Contribution of Superficial Layer Neurons to Premotor Bursts in the Superior Colliculus

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Özen, Gülden, George J. Augustine, and William C. Hall Contribution of superficial layer neurons to premotor bursts in the superior colliculus. J Neurophysiol 84: 460–471, 2000. In vitro whole-cell patch-clamp methods were used to examine the contribution of one component of intracollicular circuitry, the superficial gray layer, to the generation of bursts of action potentials that occur in the intermediate layer and that command head and eye movements in vivo. Applying a single brief (0.5 ms) pulse of current to the superficial layer of rat collicular slices evoked prolonged bursts of excitatory postsynaptic currents (EPSCs) in the cells of the intermediate layer. The EPSCs were sufficient to elicit bursts of action potentials that lasted as long as 300 ms and resembled presaccadic command bursts. To examine the contribution of neurons within the superficial layer to the production of these bursts, we determined how superficial neurons respond to the same current pulses that evoke bursts in the intermediate layer. Recordings from 61 superficial layer cells revealed 19 neurons that produced multiple action potentials following stimulation. Nine of these 19 neurons were wide- and narrow-field vertical cells, which are known to project to the intermediate layer and could contribute to producing the EPSC bursts. The remaining cells (n = 42) did not generate trains of action potentials and 21 of these showed only subthreshold potential changes in response to the stimulus. Our results indicate that most superficial cells do not directly contribute to production of the EPSC bursts, but a small number do have the properties necessary to provide a prolonged excitatory drive to the premotor neurons.

INTRODUCTION

The superior colliculus is composed of layers with sensory and motor functions, which provides an opportunity to address the fundamental problem of how sensory signals are translated into the command signals for movements. Recent in vitro experiments demonstrated a strong functional link between the superficial visuosensory and intermediate premotor layers of the superior colliculus in the tree shrew and rat (Isc et al. 1998; Lee et al. 1997). Of particular interest was the observation, in tree shrew, that applying single, brief electrical stimuli to the superficial layer of the superior colliculus evokes strong and prolonged synaptic excitation of intermediate layer premotor neurons (Lee et al. 1997). These prolonged responses may be generated by the same collicular circuitry responsible for producing the bursts of action potentials that command saccadic head and/or eye movements in vivo (Goldberg and Wurtz 1972; Munoz and Guitton 1986; Munoz et al. 1991; Sparks 1978; Sparks and Nelson 1987; Wurtz and Albano 1980; Wurtz and Goldberg 1972; Wurtz and Mohler 1976).

The present experiments examined the circuitry responsible for the excitatory postsynaptic current (EPSC) bursts that are generated in the intermediate layer. One possibility is that these prolonged synaptic responses are produced by trains of action potentials generated by neurons within the superficial layer itself. Although the superficial layer contains distinct types of neurons (Hall and Lee 1993; Isa et al. 1998; Langer and Lund 1974; Lee and Hall 1995; Lund and Lund 1972; Mooney et al. 1988; Moschovakis et al. 1988; Shi et al. 1997; Sterling 1971; Warton and Jones 1985; Warton et al. 1990), not enough is known about their synaptic connectivity or physiological properties to predict whether bursts could arise in the superficial layer itself. Thus it is not clear whether the electrical stimuli that evoke prolonged EPSC bursts in the premotor neurons do so by evoking bursts of action potentials in superficial neurons. Alternatively, recurrent axonal collaterals arising from cells in the underlying optic and/or intermediate layers of the colliculus (Hall and Lee 1997; Lee and Hall 1995; Mooney et al. 1988; Moschovakis et al. 1988) might give rise to positive feedback that prolongs and strengthens transient signals that arrive from the superficial layer (Arai et al. 1994; Pettit et al. 1999).

In the present study, we examined the origins of intermediate-layer EPSC bursts in the rat superior colliculus. First, we confirmed that rat intermediate layer neurons produce prolonged bursts of EPSCs that resemble those reported in the tree shrew (Lee et al. 1997). Second, we demonstrated that these responses are capable of generating prolonged bursts of action potentials in the intermediate layer neurons. This result suggests that one function of intracollicular circuitry may be to generate command bursts. Finally, we recorded the responses of many superficial layer neurons to the same brief stimuli and found that while most of these neurons do not produce prolonged responses, a few produce multiple action potentials and also project to the intermediate layer. Signals transmitted to the intermediate layer by this subset of visuosensory neurons may play a direct role in generating the bursts of action potentials that command saccadic orienting movements of the head and eyes.

METHODS

Forty-one Sprague-Dawley rats were used for this study. All experimental protocols were approved by Duke University Institutional Review Board for the Protection of Human Subjects and the Duke University Institutional Animal Care and Use Committee. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Recoding pipettes. The internal collicular neurons using 3–8 M V clamp recordings (Edwards et al. 1989) were obtained from individual were incubated at 37°C for 1 h and then stored at 25°C on a membrane for relating these layers in the rat to layers defined by the same criteria in the superficial and intermediate layers. The stain demonstrates the well-differentiated superficial of Sprague-Dawley rats [age, postnatal day 11–19 (P11–P19)] that were anesthetized with pentobarbital sodium. Slices 300 μm thick were incubated at 37°C for 1 h and then stored at 25°C on a membrane interface until transfer to the recording chamber. Whole-cell patch-clamp recordings (Edwards et al. 1989) were obtained from individual collicular neurons using 3–8 MΩ recording pipettes. The internal solution for the recording electrodes was composed of (in mM) 130 K-glucanote, 2 NaCl, 20 HEPES (pH 7.3), 4 MgCl₂, 4 Na₂ATP, 0.4 Na-GTP, 0.5 EGTA, and 0.3–0.5% biocytin. The liquid-junction potential between the electrode solution and the bath was calculated to be −10 mV and the membrane potential measurements presented in the present study were corrected to take this into account.

The external solution contained (in mM) 123 NaCl, 2.5 KCl, 1 NaH₂PO₄, 1.3 MgSO₄, 26.2 NaHCO₃, 11 d-glucose, and 2.5 CaCl₂. The drugs bicuculline methiodide (Sigma) and/or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (RBI) were added to this solution as indicated. After each drug application, drug-free solutions were superfused for at least 15 min before new recordings were made. To electrically stimulate the slices, brief current pulses (0.5 ms, 3–100 μA) were applied through an array of eight extracellular thin (≈20 μm diam) tungsten wire electrodes (NB Labs, Denison, TX) positioned parallel to the dorsal surface of the superior colliculus within the superficial gray layer (individual wires were placed ≈350 μm from each other so that the array was ≈2.5 mm wide). Of these eight electrodes, the one that induced the most vigorous responses was used; typically, this electrode was the one closest to the cell body (within 350 μm). The evoked responses were recorded by a Warner PC501A patch clamp amplifier, digitized with an Axon 1200 series Digidata A/D board, acquired at 4–20 kHz, and analyzed with pCLAMP 6 software (Axon Instruments).

Histology

Individual cells were labeled for subsequent morphological identification by diffusing a 5% biocytin solution into their cytoplasm from the patch pipette. Slices were then fixed in 4% paraformaldehyde and processed histologically to reveal the biocytin as described in Hall and Lee (1997) and Lee and Hall (1995).

In several brains, sections 60 μm thick were cut and stained for acetylcholinesterase activity to identify unequivocally the borders between the layers. The sections were soaked overnight in 0.1 M phosphate buffer at 4°C, rinsed briefly, and then immersed in an acetylthiocholine solution for 1 h followed by treatment with sodium sulfide solution for 1 min. The reaction was intensified with silver nitrate (Billet et al. 1999).

The acetylcholinesterase-stained section shown in Fig. 1 illustrates the nomenclature that we used for the layers of the superior colliculus in the rat. The stain demonstrates the well-differentiated superficial and intermediate layers in the rat and provides an unambiguous basis for relating these layers in the rat to layers defined by the same criteria in the tree shrew (Hall and Lee 1997), cat (Illing 1990; Illing and Graybiel 1985), and rhesus monkey (Ma et al. 1991). In the rhesus monkey, the intermediate layer defined by the dense band of cholinergic axons contains a majority of presaccadic cells (Ma et al. 1991) whereas in the tree shrew, the similarly defined layer contains mainly cells, that project to pontine gaze centers via the predorsal bundle (Hall and Lee 1997).

RESULTS

The goal of our experiments was to investigate the circuitry responsible for the prolonged and vigorous bursts of EPSCs that are generated by intermediate layer neurons in response to a single, brief electrical stimulus in the superficial layer. Because these bursts had only been described in the tree shrew (Lee et al. 1997), we first asked whether they also were generated by circuitry found in slices of the rat superior colliculus.

Responses of intermediate layer neurons

Bursts of EPSCs were recorded from four of the five intermediate gray layer neurons that responded to electrical stimulation of the superficial layer. The mean duration of the bursts evoked in these cells by single-current pulses (10–100 μA) varied from 67 ± 41 to 287 ± 24 ms (mean ± SE). Figure 2 illustrates the functional properties of the connection between the superficial and intermediate layers. The dendrites of this cell extended vertically into the overlying optic and superficial gray layers (Fig. 2A), as was described for some predorsal bundle cells in the rodent (Rhoades et al. 1987). Thus even though the axon of this cell was not filled with biocytin, the distribution and morphology of its soma and dendrites were consistent with its identity as a projection neuron.

This cell responded to the stimulus pulse in the superficial layer with a powerful burst of inward EPSCs that lasted up to 330 ms. The magnitude and duration of the currents varied according to the intensity of the stimulus, with larger stimuli producing more EPSCs (Fig. 2B, top). The synaptic responses evoked by the most intense stimulus (50 μA) were sufficient to generate unclamped action potentials (asterisks in Fig. 2B, top).

To examine the ability of the EPSC bursts to excite the intermediate layer neurons, evoked responses were also recorded under current-clamp conditions. The resultant excitatory postsynaptic potentials (EPSPs) depolarized the intermediate layer cell and, if the stimulus was sufficiently
strong (30–50 \(\mu A\)), produced multiple action potentials (Fig. 2B, bottom). In this cell, a 50-\(\mu A\) stimulus also evoked currents large enough to cause unclamped action potentials (asterisks), which are off-scale. Bottom, current clamp recordings from the same cell show that the responses are sufficient to evoke bursts of action potentials. C: EPSCs were a graded function of the intensity of the stimulus. D: the response evoked in this cell (top, 50-\(\mu A\) stimulus) was blocked by application of 10 \(\mu M\) 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (bottom).

FIG. 2. Synaptic transmission between the superficial and intermediate layers. A: this intermediate layer neuron had dendrites that extended vertically to the lower superficial gray layer. The stimulating electrode was located in the superficial layer, near the collicular surface (gray area). B: top, stimuli at three different intensities evoked inward excitationatory postsynaptic currents (EPSCs) in this cell. The 50-\(\mu A\) stimulus also evoked currents large enough to cause unclamped action potentials (asterisks), which are off-scale. Bottom, current clamp recordings from the same cell show that the responses are sufficient to evoke bursts of action potentials. C: EPSCs were a graded function of the intensity of the stimulus. D: the response evoked in this cell (top, 50-\(\mu A\) stimulus) was blocked by application of 10 \(\mu M\) 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (bottom).

In summary, brief electrical stimulation of the superficial layer evoked strong postsynaptic responses in intermediate layer cells of rat collicular slices. These synaptic responses consisted of bursts of EPSCs that were caused by activation of multiple glutamatergic synapses and were capable of eliciting prolonged bursts of action potentials. These results are similar to observations in the superior colliculus of tree shrews and permit the use of rat slices to elucidate the circuit mechanisms underlying the generation of the EPSC bursts in the intermediate layer.

Responses of superficial layer neurons

The preceding results show that some mechanism intrinsic to the superior colliculus converts a brief electrical stimulus in the superficial layer into prolonged synaptic excitation of the premotor neurons in the intermediate layer. We next wanted to determine whether neurons in the superficial layer directly activate the synaptic drive for the EPSC bursts. As mentioned in the introduction, the EPSC bursts could arise from bursts of action potentials in superficial layer neurons or could be the...
product of excitatory feedback in the deeper layers of the superior colliculus. The first hypothesis predicts that superficial layer neurons generate prolonged bursts of action potentials whereas the second hypothesis predicts that superficial layer neurons will produce transient responses. To distinguish between these two possibilities, we examined the responses of superficial layer neurons to the same stimulus that produced the EPSC bursts in the intermediate layer neurons.

Responses were detected with whole-cell current clamp recordings from a total of 61 superficial layer neurons (Fig. 3A). Although every cell responded to the stimulus at 10–100 μA intensity, the responses of these cells to even the most intense stimuli varied. Approximately one-third of the superficial layer cells (21/61) did not generate any action potentials in response to superficial layer stimulation but instead produced subthreshold synaptic potentials. Another one-third of the cells (21/61) responded to even the most intense stimuli with a single action potential. The remaining cells (19/61) produced two or more action potentials in response to maximal superficial layer stimulation. By filling the cells with biocytin, we were able to obtain information about the morphology of 23 of the 61 superficial layer cells (Fig. 3B). Two of these cells were identified as horizontal cells, 12 were wide-field vertical cells, and nine were narrow-field vertical cells.

Among the superficial cells that produced two or more action potentials in response to the stimulus, the duration of the evoked activity and the number of action potentials were quite variable as a function of stimulus intensity. When measured from stimulus onset to the time of the peak of the last action potential, the response duration ranged from a few ms to several hundred ms (Fig. 3C). The median duration of the responses of these cells to maximal stimuli was 18 ms and the mean was 124 ms. As our morphological results will demonstrate, some of this variability in response duration can be accounted for by the presence of several different types of neurons in this layer (Langer and Lund 1974; Lund and Lund 1972; Sterling 1971).

**Superficial cells that produced transient responses**

As predicted from the hypothesis that recurrent collaterals in the deeper layers are responsible for burst production, several types of superficial layer neurons produced only transient responses to the stimulus.

**WIDE-FIELD VERTICAL CELLS.** Wide-field cells in the superficial layer of the superior colliculus project to the deeper layers and therefore potentially provide monosynaptic input to the intermediate layer premotor cells (Lee and Hall 1995; Mooney et al. 1988). An example of this type of cell is shown in Fig. 4. The soma of this cell was located in the lower part of the superficial gray layer and its dendrites extended vertically and diagonally into the upper superficial gray and zonal layers (Fig. 4A). The broad dendritic field of this neuron is characteristic of wide-field vertical neurons (Lee and Hall 1995; Mooney et al. 1988). Although the axon of the cell in Fig. 4 was not well-filled, previous studies demonstrated that this cell type projects extensively to the deeper layers (Lee and Hall 1995; Mooney et al. 1988).

Of the 12 wide-field vertical cells that we identified, four responded to superficial layer stimulation with only a single action potential (Fig. 3B), even at maximal stimulus intensities.
a peak within 10 ms after the stimulus and lasted approximately 50 ms; this time course was similar to the duration of the EPSP. The EPSC decayed with a rather simple time course, which suggests that it arose from excitatory inputs that are small in number and/or synchronized with each other. Like the EPSP, the EPSC was blocked by CNQX (Fig. 4C).

In the presence of CNQX, a small outward current (Fig. 4C, inset) could sometimes be detected. This current was blocked by bath application of 10 μM bicuculline (not shown), a GABA<sub>A</sub> antagonist (Yoon et al. 1993). This indicates that the same stimulus that excited the wide-field cell also activated inhibitory GABAergic inputs. In the absence of CNQX, bicuculline increased EPSC amplitude and caused the appearance of multiple peaks on the EPSC trace (Fig. 4D, bottom). In current clamp recordings, the bicuculline treatment caused these cells to respond to superficial layer stimulation with a prolonged burst of action potentials (Fig. 4E). Thus when synaptic inhibition is removed by bicuculline, multiple excitatory inputs allow these cells to fire prolonged bursts of action potentials, which suggests that the transient responses of superficial layer cells are constructed at least in part by intracollicular GABAergic circuitry.

In summary, one-half of the wide-field vertical cells (n = 6) responded to superficial layer stimulation with an EPSP (n = 2) or a single action potential (n = 4). Because such cells generated only one short-latency action potential even in response to the most intense stimuli, the brief duration of the responses is independent of the stimulus parameters and, instead, seems to be limited by synaptic inhibition. Thus these cells are unlikely to be directly responsible for the prolonged bursts of EPSCs that are evoked in intermediate layer neurons by the same stimuli.

NARROW-FIELD VERTICAL CELLS. Narrow-field vertical cells comprise a second type of superficial layer cell that has extensive projections to the deeper layers (Hall and Lee 1993; Langer and Lund 1974; Lee and Hall 1995; Mooney et al. 1988). We recorded from nine identified narrow-field vertical cells. Similar to the wide-field cells, most of the narrow-field cells (6/9) generated either no spikes (n = 2) or only one spike (n = 4) in response to superficial layer stimulation. An example of such a narrow-field cell is illustrated in Fig. 5A. The soma of the cell was located in the lower part of the superficial gray layer and its apical dendrites extended superficially to form a narrow, conical field. Its axon gave rise to several collaterals in the lower superficial gray and optic layers before branching into the intermediate gray layer. Figure 5B illustrates that the cell discharged a single spike following superficial layer stimuli as intense as 100 μA. Under current-clamp conditions, bath application of 10 μM CNQX blocked the spike and underlying EPSP and revealed that a small hyperpolarizing inhibitory postsynaptic potential (IPSP) also was evoked by the stimulus (Fig. 5B). These results suggest that the superficial layer stimuli activated both excitatory and inhibitory inputs.
inhibitory synaptic inputs to the narrow-field cells, as was the case for the wide-field cells. When these cells were voltage-clamped at a holding potential of −65 mV, a biphasic synaptic current was revealed (Fig. 5C). This response consisted of an initial EPSC approximately 100 pA in amplitude and 15 ms in duration followed by a smaller and longer-lasting net outward current (Fig. 5C, inset). Treatment with 10 μM CNQX eliminated the EPSC but uncovered a train of large outward currents that lasted up to 150 ms (Fig. 5C).

Similar results from six cells indicated that most narrow-field vertical cells responded to brief stimulation with a single action potential or with subthreshold EPSPs and therefore are unlikely to contribute directly to the prolonged bursts of EPSCs in intermediate layer neurons. As was the case for the wide-field cells, the duration of the excitatory responses of these narrow-field cells appeared to be limited by bicuculline-sensitive GABAergic inputs.

**OTHER CELLS.** Twenty additional cells that generated either no spikes (n = 17) or only one spike (n = 13) in response to superficial layer stimulation could not be unequivocally identified morphologically either because the area around the cell body was stained with biocytin or because the dendrites and axon were insufficiently filled. Regardless of their type, their brief responses indicate that these cells cannot be directly responsible for the prolonged bursts of EPSCs in the intermediate layer.

Superficial cells that produced multiple action potentials

Nineteen superficial layer neurons produced two or more spikes in response to the stimulus (Fig. 3A). Eleven of these 19 cells were defined morphologically. Such neurons are potential sources for the EPSC bursts in the intermediate layer should belong to this group, so their morphology and the temporal properties of their responses will be described in detail.

**HORIZONTAL CELLS.** Two cells that produced prolonged trains of action potentials resembled the GABAergic horizontal cells that were described in previous studies (Langer and Lund 1974; Mize et al. 1982; Mooney et al. 1988; Warton et al. 1990). A drawing of one of these cells is shown in Fig. 6A. The cell had a dendritic field that extended in both directions from the cell body, tangential to the dorsal surface of the superior colliculus, and a small (8–10 μm) cell body that was located within 100 μm of the surface.

Brief stimulation (0.5 ms at 50 μA) of the superficial layer
evoked a vigorous and prolonged burst of spikes in this cell (Fig. 6B, top). The response consisted of a large, prolonged depolarization with numerous spikes superimposed. The duration of this burst response lasted as long as 350 ms and the maximum spike frequency was approximately 65 Hz. Treatment with 10 μM CNQX completely eliminated these bursts and left a small hyperpolarizing potential (Fig. 6B, bottom), showing that the spikes were driven by excitatory synaptic input.

Both horizontal cells were voltage clamped so that the synaptic currents underlying these vigorous responses could be examined. Varying the postsynaptic holding potential showed that the synaptic response consisted of several components. At a holding potential of −50 mV, the net current was outward and long-lasting (Fig. 6C, top). However, at −75 mV the postsynaptic response consisted of a large, rapidly decaying inward current (Fig. 6C, bottom). These results indicate that horizontal cells receive a mixture of synaptic input: an initial excitatory input as well as second input with a reversal potential near the resting potential that may be inhibitory.

In summary, superficial layer stimulation activated excitatory and inhibitory inputs whose net effect was to cause horizontal cells to produce prolonged bursts of action potentials. However, the responses of these cells are unlikely to be directly responsible for the EPSC bursts in the intermediate layer because evidence suggests that 1) they are GABAergic and 2) they do not project out of the superficial layer (Langer and Lund 1974; Sterling 1971).

**WIDE-FIELD CELLS.** Six of 12 wide-field vertical cells produced two or more action potentials in response to the superficial layer stimulus. The duration of the trains of action potentials that we recorded from these six wide-field cells ranged from 35 to 250 ms in response to stimuli of up to 90 μA. A typical example of such cells is shown in Fig. 7A. This cell responded to a 60-μA stimulus with brief bursts of action potentials (Fig. 7B). These spikes began shortly after the stimulus and lasted for up to 50 ms. The response of this wide-field cell consisted of up to five action potentials and the maximum frequency of spikes during the bursts was 167 Hz. Voltage clamp recordings demonstrated that the evoked response included a brief EPSC that peaked within 10 ms and decayed within 50 ms. This EPSC was very large, more than 200 pA, and often was sufficient powerfully to produce unclamped action potentials (Fig. 7C, asterisk).

**NARROW-FIELD VERTICAL CELLS.** Three of the nine identified narrow-field vertical cells generated two or more action potentials in response to superficial layer stimulation. Two of these cells showed responses with shorter duration than those of the EPSC bursts in the intermediate layer, whereas one cell in this group generated prolonged trains of action potentials that increased in frequency and duration with the intensity of stimulus. A drawing of this cell is shown in Fig. 8A. The cell was located in the upper part of the superficial layer and gave rise to an axon with multiple terminals in the optic and intermediate layers. Stimuli in the range of 50–80 μA evoked trains of action potentials that lasted an average of 163 ± 23 ms (Fig. 8B). In response to a 100-μA stimulus, this cell produced a burst of 11 action potentials that lasted 626 ms (not shown). Voltage clamp measurements showed that the burst was triggered by a short-latency EPSC with multiple peaks followed by a small outward current (Fig. 8C). The EPSC lasted approximately 20 ms, far shorter than the duration of the action potential burst (Fig. 8B), which suggests that the cell may have had some intrinsic capacity to produce bursts of action potentials in response to brief currents.

Our results indicate that 33% of the narrow-field cells (n = 3) and 50% of the wide-field vertical cells (n = 6) receive sufficient excitatory drive to produce multiple action potentials in response to a brief stimulus in the superficial layer. Because these neurons project to the optic and intermediate layers, they are potential candidates for the presynaptic source of the prolonged EPSC bursts in intermediate layer cells. Finally, it is important to note that there was no correlation between the age of the rats and the frequency of occurrence of cells that generated two or more action potentials.

**Temporal profiles of the responses in the superficial and intermediate layers**

To examine more quantitatively the temporal correlation between activity in superficial layer and intermediate layer neurons, we compared the timing of the EPSC bursts recorded in the intermediate layer with the timing of action potentials generated by the wide- and narrow-field cells of the superficial layer.
of discharge for only those narrow- and wide-field cells that generated two or more action potentials (n = 9) has a temporal profile that resembles the temporal pattern of EPSC bursts in the intermediate layer, but the more gradual decline in the activity of these cells appears to be largely, but not entirely, due to the contribution of the single narrow-field cell illustrated in Fig. 9D. The narrow-field cell in Fig. 9D exhibited a temporal profile of action potentials that closely resembled the timing of the responses in the intermediate layer and, thus, of all the cells in our sample, can most adequately account for the prolonged bursts of EPSCs.

**DISCUSSION**

Bursts of action potentials that are generated by premotor cells in the intermediate gray layer in vivo comprise the command signal for orienting movements of the head and eyes (Goldberg and Wurtz 1972; Munoz and Guitton 1986; Munoz et al. 1991; Sparks 1978; Sparks and Nelson 1987; Wurtz and Goldberg 1972; Wurtz and Mohler 1976). In the tree shrew slice preparation (Lee et al. 1997), and now in rat collicular slices, we found that the application of a single current pulse, 0.5 ms in duration, to the superficial layer can produce a prolonged burst of EPSCs in intermediate layer cells. In the present experiments we also found that, even in a slice preparation, these EPSCs are capable of producing prolonged bursts of action potentials in intermediate layer neurons. These prolonged responses indicate that circuitry within the colliculus is capable of amplifying and prolonging responses triggered by transient inputs. Thus the results suggest that one function of the intrinsic circuitry of the superior colliculus may be to generate the prolonged bursts that command orienting movements in vivo. The results also suggest that this circuitry is preserved in collicular slices, making possible the detailed patch clamp analysis of its mechanisms.

Our experiments demonstrated that the same current pulses that, when delivered to the superficial layer, generate the prolonged bursts in the intermediate layer neurons also evoke a variety of responses in the superficial layer cells. The majority of superficial cells responded with subthreshold postsynaptic currents or only one action potential, regardless of the intensity of the stimulus. However, a smaller subset of superficial neurons (19/61) produced two or more action potentials. Since none of these neurons are types known to project to the intermediate layer, they are potential sources of direct, monosynaptic inputs that could contribute to the EPSC bursts in the intermediate layer cells. However, all but one of these cells exhibited responses that were shorter than the duration of the intermediate layer bursts. Thus the results suggest that while...
the superficial layer may be included in the circuitry that enhances and prolongs transient inputs to generate EPSC bursts, other sources, such as the recurrent circuitry within the deeper layers (Pettit et al. 1999), may also play an important role.

The use of rat superior colliculus for in vitro studies

The decision to use rats for these experiments was initially based on previous studies that demonstrated that the laminar organization of the superior colliculus is similar in most mammals. In support of these studies, we illustrated the superficial and intermediate layers in the rat with an acetylcholinesterase-stained section (Fig. 1). The section shows the distinct differentiation of these layers in the rat and also provides a basis for relating them to the layers defined by the same criteria in the cat (Graybiel 1978; Illing and Graybiel 1985), tree shrew (Hall and Lee 1997), and rhesus monkey (Ma et al. 1991). Further evidence for a similar laminar organization across species was provided by studies that demonstrated projections from the superficial to the intermediate layer in species as diverse as the tree shrew (Hall and Lee 1997; Lee and Hall 1995), hamster (Mooney et al. 1988), and squirrel monkey (Moschovakis et al. 1988). Finally, our experiments provided evidence for a strong functional connection between these layers by demonstrating that, as in the tree shrew (Lee et al. 1997), stimulation of the superficial gray layer in the rat evokes powerful synaptic responses in intermediate layer cells (Fig. 2).

Rats between the ages of P11 and P19 were used because it is more practical to obtain patch clamp recordings in slices from young animals (Aitken et al. 1995; Lipton et al. 1995; Plant et al. 1995). However, the use of young animals raises the question of whether our observations also apply to adults. Several lines of evidence support the argument that circuits with properties similar to those we described in young animals are present in adults. First, both the morphologies and the interlaminar projections of the cells that we filled with biocytin are very similar to those in adults (Hall and Lee 1993; Langer and Lund 1974; Lee and Hall 1995; Lund and Lund 1972; Mooney et al. 1988). Second, glutamatergic receptor activity changes from immature to adult levels by the time that eyes are opened (~P11–P14) (Shi et al. 1997). Third, while there are suggestions that the development of collicular GABAergic pathways may be incomplete at this age (Grantyn et al. 1984; Lund and Lund 1972; Mize 1992; Mize et al. 1982; Shi et al. 1997; Warton and McCart 1989; Warton et al. 1990), our whole-cell recordings provide unequivocal evidence of strong bicuculline-sensitive inhibition at the ages used in our experiments. Specifically, in recordings from seven cells in animals with age ranges from P11 to P17, large outward currents that

\[ \text{FIG. 9. Temporal profiles of responses evoked in superficial and intermediate layer neurons.} \]

A: time of occurrence of EPSCs recorded from 4 intermediate layer cells. B: timing of responses recorded from all 16 wide- and narrow-field cells in the superficial layer that fired at least one action potential in response to superficial layer stimulation (except for the one narrow-field cell illustrated in D). C: temporal distribution of action potentials evoked in 9 narrow- and wide-field superficial layer cells that generated two or more action potentials in response to brief electrical stimulus in the superficial layer. D: temporal pattern of responses observed in a single narrow-field cell that generated prolonged trains of action potentials. The total number of action potentials is plotted as a function of discrete latencies of each action potential generated by SGS cells in B, C, and D.
could be blocked with bicuculline were revealed in the presence of CNQX, and the application of bicuculline strongly enhanced the excitatory responses of these cells in the absence of CNQX. Moreover, for those cells that fired two or more action potentials, neither the number of action potentials nor their frequency showed any dependence upon the age of the animal. Finally, the phasic responses that predominate in the superficial layer of young rat slices are also characteristic of superficial cells in vivo in the adult (Goldberg and Wurtz 1972; Sefton 1969). In the slices, these phasic responses appeared to be mediated, at least in part, by intracollicular inhibition because treatment of the slices with bicuculline both amplified the inward currents of the superficial cells and converted their responses from one or two action potentials to prolonged bursts (Fig. 4E). Thus it is likely that our observations describe mechanisms that operate in the superior colliculus of adult rats and other species.

The relationship between activity in the superficial and deep layers

The goal of our work was to understand the circuitry responsible for the EPSC bursts that occur in intermediate layer neurons following stimulation of the superficial layer (Lee et al. 1997). The two hypotheses that we considered predicted that superficial layer neurons would respond to brief stimulation with either transient or prolonged responses. In fact, we observed both types of responses. While most superficial layer cells responded phasically to the stimulus, 19 of 61 cells produced multiple action potentials, which could account for the generation of the EPSC bursts in the intermediate layer. Two of the superficial cells that exhibited prolonged and high-frequency trains of action potentials were horizontal cells and eight of them were morphologically unidentified cells. Since evidence from previous studies indicates that horizontal cells are GABAergic neurons that do not project to the intermediate layer (Langer and Lund 1974; Sterling 1971; Warton et al. 1990), they are unlikely to contribute directly to the EPSC bursts. Instead, they may contribute to the trains of outward currents that we recorded from superficial cells in the presence of CNQX (Figs. 4 and 5). The unidentified cells may contribute to the EPSC bursts either directly or indirectly depending on their type.

In contrast, both wide- and narrow-field cells project to the underlying layers (Figs. 7A and 8A) (Lee and Hall 1995; Mooney et al. 1988) and are in a position to make monosynaptic contacts with intermediate cells that generate the bursts. Comparison of the temporal profiles of the EPSC bursts with the temporal distribution of the evoked responses of the wide- and narrow-field cells (Fig. 9) provides some support for both hypotheses about the synaptic relationships between the superficial and intermediate layers.

Our observation of a superficial layer neuron that produced prolonged responses that tracked the time course of EPSC bursts (Fig. 9D) indicates that the superficial layer is capable of generating prolonged activity, which is consistent with the hypothesis that such activity produces the EPSC bursts. However, because the majority of the cells produced either subthreshold responses, only one action potential, or bursts of two or more action potentials of shorter duration than the duration of EPSC bursts in the intermediate layer, if this hypothesis is correct then superficial cells with prolonged responses must make a disproportionately large contribution to synaptic excitation of the premotor neurons. The relative contribution of each cell type will depend not only on the proportion of each type in the superficial layer but also on the number of action potentials produced by each cell and the strength of the synaptic connections between these cells and the premotor neurons.

Alternatively, the observation that the responses of most superficial layer neurons decline more rapidly than do the EPSC bursts in the intermediate layer implies that their output
must be further amplified by circuitry in the deeper layers, in the optic layer, or, more likely, by recurrent excitation in the intermediate layer (Arai et al. 1994; Pettit et al. 1999). The same circuitry also may amplify signals that reach the intermediate layer from other sources such as the frontal eye fields (Schlag-Rey et al. 1992) and the auditory and somatosensory systems (Sparks and Nelson 1987).

**A model of superficial layer connectivity**

Although our experiments were not designed to determine intrinsic synaptic connections, the responses generated by the various collicular cell types suggest a minimal model for the intrinsic circuitry of the superficial layer (Fig. 10). The horizontal cells from which we recorded are representative of a population of inhibitory interneurons that may contact wide- and narrow-field cells, and also each other, through dendrodendritic synapses (Langer and Lund 1974; Sterling 1971; Warton et al. 1990). The proposed contacts of horizontal cells with narrow- and wide-field cells could account for the complex outward currents observed in the latter cell types in the presence of CNQX and could contribute to the transient nature of their responses in normal conditions. The output signals of the superficial layer are transmitted to the intermediate layer by axons of the wide- and narrow-field cells (Iida et al. 1998; Lee et al. 1997). According to the model, a small subset of these projecting cells may have relatively little input from local inhibitory neurons and may be able, perhaps through local recurrent circuitry, to generate high-frequency and prolonged trains of action potentials. These trains of spikes may combine with the excitatory local circuitry within the intermediate layer (Pettit et al. 1999) to generate the bursts of action potentials that command saccades. Future experiments could use in vitro methods to test these ideas by directly measuring the influence of individual superficial cells on intermediate cells.

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