Local Excitatory Circuits in the Intermediate Gray Layer of the Superior Colliculus

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Pettit, Diana L., Matthew C. Helms, Psyche Lee, George J. Augustine, and William C. Hall. Local excitatory circuits in the intermediate gray layer of the superior colliculus. J. Neurophysiol. 81: 1424–1427, 1999. We have used photostimulation and whole cell patch-clamp recording techniques to examine local synaptic interactions in slices from the superior colliculus of the tree shrew. Uncaging glutamate 10–75 μm from the somata of neurons in the intermediate gray layer elicited a long-lasting inward current, due to direct activation of glutamate receptors on these neurons, and brief inward currents caused by activation of presynaptic neurons. The synaptic responses occurred as individual currents or as clusters that lasted up to several hundred milliseconds. Excitatory synaptic responses, which reversed at membrane potentials near 0 mV, could be evoked by uncaging glutamate anywhere within 75 μm of an intermediate layer neuron. Our results indicate the presence of extensive local excitatory circuits in the intermediate layer of the superior colliculus and support the hypothesis that such intrinsic circuitry contributes to the development of presaccadic command bursts.

INTRODUCTION

The intermediate gray layer of the superior colliculus commands saccadic eye movements by generating high-frequency bursts of action potentials in spatially restricted populations of cells. The spatial and temporal properties of these bursts determine the time of onset, amplitude, and direction of an impending saccade (Munoz and Wurtz 1995a,b; Sparks 1978). This causal relationship between the pattern of activity in a population of nerve cells and a defined behavior has aroused interest in understanding the neural processing that underlies the spatiotemporal properties of these bursts. Most models of presaccadic bursting include a wide ranging network of inhibitory connections within the intermediate layer that help shape the distribution of electrical activity. However, these models differ in the predicted role of local excitatory connections. Some models postulate that local recurrent excitatory circuitry produces an increase in excitation among neighboring cells. This increase in local excitation, in concert with the inhibitory connections, determines which cells produce the high-frequency command bursts (Arai et al. 1994; Van Opstal and Van Gisbergen 1989). In contrast, a second type of model does not require intrinsic excitatory connections and suggests that extrinsic synaptic inputs, such as those arising from the frontal eye fields, impose the pattern of excitation (Schlag-Rey et al. 1992). One way to evaluate these models is to examine intrinsic synaptic circuits; the presence of robust local excitation would provide a physiological substrate for intrinsic excitatory circuitry postulated to contribute to the production of presaccadic bursts.

METHODS

Collicular slices

Coronal or parasagittal slices (300 μm thick) were prepared from the superior colliculus of 13- to 20-day-old tree shrews. The well-defined laminar organization of the colliculus in this species made it possible to visually select and record from single neurons in identified layers. Slices were superfused at room temperature with oxygenated physiological saline (in mM: 119 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 1 NaH₂PO₄, 26.2 NaHCO₃, and 11 glucose) containing 100–150 μM carboxy-nitrobenzyl–caged glutamate (Molecular Probes, Eugene, OR). In some experiments, 1 μM tetrodotoxin was added to the saline to block synaptic transmission. Whole cell patch-clamp recordings were made from 27 intermediate and deep layer neurons, as described in Lee et al. (1997). Recordings were accepted only if the holding current was <100 pA when the membrane potential was voltage clamped at −60 mV. The patch pipette solution contained (in mM) 100 gluconic acid, 2–10 EGTA, 5 MgCl₂, 2 ATP, 0.3 GTP, and 40 HEPES; pH to 7.2 with CsOH. During the experiment, a fluorescent dye, Oregon Green 488 BAPTA-1 (200 μM; Molecular Probes, Eugene, OR), was dialyzed from the patch pipette into the neuron to visualize the soma and dendrites of individual cells with a confocal microscope (Nornan Odyssey) during the experiment. To characterize the cell morphology in more detail, biocytin (10%) was included in the pipette solution for 16 of these neurons. The slices were fixed and a diamino-benzidine reaction was performed after the experiment to reveal the biocytin. 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.
**Results**

Electrical recordings from the cell bodies of intermediate and deep gray layer neurons were used to detect responses evoked by photostimulation (n = 27). For all of the cells, uncaging glutamate at distances 10–75 μm from the soma elicited inward currents with two distinctive components (Fig. 1A). The first component consisted of a long-lasting inward current with a duration of several hundred milliseconds (Fig. 1A, top trace). Such responses were insensitive to 1 μM tetrodotoxin, which blocks voltage-gated sodium channels and, therefore, the action potentials that require these channels (Fig. 1B). This component closely resembled responses mediated by glutamate receptors (Pettit et al. 1997) and probably arose from direct activation of glutamate receptors on the neurons from which recordings were made. Consistent with this interpretation, and the range of action of uncaged glutamate (Katz and Dalva 1994; Pettit et al. 1997), these responses were always observed at locations within 20 μm of the somata or dendrites of the collicular neurons, but were often absent from more distant sites (Fig. 1A, bottom trace).

The second component consisted of brief currents with a half-maximal duration of 4.7 ± 0.2 ms (mean ± SE, n = 42 responses). These brief currents could be evoked even at locations that evoked no direct responses (Fig. 1A, bottom trace) and were blocked by application of tetrodotoxin (1 μM; Fig. 1B). These properties indicate that the brief currents arose from excitation of neurons innervating the patch-clamped cells. The synaptic currents occurred with a wide range of latencies and occurred as isolated events or in clusters with durations up to hundreds of milliseconds (Fig. 1A, bottom trace). The frequency of these events varied within a range of 20–40 Hz. Typically the greatest number of responses occurred within the first 50 ms after the light flash, presumably reflecting the time course of the glutamate-induced depolarization of the stimulated presynaptic cells. There was a mean of 8.8 ± 0.5 synaptic events/stimulus, although this may be an underestimate due to superimposition of synaptic responses. This is a very high density of evoked responses in comparison with other brain areas where photostimulation has been attempted, such as visual cortex (Callaway and Katz 1993; Dalva and Katz 1994; Sawatari and Callaway 1996).

At a holding potential of −60 mV, the evoked synaptic currents had a mean amplitude of 36 ± 1.9 pA (n = 56 responses). We conclude that these currents were excitatory because they reversed their polarity when the holding potential was depolarized to positive potentials (Fig. 1C). Further evidence of their excitatory nature is their decay time constant of 4.1 ± 0.3 ms, which is consistent with the decay of glutamate-mediated synaptic currents (Hestrin 1993), whereas GABA-mediated currents typically decay much more slowly (Jones and Westbrook 1995). In addition, any chloride-mediated inhibitory currents would likely escape detection because of the small difference between the chloride reversal potential (−66 mV) and the holding potential (−60 mV).

The spatial arrangement of functional synaptic connections was examined for seven neurons whose anatomy (confirmed with biocytin histology) was consistent with their identity as premotor neurons (Hall and Lee 1997). That is, they were located in the intermediate layer and had large (>20 μm diam) somata and multipolar dendritic arbors. In other studies, neu-
rons with these features have been shown to generate command signals for saccades (Moschovakis et al. 1988). Photostimulating at sites along several axes within the field of the microscope objective revealed an isotropic excitatory surround of synaptic transmission (Fig. 2). This excitatory surround was characteristic of all of the recordings from intermediate layer neurons with these features.

**FIG. 2.** Local photostimulation evoked synaptic currents. The spatial pattern of synaptic inputs is illustrated by overlaying a camera lucida drawing of a biocytin-filled intermediate layer neuron with the direct and synaptic current responses evoked by photostimulation. The site of glutamate uncaging is indicated by the location of the peaks of the current responses. The brief synaptic responses were evident along all axes and at all sites of stimulation.

**FIG. 3.** Widespread spatial distribution of local excitatory synaptic inputs. A and B: relationship between the location of the uncaging light spot and the number of synaptic responses evoked at each location when scanning laterally within the intermediate layer (A; Intralaminar) and more superficially (B; Superficial). The mean number of events/stimulation was calculated by measuring all synaptic events whose amplitude exceeded twice the baseline noise. C: relationship between photostimulus location and number of evoked synaptic responses. At each location, the number is the sum of responses along all scanning axes. D: spatial relationship of the mean amplitude of synaptic currents evoked along all axes.
neurons (Fig. 3). When the light beam was moved laterally within the intermediate layer, synaptic events were evoked at every location. In fact, over a range of 50 μm, the number of events evoked by a light flash was remarkably constant (Fig. 3A), suggesting a constant density of local excitatory inputs converging on the premotor cells. Likewise, when the light beam was placed at sites along a superficial axis perpendicular to the surface of the colliculus, responses could be detected from each location within 75 μm of the soma (Fig. 3B). Stimulating along axes with other orientations also evoked very similar synaptic responses. Combining responses from all axes reveals that there was no significant spatial gradient in the distribution of synaptic inputs within 75 μm of a given intermediate layer neuron (Fig. 3C). Similarly, the amplitude of the synaptic responses did not vary systematically with distance, either between or within axes (Fig. 3D). Responses evoked near cell bodies were somewhat smaller than those observed for regions distant from the cell body, but this may be a consequence of the large direct responses that were generated when the light spot was close to the target neuron. Our results indicate that there is a high and relatively constant density of strong synaptic excitation surrounds each intermediate layer neuron.

**DISCUSSION**

Previous studies that used electrical stimulation suggested a rich plexus of intrinsic synaptic circuitry in the intermediate layer. For example, electrical stimulation of the intermediate layer produced transynaptic excitation of premotor neurons (McIlwain 1982) and lateral inhibitory interactions (Meredith and Ramoa 1998; Munoz and Istvan 1998). However, the likely stimulation of fibers of passage made it difficult for such experiments to distinguish between extrinsic and intrinsic sources of synaptic input to the cells of this layer. Because the uncaged glutamate does not activate axons (Callaway and Katz 1993) and because slicing the colliculus eliminated most extrinsic sources of synaptic input, we were able to selectively activate local inputs. We find that there are indeed extensive excitatory synaptic interactions among the neurons of the intermediate gray layer. These local excitatory circuits could provide the substrate for positive feedback that sustains and intensifies the low-frequency activity that precedes the command burst for a saccade (Glimcher and Sparks 1992; Munoz and Wurtz 1995a). They may also contribute to the prolonged bursts of excitatory postsynaptic currents seen in intermediate layer neurons in response to electrical stimulation of sensory inputs from the superficial layer (Lee et al. 1997). Premotor cells in the intermediate layer give rise to local axonal arbors that may underlie these interactions (Hall and Lee 1997; Moschovakis et al. 1988).

Our results support the hypothesis that intrinsic excitatory interactions contribute to the development of presaccadic command bursts in the superior colliculus. Future work will be needed to address the contributions of intrinsic inhibitory connections and extrinsic inputs. It is probable that all of these sources of synaptic input work in concert to shape the spatiotemporal profile of electrical activity in the intermediate layer, and the present results demonstrate that photostimulation will be useful for analyzing their relative contributions.

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