Repetitive Spikes in Photoreceptor Axons of the Scorpion Eye

Invertebrate Eye Structure and Tetrodotoxin

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ABSTRACT A graded depolarization accompanied by nerve impulses can be recorded from the scorpion lateral and median eyes in response to light. Electron microscopy shows that axons forming the optic nerve arise directly from the photoreceptors. Thus, photoreceptors must respond both by the generation of a slow receptor potential and the initiation of spikes. The latency of the first spike, and the maximal and mean discharge frequencies were a function of light intensity. Spikes were abolished by tetrodotoxin. Repetitive firing to light therefore appears to be a normal response of scorpion photoreceptors and is the result of regenerative Na influx in the cell membrane.

INTRODUCTION

Many sensory receptors encode information about the stimulus by a pattern of nerve impulses. Impulse activity has not been detected in receptor cells of the vertebrate eye or in most arthropod eyes where transmission seems to occur through decremental conduction. Receptor cells in insects and arachnids typically respond to low-level illumination with a sustained depolarizing potential (1, 2), but a single spike occurs with high-stimulus intensities in a few species (3, 4). In the dragonfly, such regenerative responses seem to play no role in transmission, and the same may be true for other photoreceptors responding with a single spike to strong illumination (4). Trains of spikes from retinula cells of the drone honeybee (3) during intense illumination are probably an experimental artifact (5), and propagated activity in the lateral eye of Limulus (6) is secondary to the spread of depolarization from retinula to eccentric cells (7). In the present communication we report repetitive spikes superimposed on graded depolarizations from scorpion optic nerve fibers. Since photoreceptors give rise to these axons, they must respond both by the generation of slow receptor potentials and the initiation of spikes.
MATERIALS AND METHODS

Two species of scorpions, Centruroides sculpturatus and Hadrurus arizonensis have been studied. Both have three pairs of lateral eyes and two median eyes located on the prosoma (8).

Material for electron microscopy was prepared by immersing the prosoma in fixative after removing glandular and connective tissue from the eyes. A fixative with the following composition was found to give good preservation at pH 7.6: H2O, 110 cm3; sodium cacodylate, 2.1 g; sodium chloride, 3.0 g; polyvinyl pyrollidine, 3.0 g; paraformaldehyde, 4.0 g; glutaraldehyde (25%), 2.0 cm3; calcium chloride (1.5%), 20 drops.

After fixation for 4–6 h at room temperature the tissue was washed in buffer, postfixed in 2 % osmic acid in cacodylate buffer for 2 h, dehydrated in graded series of alcohol, and embedded in Araldite. The prosoma containing the eyes was carefully oriented, and transverse ultrathin sections were taken through the median and lateral eyes. The blocks were then reoriented and ultrathin sections taken through the photoreceptors tangential to the lens. Photo montages of the entire eye were prepared for both types of eyes by means of electron microscopy and the individual photoreceptors mapped.

Recordings were made from optic nerves in the intact animal, or in the prosoma excised and mounted with paraffin wax in a Lucite chamber containing scorpion Ringer’s (9). Optic nerve filaments from the lateral or median eyes were cut near the optic lobes, placed on cotton wick Ag-AgCl electrodes, and covered with mineral oil. Recording electrodes were connected to a DC amplifier, and the electrical events displayed on an oscilloscope and stored on tape. Light for stimulation was provided by a 6-V, 15-W microscope lamp connected to a fiber optic cable (0.2 mm in diameter) one end of which was placed on the lens of the eye. Stimulus intensity was adjusted by calibrated neutral density filters, and the stimulus was controlled by an electromagnetic shutter driven by a pulse generator. The light stimulus was recorded by a photoelectric cell.

RESULTS

Electron microscopic montages of the entire median and lateral eye reveal a difference in size but a basic similarity in the morphology of photoreceptor cells (Figs. 1, 4). Numerous examples of photoreceptors giving rise to an axon were observed. There were no signs of axon collaterals, synaptic contact, or second-order neurones in either type of eye. In the median eye, rhabdomeres unite to form pentagonal rhabdomes (Fig. 2), but rhabdomeres of lateral eye photoreceptors are irregular and establish no regularly repeating unit (Fig. 5). Zonulae occludentes unite adjacent nonrhabdomeric portions of photoreceptors where there is a lack of interposition of glial cells (Fig. 2, inset). Most axons are separated by sheet-like glial processes, and direct apposition has only been observed near the optic nerve where the glial sheath
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is incomplete (Figs. 3, 6). The axon, arising from the base of photoreceptor cells, joins other axons to form an optic nerve. The axoplasm lacks filaments and microtubules but contains ribosomes, mitochondria, and a textured cytoplasmic matrix (Figs. 3, 6). The density of the cytoplasmic matrix is similar in axons of the median eye (Fig. 3) but is variable in axons of the lateral eyes. As shown in Fig. 6, large axons are light and small axons dark. Similar differences in density also distinguish soma of certain lateral eye photoreceptors (Fig. 5 numbers 1, 3). Glial cells ensheathing axons contain little pigment while those intercalated between photoreceptors contain pigment granules similar to those in the photoreceptors. These granules are concentrated in distal portions of the photoreceptors in the light-adapted state.

Low-level illumination of the lateral or median eyes produced a slow negative potential. This potential reached a maximum after a short flash, but prolonged illumination (Fig. 7 A) produced a response composed of a transient initial peak and a steady depolarization of smaller amplitude. When light was switched off, rapid repolarization was followed by a slow return to control values. A massive discharge of spikes superimposed on the slow depolarization is shown in Fig. 7 B. Spikes superimposed on a slow depolarization were also observed in the lateral eye nerve. They were more easily seen when the lateral eye nerve was teased apart and recordings made from filaments containing only a few nerve fibers. Illumination of the lateral eye with long flashes of light (5–30 s) produced spike discharges lasting for the duration of the stimulus. They consisted of an initial burst followed by a steady discharge of spikes (Fig. 7 C–G). The latency of the first spike in the discharge, the maximal instantaneous frequency, and the mean frequency per second during the steady-state period were a function of light intensity.

Short light flashes (25–200 ms) elicited a series of spikes that outlasted the stimulus. The number of spikes per flash was reduced when flashes of constant intensity were applied every 10 s to dark-adapted eyes. If background light was increased while the flashes were being delivered, the number of spikes per flash was reduced approximately in proportion to the log of the intensity of the adapting light. This effect was reversed 20–30 s after switching off the adapting light. When the intensity of light in the flash was increased in each successive flash, the number of spikes per flash increased and the latency of the first spike decreased.

Low frequency spontaneous activity was present in most dark-adapted preparations. There was a pause in such activity after stimulation, and repeated strong illumination abolished spontaneous activity. Tetrodotoxin (10⁻⁴ g/liter) added to the bathing fluid totally abolished spikes in response to light within 5 min and indicates that spikes are normally the result of a
regenerative Na\(^+\) influx. It should be noted that tetrodotoxin had little effect on the slow negative depolarization which persisted at the same or slightly reduced amplitude.

The spikes in the median eye in response to illumination were only ob-
served in preparations in which the eye was left in the intact animal (Fig. 7 B). Propagated activity was difficult to obtain in excised preparations and was only observed in a few instances with high intensity illumination. In all cases, higher intensities than those used to stimulate the lateral eye were required to elicit these propagated responses, and they soon disappeared despite the persistence of slow potentials. The recorded activity was always composed of low voltage spikes, and the nerve was easily damaged when an attempt was made to dissect it into small bundles of axons.

**DISCUSSION**

The graded response to illumination of the scorpion eye is similar to that recorded from insects and arachnids, but the presence of repetitive spikes is exceptional. This study confirms earlier reports that axons of scorpion median and lateral eyes arise directly from photoreceptor cells (8, 10). Montages of the entire median and lateral eyes allowed us to follow optic nerve fibers from the base of photoreceptor cells and to rule out the existence of axon collaterals or synapses with other cells. Therefore, the same cells which produce generator potentials must also initiate nerve impulses. The principal question is whether the recorded impulses are normal phenomena or whether they are the result of "unusual" experimental conditions. In the honeybee, spikes appeared during impalement, towards the end of a long experiment, or at the beginning of responses to steps of light in strongly light-adapted preparations (5). Such conditions did not exist in our experiments.

**FIGURE 1.** Schematized scale drawing of photoreceptors in the median eye. The rhabdomeric (R) process is long, contains pigment granules (G), and is regularly apposed to the processes of other photoreceptors. The axon emerges from the base of the cell and unites with axons of other photoreceptors near the postretinal membrane (PRM) to form an optic nerve. Dashed lines indicate plane of section for Figs. 2 and 3.

**FIGURE 2.** Rhabdomeres arising from five photoreceptors (numbered) unite in the median eye to form a pentagonal rhabdome. Nonrhabdomeric surface of adjacent photoreceptors (arrows), not separated by glial cell processes, are united by zonulae occludens (inset). $\times$ 4,700.

**FIGURE 3.** Axons of photoreceptors in the median eye are relatively uniform in size and separated by processes of glial cells (G). $\times$ 4,700.

**FIGURE 4.** Schematized scale drawing of a photoreceptor in the lateral eye. Rhabdomeric portions of these cells are short and irregular. The axon arises from the base of the cell and penetrates the postretinal membrane in small bundles which later form an optic nerve. Dashed lines indicate plane of section for Figs. 5 and 6.

**FIGURE 5.** Rhabdomeric portions of photoreceptors (numbered) in the lateral eye give rise to irregular aggregates of microvilli. Glial cells (G) containing pigment granules separate most photoreceptor processes. $\times$ 4,700.

**FIGURE 6.** Photoreceptor axons in the lateral eye range from large, lightly staining profiles (asterisks) to small electron-dense processes (arrows). Axons in this small bundle near the postretinal membrane are ensheathed by processes of glial cells (G). $\times$ 4,700.
where the cells were not impaled, where propagated responses consistently followed illumination of the lateral eyes, and where such activity was reduced in a predictable fashion with light adaptation. Since spikes of similar shape and amplitude occur repeatedly, it seems unlikely that trains of spikes in scorpion optic nerve filaments are obtained from asynchronous activation of different fibers.

It has been established that tetrodotoxin blocks action potentials by blocking the sodium-carrying system of the membrane; it does not block generator potentials in sensory cells, including photoreceptors (11, 4). The fact that tetrodotoxin applied to the scorpion eye blocks nerve impulses without affecting the slow depolarizing potentials, leads us to conclude that the process responsible for the development of graded potentials in scorpion photoreceptors is different from that involved in the production of conducted spikes. Our results therefore suggest that scorpion cells, in contrast with other arthropod photoreceptors, exhibit graded depolarization and
repetitive firing as a normal mode of response to a light stimulus. Both types of responses appear to be important for encoding information about light intensity and for transmitting it to second-order cells.

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