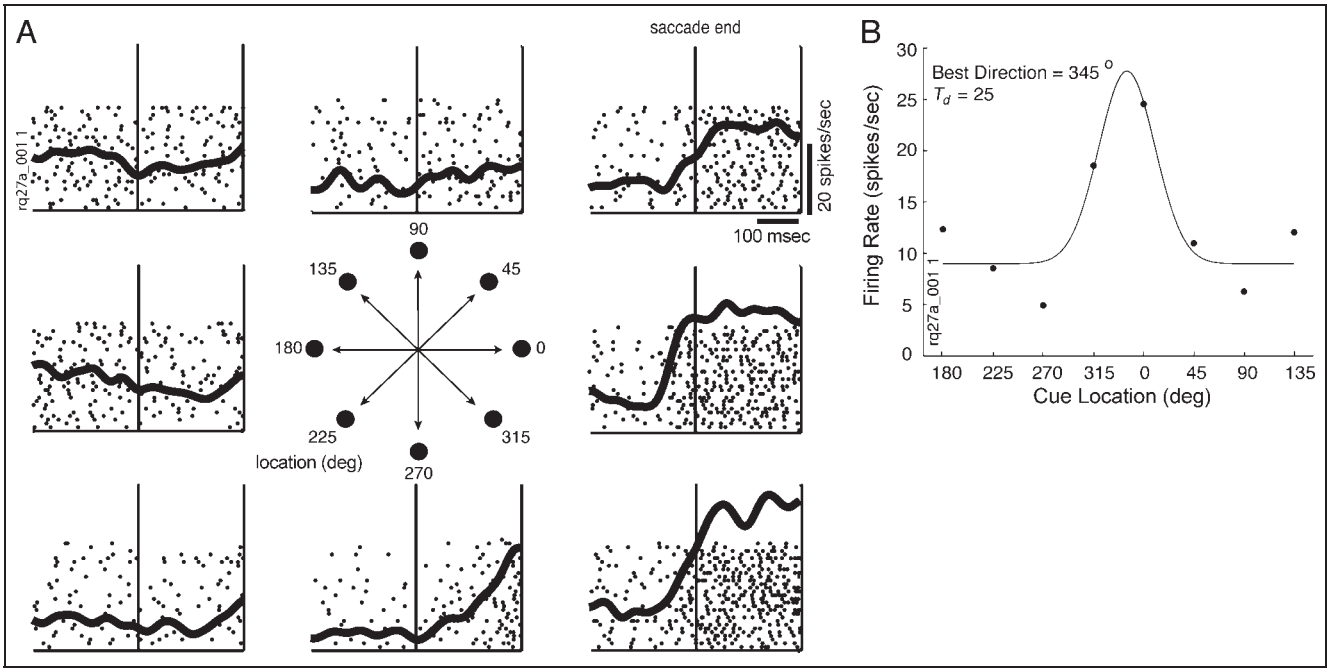

Figure 9. Single DLPFC cortico-tectal neuron showing significant effects during the postsaccade epoch. (A) Single-neuron activity, plotted as in Figures 4, 6, and 7. Rasters and spike density functions aligned on saccade end. This neuron showed directionally selective saccade-related activity, responding most strongly following saccades directed to the visual field ipsilateral to the recorded hemisphere (ANOVA, $p < .05$). (B) Gaussian tuning function for activity in the postsaccadic epoch for neuron shown in A. Function was fit to the mean postsaccadic activity for each direction (see Methods). This neuron was well tuned with a best direction of 345° and a width of 25°.

memory in particular, as a model system in which to investigate the detailed nature of the neural signals sent from the DLPFC to the SC during a memory-guided saccade task. We found that DLPFC neurons sent a mixture of signals to the SC, including cue, delay, saccade, and postsaccadic activity. These signals consisted of both upward and downward modulations in relation to baseline activity, and were spatially tuned in some cases. These signals are consistent with those observed in previous studies investigating the response properties of the general population of DLPFC neurons in this task (Funahashi et al., 1989, 1990, 1991). Although we did not find that signals conveyed by the DLPFC to the SC are specialized in relation to the general population of DLPFC neurons, our data provide the first direct evidence that mnemonic signals are sent directly from the DLPFC to an oculomotor structure, supporting a role of the DLPFC in the direct modulation of oculomotor output via an influence on the SC. These data therefore provide direct support for a role of the DLPFC in the modulation of brain areas to which it is anatomically connected.

We found that single DLPFC cortico-tectal neurons carried task-related signals about multiple aspects of the memory-guided saccade task. The majority of neurons we recorded exhibited some form of selectivity in more than one epoch. This type of organization is consistent with a continuous multistage processing model (Sommer & Wurtz, 2000), in which signals from each temporal stage of a task requiring a sensorimotor transformation can be sent independently to a target structure, in this case, the SC. This suggests that the SC receives information from the population of DLPFC output neurons at each stage in the memory-guided saccade task, and integrates this information together with input from other sources into a movement command. This is consistent with other studies of the output properties of cortico-tectal neurons (LIP: Paré & Wurtz, 2001; FEF and LIP: Wurtz, Sommer, Paré, & Ferraina, 2001; FEF: Sommer & Wurtz, 2000; FEF and LIP: Ferraina, Paré, & Wurtz, 2002), which have suggested that neural activity is transformed from visual and cognitive at the cortical level to motor biased in the SC.

Possible Modulatory Effects of DLPFC Inputs on SC Neurons

One way in which to attempt to elucidate the modulatory role of neural signals sent from DLPFC cortico-tectal neurons is to compare the properties of those neurons with SC neurons with known response properties. Based on this logic, if the responses of DLPFC output neurons are at least qualitatively similar to those of defined classes of SC neurons, some understanding of the nature of the output signal can be obtained. In this case, a difficulty with this approach is that the response properties of the cortico-tectal neurons we recorded share some similarity to those of multiple classes of SC neurons. For example, delay activity has been observed in the quasi-visual neurons described by Mays and Sparks

(1980), in mnemonic motor neurons (Kojima et al., 1996), and in buildup neurons (Munoz & Wurtz, 1995).

An alternative approach would be to rule out direct input to some classes of neurons on the basis of the response properties we observed in cortico-tectal DLPFC neurons. For example, we found no strong correlation between the presaccadic activity of DLPFC output neurons and SRTs. This finding is inconsistent with a direct input to SC saccade neurons, the presacccadic activity of which has been shown to be positively correlated with SRT (Dorris & Munoz, 1998; Dorris et al., 1997). Based on this exclusionary logic, it seems reasonable to conclude that cortico-tectal DLPFC neurons do not play a direct role in saccade initiation but have a modulatory influence on SC activity. Previously (Johnston & Everling, 2006b), we suggested that DLPFC output neurons might modulate SC activity via the extensive network of SC inhibitory interneurons (Munoz & Istvan, 1998). Our findings here are consistent with this conceptualization, however, a direct assessment of the influence of DPLFC neurons on SC neurons would be required to fully substantiate this argument.

Comparison with Other Cortical Areas Sending a Direct Projection to the SC

Another way in which to derive an understanding the nature of the signals sent from the DLPFC to the SC is to compare these output signals with those sent from other cortical areas to the SC during the same task. In this way, it should be possible to determine whether the output signals sent from the DLPFC to the SC are unique or overlap with other output signals. Both FEF and LIP send direct projections to the SC (Selemon & Goldman-Rakic, 1988; Stanton et al., 1988; Leichnetz et al., 1981), and the response properties of neurons in both areas have been extensively investigated in memory-guided saccade paradigms (Barash, Bracewell, Fogassi, Gnadt, & Andersen, 1991; Funahashi et al., 1989; Gnadt & Andersen, 1988; Bruce & Goldberg, 1985). Spatially tuned visual, delay, and saccade-related activity have been observed in these areas, and studies of identified cortico-tectal neurons have shown that this activity is transmitted to the SC (FEF: Sommer & Wurtz, 2001; Segraves & Goldberg, 1987, LIP: Paré & Wurtz, 1997, 2001, both: Wurtz et al., 2001). These signals share a great deal of similarity with those we observed in DLPFC cortico-tectal neurons, suggesting that there is substantial overlap in the signals sent from the FEF, LIP, and DLPFC to the SC. This is perhaps not surprising given the extensive interconnections between these areas (Petrides & Pandya, 1999; Stanton, Bruce, & Goldberg, 1993, 1995; Selemon & Goldman-Rakic, 1988), the similarity of neuronal responses during this task in the general populations of neurons in these areas (Sommer & Wurtz, 2000; Paré & Wurtz, 1997), and the fact that responses between these cortical areas exhibit some interdependence (Chafee & Goldman-Rakic, 2000). Based on this, it seems evident that the pathway between the DLPFC and the SC is but one link in a massively interconnected cortical and subcortical network subserving spatial working memory performance.

A potential functional difference between DLPFC cortico-tectal neurons, and those of FEF and LIP, may be a role of DLPFC output neurons in maintaining SC delay activity during memory-guided saccades. Delay activity in both FEF (Wurtz et al., 2001) and LIP (Paré & Wurtz, 2001) has been shown to be strongly dependent on the presence of a visual stimulus. Activity during delay periods is greater in both of these areas during delayed saccade tasks, in which the visual stimulus remains on throughout the delay period, than in memory-guided saccade tasks, where the stimulus is absent. This visual dependence is absent in SC neurons (Paré & Wurtz, 2001), suggesting that it may arise from a different cortical input. Given the established role of the DLPFC in tasks requiring working memory (Miller & Cohen, 2001), the deleterious effects of DLPFC lesions (Funahashi, Bruce, & Goldman-Rakic, 1993; Funahashi, Chafee, & Goldman-Rakic, 1993) and inactivation (Sawaguchi & Iba, 2001) on memory-guided saccade performance, and the fact that DLPFC delay activity reaches the SC suggests that it may provide such an input. This role must remain speculative, however, as we did not compare delay activity during memory-guided and visually guided delayed saccades here.

The antidromic latency of cortico-tectal DLPFC neurons we observed here was longer (mean = 6.2 msec) than those reported for FEF (Segraves & Goldberg, 1987, mean = 2.25 msec; Sommer & Wurtz, 1998, mean = 2.1 msec) or LIP (Paré & Wurtz, 2001, 1.9 msec). It is tempting to speculate that this difference in transmission time is somehow related to differences in the functional connectivity of these areas, however, the only statement that can be made regarding this finding is that the axons of DLPFC–SC projection neurons are smaller or less myelinated than those of cortico-tectal output neurons of FEF or LIP. Such a conclusion seems reasonable, at least in comparison to FEF, as it has been shown that the density of large layer V output neurons is high in the FEF and declines substantially in area 8 anterior to the FEF (Stanton, Deng, Goldberg, & McMullen, 1989), an area typically considered part of the DLPFC. Thus, it seems likely that the DLPFC neurons we identified had smaller cell bodies, and thus, smaller axons than FEF neurons, which could account for the increased latencies we observed.

The DLPFC has been shown to send direct connections to pontine nuclei in the brainstem, which pass through the SC (Selemon & Goldman-Rakic, 1988). With the technique employed here, we cannot unequivocally reject the possibility that we activated axons passing through the SC, and thus, the possibility that we activated these fibers of passage. Another possibility is that

the axons of some DLPFC neurons terminated in areas rostral to the SC, for example, the pretectum (Simpson, Giolli, & Blanks, 1988; Leichnetz et al., 1981). This may have occurred as a result of current spread to such areas due to the rostral location of our stimulating electrodes in the SC, and the current levels applied. We feel that this is unlikely based on two lines of evidence. First, based on our calculations of the current spread, encroachment of current on areas rostral to the SC would be minimal for the majority of neurons we recorded here, making it unlikely that these neurons were activated by current outside of the SC. Further, we found no differences in response properties between neurons antidromically activated by low or high currents. This evidence, although circumstantial, suggests that the DLPFC neurons we report here terminated in a common subcortical target, the SC. Second, it is generally thought that the pretecum is involved in slow eye movements such as smooth pursuit, and optokinetic nystagmus, rather than saccades. For example, stimulation of the nucleus of the optic tract (NOT), a prominent pretectal nucleus, does not evoke saccades (Schiff, Cohen, & Raphan, 1988), and lesions of this area affect slow eye movements but leave saccades unaffected (Kato et al., 1986). Electrophysiological studies have also obtained results consistent with these findings (Mustari & Fuchs, 1990). The response properties of this area are inconsistent with those we observed here, and those from previous studies showing saccade-related activity in DLPFC neurons (Funahashi et al., 1991; Boch & Goldberg, 1989).

In summary, we characterized for the first time the output signals sent from the DLPFC to a target structure during a spatial working memory task. We found that cue, delay, saccade, and postsaccadic activity are sent to the SC, supporting a role of the DLPFC in the modulation of other brain areas. The nature of these signals overlaps extensively with those sent from other cortical areas to the SC. We conclude that the DLPFC–SC pathway is part of a distributed network of cortical and subcortical areas that are critical for spatial working memory performance. A direct comparison between the activity of simultaneously recorded DLPFC–SC projection neurons and SC neurons may provide further information regarding the specific manner in which DLPFC input modulates the activity of SC neurons. This, in turn, may provide further insight into the physiological mechanisms underlying prefrontal modulation of target areas.

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