Organization of the Intermediate Gray Layer of the Superior Colliculus. I. Intrinsic Vertical Connections

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INTRODUCTION

The juxtaposition of layers with sensory and motor functions makes the superior colliculus especially useful for studying mechanisms that underlie sensorimotor integration. Investigations since the 1970s using extracellular recording demonstrated that cells within its superficial layers (SGS/SO) have visuo-sensory properties. That is, they receive input from the retina and visual cortex and are activated by the onset of visual stimuli (Goldberg and Wurtz 1972a,b; Huerta and Harting 1984). In contrast, the intermediate layer (SGI) includes cells that have properties of motor cells. These cells project to the brain stem gaze centers, and generate “bursts” of action potentials that reliably precede the onset of saccades (Raybourn and Keller 1977; Sparks 1978). The extracellular recordings also demonstrated that SGS and SO contain a map of visual space that is in register with a map of saccade vectors in SGI (Schiller and Stryker 1972). Registration of sensory and motor maps is consistent with a simple model that postulates that stimuli at one locus within the visual field maximally activate a region of SGS and SO, which in turn, gives rise to columnar projections to the region of SGI that shifts gaze toward that same locus (Mohler and Wurtz 1976; Schiller and Koerner 1971).

Even though registry of the sensory and motor maps was established more than 30 years ago, methodological limitations made it impossible to determine whether circuitry for aligning the maps resides within the colliculus, as the model proposes, or instead, whether the alignment is imposed by pathways that arise from extracollicular sources (Edwards 1980). Now, advancements in in vitro techniques provide opportunities to examine intrinsic circuitry with the precision necessary to test such models. In initial in vitro experiments, electrical stimulation in SGS combined with whole cell recording in SGI provided data consistent with a pathway from the superficial to the deeper layers (Isa et al. 1998; Lee et al. 1997; Özen et al. 2000). However, even focal electrical stimulation might activate axons of passage. Thus these experiments did not eliminate the argument that the connection between the layers is less direct than the columnar link proposed by the model.

To resolve the issue, we recorded from SGI cells while stimulating SGS or SO using the photolysis of “caged” glutamate. In contrast to electrical stimulation, photostimulation avoids fiber of passage (Callaway and Katz 1993). Our results establish that neurons intrinsic to the superior colliculus project in a spatially tuned fashion from SGS and SO to SGI, and thus support the proposed model. The spatial precision of the results also revealed two additional features of SGI. First, the results demonstrated projections to single SGI cells arising from extensive regions of SGS that represent large portions of the visual field. The breadth of these regions implies that the pathway to SGI has functions in addition to signaling the loci of visual stimuli. Second, the results demonstrate that sublayers of SGI differ in the amount of their inputs from SGS and SO; neurons in the middle of the intermediate layer (SGIb) were less likely to respond to visual layer photostimulation than were those in sublayers just above and below them. However, focal stimulation within SGIa did evoke responses within SGIb, indicating that SGIb neurons may receive input from the visual layers indirectly. These results demonstrate a columnar pathway that may mediate visually guided orienting movements, but the results also reveal spatial attributes of the pathway which imply that it also plays a more complex role in visuomotor integration.

METHODS

Colliculus slices

Coronal or parasagittal slices 300–400 μm thick were prepared from the superior colliculi of 28 14- to 28-day-old rats. The animals were anesthetized with intraperitoneal sodium pentobarbital according to National Institutes of Health and institutional (IACUC) guidelines consistent with a simple model that postulates that stimuli at one locus within the visual field maximally activate a region of SGS and SO, which in turn, gives rise to columnar projections to the region of SGI that shifts gaze toward that same locus (Mohler and Wurtz 1976; Schiller and Koerner 1971).

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recovery period, the slices were maintained at room temperature with a chamber at 37°C and sectioned with a microslicer vibratome. The sections were immersed such that the collicular hemispheres were isolated, mounted and sectioned with a microslicer vibratome. The sections were immediately transferred to an oxygenated membrane interface-style storage chamber at 37°C and allowed to recover for 30–45 min. After the recovery period, the slices were maintained at room temperature with oxygenated ACSF until used for recording. For whole cell patch-clamp recordings of postsynaptic responses, a recirculating bath containing ACSF and 100 μM CNB-caged glutamate (G-7055, Molecular Probes, Eugene, OR) was superfused over the slice, which was restrained in the recording chamber by a U-wire crossed with nylon threads.

Nomenclature

The nomenclature for the layers that we employ follows that of Weiner (1986) and Bickford and Hall (1989). Figure 1 is a photograph of an unstained slice and illustrates these layers as they appear in the living slices during a patch-clamp experiment. The superior colliculus (SC) is composed of well-differentiated, alternating gray and white layers that can be easily identified in the living slice. Layers indicated by the white letters in the figure contain rostrocaudally or mediolaterally oriented fibers that make the layers appear opaque in the unstained slice. In contrast, “gray” layers occupied principally by cell somas and dendrites are lighter in the living slice. Importantly, even in the living slices, three sublayers can be distinguished in the intermediate gray layer, with a middle tier (SGIb) marked by the presence of rostrocaudally oriented fibers as described by Kanaseki and Sprague (1974). The following abbreviations are used for anatomical structures in the figures and text: SGS, stratum griseum superficiale or superficial gray layer; SO, stratum opticum or optic layer; SGI, stratum griseum intermediale or intermediate gray layer; SAI, stratum album intermediale or intermediate white layer; SGP, stratum griseum profundum or deep gray layer; SAP, stratum album profundum or deep white layer; CG, central gray.

Pharmacology

In four experiments, 1 μM TTX was added to the ACSF to block synaptic transmission. This and other pharmacological manipulations were not used routinely since two aspects of our method introduce practical limitations to the use of pharmacology. First, use of a recirculating bath for the delivery of agents to the slice dictates that application of pharmacological agents must be reversed by draining and replacing the entire bath solution with a drug-free and caged glutamate-free solution before reintroducing caged glutamate to the bath. The time consumed by this procedure greatly reduced the number of tests that could be performed on each cell. Second, glutamate receptor antagonists cannot be bath applied to selectively block glutamatergic synaptic transmission since they would also block the direct response of the presynaptic neurons to glutamate photolysis.

Patch-clamp recordings

These results are based on whole cell patch-clamp recordings from 33 neurons. Recording methods similar to those described in previous work (Lee et al. 1997; Petit et al. 1999) were employed for this study. One notable difference was the composition of the internal solution for the experiments in this study. The patch pipette solution contained (in mM) 126.0–132.0 K-glucuronate, 1.0 NaCl, 1.0 d-glucuronic acid Na salt, 0.2–1.0 EGTA, 2.0 MgCl₂ · 6 H₂O, 2.0 d-glucuronic acid hemi-Mg salt, 4.0 Na₂ATP(2.5 H₂O), 0.4 NaGTP, and 20 HEPES; pH was adjusted to 7.28–7.33 with 8N KOH. With this combination of internal and external solutions, the reversal potential for chloride was −82 mV. All holding potentials include correction for a −13 mV calculated junction potential. Thus at typical holding potentials of −68 and −53 mV, the driving force for chloride conductance was sufficient to facilitate the detection of chloride mediated outward synaptic currents. Recordings were accepted only if the holding current was <100 pA when the membrane potential was voltage clamped at −68 mV. To permit characterization of cell morphology after the experiment, biocytin (3–4%) was included in the pipette solution and diffused into the cell during the recording. After the recording, the slices were fixed overnight in 4% paraformaldehyde and a 3,3′-diamino benzidine tetrahydrochloride (DAB) reaction was performed to reveal the biocytin. For 22 of 33 cells, the biocytin allowed us to precisely identify the laminar location of the recorded cell. In the case of the more complete fills, the biocytin also revealed the spatial relationship between the stimulation site and the dendritic field of the cell and the contribution of its axons to major efferent pathways of the superior colliculus.

Photostimulation system

The system used for photostimulation is depicted in Fig. 2. In brief, UV light from an argon ion laser was directed via laser mirrors to dichroic mirrors in the epifluorescence pathway of a Nikon upright microscope, delivered through a 40× (0.8 NA) water immersion objective, and focused on the slice (Fig. 2, A and B). Figure 2A is a schematic that provides a view from above of the components of the system that direct light from the laser into the microscope. Figure 2B is a greatly magnified front view of the laser light leaving the microscope objective and entering the brain slice. With this arrangement, glutamate was photolyzed consistently over an area 50 μm in diameter in the slice at the focal plane (Fig. 2C). Prior to the first illumination of the 40× objective for a given experiment, laser power was adjusted to 2 mW output beneath the objective with a power meter. For uncaging events during the course of an experiment, an electronic shutter was used to vary the duration of the light pulse (2–20 ms, mode 10 ms) and thus the total amount of photostimulation. For a given pulse duration, we assume a reproducible light density and concentration of “caged” glutamate for each photostimulation event. The laser beam was aligned in the center of the objective and re-
moned stationary in the field of view. The distance between the uncaging spot and the recorded cell was varied by a motorized microscope stage that held the recording chamber and patch-clamp manipulator. For each recorded data file, measurements of approxi-

mately 1 s in duration were taken for each of four holding potentials, with intersweep intervals of ≥5 s to guarantee that responses did not accommodate. Multiple (typically 3) data files were recorded for each photostimulation site. The time interval between stimuli and number of files collected per site both determined the number of sites that could be sampled per slice. An average of 12 sites were tested per neuron.

Spatial pattern of photostimulation

The spatial distribution of photostimulation sites in the slice followed two general patterns. In the first pattern, sites were stimulated along an axis extending superficially from the recorded SGI cell and normal to the dorsal surface of the slice. Sites in this axial pattern were confined to a column that encompassed several layers, and were used to measure the synaptic contributions of each layer superficial to the recorded cell’s soma. In the second pattern, stimulation sites were arranged in an arc following the curvature of the superficial layers at a uniform depth relative to the collicular surface. This second pattern was used to measure the mediolateral and rostrocaudal extent of “off-axis” presynaptic input to the recorded cell from a given layer. Along the vertical axis, an average of 5 sites (1–19 sites, min-max) were examined per cell, with the most distant attempt per cell at 480 ± 40 (SE) μm from the soma of the recorded neuron. For some recordings in upper SGI, the location of the soma precluded stimulation attempts beyond this distance. However, for stimuli near the dorsal surface of the superficial layers of the SC and recording sites in the lowest sublayer of SGI, it was possible to stimulate nearly 1 mm above the recorded cell soma.

Data analysis

Measurements of evoked current amplitudes, durations, and integrals were made using Clampex 8.2 (Axon Instruments), Synaptosoft’s Mini Analysis, or Microcal’s Origin software. To determine the probability of evoking excitatory and inhibitory synaptic responses from a given site, voltage-clamp traces over a range of holding potentials were analyzed together for single pCLAMP files that represented one of several photostimulation attempts at a given site for a given cell. These files were scored as yes/no for inhibition and excitation based on the presence or absence of inward and outward currents at the various holding potentials. Chloride-mediated inhibitory postsynaptic currents (IPSCs) were inward at holding potentials below the chloride reversal potential (∼82 mV), essentially absent around the reversal potential, and outward at more depolarized holding potentials. Excitatory postsynaptic currents (EPSCs) were inward at all holding potentials. Potassium-mediated IPSCs were outward at all holding potentials. In all cases, the individuals scoring a synaptic response were blind to the identity of the neuron or photostimulation site. After scoring all individual files, IPSC and EPSC probability scores for all of the files at a given site for a given cell were averaged to determine the mean probability of evoking excitation and inhibition at a given site. Those mean scores were used for further analyses and calculations.

RESULTS

Efficacy of photostimulation

The use of photostimulation for circuit analysis depends on the release of enough glutamate molecules from their molecular cages to evoke action potentials in cells presynaptic to the recorded cell. To assess the efficacy of the photostimulus in our experiments, glutamate was uncaged directly over the somas and dendrites of 25 cells while recording from each of them in current-clamp mode. In more than 95% of these cells, 10 ms of 2 mW UV light was sufficient to evoke action potentials. A typical response is illustrated in Fig. 3A. This neuron generated 11 action potentials over a period of 800 ms. Figure 3B is a histogram illustrating the average response per cell to direct stimulation for a subset of neurons that exhibited no activity in the absence of uncaged glutamate (19 of 25). After uncaging, they fired an average of 4.7 ± 1.0 action potentials during the 2 s period following the photostimulus. The cells in this group varied in responsiveness, with their average evoked responses ranging from 1 to 20 action potentials. For the remaining six neurons that were spontaneously active, photostimulation increased their average discharge rate from 5.7 ± 2.2 spikes/s in the absence of uncaged glutamate to 11.8 ± 5.0 spikes/s, which was an increase of 110% for the 2 s interval following stimulation. However, the peak effects of direct photostimulation were even more pronounced. While baseline spontaneous ac-
Activity was fairly constant at low frequencies, firing rates were often potentiated by an order of magnitude immediately following stimulation, and it was not uncommon for these neurons to attain peak instantaneous firing rates of 200–400 Hz at room temperature (Fig. 3C). These robust direct responses indicate that the photostimulus effectively evokes action potentials and thus provides a reliable tool for assessing synaptic relationships in the slice.

Figure 4 illustrates the spatial resolution of photostimulation. When the laser uncaged glutamate directly over the recorded cell, at the locus denoted “A,” the response recorded in voltage clamp was a long-lasting inward current resulting from direct activation of glutamate receptors expressed by the cell (Fig. 4A). At Site B, the responses were brief, synaptically mediated inward currents caused by photoactivation of neurons presynaptic to the patch-clamped neuron (see also Fig. 6C). The spatial resolution of the method is also indicated by stimulation Site C, which even though only 50 μm from B, evoked no response.

**Photostimulation along the vertical axis evokes EPSCs**

For each of the 33 collicular neurons illustrated in Fig. 5 (4 SO and 29 SGI), photostimulation sites were tested along a vertical axis extending superficially from the recorded cell, normal to the surface of the slice. Overall, 75% of the neurons in SO and 41% of those in SGI responded to stimulation in SGS, and 47% the SGI cells responded to stimulation in SO. An example of one such neuron is depicted in Fig. 6A for a cell with a soma located within SGIc. For this particular cell, the photostimulus was applied to 10 sites in SGS and SO, ranging between 420 and 780 μm from the recorded cell soma. The circled numbers adjacent to the current traces in Fig. 6B identify responses with the stimulus sites numbered in Fig. 6A. These responses demonstrate that EPSCs could be evoked from all 10 SGS and SO sites. The EPSCs had latencies ranging between 25 and 30 ms and often occurred in clusters lasting more than 100 ms. Peak amplitudes ranged from 10 to 300 pA (median 19 pA for all cells). Figure 6C shows that the addition of 1 μM TTX blocked the EPSCs evoked from site 6. Since TTX blocks the sodium channels that mediate action potentials in the presynaptic cells but does not interfere with responses evoked by directly stimulating glutamate receptors on the recorded cell, the experiment in Fig. 6C confirms that the evoked currents were synaptically mediated.

**Sublayer specificity**

The responsiveness of the recorded cells varied as a function of their location in SGI. Overall, for the sample of SGI cells recorded in voltage-clamp mode, 17 of 28 (61%) were excited synaptically by stimulation in SGS or SO. Moreover, in the subset of SGI cells for which stimulation was attempted in SGS (n = 22 cells), responses were evoked in nine cells (41%). However, comparisons of the spatial distributions of the responsive and unresponsive cells indicate that sublaminar differences in cell location, rather than a decrease in response...
probability as a function of distance (which could be an artifact of in vitro slice recording), account for most of this variation in responsiveness. Specifically, most of the unresponsive cells were located in sublayer SGIb.

Data demonstrating the differences among the sublayers are summarized in Table 1. The left half of the panel shows the percentage of cells within a given SGI sublayer that responded to photostimulation sites in SGS and SO along the vertical axis superficial to the recorded cell. The right half illustrates the percentage of visual layer photostimulation sites in SGS and SO, pooled for all of the cells from a given sublayer that evoked EPSCs in recorded cells. For stimulation sites in SGS and SO, only 2 of 10 SGIb cells (20%) exhibited evoked EPSCs. Because SGIc is such a thin sublamina, too few cells were recorded there (n/11005 5 cells total, 3 with stimulation sites in both SGS and SO) to establish a statistically significant difference between it and SGIb. However, when the results from cells isolated in SGIc were combined with those from SGIa, 9 of 14 (64%) generated evoked responses to SGS or SO stimulation, and a significant difference was found between these two sublamina and SGIb (P = 0.03). Even more robust differences (P < 0.0001) were found when the results were expressed as the percentage of SGS and SO sites that evoked EPSCs. Whereas only 14% of the sites evoked responses in SGIb cells, 56% and 87%, respectively, of the stimulus sites evoked responses in SGIa and c neurons.

For the cells and sites that demonstrated vertical connections, peak amplitude measurements were taken to determine whether there were any obvious differences in evoked activity by sublayer. The peak amplitude data were collected for each cell and averaged for all cells in a given layer or sublayer. At the chloride reversal potential (~82 mV), data were as follows: SO 40 ± 14 (SE) (n = 3 cells), SGIa 22 ± 3 (n = 7 cells), SGIb 49 ± 17 (n = 2 cells), and SGIc 58 ± 6 pA (n = 2 cells). This result shows that, when present, the SGIb responses were within the range of those responses seen in other layers.

These differences among SGI sublayers in responsiveness are further illustrated in Fig. 7. Figure 7A shows graphically that the mean probability of evoking a synaptic response by stimulation above the cell in SGS and SO is significantly (P = 0.001) lower if the recorded neuron resides in SGIb. Figure 7B shows three histograms that are scaled and normalized to reveal the distribution of distances above the recorded neuron at which EPSCs could be evoked along the vertical axis. Here,}

### Table 1. Intralaminar differences in responsiveness

<table>
<thead>
<tr>
<th>Cell body location</th>
<th>Connected cells</th>
<th>Recorded cells</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>SGIa</td>
<td>7</td>
<td>11</td>
<td>64%</td>
</tr>
<tr>
<td>SGIb</td>
<td>2</td>
<td>10</td>
<td>20%</td>
</tr>
<tr>
<td>SGIc</td>
<td>2</td>
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<table>
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<tr>
<th>Connected sites</th>
<th>Attempted sites</th>
<th>Percentage</th>
<th>Cell body location</th>
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<td>17</td>
<td>59%</td>
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<td>23</td>
<td>41</td>
<td>56%</td>
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<td>3</td>
<td>21</td>
<td>14%</td>
<td>SGIb</td>
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<td>13</td>
<td>15</td>
<td>87%</td>
<td>SGIc</td>
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<td>39</td>
<td>77</td>
<td>51%</td>
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<tr>
<td>49</td>
<td>94</td>
<td>52%</td>
<td>Total</td>
</tr>
</tbody>
</table>

Data are organized into the percentages of cells that exhibited EPSCs (left) and of stimulation sites (right) that evoked EPSCs. A single row consists of data pooled together for all recorded cells of a given layer. The table summarizes data for the subset of stimulus attempts that was restricted to the visual layers (SGS and SO).
excitatory inputs from remote sites were more frequent for cells that lie in SGIa and SGIc than for those in SGIb. Finally, Fig. 7C shows the contributions of the other layers to direct and synaptic responses of neurons in SGIb. The figure shows that SGIb cells are frequently excited by stimulation at sites within SGIb or SGIa but only very rarely at sites in SGS and SO.

**Stimulation across the visual layers**

Measurements of postsynaptic responses to off-axis stimulation were made for a subset of neurons that responded to stimulation in SGS and SO along the vertical axis. The results are summarized in Fig. 8 for four SGI cells. EPSCs were recorded from sites as far away as 1,000 µm from the recorded SGI cell and approximately 800–900 µm lateral to the vertical axis that ascends from the cell soma perpendicular to the colliculus surface. Figure 8, A and B, depicts pooled data for these cells, measured as the normalized peak inward postsynaptic current (PSC) or the integral of PSCs, respectively. With either measurement, the resulting histogram is essentially Gaussian with a half-width of more than 1,000 µm and peak values concentrated along the vertical axis. As an example, Fig. 9 illustrates a cell located in SGIc that responded vigorously to stimuli at sites in SGS over 600 µm from the vertical axis.

**Morphological identification of the recorded neurons**

For 22 of the 33 recorded cells, a biocytin-filled soma could be clearly identified to establish the soma location within the slice. For all of these cells, the location confirmed low-magnification photographs taken during the recording that showed the tip of the whole cell patch-clamp pipette in contact with the cell soma. For 14 of 22 cells, features of dendrites and axons could be identified as well. Classifying these cells by sublayer, two were SO cells, six were SGIa cells, four were SGIb cells, and two were SGIc cells. Figure 9 illustrates the pattern of synaptic connections for a biocytin-labeled neuron that resided within the lower part of SGIc. A grayscale photograph of the cell taken after histological processing for biocytin (Fig. 9B, inset) reveals the beginning segments of commissural (single arrow) and predorsal bundle (double arrows) components of the axonal arbor. Several recurrent collateral branches arise at points between these horizontally oriented axonal components and the cell soma (Fig. 9, A and B, *). All of these morphological features are also characteristics of premotor cells called “T” cells in cats and primates (Moschovakis and Karabelas 1985; Moschovakis et al. 1988a,b).

The location of the cell and its processes with respect to the collicular layers are illustrated in Fig. 9A by the flattened drawing of the neuron (yellow) superimposed on a photograph of the slice taken prior to the patch-clamp recording. Spots
depicting photostimulation sites also are indicated on the photograph. For this neuron, the photostimulus was applied at 22 sites (Fig. 9A) throughout the superficial, optic, and intermediate layers. Figure 9, C and D, illustrates the evoked responses of this neuron to stimulation at the photostimulation sites depicted in Fig. 9A. Comparison of the cell’s morphology with the photostimulation sites indicates that even the most distal apical tips of this neuron’s dendrites are located more than 200μm from the most proximal stimulus site, and thus confirms that long distance synaptic connections can be detected in the slice.

**Inhibitory influences**

While nearly one-half of the neurons within SGI responded with EPSCs to stimulation along the vertical axis, IPSCs were evoked following stimulation at only 19% (15 of 77) of the SGS and SO vertical axis sites. Most frequently, inhibition was encountered following stimulation within SGI, close (<200μm) to the recorded cell (red traces at the holding potential of -53 mV in Fig. 9), or off-axis at great distances from the cell following SGS stimulation (data not shown). The IPSCs were rare despite efforts to increase the likelihood of observing outward currents. For example, recordings were made while the cells were voltage-clamped at depolarized holding potentials (-43 mV) to maximize the driving force for chloride- and potassium-mediated conductances. Moreover, for all but two cells in these experiments, the driving force for chloride was increased to maximize the likelihood of seeing inhibition by altering the pipette solution composition to shift the chloride reversal potential ($E_{Cl}$) to -82 mV from the more common $E_{Cl}$ of -65 mV. It is possible, however, that some IPSCs were obscured by EPSCs, and when stimuli were located very close...
to the soma of the recorded cell, by the cell’s direct response to the uncaged glutamate. These issues will be addressed more fully in future studies.

**Discussion**

In this study, photostimulation was used to test for the hypothesized pathway between the visual and motor layers of the superior colliculus. The results provided convincing evidence that both SGS and SO give rise to columnar excitatory projections to SGI. The results also demonstrated additional features of this pathway: (1) widespread regions of SGS project to single SGI neurons and (2) SGS and SO project preferentially to upper and lower sublayers of SGI while avoiding the intercalated sublayer, SGIb. Before discussing the implications of these results, we will consider the strengths and limitations of the photostimulation method.

**Strengths and limitations of the method**

The utility of the in vitro approach to study synaptic circuitry depends on reliable, localized activation of presynaptic neurons. Recordings from cells exposed to uncaged glutamate indicated that photostimulation activates cells with high reliability. For example, in current-clamp mode, responses to direct photostimulation consisted of bursts of as many as 20 action potentials that sometimes reached instantaneous frequencies as high as 200-400 Hz. This level of activation was sufficient to evoke large EPSCs in SGI.

However, even the most efficacious stimuli lack utility if they cannot be harnessed to activate only the desired population of neurons. The present results suggest that photostimulation activated cells restricted to a small volume of tissue while avoiding fibers of passage. This spatial precision was demonstrated by the large changes in response magnitude that were measured following small changes in the stimulus site (Fig. 4). Because of this precision, photostimulation could eliminate cells other than those in SGS and SO as responsible for the evoked responses. The alternatives that were eliminated include (1) cells with axonal branches to both the superficial and intermediate layers that might be antidromically activated by electrical stimulation, and (2) cells outside of SGS and SO that give rise to fibers of passage that might be activated by an electrical stimulus.

Despite its strengths, the photostimulation method has the limitation of not distinguishing with certainty between monosynaptically and polysynaptically mediated evoked responses. Commonly, temporal properties such as response latency and the ability to follow high-frequency presynaptic stimulation obscure these properties. These factors include laser shutter opening time, the time required for the release, diffusion and binding to receptors of uncaged glutamate, and time for the depolarization of the presynaptic membrane. These photostimulation specific factors are in addition to conduction times and synaptic delays that occur regardless of the nature of the stimulus. However, even though these factors confound temporal measurements, three lines of evidence indicate the EPSCs evoked in SGI were mediated by monosynaptic, and perhaps disynaptic, pathways. First, axon terminals from SGS and SO cells spatially overlap the dendrites of SGI neurons (Lee and Hall 1995; Mooney et al. 1985; Moschovakis and Karabelas 1985; Moschovakis et al. 1988a). Second, while the latencies of the SGI EPSCs (25–30 ms) seem long, they are consistent with a monosynaptic connection after subtracting from them the latencies of the presynaptic action potentials evoked in SGS (latency = 12–15 ms, with peak firing frequency following by several milliseconds). Finally, current-clamp recordings showed that the threshold for generating action potentials is rarely reached in postsynaptic cells. However, the occasional suprathreshold postsynaptic responses that were evoked make it impossible to completely rule out a disynaptic contribution.

**Laminar origins and terminations of the pathway**

Previous anatomical studies (Hall and Lee 1993, 1997; Lee and Hall 1995) proposed that SGS cells influence SGI cells either monosynaptically or after intervening relays in SO, which in turn, projects to SGI. Our current results are consistent with both alternatives. We found that SGS photostimulation evoked EPSCs in SGI, but SGS stimulation also evoked robust responses in SO, and in turn, SO stimulation evoked responses in SGI. Furthermore, SGIa cells, which receive input from SGS and SO, project to SGIb and SGIc. Taken together, these results suggest a cascade of descending projections, some direct and some indirect, which link SGS and SO with SGI (Fig. 10). It is possible that the somas of some of the presynaptic cells resided in the intermediate or deep layers of the colliculus while their dendrites extended into SGS and SO (Rhoades et al. 1987). However, the spatial precision of the photostimulation method demonstrates unequivocally that they received input within the retinal recipient layers, SO and SGS, and terminated synaptically on cells in SGI. Thus regardless of the location of the presynaptic cell somas, the results demonstrate a synaptically mediated functional link between the visual and the premotor layers of the superior colliculus.

**FIG. 10.** Summary schematic of synaptic connections between SGS, SO, and SGI. Neurons in SGS project to SO and to the most dorsal and ventral sublayers of SGI (SGIa and c), but are much less likely to project to SGIb. SO cells respond to stimulation in SGS and project to SGIa and c. SGIb receives projections from SGIa. Responses to off-axis photostimulation in SGS suggest that both wide-field and narrow-field neurons in this layer project to SGI.
link completes the shortest known pathway linking the visuo-
sensory and oculomotor systems, and may mediate short la-
tency orienting movements to visual targets (Fischer et al.
1993).

Implications of breadth of the projection

Although the largest postsynaptic responses were evoked by
photostimulation in a vertical column (~500 μm diam) above
the recorded cell, the effective area for evoking responses in a
single SGI cell was much more extensive (~2 mm diam). One
interpretation of these results is that both narrow-field (NF) and
wide-field (WF) cells in the superficial layers project to single
SGI cells.

According to this interpretation, NF cells, which have small
(2–15°) receptive fields (Langer and Lund 1974), are respon-
sible for the robust responses that are evoked when the pho-
tostimulation is in the column above the recorded cell. The
dendritic fields of NF cells would sample input from small
regions of the visual field, and their descending interlaminar
projections, which also are columnar (~150 μm radius), would
be expected to influence a small region of SGI cells (Fig. 10).
Thus NF neurons seem organized to signal the location of
visual targets to those SGI cells that shift gaze toward that
location.

In contrast, WF cell dendritic fields can span an area over 2
mm in diameter and correspond in size to the off-axis region
that photostimulation demonstrated is presynaptic to single
SGI cells. Thus each WF cell potentially samples input from a
large portion of the visual field (Langer and Lund 1974; Major
et al. 2000). The WF neurons may respond best to moving
stimuli and increase the motion sensitivity of SGI cells (Hum-
phrey 1968; Major et al. 2000; Mooney et al. 1985; Schiller
and Koerner 1971). Taken together, narrow and wide field
pathways may track the location of moving targets and transmit
this information to SGI cells that command corresponding
shifts in gaze.

Implications of sublayer specificity of the projection

The percentage of recorded neurons that could be excited by
photostimulation in SGS (50%) was less than that (77%) found
in our earlier experiments using electrical stimulation and
recording primarily from cells in SGIs (Lee et al. 1997). How-
ever, by recording from all three SGI sublayers in this
study, significant intralaminar variations in responsiveness
were found that could account for most of this difference.

Specifically, only a small percent (20%) of SGIb cells re-
sponded to either SGS or SO photostimulation. In contrast, the
majority of SG1a (64%) and c (67%) cells responded to the
same stimuli. These sublaminal differences cannot be ex-
plained as a function of distance between stimulus and record-
ingsite, since cells in SG1c are farther from the superficial
layers than are SGIb cells yet were more likely to respond.
Moreover, for the SGI cells that did respond, neither response
probability nor amplitude decreased monotonically with dis-
tance (see Figs. 6, 7B, and 9). Both of these observations
suggest that the variations in responsiveness reflect a precise
spatial organization of SGI rather than the loss of connections
served in the slices.

The similarity of projections to cells of upper and lower
sublayers in SGI and the difference from those to SGIb are
consistent with findings from the anatomical literature. For
example, in the cat, SGIb receives input from cortical somato-
sensory areas, while both SG1a and c receive input from visual
areas (Harting et al. 1992). These results fit with our results in
two ways: first, sublayers a and c share inputs which are
distinct from those to SGIb, and second, relative to sublayer b,
sublayers a and c are more directly associated with the visual
system. Other inputs also distinguish SGIa and c from b. For
example, studies of the cat show the medial frontal eye fields
project to superficial and deep sublayers of SGI, whereas
lateral fields project to the middle sublayer (Harting et al.
1992). Similarly, dorsal substantia nigra pars reticulata termi-
nates preferentially in SGIa and SGIc, while the ventral part of
nigra projects to SGIb (Harting and Van Lieshout 1991).

Finally, cholinergic projections from the brain stem parabra-
chial region terminate primarily in SGIb (Harting and Van

The sublayers also give rise to different efferent pathways.
For example, in rodents, the efferent cells that project to the
brain stem gaze centers are primarily in SGIb (Bickford and
Hall 1989; May and Hall 1984), whereas cells that contribute
to the collicular commissure are preferentially in sublayers a
and c. A similar distinction may apply to the primate, in which
“T-cells,” which contribute to the collicular commissure, tend
to be superficial and deep in SGI, whereas “X-cells,” which
project heavily to the gaze centers, are more common in a
middle zone (Moschovakis and Karabelas 1985; Moschovakis
et al. 1988a).

Thus different types of experiments suggest that SGI has
sublayers that are preferentially associated with particular sen-
sory and efferent systems. However, further studies are re-
quired to determine how these sublayers differentially influ-
ence the oculomotor system.

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