INTRODUCTION

The frontal eye field and superior colliculus are components of a network of cortical and subcortical structures acting together to control the generation of voluntary saccades and fixation. Each area makes a contribution to the normal control of eye movements with considerable overlap in function between structures. The role of the frontal eye field and superior colliculus in the generation of eye movements has been studied extensively (for reviews, see Goldberg and Segraves 1989; Guitton 1991; Schall 1997; Sparks and Hartwich-Young 1989; Wurtz and Munoz 1995). In both structures, depending on the location of the electrode site within the topographic map of eye movement space, low-threshold microstimulation evokes contraversive saccades or suppresses saccades and produces active fixation (Bruce et al. 1985; Burman and Bruce 1997; Munoz and Wurtz 1993b; Robinson 1972; Robinson and Fuchs 1969).

In addition, reversible inactivation of the frontal eye field or the superior colliculus interferes with the generation of saccades and fixation (Dias and Segraves 1999; Hikosaka and Wurtz 1985, 1986; Schiller et al. 1987; Sommer and Tehovnik 1997). Surgical ablation of only the frontal eye field or superior colliculus produces mild to moderate deficits in oculomotor behavior while combined lesions of the frontal eye field and superior colliculus produce profound oculomotor deficits, suggesting that there are both serial and parallel contributions from these areas to the control of eye movements (Deng et al. 1986; Schiller et al. 1987). Elaborating on the relative contributions of serial and parallel inputs of the frontal eye field and superior colliculus to the control of saccades, a recent report indicates that, in spite of direct links between the frontal eye field and oculomotor centers in the brain stem, it is the indirect, serial pathway from frontal eye field to colliculus to brain stem oculomotor centers that is the most important for the control of eye movements (Hanes and Wurtz 2001).

Neurons within both structures exhibit a variety of eye-movement-related activity. During voluntary, purposive saccades, frontal eye field neurons may respond to visual stimuli within the neuron’s receptive field as well as produce movement-related activity associated with the generation of a saccade of a preferred amplitude and direction (Bruce and Goldberg 1985; Mohler et al. 1973; Schall 1991). In contrast to brain stem oculomotor centers, which are involved in the direct control of oculomotor neurons, the frontal eye field appears to be involved in a higher level of control of eye movements. Neurons in the frontal eye field show activity related to the process of saccade target selection during both visual search tasks and scanning of natural images (Bichot et al. 1996; Burman and Segraves 1994; Schall and Hanes 1993; Schall et al. 1995; Thompson et al. 1996). Within the deep layers of the superior colliculus, saccade-related activity has been characterized as including...
a prolonged anticipatory-like activity prior to the onset of the saccade and/or a discrete burst of activity associated with the onset of the saccade (Glimcher and Sparks 1992; Mays and Sparks 1980; Mohler and Wurtz 1976; Munoz and Wurtz 1995b; Sparks 1978; Sparks et al. 1976). In this report, we define deep layers as all collicular layers located below the superficial layers (superficial gray and stratum opticum), including the intermediate and deep gray layers. Both frontal eye field and superior colliculus neurons have been shown to generate preparatory set activity that is directly correlated with saccadic reaction time (Glimcher and Sparks 1992; Dorris and Munoz 1998; Dorris et al. 1997; Everling et al. 1999; Everling and Munoz 2000).

As part of the overall network responsible for the control of voluntary saccades, the deep layers of the superior colliculus receive input from both cortical and subcortical structures involved in this process (Grantyn 1988; Moschovakis et al. 1996). The signals sent from the frontal eye field to the superior colliculus have been characterized. It has been known for some time that frontal eye field neurons with fixation and saccade-related activity project to the deep layers of the superior colliculus, providing information to the superior colliculus concerning the maintenance and release of fixation and the generation of the saccade (Segraves and Goldberg 1987). More recently, this finding has been confirmed and extended to include corticocortical neurons with visually driven activity located primarily within lateral portions of the frontal eye field (Sommer and Wurtz 2000). Thus there appears to be little selectivity in the output of frontal eye field activity that is sent to the superior colliculus. Frontal eye field input provides collicular neurons with an upper level eye movement signal relaying information concerning fixation, anticipatory activity prior to the appearance of a target, the presence and selection of visual targets, and movement-related activity for saccades of specified amplitude and direction (Schlag-Rey et al. 1992; Segraves and Goldberg 1987; Sommer and Wurtz 2000).

Is there selectivity in the types of superior colliculus neurons that receive this frontal eye field input? This study attempts to answer this question by identifying superior colliculus neurons that receive direct excitatory frontal eye field input. Extracellular recordings were made simultaneously in the frontal eye field and superior colliculus of rhesus monkeys, and antidromic excitation of frontal eye field neurons from the colliculus was used to isolate interconnected cortical and collicular sites. Microstimulation at the frontal eye field site was then administered to orthodromically activate superior colliculus neurons that received short-latency frontal eye field input. The activity of collicular neurons receiving direct frontal eye field input was then characterized during visuomotor tasks. This identification and characterization of the types of superior colliculus neurons that receive excitatory frontal eye field input contributes to our understanding of how these components of the oculomotor network interact to control the generation of saccades. This work also provides insight into the involvement of the different functional types of superior colliculus neurons in the generation of voluntary saccades.

A preliminary report of this study has been published (Helminski and Segraves 1996).

Methods

Two female adult rhesus monkeys (Macaca mulatta) weighing between 5 and 8 kg were used for these experiments. The monkeys were identified as MK04 and MK05. Northwestern University’s Animal Care and Use Committee approved all procedures for training, surgery, and experiments performed with these monkeys.

Preoperative training

The monkeys were trained preoperatively in a simple visual fixation task with liquid reward. The preoperative training taught monkeys to get in and out of a primate chair and attend to a visual stimulus. Monkeys were seated in a primate chair positioned in front of a tangent screen. Each trial began when the monkey touched a metal bar in front of her, causing a spot of light to appear on the screen. When the light dimmed, the monkey was required to release the bar to obtain a liquid reward. Preoperative training was completed when the monkey was ready to progress to more difficult oculomotor tasks that required monitoring of eye position.

Surgery

Two surgeries were performed on each animal under aseptic conditions. Prior to surgery, the monkey was anesthetized and tranquilized with ketamine hydrochloride (10 mg/kg im) and methohexitol sodium (11 mg/kg iv or to effect), and secretions were reduced by administration of atropine sulfate (0.05 mg/kg im). For the duration of the surgery, anesthesia was maintained with halothane inhaled through an endotracheal tube.

During the initial surgery, a subconjunctival wire coil was implanted in the left eye to measure eye position using the magnetic search coil technique (Judge et al. 1980; Robinson 1963). Two trephine holes were made through the skull to enable a microelectrode to be inserted through the dura to record neuron activity from the frontal eye field and superior colliculus. Slots were cut through the skull extending away from the trephine holes. Stainless steel bolts were positioned in the slots and secured with stainless steel nuts and washers. Thin, curved strips of stainless steel were used to interconnect the bolts. The stainless steel bolts and strips functioned to enhance the bond between the skull and the dental acrylic. Recording cylinders were positioned over the left frontal eye field and superior colliculus trephine holes and bonded to the skull with dental acrylic. The frontal eye field cylinder was centered at stereotaxic coordinates anterior ±25.0 mm and lateral 20.0 mm. The orientation of the frontal eye field cylinder was adjusted to allow penetrations to be made roughly parallel to the bank of the arcuate sulcus. The superior colliculus cylinder was tilted posteriorly, 38° from vertical in the sagittal plane and oriented so that a line of projection through its center would pass through a point on the midline 15 mm above and 1 mm posterior to the inter-aural line. This location enabled us to record from both left and right superior colliculi. A steel receptacle to fix the monkey’s head during recording sessions and a connector for the eye coil were fixed in place and bonded to the skull with dental acrylic. For both monkeys, a right frontal eye field recording cylinder was later added during a second surgery.

To prevent infection, the monkey was given the antibiotic cefazolin (25 mg/kg iv) prior to and immediately after surgery as well as twice a day for 1 wk after surgery (25 mg/kg im). The analgesic buprenorphine hydrochloride (0.01 mg/kg im) was administered to the monkey at the end of surgery and twice a day for 3–4 days after surgery.

Postoperative training

Postoperative training began 10–14 days after surgery. The monkey was seated in a primate chair with its head restrained and centered within horizontal and vertical magnetic field coils. All behavioral
control and data collection were performed via a PDP-11/73 (Digital Equipment). With the room dimly lit, visual stimuli originating from light-emitting diodes as well as laser diodes were rear-projected onto a tangent screen in front of the monkey. Stimuli consisted of a stationary and a movable light where the movable stimulus was positioned by a pair of servo-controlled mirror galvanometers (General Scanner) driven by computer generated analog signals. Eye position was measured by the magnetic search coil system with a phase-sensitive detector (C.N.C. Engineering).

**Visuomotor tasks**

Monkeys were trained to perform visuomotor tasks for a liquid reward (Fig. 1). Visual stimuli were generated by using two independent light-emitting or laser diodes. The first was a stationary light located at the center of the tangent screen. This light was used as the initial fixation point for the monkey. The second was a movable light positioned by mirror galvanometers that was used as either a visual stimulus or a saccade target. Each visuomotor task began after the monkey had fixated the central light and maintained fixation of this light for a randomly varied interval of 100–400 ms.

The first two visuomotor tasks required the monkey to maintain fixation of the fixation point during the task.

**FIXATION TASK** (FIG. 1A). The monkey held fixation for the duration of the trial. During the trial, the fixation light was turned off for brief periods of time. This enabled the task to be used to distinguish between fixation-related activity and visual activity driven by the foveal stimulus. If a neuron’s activity was primarily related to fixation behavior, we would expect a change in firing rate that was maintained throughout the period of time that the monkey fixated the central light. If instead, a neuron’s activity was primarily related to sensory visual input, then we would expect to see changes in firing rate coinciding with the appearance and disappearance of the fixation light.

**VISUAL NO-SACCade TASK** (FIG. 1B). The monkey held fixation while a single stimulus was briefly flashed in the periphery. The location of the stimulus on the screen could be varied within the monkey’s visual field either by explicitly entering desired stimulus coordinates into the software controlling stimulus position or by joystick readout. The task was used to identify a neuron’s visual receptive field by delineating the area of tangent screen where visual stimuli produced increased neuron firing.

The last four visuomotor tasks required the monkey to make saccades from the fixation point to a target location. For all but the gap task, once the center of a response field was identified, a single target positioned at the center of that response field was used.

**VISUALLY GUIDED SACCADE TASK** (FIG. 1C). After the monkey maintained fixation for 700–1,000 ms, the fixation point was turned off at the same time that a target appeared in the periphery. When the target appeared, the monkey looked at the target. The location of the target on the tangent screen could be varied within the monkey’s visual field. The task was used to identify a saccade-related neuron’s movement field and preferred movement vector.

**OVERLAP TASK** (FIG. 1D). During the overlap task, the fixation point and the target were illuminated together for 500 or 1,000 ms. The monkey maintained fixation as long as the fixation point was on, a period of 1,500 ms. To begin the task, the fixation point was turned on. After 500 (or 1,000) ms, the target was turned on. Then, at 1,500 ms, the fixation point was extinguished, and the monkey was required to look at the target. This task was used to distinguish between visual- and saccade-related neuronal activity when the monkey was required to make a saccade to a target.

**GAP TASK** (FIG. 1E). The monkey began this task by fixating the central light. The fixation light was kept on for 1,000 ms and then extinguished. At 400 ms after the disappearance of the fixation light, a target light was turned on, and the monkey was rewarded after making a saccade to this light. For this task, the target would appear at one of two positions with equal likelihood. The first target position was located at the center of the cell’s response field. The second target position was positioned at a location with identical radius but with angle rotated 180° from the first target. This task reduces saccadic latency by removing the visual stimulus for active fixation prior to the appearance of the peripheral target light.

**MEMORY-GUIDED SACCADE TASK** (FIG. 1F). In this task, the monkey was required to make a saccade to a remembered target location. After the first 600 ms of a 1,000-ms fixation period, a peripheral target stimulus was turned on for a period of 300 ms. Then 100 ms after the target stimulus was turned off, the fixation point was extinguished, and the monkey was rewarded for making a saccade to the location on
the tangent screen where the target had appeared. The separation that this task provided between when the visual target stimulus was present and when the monkey was allowed to make a saccade made it possible to distinguish between visual- and saccade-related neuronal activity.

In this study, the location of visual stimuli and targets are expressed in polar coordinates, radius and angle, where radius is equal to the distance, in degrees of arc, of the target or stimulus from the central fixation point. An angle of 0° describes a rightward horizontal direction, and a 90° angle describes an upward vertical direction.

In all tasks, the error window for comparison of eye position to fixation point and target position was ±3–5° in the horizontal and vertical directions.

Microelectrode recording and stimulation

Single-unit recordings were obtained using epoxy-insulated tungsten microelectrodes (A-M Systems) with wire diameters of 0.203 and 0.254 mm for the frontal eye field and 0.127 and 0.203 mm for the superior colliculus. Electrode impedance ranged from 0.6 to 1.5 MΩ measured at a frequency of 1 kHz. Hydraulic microdrives (Narishige) were used to advance the electrodes, and the isolation of the action potentials of single neurons was accomplished using a computer-based waveform discriminator (Signal Processing Systems, Prospect, Australia).

Electrodes were introduced into the brain through guide tubes held within a plastic grid fastened within the recording cylinder (Crist et al. 1988). The use of guide tubes penetrating the dura allowed for the use of finer electrodes that could not penetrate the dura mater alone. In addition, guide tubes helped to increase electrode stability and facilitated the making of repeated penetrations through the same neural sites. When a guide tube was not being used, a stainless steel wire, coated with antibiotic (3% tetracycline HCL) was inserted into it to prevent infection.

To identify the frontal eye field, electrode penetrations were made in the region of the arcuate sulcus. During each penetration, unit activity was characterized during visuomotor tasks at intervals of 50–100 μm in depth, and electrical stimulation was applied every 200–250 μm in depth. The frontal eye field (FEF) was physiologically defined as the area of cortex located primarily on the rostral bank as well as the fundus of the arcuate sulcus where saccades could be electrically evoked with stimulus thresholds of <50 μA (Bruce and Goldberg 1985; Bruce et al. 1985). Electrical stimuli consisted of 70-ms trains of biphasic pulses (−0.2-ms pulse followed by +0.2-ms pulse) applied at a frequency of 330 Hz. The output of the stimulator was connected to the electrode through constant-current optical isolators. Maximum current intensity used was 75 μA. Threshold was defined as the current intensity at which saccades were evoked in response to 50% of the stimulus trains. In these experiments, all active sites that had FEF-like activity and low thresholds for evoking saccades were tested for the effects of orthodromic stimulation on the ipsilateral superior colliculus.

Projections from the FEF to the superior colliculus connect matching sites within each topographical representation (Komatsu and Suzuki 1985; Segraves and Goldberg 1987; Stanton et al. 1988b). To map the topography of the superior colliculus, a microelectrode was advanced and neuron activity was characterized during visuomotor tasks at intervals of 50–100 μm. Visual receptive fields and movement fields were determined at each depth. These were compared with visual receptive field (Cynader and Berman 1972) and motor maps (Robinson 1972) to determine the location of the electrode within the collicular topography. Once the relationship of collicular topography to recording grid location was understood, we positioned a guide tube at a site that corresponded to the current recording site in the FEF. A comparison of the saccade amplitude for response fields of pairs of FEF and superior colliculus sites where antidromically activated cells were isolated yielded a correlation coefficient of 0.80 (Pearson’s product moment).

Antidromic excitation

Interconnected FEF and superior colliculus sites were identified using antidromic excitation (Fig. 2). All pairs of interconnected FEF and superior colliculus sites were ipsilateral to one another. With the cortical electrode positioned at the surface of the FEF and the collicular electrode positioned at a depth where both visual and saccade-related activity were observed, the FEF electrode was advanced in 50-
to 100-μm increments. At each depth, neuronal activity was characterized during visuomotor tasks to monitor the progress of the penetration through the cortex, and the superior colliculus was stimulated in an attempt to antidromically excite FEF neurons. Stimuli used for antidromic excitation were single biphasic pulses (~0.1-ms pulse followed by +0.1-ms pulse) and had a maximum negative pulse intensity of 1 mA. The amplitude of the positive-going component of the biphasic pulse was always less than the negative component and was adjusted to produce the minimum stimulus artifact. Based on a review by Ranck (1975) of several studies of mammalian CNS stimulation, the passive spread of current from a 1-mA source could excite cell bodies and myelinated axons at a distance of ≤2 mm from the electrode tip. The criteria for antidromic identification were a constant response latency to antidromic excitation and the ability to collide spontaneous and antidromically evoked spikes (Bishop et al. 1962; Fuller and Schlag 1976). Antidromic latency was defined as the time interval between the onset of the stimulus and the appearance of the antidromic spike. Threshold was defined as the current intensity at which antidromic spikes were obtained in response to ~50% of the stimulus pulses. Once an antidromically excited neuron was identified, its activity was characterized during visuomotor tasks.

Current-source-density analysis

Extracellular field potential and current-source-density analysis were used to examine the vertical distribution of FEF terminations in the superior colliculus. A FEF electrode was positioned at a site where cells projecting to the superior colliculus had been localized by antidromic excitation, and a collicular electrode was positioned with its tip just above the dorsal surface of the superior colliculus. The collicular electrode was then advanced in 100-μm increments. After each advance, an electrical stimulus was applied through the FEF electrode and evoked field potentials were recorded from the collicular electrode. The cortical stimulus was a single biphasic pulse where a ~0.1-ms pulse was followed by +0.1-ms pulse. As was the case for the stimuli used to search for antidromically excitable neurons, the maximum amplitude of the negative pulse was 1 mA with the positive pulse adjusted <1 mA to make the stimulus artifact as small as possible.

A current-source-density analysis was used to summarize the activity of many individual neurons enabling us to assess the global pattern of activity at a recording site. This analysis helped us to localize the site of dominant synaptic input within the vertical distribution of field potentials providing better localization of neuronal events than would be possible with field potentials alone. Based on established techniques (Ferster 1990; Freeman and Nicholson 1975; Mitzdorf and Singer 1978; Nicholson and Freeman 1975), current-source-density was defined as being directly proportional to the second spatial derivative of the field potential at a point (x, y, z)

\[
I_{(x,y,z)} = -\sigma \nabla^2 V_{(x,y,z)}
\]  

where \(\sigma\) is the resistivity of the medium, \(V\) is the voltage, and \(I\) is the conductance.

Two assumptions were made based on the findings of Nicholson and Freeman (1975). The first was that conductivity in the extracellular space did not change significantly with superior colliculus depth. The second was that the spread of conductivity occurs symmetrically in both the x and the y directions. In the present experiment, the x and y directions ran parallel to the plane of the surface of the superior colliculus. Variation only occurred in the z direction, perpendicular to the superior colliculus surface. Therefore Eq. 1 was simplified to

\[
I_{(z)} = -\sigma \frac{dV_{(z)}}{dz}
\]  

Evoked potentials were measured at finite points. Thus the second derivative was approximated by a finite-difference equation as described by Freeman and Nicholson (1975)

\[
I_{(z)} = -\sigma \frac{V_{(z+\Delta z)} - 2V_{(z)} + V_{(z-\Delta z)}}{(n \cdot \Delta z)^2}
\]  

The expression \((V_{(z+\Delta z)} - 2V_{(z)} + V_{(z-\Delta z)})\) could be determined from our experimental measurements, \(\sigma\) and the denominator \((n \cdot \Delta z)^2\) were assumed to be constant, therefore \((V_{(z+\Delta z)} - 2V_{(z)} + V_{(z-\Delta z)})\) was directly proportional to current density. Previous studies showed that when field potentials were recorded at 50-μm increments in depth, \(n\) should be 5 for optimal spacing to reduce noise and maximize the sinks and sources (Mitzdorf and Singer 1978). In our experiments, \(n\) was chosen to be 3 because the separation in recording sites \((\Delta z)\) was 100 μm. Therefore \(V_{(z)}\) was the field potential recorded at depth \((z)\), and \(V_{(z+\Delta z)}\) and \(V_{(z-\Delta z)}\) were the field potentials recorded at depths 300 μm dorsal and 300 μm ventral to depth \((z)\).

Orthodromic excitation

Orthodromic excitation was used to identify superior colliculus neurons which received FEF input (Eq. 3). A FEF microelectrode was positioned with its tip within a site where corticocollicular cells had been identified, and another microelectrode was positioned just above the superior colliculus (Fig. 3A). The superior colliculus electrode was advanced in 50- to 100-μm increments through the entire depth of the superior colliculus, including both superficial and deep layers. At each depth, the FEF was stimulated in an attempt to orthodromically excite collicular cells. The stimulus consisted of one or two biphasic pulses, ~0.1-ms pulse followed by ~0.1-ms pulse, applied 1–4 ms after the superior colliculus neuron fired spontaneously or in response to a brief visual stimulus in its response field (Fig. 3, B and C). The twin pulse had a 3-ms separation between pulses. Orthodromically driven neuronal spikes were recognized by their relatively fixed response latency following the FEF stimulus. Threshold for orthodromic excitation was defined as the current intensity at which orthodromic spikes were obtained in response to ~50% of the stimulus pulses.

Data analysis

During the experiments, behavioral and neuronal activity was sampled at 1 kHz and was stored in the laboratory computer. After the experiment, the data were transferred to a UNIX-based workstation (Sun Microsystems) for further analysis.

Neuron activity was displayed in rasters and histograms aligned to specific events occurring within the behavioral paradigm to evaluate the relationship between neuron activity and those events. Neuronal activity was also plotted as continuous spike-density functions (Richmond et al. 1987; Sanderson and Kobler 1976). To generate a spike density function for each trial, a Gaussian pulse of fixed width (\(\sigma = 10\) ms) was substituted for each spike, and then all of the Gaussians were summed together to produce a continuous function expressing the expected spike frequency for any point in time during the trial. A Poisson spike train analysis technique (Hanes et al. 1995; Legédny and Salcman 1985) was used to identify statistically significant changes in neuronal firing.

The activity of FEF neurons during visuomotor tasks was classified according to the criteria of Bruce and Goldberg (1985). The activity of superior colliculus neurons during visuomotor tasks was classified using published criteria as a basis (Glimcher and Sparks 1992; Mays and Sparks 1980; Mohler and Wurtz 1976; Munoz and Wurtz 1995a; Sparks 1980; Sparks et al. 1976). For both FEF and superior colliculus, visual activity was defined as a fixed, short-latency response to a visual stimulus regardless of whether or not the stimulus would become the target for a saccade. The visual no saccade and memory-guided saccade tasks were the primary tasks used to identify visual activity. For both regions, saccade-related burst activity was identified in the memory-guided saccade task because of the advantage it provides of separating the time when the target stimulus is flashed and
the time when the saccadic eye movement is allowed by \( \geq 100 \) ms. Saccade-related burst activity was defined as a high-frequency burst of activity peaking near the beginning of the saccade. For prelude/build-up activity in the superior colliculus, we relied on the use of the gap task. During the gap task, neurons with prelude/build-up activity increased their firing rates during the gap period regardless of where the target would appear in order for it to be classified as prelude/build-up.

**Histology**

On completion of all experiments, monkeys MK04 and MK05 were given the analgesic ketamine hydrochloride (10 mg/kg im). Three to four reference guide tubes were inserted into each recording grid through the dura and into the brain. Each monkey was given a lethal dose of pentobarbital sodium and was perfused transcardially with saline followed by 10% formalin. The brain was removed and photographed. For monkey MK04, the brain was frozen sectioned at 50 \( \mu \)m. The location of tracts left by the reference guide tubes were used to assist in aligning the plane of sectioning parallel to the trajectories of electrode tracts. Sections through the superior colliculus were made in the coronal plane. Sections through the arcuate sulcus were made in the plane running rostrocaudally, parallel to the principal sulcus. These sections helped to confirm that our penetrations were, in fact, through the FEF and superior colliculus. Although a complete historical processing was not performed on monkey MK05, gross observation of the brain’s surface confirmed that the FEF penetrations were through the anterior bank of the arcuate sulcus.

**RESULTS**

**Interconnected FEF and superior colliculus sites**

Antidromic excitation of FEF neurons by superior colliculus microstimulation was used to identify 15 pairs of interconnected FEF and superior colliculus sites in two rhesus monkeys (see Table 1). In the process of identifying these pairs of interconnected sites, 22 FEF neurons were antidromically excited by superior colliculus stimulation and their activity was characterized with visuomotor tasks. Ninety-one percent (20/22) of the corticotectal neurons exhibited saccade-related activity during the memory-guided or overlap tasks. Of these neurons, 30% (6/20) also had visually related activity. One of the corticotectal neurons had only visually related activity, and the other had only saccade-related activity.

**TABLE 1. Interconnected sites and orthodromically excited superior collicular neurons**

<table>
<thead>
<tr>
<th>Activity Type</th>
<th>MK04</th>
<th>MK05</th>
<th>Total</th>
<th>Percent of Total</th>
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</thead>
<tbody>
<tr>
<td>Interconnected sites</td>
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<td>5</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Orthodromically excited</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>superior collicular neurons</td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Visual</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual &amp; prelude/build-up</td>
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<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Visual &amp; prelude/build-up &amp; burst</td>
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<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Visual &amp; burst</td>
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<td>1</td>
<td>12</td>
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<td>2</td>
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<td>4</td>
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<tr>
<td>Burst</td>
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<tr>
<td>Total</td>
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<td>45</td>
<td>1</td>
<td>83</td>
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</table>

FIG. 3. Orthodromic excitation. A: a microelectrode was positioned with its tip in the FEF and was used to electrically stimulate the FEF, thus providing orthodromic stimulation to the superior colliculus. A 2nd microelectrode was positioned within the superior colliculus and was advanced in depth through the colliculus, while stimuli were applied to the FEF until an orthodromically driven superior colliculus neuron was isolated. B: example of a single stimulation/recording trace where the FEF was stimulated with a single stimulus pulse 3 ms after the superior colliculus neuron spontaneously fired (1st arrow), the collicular neuron was orthodromically excited with a latency of \(-4\) ms (2nd arrow). C: example of a 2nd neuron obtained from a different pairing of recording and stimulation sites from the example shown in B. Here, the FEF was stimulated with a twin pulse 1 ms after the superior colliculus neuron fired spontaneously, and the collicular neuron was orthodromically excited with a latency of \(-4\) ms.
one of the corticotectal neurons responded only to novel visual stimuli and was unresponsive in standard oculomotor tasks, both of these neurons were recorded from our most lateral recording sites where saccades could be evoked with thresholds of <50 μA. At points 0.5 mm lateral to these sites, the threshold for evoking a saccade exceeded the 50-μA criteria for defining the FEF. The mean response latency to antidromic excitation was 2.00 ± 0.43 ms (range: 1.5–3.0 ms), and the mean threshold for antidromic excitation was 223 ± 187 μA (range: 10–700 μA; Table 3). These findings regarding cell activity type, as well as latency to antidromic excitation and threshold are consistent with earlier reports (Everling and Munoz 2000; Segraves and Goldberg 1987; Sommer and Wurtz 2000). In addition to finding the corticotectal cells types reported here, Sommer and Wurtz (2000) found a prevalence of corticotectal neurons with exclusively visual activity in more lateral regions of the FEF. Our failure to isolate a high percentage of FEF sites where visual corticotectal cells were prevalent suggests that our stimulation sites were confined to the medial FEF.

Localization in depth of FEF input to the superior colliculus

Once an antidromically activated site was identified, orthodromic excitation was used to determine the location in depth within the superior colliculus that received excitatory FEF input. This was done for monkey MK04 alone. Our findings were then compared with published results from anatomical tracing studies. Orthodromically evoked superior colliculus field potentials were recorded from two interconnected FEF and superior colliculus sites in this monkey. Field potentials were averaged by calculating the mean of 50 individual responses and are shown in series dorsoventrally through the superior colliculus (Fig. 4A). An increase in the amplitude of evoked field potentials occurred from ~1,100–1,700 μm below the level where visually related background activity was first observed (0 μm). To refine the broad spatial distribution of field potentials that results from the linear addition of overlapping potentials, we used a current-source-density analysis that provided a better localization of the site of FEF input (Fig. 4B). Visual inspection of this current-source-density analysis showed that maximum current densities occurred from ~1,200–1,500 μm below the level where visually related background activity was first observed. When the patterns of field potentials and current-source-density were compared with histograms of neuronal activity during the memory-guided saccade task at each depth, it was found that recording sites between 0 and 1,000 μm exhibited primarily visually related activity, sites located from 1,100 to 1,700 μm exhibited primarily saccade-related activity and recording sites located between 1,800 and 2,000 μm exhibited no saccade-related neuronal activity. Saccade-related activity was first observed at a depth of 700 μm, and at 1,100 μm, the peak frequency of saccade-related activity was greater than the peak frequency of visually related activity. At 1,800 μm, only very weak saccade-related background modulation was found.

Our field potential and current-source-density analysis of these recording sites, helped us to identify the depths at which FEF input was concentrated within the superior colliculus. The FEF sites that we sampled did not appear to project to sites containing purely visual neurons in the superficial layers of the superior colliculus, instead, they projected selectively to recording sites containing saccade-related neurons in the deeper layers of the superior colliculus, the stratum griseum intermediate and profundum. These findings confirm previous reports from anatomical tracer studies that the FEF neurons terminate primarily in the deeper layers of the superior colliculus (Astruc 1971; Fries 1984; Huerta et al. 1986; Komatsu and Suzuki 1985; Kunzle and Akert 1977; Kunzle et al. 1976; Leichnetz et al. 1981; Stanton et al. 1982, 1988b). Our next step was to isolate and characterize individual superior colliculus neurons that were orthodromically excited by FEF stimulation.

Orthodromically excited neurons

Physiological classification. Orthodromic stimulation from all antidromically activated sites was used to identify superior colliculus neurons that received FEF input and the activity of the neurons was characterized with visuomotor tasks. A total of 83 orthodromically excited superior colliculus neurons were isolated and their activity characterized (Table 1). All of these neurons increased their discharge rates in association with saccadic eye movements made in the visually guided saccade
task. All of the orthodromically excited superior colliculus neurons also exhibited activity aligned to the beginning of the saccade, in the memory-guided or overlap tasks, with or without activity aligned to the visual stimulus. None had visual activity alone.

For each identified orthodromically excited neuron, a visually guided saccade task was used to identify the neuron’s response field and preferred eye movement vector. Once the preferred movement vector was determined, the memory-guided saccade task and overlap task were used to distinguish between visual and saccade-related activity. Next, the gap task was used to distinguish between two types of presaccadic activity: prelude/build-up activity prior to the onset of the saccade and a high-frequency burst of activity associated with the onset of the saccade. The gap task helped us determine the duration of the prelude/build-up of neuronal activity during the delay period between the times when the fixation point was extinguished and the target was illuminated. Target position was randomly alternated between two target locations, corresponding to the endpoint of the neuron’s preferred movement vector as well as the end point of a vector of the same amplitude but in the opposite direction.

**Burst activity.** Most of the superior colliculus cells driven orthodromically from the FEF exhibited saccade-related burst activity during memory-guided and overlap tasks (93%, n = 77). In the gap task, these cells exhibited a high-frequency burst of activity associated with the onset of the saccade in the direction of the preferred movement vector and no significant burst activity for saccades made in the opposite direction.

**Prelude/build-up activity.** During the gap task, 25% (n = 21) of the orthodromically driven superior colliculus neurons exhibited prelude/build-up activity prior to the onset of the saccade in the direction of the preferred movement vector as well as for saccades in the opposite direction. This activity was often accompanied by a high-frequency burst of activity beginning prior to the onset of the saccade in the direction of the preferred movement vector but not for saccades made in the opposite direction. Seventy-one percent (15/21) of the neurons with prelude/build-up activity exhibited a high-frequency burst of activity for saccades made in the preferred direction.

An example of the activity of an orthodromically excited neuron with prelude/build-up activity followed by a saccade-related burst of activity is shown in Fig. 5. During the gap task, prelude/build-up activity was elicited for saccades in the direction of the preferred movement vector (Fig. 5A) as well as for the opposite direction (Fig. 5B). The peak of the high-frequency burst of activity occurred 84 ms prior to the onset of the saccade made in the preferred movement direction (Fig. 5A). No burst of activity was elicited for saccades made in the opposite direction (Fig. 5B).

**Visually related activity.** In contrast to the frequent occurrence of saccade-related burst as well as prelude/build-up activity in the population of orthodromically driven neurons, none of the orthodromically driven superior colliculus neurons exhibited exclusively visually related activity during the memory-guided or overlap tasks. With each penetration, an attempt was made to identify orthodromically driven neurons within the superficial layers of the superior colliculus. None was found. This was in agreement with our initial field potential and current-source-density findings that orthodromic stimulation produced little effect in the pattern of evoked activity within the superficial layers of the colliculus. FEF driven trans-synaptic potentials were first observed within the transition region between the superficial layers and the deeper layers of the colliculus. Although neurons with visually related activity were found in this transition region, these neurons always had an additional component of their activity that was primarily aligned to the time of saccade onset. Of the orthodromically
driven superior colliculus neurons found, 30% (n = 25) exhibited visual activity in combination with prelude/build-up and/or burst activity. Of these neurons, 8% (n = 2) combined visual activity with prelude/build-up activity alone, 44% (n = 11) exhibited visual activity in combination with both prelude/build-up and burst activity, and 48% (n = 12) exhibited visual activity in combination with burst activity alone.

In summary, all orthodromically driven neurons exhibited prelude/build-up and/or burst activity. During the gap task, 25% of the orthodromically driven neurons had prelude/build-up activity while 93% of the orthodromically driven neurons had a high-frequency burst of activity associated with the onset of the saccade. No orthodromically driven neurons had purely visually related activity, although 30% had a combination of visual activity with prelude/build-up or burst activity.

### Relationship of activity of orthodromically driven collicular neurons to saccade timing

The saccade-related neuron population within the superior colliculus can be divided based on the timing of the fall of activity relative to the end point of the saccade (Waitzman et al. 1991) with neurons the activity of which ends with the end of the saccade referred to as clipped and neurons the activity of which continues for a short period beyond the end of the eye movement called unclipped. These two forms of cell activity have important roles in a number of models of oculomotor control (for example, Arai et al. 1994; Quaia et al. 1999; Van Opstal and Kappen 1993; Waitzman et al. 1991).

To determine whether or not the FEF projection to the superior colliculus was selective for one of these two subpopulations of saccade-related neurons, we compared the timing of spike discharges of orthodromically driven superior colliculus neurons to the timing of the beginning and end of the saccades. Calculations were made from plots of mean spike density during the memory-guided saccade task. The baseline activity was calculated as the mean of activity that occurred during the 200-ms period prior to the disappearance of the fixation point. We measured the time of peak activity relative to the onset of the saccade, the decrement of activity during the saccade, and the time of the return of activity to baseline level relative to the end of the saccade.

Sufficient data to perform this analysis were available for 33 orthodromically driven neurons (Fig. 6 and Table 2). We found that of the neurons with burst activity, 30% had a 90% decrease in activity prior to the end of the saccade. Of the remaining neurons with burst activity, the neurons’ activity did not decrease below the baseline level relative to the end of the saccade.

### Table 2. Timing of spike discharges relative to the timing of the saccade

<table>
<thead>
<tr>
<th>Activity Type</th>
<th>Sample</th>
<th>Time of Peak Activity Relative to Onset of Saccade</th>
<th>Percent Decrement of Activity During</th>
<th>Time of End Activity Relative to End of Saccade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prelude/build-up</td>
<td>2</td>
<td>-6.5 ± 21.9</td>
<td>93.8 ± 29.8</td>
<td>12 ± 17</td>
</tr>
<tr>
<td>Prelude/build-up &amp; burst</td>
<td>3</td>
<td>-32 ± 43.5</td>
<td>75.5 ± 21.8</td>
<td>27 ± 24.4</td>
</tr>
<tr>
<td>Burst</td>
<td>28</td>
<td>4.5 ± 14</td>
<td>70.6 ± 30.2</td>
<td>30 ± 32.8</td>
</tr>
</tbody>
</table>

Values are means ± SD.
orthodromically driven neurons, the Pearson correlation coefficient was calculated comparing percent decrement of activity during the saccade to the timing of peak activity relative to the onset of the saccade (Fig. 6B), if the time of peak activity occurred prior to the onset of the saccade to the time of peak activity relative to the onset of the saccade. For a comparison of the decrement of activity during the saccade to the time of peak activity relative to the onset of the saccade (Fig. 6C), if the time of peak activity occurred prior to the onset of the saccade the percent decrement of activity during the saccade was greater than when the time of peak activity occurred after the saccade onset. For this sample of 33 orthodromically driven neurons, the Pearson’s product moment correlation coefficient was calculated comparing percent decrement of activity during the saccade to the timing of peak activity relative to the onset of the saccade and was found to be significant using a t-test ($P < 0.01$). Thus the FEF projection to the superior colliculus does not appear to be selective for cells with saccade-related activity based on the timing of the fall of activity relative to the end point of the saccade.

**Orthodromic latencies and thresholds**

We compared the orthodromic response latencies and thresholds for the different types of cells activated in our study to reveal similarities or differences in these parameters between the different cell types (Table 3). A single pulse was used to evoke an orthodromic response in 61 superior colliculus neurons and a twin pulse was used to evoke a response in 22 neurons. A t-test was used to determine if there were significant differences in the mean response latencies and thresholds for the single- and twin-pulse groups. If the response latency was calculated from the onset of the second twin pulse, no significant difference was found between response latencies and thresholds evoked by single or twin pulses. Moreover, no significant differences were noted in mean response latencies and thresholds between orthodromically driven neurons with prelude/build-up activity or burst activity. This suggests that neurons with prelude/build-up and burst activity received the same type of excitatory connections from the FEF.

We divided orthodromic latencies into two groups, based on whether latency was less than or $\geq 6$ ms. The range (prelude/build-up: 2.8–4.7 ms; burst: 2.0–5.8 ms) and mean response latencies for orthodromic excitation of the group with latencies $<6$ ms were slightly longer than the range of response latencies reported for antidromic excitation of FEF neurons by collicular stimulation (range: 0.8–5.0 ms, mean: 2.49 ms, Everling and Munoz 2000; range: 1.0–6.0 ms, mean: 2.25 ms, Segraves and Goldberg 1987). For the 22 corticocortical neurons isolated in these experiments, antidromic latencies ranged 1.5–3.0 ms. One would expect the latency of orthodromic stimulation of a monosynaptic pathway to be $\geq 0.5$ ms longer than for antidromic stimulation due to the introduction of a synaptic delay in the orthodromic direction. Thus it is likely that the group with orthodromic latencies of $<6$ ms represents monosynaptic input to saccade-related neurons in the superior colliculus. For the small number of neurons with mean orthodromic latencies $>6$ ms (range: prelude/build-up: 7.0–10.5; burst: 7.2–8.2), it is likely that these latencies represent activations of the collicular cells by multisynaptic pathways.

The distribution of neurons with prelude/build-up and burst activity versus the latency of the orthodromically evoked response is shown for responses evoked with a single pulse (Fig. 7A) and twin pulse (Fig. 7B). The response latencies were unimodally distributed, suggesting a homogeneous form of excitatory input to these neurons.

The distribution of orthodromic thresholds for neurons with prelude/build-up and burst activity is shown for activity evoked with both single (Fig. 8A) and twin pulses (Fig. 8B). The low threshold for orthodromic excitation and the required precise

<table>
<thead>
<tr>
<th></th>
<th>Latency, ms</th>
<th>Threshold, $\mu$A</th>
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<tbody>
<tr>
<td><strong>Orthodromic response</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency $&lt; 6$ ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelude/build-up</td>
<td>3.79 ± 0.53 (19)</td>
<td>474 ± 176 (17)</td>
</tr>
<tr>
<td>Burst</td>
<td>3.86 ± 0.92 (52)</td>
<td>403 ± 148 (44)</td>
</tr>
<tr>
<td>Total neurons</td>
<td>(71)</td>
<td>(61)</td>
</tr>
<tr>
<td>Latency $&gt; 6$ ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prelude/build-up</td>
<td>8.75 ± 2.47 (2)</td>
<td>450 ± 70.7 (2)</td>
</tr>
<tr>
<td>Burst</td>
<td>7.65 ± 0.42 (4)</td>
<td>500 ± 158 (4)</td>
</tr>
<tr>
<td>Total neurons</td>
<td>(6)</td>
<td>(6)</td>
</tr>
</tbody>
</table>

Values are means ± SD; number of neurons in parentheses.

<6 ms were slightly longer than the range of response latencies reported for antidromic excitation of FEF neurons by collicular stimulation (range: 0.8–5.0 ms, mean: 2.49 ms, Everling and Munoz 2000; range: 1.0–6.0 ms, mean: 2.25 ms, Segraves and Goldberg 1987). For the 22 corticocortical neurons isolated in these experiments, antidromic latencies ranged 1.5–3.0 ms. One would expect the latency of orthodromic stimulation of a monosynaptic pathway to be $\geq 0.5$ ms longer than for antidromic stimulation due to the introduction of a synaptic delay in the orthodromic direction. Thus it is likely that the group with orthodromic latencies of $<6$ ms represents monosynaptic input to saccade-related neurons in the superior colliculus. For the small number of neurons with mean orthodromic latencies $>6$ ms (range: prelude/build-up: 7.0–10.5; burst: 7.2–8.2), it is likely that these latencies represent activations of the collicular cells by multisynaptic pathways.

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![Fig. 7](http://jn.physiology.org/)

**FIG. 7.** Response latencies to orthodromic excitation. The FEF was stimulated with either a single pulse (A, $n = 22$) or a twin pulse (B, $n = 61$) to evoke an orthodromic superior colliculus response.
The topographical alignment of FEF stimulation site with collicular recording site (see METHODS) argue strongly for the view that our FEF stimulation excited a direct monosynaptic projection to the colliculus.

Location of orthodromically driven neurons within superior colliculus

The location in depth within the superior colliculus of 75 of the 83 orthodromically driven neurons is plotted (Fig. 9). The depth of each neuron is measured relative to the depth at which multiunit visual activity was first encountered when entering the superior colliculus. The radius plotted on the x axis for each neuron is equal to the amplitude of the neuron’s preferred movement vector. The amplitude of the movement vector did not appear to influence the depth at which orthodromically driven cells were found. Overall, neurons with prelude/build-up activity were found at a slightly greater average depth than neurons with burst activity. In monkey MK04, at a depth between 2.0 and 2.5 mm below the onset of visually related activity, three of the six orthodromically driven burst neurons that were found exhibited a high, spontaneous discharge rate, a high-frequency burst of activity associated with the onset of the saccade in the direction of the preferred movement vector, a cessation of activity during the saccade in the opposite direction, and no prelude/build-up activity. In the course of electrode penetrations made through the superior colliculus, this type of neuron was consistently found below neurons with prelude/build-up activity.

Preferred movement vectors of orthodromically driven neurons

The optimal saccade amplitudes and directions for the orthodromically driven superior colliculus neuron population are shown in Fig. 10. For monkey MK04, the amplitude of the preferred movement vector for orthodromically driven neurons with prelude/build-up activity (Fig. 10A), or burst activity (Fig. 10B), ranged from 3 to 22° in the right hemifield and from 1 to 23° in the left hemifield. For the sample of neurons from monkey MK05, the amplitude of the preferred movement vector for neurons with prelude/build-up activity (Fig. 10C) and burst activity (Fig. 10D) ranged from 4 to 19° in the left hemifield. For the single cell with burst activity whose movement field was in the right hemifield, the amplitude of the preferred movement vector was 8° and angle was 4°. This neuron exhibited a high, spontaneous discharge rate, a high-frequency burst of activity associated with the onset of the saccade in the direction of the preferred movement vector, a cessation of activity during the saccade in the opposite direction, and no prelude/build-up activity. In the course of electrode penetrations made through the superior colliculus, this type of neuron was consistently found below neurons with prelude/build-up activity.
The FEF and superior colliculus, along with supplementary eye field, posterior parietal cortex, and oculomotor thalamus, are components of a cortical and subcortical network involved in the upper level of control of saccadic eye movements. A number of attempts have been made to identify the contributions of these different areas to the generation of saccades by identifying the functional classes of neurons that relay information from one component of the network to the next. Examples of this form of approach included studies of corticocortical cells in the FEF (Everling and Munoz 2000; Segraves and Goldberg 1987; Sommer and Wurtz 2000) and parietal cortex (Pare and Wurtz 1997, 2001), connections between posterior parietal cortex and FEF (Ferraina et al. 2002) as well as studies of multisynaptic pathways from the superior colliculus to FEF via a thalamic waypoint (Sommer and Wurtz 1998, 2002). There are several well-known classes of neurons within the layers of the superior colliculus where FEF projections are known to terminate. In addition, each neuronal class may fulfill multiple functions in the preparation of movements of the eyes as well as the head. By identifying the classes of neurons that are the target of the major FEF input to the colliculus, the present findings further our understanding of the functional organization of this complex network at both the cortical and subcortical level. We found that the excitatory effects of FEF stimulation were not present in the region containing purely visual neurons in the superficial layers of the superior colliculus. Instead, the FEF appears to provide direct excitatory input to saccade-related neurons located within the deep layers of the superior colliculus, including direct inputs to neurons with prelude/build-up activity as well as those with saccade-related bursts of activity.

**Are FEF projections to the colliculus directed to a subset of the neuron population?**

The activities of neurons within the deep layers of the superior colliculus have been divided into three basic functional types. These include fixation, prelude/build-up, and burst activity. A number of recent reports and reviews have described these activities in detail, and they are summarized briefly here. Fixation activity is characterized by increased activity during fixation followed by a pause in activity before the onset of a saccade and a resumption of firing at the end of the saccade (Munoz and Wurtz 1993a). Prelude/build-up activity consists of an increase of activity beginning several hundreds of milliseconds before the beginning of a saccade (Glimcher and Sparks 1992; Mays and Sparks 1980; Munoz and Wurtz 1995a). It appears to be related to the anticipation of or preparation for a saccade toward the cell’s movement field. The level of prelude/build-up activity is related to the likelihood that a saccade will be made into the cell’s movement field (Basso and Wurtz 1998; Horwitz and Newsome 1999, 2001; Ratcliff et al. 2003), and increased levels of prelude/build-up activity appear to facilitate the initiation of a saccade, decreasing saccade latency (Dorris and Munoz 1998; Dorris et al. 1997). Besides representing activity characteristic of the preparatory stages of generating a saccade, it has been proposed that prelude/build-up activity is responsible for the drive required to generate the saccade-related burst activity—the third major form of activity seen within the saccade-related layers of the superior colliculus (Optican 1994). Burst activity rises quickly to a peak of several hundred spikes per second at the start of the saccade (Sparks 1978). This activity falls rapidly during the eye movement. Burst activity that reaches baseline activity by the end of the saccade has been referred to as clipped activity by Waitzman and colleagues (Waitzman et al. 1991), and cells whose bursts end shortly after the end of the saccade have been called unclipped. Both prelude/build-up and burst activities are tuned to saccades within a restricted field of amplitudes and directions.

We found that saccade-related superior colliculus neurons that had prelude/build-up activity and/or a burst of activity prior to the onset of the saccade received excitatory FEF input. To determine whether there was a bias in the types of neurons receiving FEF input, we compared the distribution of activity types in the neuronal population receiving direct FEF input with the distribution of activity types in the overall population (Munoz and Wurtz 1995a) and found the distribution of prelude/build-up and burst activities to be essentially the same in the two populations (Fig. 11). In addition, we compared the relationship of the activity of orthodromically driven collicular neurons to saccade timing. Our sample of neurons receiving...
direct FEF input included neurons the activity of which fell to near baseline by the end of the saccade as well as neurons whose activity did not reach baseline levels until after the saccade had ended.

We limited the anterior-posterior extent of our recordings to portions of the superior colliculus with response fields whose centers corresponded to the response fields of FEF sites where corticotectal cells were isolated. At the most rostral portion of this zone, we found cells with activity related to saccades of just 1–2° amplitude, but we did not look for orthodromically driven cells in parts of the colliculus with fixation-related activity. As a result, we cannot report on the effects of FEF input on collicular neurons with fixation-related activity. Because the maximum amplitude of response field center for identified FEF corticotectal neurons in this study was slightly <20°, we estimate that our search for orthodromically driven cells was restricted to approximately the rostral half of the superior colliculus (Robinson 1972).

Our investigation focused on collicular cells that were orthodromically excited by the FEF, and we did not characterize cells, isolated by our electrodes, that could not be orthodromically driven. For this reason, we cannot rule out the possibility that there are cells with burst and prelude/build-up activity that do not receive direct FEF input. This negative finding, however, would not alter our primary conclusion that the FEF projection does not select between burst and prelude/build-up activity types. In addition, we cannot rule out the unlikely possibility that there were cells in the SC sites that we sampled that did not match the criteria for burst and prelude/build-up cells and also did not receive FEF input.

**FEF projects directly to collicular output neurons**

Evidence for the projection of FEF neurons to both burst and prelude/build-up neurons in the colliculus demonstrates that the FEF input exerts its effects on all stages of processing within the deep layers of the colliculus, including input to cells that comprise the collicular output (Istvan et al. 1994). An early proposal for the relationship between prelude/build-up cells and burst cells suggested that burst cells generated a saccade command that was output to brain stem oculomotor centers and that prelude/build-up cells provided an early drive that helped to select and develop burst cell activity (Optican 1994). In this form of hierarchical organization, one might expect FEF input to drive prelude/build-up cells that in turn drove burst neurons. Our results do not support this proposed pattern of organization. In a more recent proposal, Quaia and colleagues (1999) argue against a hierarchical relationship between prelude/build-up and burst cells, providing theoretical support for the possibility that both forms of activity have direct input to brain stem oculomotor circuitry. In their model, Quaia and colleagues propose that the FEF movement cells provide a saccadic command to cells with both prelude/build-up and burst activity. The Quaia model is consistent with the results of the present study, which demonstrates that FEF sites with saccade-related activity provide monosynaptic input to both prelude/build-up and burst activity types. Moreover, the similarity between the proportions of prelude/build-up and burst cells receiving direct FEF input, and the proportions of prelude/build-up and burst cells sampled in the general population of collicular neurons suggests that there is no bias among corticotectal neurons for one cell type over the other. Burst and prelude/build-up cells receive FEF input with equal likelihood. Of course, we have not demonstrated that FEF input is solely responsible for the saccade-related drive provided to burst neurons. For example, we cannot rule out the possibility, and it is very likely the case that burst neurons receive some of their saccade-related drive from prelude/build-up neurons, as well as other cortical areas. Likewise, the input to prelude/build-up neurons is almost certainly derived from sources in addition to the FEF input, with a prominent cortical source coming from posterior parietal cortex (Paré and Wurtz 1997, 2001. Finally, as demonstrated here as well as in earlier reports, it is important to point out that many collicular neurons have a combination of both prelude/build-up and burst activities, obviating the possibility for interaction between these two components.

Both anatomical and electrophysiological studies suggest that the output neurons within the superior colliculus are organized into two functional sublayers. Two morphologically distinct types of output neurons have been identified in the superior colliculus stratum opticum and intermediate gray layers of squirrel monkeys (Moschovakis et al. 1988a). T neurons located slightly more dorsally within the superior colliculus exhibit saccade-related burst activity during spontaneous eye movements, whereas the X class of neurons located more ventrally within the superior colliculus do not fire in association with spontaneous saccades and are believed to be analogous to the tecto-reticulo-spinal neurons identified in cats and may be the equivalent of prelude/build-up cells identified in macaque monkeys (Grantyn and Berthoz 1985; Moschovakis et al. 1988b; Munoz and Guillon 1985, 1986; Munoz and Wurtz 1995a). Both T and X neurons contribute to the efferent projections of the superior colliculus. In macaque monkeys, output neurons of the superior colliculus comprise at least three groupings distributed in the deep layers (May and Porter 1992). Together, the projections of these different groupings reach both medial and lateral portions of the pontine reticular formation as well as the spinal cord and are believed to subserve orienting movements that include eye as well as head movements. For eye movements, macaque collicular outputs make monosynaptic connections with saccade-related burst neurons as well as omnipause neurons in the pontine reticular formation.
(Gandhi and Keller 1997; Raybourn and Keller 1977). Due to the wide range of collicular depths where orthodromically excited neurons were found in the present study, it is likely that the FEF provides direct input to all functional sublayers within the deep layers of the superior colliculus and thereby exerts an effect on both eye and head-orienting movements.

In addition to its strong projection to the superior colliculus, the FEF projects directly to oculomotor regions of the pons (Huerta et al. 1986; Stanton et al. 1988a), and the signals that the FEF sends by way of these two projections are very similar (Segraves 1992; Segraves and Goldberg 1987; Sommer and Wurtz 2000). Although the corticotectal projection of the FEF is much more substantial than its corticopontine projection, studies of the effects of surgical ablation of the FEF or superior colliculus have supported the notion that the corticopontine or tectopontine pathways alone could function to allow the generation of saccades (Albano et al. 1982; Latto and Cowey 1971a,b; Lynch 1992; Schiller and Chou 1998; Schiller et al. 1980, 1987; Wurtz and Goldberg 1972). Recently, however, Hanes and Wurtz (2001) examined the effect of muscimol inactivation of a portion of the superior colliculus on the ability to evoke saccades by electrical stimulation of the FEF. They found that when the topographies of the cortical and collicular sites were closely matched, saccades could not be evoked by FEF stimulation after the collicular site was inactivated. When the stimulation and injection site did not represent the same saccade vector, saccades evoked by FEF stimulation reflected the loss of the vector component represented by the inactivated collicular site. This is strong evidence supporting a dominant role of the serial pathway from FEF to colliculus to brain stem in the generation of saccades, putting emphasis on the FEF inputs to prelude/build-up and burst cells that we have identified in this report.

Topography of FEF to colliculus projections

The direct connections between the FEF and superior colliculus are topographically organized providing point-to-point connections between the well-developed maps of these two regions (Komatsu and Suzuki 1985; Segraves and Goldberg 1987; Sommer and Wurtz 2000; Stanton et al. 1988b). Schlag-Rey and colleagues (1992) have demonstrated the precision of these topographically organized connections by showing that microstimulation of the FEF, eliciting a saccade of a given vector, excites saccade-related collicular cells, which encode the same vector while, at the same time, inhibiting neurons whose saccade representation does not match that of the cortical stimulation site. Topographical alignment of FEF stimulation site with collicular recording site was critical to the success of the current experiments. It is highly likely that direct FEF projections play an important role in the selective excitation observed by Schlag-Rey and colleagues. This excitation is also facilitated by a release of inhibition effected by the FEF to basal ganglia to colliculus pathway (Hikosaka and Wurtz 1983). Because all layer V pyramidal cells that make up the FEF’s corticopontine pathway are likely to be excitatory (Hendry et al. 1987; Schwartz et al. 1985), it is unlikely that the monosynaptic FEF projection is directly involved in the inhibition that was observed by Schlag-Rey and colleagues (1992). This inhibitory activity may arise from intrinsic collicular activity as well as by way of GABAergic input to the colliculus from the substantia nigra and other subcortical sources (Appell and Behan 1990; Munoz and Istvan 1998).

Location of cortical stimulation sites within the FEF

Our primary criteria for localizing a stimulation site to the FEF was that it be a site in the region of the rostral bank and fundus of the posterior arcuate sulcus where saccades could be evoked with thresholds of }=50 }A. As noted in Results, the majority of corticotectal neurons identified in this study had saccade-related activity, sometimes in combination with visual activity (30%). Only a single corticotectal neuron with exclusively visual activity was found in our experiments. The prevalence of neurons with saccade-related activity is similar to the original examination of corticotectal cells in the FEF by Segraves and Goldberg (1987). In a more recent examination of the distribution of corticotectal cells in the FEF, Sommer and Wurtz (2000) found a prevalence of corticotectal neurons with presaccadic bursts in a medial segment of the stimulation-defined FEF. In a more lateral segment coding for relatively smaller saccades, however, they found a large proportion of corticotectal neurons with visual responses. This regional distribution of corticotectal neurons with differing activities suggests that the FEF might be further subdivided into separate functional regions or areas. Anatomical support for these subdivisions was provided earlier by Stanton and colleagues (1988b), who found that collicular projections from the medial FEF region terminated primarily in the intermediate and deep gray layers of the superior colliculus, whereas projections from the lateral FEF preferentially ended in superficial and intermediate gray layers. In the present experiments, the preponderance of saccade-related activities for corticotectal neurons that we identified, as well as the localization of their terminations to the intermediate and deep gray layers of the superior colliculus, suggests that our FEF recording and stimulation sites were confined to the medial FEF region. As a result, the experiments reported here do not shed light on the characteristics of collicular cells, potentially in the superficial layers, that might receive direct input from corticotectal visual cells in the lateral FEF.

Corticotectal projections and the sensorimotor network

The characterization of corticotectal cells in the FEF and lateral intraparietal area (LIP) demonstrates that the deep layers of the colliculus receive a rich combination of signals corresponding to the response to visual stimuli, delay period, and preparatory set activity and to preparation and specification of saccades (Everling and Munoz 2000; Paré and Wurtz 1997, 2001; Segraves and Goldberg 1987; Sommer and Wurtz 2000, 2001). Although the issue of whether a significant number of FEF neurons with purely visual response fields project to the deep layers of the superior colliculus awaits investigation, our experiments demonstrate that the deep layers receive visual input from FEF cells that combine visual with movement-related activity. Our results show that the input coming from the FEF does not appear to be directed to a subpopulation of neurons in the deep layers. This finding is supportive of the “continuous multistage system” proposed by Sommer and Wurtz (2000) that emphasizes the highly parallel nature of processing taking place within the cortical and subcortical...
network involved in the control of saccades and proposes that a visuomotor transformation proceeds at multiple sites within the network.

In addition to contributing to the output signal of the superior colliculus, the saccade-related signal that the colliculus receives from the FEF may also be used as a form of corollary discharge to update the activity fields of spatially accurate cells. Walker and colleagues (1995), in describing a predictive response in the superior colliculus, noted that, unlike cells in the deep layers, cells in the superficial layers failed to show predictive receptive field shifts. Their observation reinforces the results of this report demonstrating that the localization of evoked activity and orthodromically driven cells does not indicate a significant input of the FEF to the superficial layers of the superior colliculus.

The collective finding that FEF input to superior colliculus contains multiple signals that are sent to all classes of cells in the deep layers underscores the idea that concurrent processing for the generation of a saccade is taking place at multiple sites within the system. At the same time, as others have pointed out, this system is not entirely without hierarchical relationships, and a progression of stages of sensorimotor processing is evident from LIP to FEF and from LIP and FEF to superior colliculus (Ferraina et al. 2002; Paré and Wurtz 2001). To advance our understanding of sensorimotor processing in this system, future investigations will need to employ techniques that allow one to monitor concurrent activity from multiple stages in this network.

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REFERENCES


