LOCALIZATION OF THE PHOTOCURRENT OF *LIMULUS* VENTRAL PHOTORECEPTORS USING A VIBRATING PROBE

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ABSTRACT We have used a vibrating probe to determine the profile of electrical current density around ventral photoreceptors of the horseshoe crab following flashes of light that uniformly illuminated the entire surface of the photoreceptor's cell body. The vibrating probe signal indicated that the density of inward current was greatest at the distal region of the cell, the region that is expected to contain the light-sensitive rhabdom. The density of inward current typically declined at the midpoint of the cell body and then reversed to an outward current flow in the proximal region of the cell body, close to the axon. The profile of local sensitivity of the photoreceptor to light closely matched the profile of inward current density, suggesting that the light-activated conductance is localized to the light-sensitive region of the cell.

INTRODUCTION

Limulus ventral photoreceptors respond to light with a depolarization that is caused by current flowing into the cell through ionic channels (Millecchia and Mauro, 1969; Bacigalupo and Lisman, 1983). Excitation by light is thought to be mediated by the release of an "internal transmitter" within the receptor cells (Cone, 1973).

Clear functional and anatomical divisions have recently been shown within ventral photoreceptors (Stern et al., 1982; Calman and Chamberlain, 1982). The sensitivity of the cell body to light is not uniformly distributed, but it is confined to a specialized lobe, the rhabdomeral-lobe (R-lobe). Usually, the R-lobe, which contains the microvilli that bear visual pigment, is located at the end of the cell body, distal from the axon. Components of the cascade of visual excitation that are "downstream" from rhodopsin may also be localized to the R-lobe. This may explain why the putative internal transmitter, inositol (1, 4, 5) trisphosphate (for review see Berridge and Irvine, 1984), is more effective in exciting the cell when injected into the R-lobe, rather than the A-lobe (Fein et al., 1984; Brown et al., 1984). The channels that are activated by the light-induced release of transmitter may therefore be localized to the R-lobe. In general, channels are not uniformly distributed in the membranes of animal cells (for review see Almers and Stirling, 1984). Here, we directly demonstrate localization of the photocurrent to the R-lobe.

We have used a vibrating probe (Jaffe and Nuccitelli, 1974) to determine the spatial profile of the current elicited by diffuse light flashes that uniformly illuminated the entire surface of the photoreceptor. When placed close to the cell membrane, the vibrating probe measures the electrical field due to current entering or leaving the cell. The vibrating probe provides an alternative to suction or "patch" electrode techniques used to measure transmembrane current. The irregular surface of ventral photoreceptors has made patch electrode measurements difficult (Bacigalupo and Lisman, 1983). We find that the density profile of the photocurrent closely matches the profile of sensitivity of the cell body to light. The light-activated conductance therefore appears to be localized to the light-sensitive region of the cell. Our result supports previous studies showing comparable localization of photocurrent within *Limulus* and other invertebrate photoreceptors (Hagins et al., 1962; Lasansky and Fuortes, 1969; Fein and Charlton, 1975*a*).

METHODS

Ventral photoreceptors of the horseshoe crab, *Limulus polyphemus*, were pinned to Sylgard (Dow Corning Corp., Midland, MI) in a dish filled with artificial seawater, pH 7. The nerves were rotated so as to expose, on the side of the nerve, photoreceptor cell bodies (Fein and Charlton, 1975*b*). The nerves were treated with 1% pronase (Calbiochem, La Jolla, CA) for 1 min and were viewed under infra-red illumination from above at a magnification of 320X. A photoreceptor cell body was cleaned of glia on the side that projected out from the nerve using a suction micropipette (Stern et al., 1982). The cell was left attached to the nerve by glial connections to the side of the cell body that was closer to the nerve. The

cell could then be impaled with a conventional glass microelectrode, connected to a DC amplifier. An Ag/AgCl electrode connected the bath to a current-to-voltage converter.

Stimulation was delivered from a conventional optical bench that projected light onto the preparation from below (Fein and Charlton, 1975*b*). Two forms of illumination could be delivered. A diffuse light, which uniformly illuminated the entire cell body of the photoreceptor and surrounding nerve, was used in conjunction with the measurements with the vibrating probe. Alternatively, a moveable 5-μm spot of light was used to determine the local sensitivity of the photoreceptor. The light spot could be viewed from above the preparation and appeared to be scattered to a diameter of 10–15 μm by passage through the photoreceptor. The white light from the tungsten source was filtered to remove infra-red (model BG-18 filter; Schott Inc., Duryea, PA). The maximum available light intensity at the surface of the preparation through either form of illumination was 45 mW/cm^2. Light intensities quoted in this paper are the $\log_{10}$ attenuation from this maximum.

A platinized, steel vibrating probe (Scheffey, 1986; Scheffey and Katz, 1986) having an exposed tip diameter of 10–15 μm was then brought close to the surface of the photoreceptor and aligned so as to vibrate in a line perpendicular to the photoreceptor's surface. The distance between the cell surface and the closest excursion of the probe was 10 μm. The probe vibrated at 250–900 Hz over an excursion of 15 μm, the exact frequency being determined by the resonance of the piezoelectric driver. The center of vibration was therefore ~18 μm from the cell surface. The probe electrode and a separate reference electrode, made from platinized wire and placed in the bath 1 cm from the preparation, were connected to a differential amplifier with output to a lock-in amplifier (model 5204; Princeton Applied Research, Princeton, NJ). The probe was calibrated by measuring the signal elicited by the passage of current through a microelectrode placed 20 μm from the tip. Jaffe and Nuccitelli (1974) show that the probe's response to current passed through a microelectrode conforms within 10% to that theoretically expected, due to the current density flowing. Scheffey et al. (1983) have analyzed the performance of a similar vibrating probe to the design used in this study. They reported that the probe localizes point current sources placed 7–15 μm from the probe with a resolution comparable to the probe excursion (4–7 μm in their paper, 15 μm here). Errors in determining the local current density due to the electrical blocking action of the probe and the local nonuniformity of the electric field around the source were estimated by Scheffey et al. (1983) as being <10%.

The output of the lock-in amplifier was filtered with a 2-pole low-pass RC filter with a corner frequency of 1.6 Hz. The corner frequency of the filter was chosen to coincide with the frequency, ~2Hz, at which the power spectrum of the response of ventral photoreceptors declines to one half (Wong et al., 1982). This choice of filter produced the best ratio of signal-to-noise, but it may have attenuated somewhat the peak response to light.

RESULTS AND DISCUSSION

Fig. 1 illustrates a typical experiment, in which a single, isolated photoreceptor was impaled by a glass microelec-

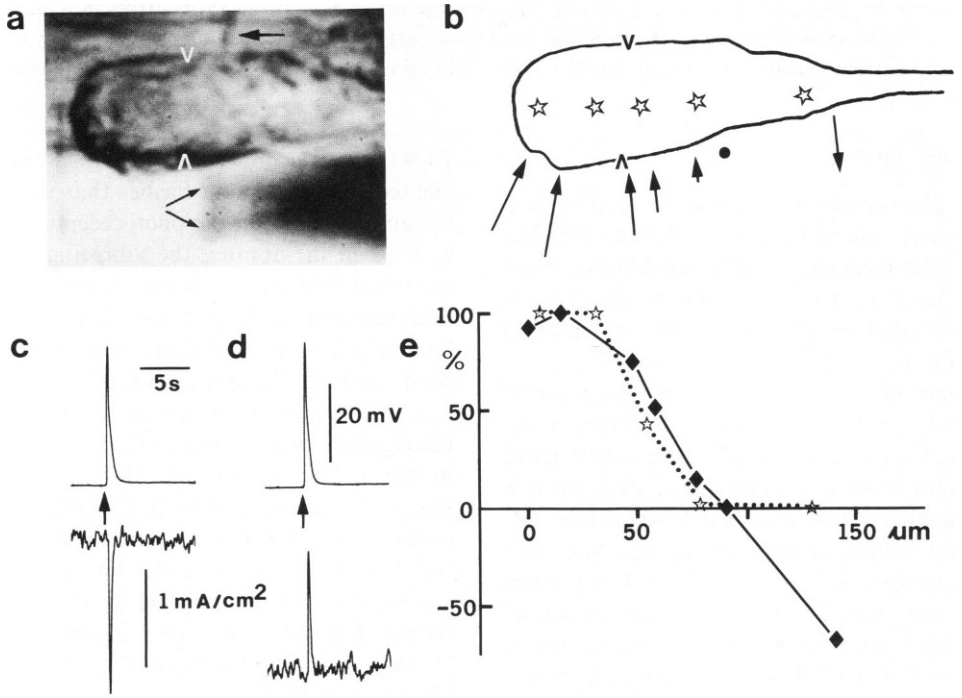

FIGURE 1 (*a*) Video recording of a ventral photoreceptor attached to the side of the ventral nerve. The upper horizontal arrow indicates the position of an intracellular glass microelectrode that impaled the cell. Lower arrows indicate the excursion of the vibrating probe below the cell. The white "vees" mark the probable junction of the R-lobe with the arhabdomeral lobe, as indicated by small indentations in the cell body. The scale of the photograph is given by the abscissa in *e*. (*b*) Sketch of the photoreceptor, showing positions (stars) at which a 5-μm diameter spot of light was placed to determine the local sensitivity to light. Length and direction of the arrows indicate the relative magnitude and sign of the vibrating probe signal. The direction of the arrow also gives the line of probe vibration. The dot indicates lack of a detectable probe signal at that position. Continuous illumination with the light spot, intensity -3.3 $\log_{10}$ units when centered on the most sensitive region of the cell, elicited steady responses of amplitude 3 mV. (*c*, *d*) Intracellular voltage recordings (*upper*) and vibrating probe signals (*lower*) in response to a bright, diffuse light flash that uniformly illuminated the entire photoreceptor. The probe signals were recorded from the positions at the extreme left (*c*)and right (*d*) of the cell body as displayed in *b*. The flash was of duration 100 ms, intensity -1 $\log_{10}$ unit. (*e*) The positions of the probe and light spot in *b* are projected onto a linear abcissa and the peak amplitude of the probe signal (diamonds) and the local sensitivity (stars) are shown at each position, expressed as a percentage of their respective maxima.

trode throughout the experiment. The intracellular electrode monitored the transmembrane depolarization caused by light to check that the cell's sensitivity to diffuse light remained constant for each probe position. The vibrating probe was then brought close to the photoreceptor's surface. Fig. 1 *a* shows the photoreceptor, probe, and intracellular electrode in position. The magnitude of the probe signal following diffuse flashes of light that illuminated the photoreceptor's entire surface was determined at several sites along the photoreceptor's surface (Fig. 1 *b*). The vibrating probe was much less sensitive than intracellular recording, requiring flash intensities that saturated the intracellular response to obtain significant signals.

Even though the illumination was uniform across the entire surface of the cell body, the probe signal was not. The probe signal indicated a high density of current flowing into the cell at the distal end of the receptor, which rapidly declined at the midpoint of the cell body and reversed to indicate an outward current close to the axon. Examples of light-induced depolarizations and probe signals when the probe was at the distal and proximal ends of the receptor are shown in Figs. 1 *c* and *d*, respectively.

The high current densities (~1 mA/cm^2) that flow into the distal end of the cell are consistent with voltage-clamp measurements of the transmembrane current (Millecchia and Mauro, 1969). If the R-lobe is assumed to be a cylinder, closed at one end, of diameter and length 50 μm, then a uniform current density across the cylinder of 1 mA/cm^2 would indicate a peak current into the R-lobe of ~100 nA. The flash intensity used in our experiment would be expected to saturate the receptor's response to light. When ventral photoreceptors are voltage-clamped to their resting potential, saturating flashes elicit currents of up to 1,000 nA (Lisman and Brown, 1975). This figure sets an upper limit to the current flowing into the R-lobe when the cell is not voltage-clamped, which will be reduced as a result of the loss in driving force associated with the depolarization. Millecchia and Mauro (1969) estimated from their voltage-clamp data that peak currents of tens of nanoamperes would be expected to flow into the cell through the light-sensitive conductance and out through the voltage-sensitive conductance during the unclamped response to bright light.

After finishing the measurements with the probe, the cell was illuminated with a dim, moveable 5-μm diameter spot of light. The sensitivity of the cell to the spot was determined at the positions of the spot indicated by the stars in Fig. 1 *b*. Because the cell body is within a few percent of being isopotential (Brown et al., 1979), the changes in potential elicited by the spot accurately reflect the local sensitivity to light of the areas illuminated. Fig. 1 *e* plots the local sensitivity of the cell to the spot, determined by the light-intensity required to elicit a small criterion response. The local sensitivity is highest at the distal end of the receptor. Local sensitivity rapidly declines 50 μm from the distal end, falling 1.8 $\log_{10}$ units at a point 80 μm from the distal end. The local relative sensitivity profile closely matched the profile of inward current

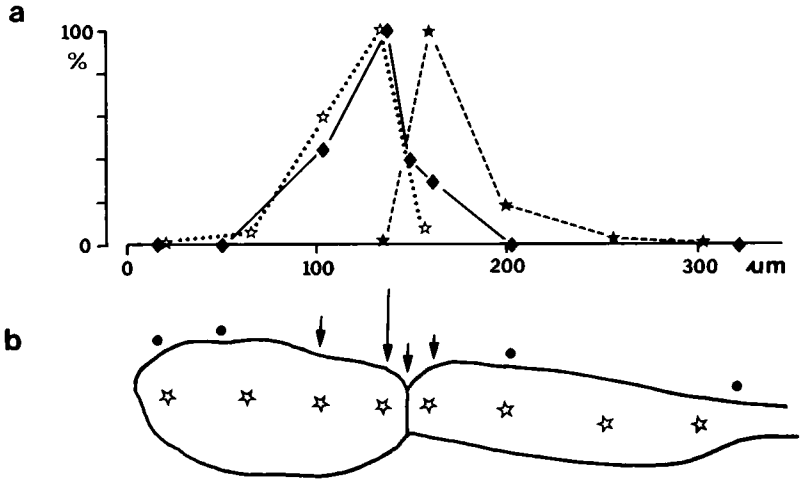

FIGURE 2 (*a*) The local sensitivity (stars) to dim 5 μm diameter light spots plotted at various positions along a cluster of two cells and the amplitude of the vibrating probe signal (diamonds) following diffuse light flashes that uniformly illuminated the entire surfaces of both cells. The open stars show the sensitivity of the cell on the left of the figure, the filled stars show the sensitivity of the cell on the right. Both the local sensitivity and the probe signal are expressed as a percentage of their respective maxima. The maximum probe signal was calibrated as 3 mA/cm^2. No outward current detectable above the background noise was found with the probe in the areas of the cell bodies shown in the figure. (*b*) Sketch of the cluster of two cells. The stars show positions at which a 5-μm diameter spot of light was placed to determine the local sensitivity of the cells to light. Length and direction of arrows indicate the relative magnitude and sign of the vibrating probe signal. The direction of the arrows gives the line of probe vibration. The dot indicates lack of a detectable probe signal at that position. The axon of the left-hand cell was not visible, but it probably enters the ventral nerve at the extreme left, under the cell body, and traverses the field of view beneath the photoreceptor cell bodies. The intensity of the diffuse flash used to determine the vibrating probe signals was -1 $\log_{10}$ unit, duration 100 ms. The spot elicited a criterion response of 3 mV when continuously illuminating the cell. The spot intensity was -3.7 and -4 $\log_{10}$ units when centered on the most sensitive region of the left-hand cell and right-hand cell, respectively.

elicited by diffuse illumination as indicated by the vibrating probe signal, which is also plotted in Fig. 1 *e*.

In Fig. 2 we illustrate a similar experiment performed on a cluster of two photoreceptors. For this experiment, measurements with the probe were completed before the determination of the local sensitivity profiles by impaling each of the photoreceptors with a microelectrode. As with single cells, the profile of local sensitivity of the two cells, determined with the 5-μm diameter spot of light, closely matched the magnitude of the probe signal elicited by diffuse light flashes that uniformly illuminated the entire surfaces of both receptors. The maximum probe signal indicates that most of the inward current flows close to the cleft between the two cells. Calman and Chamberlain (1982) showed that, in similar clusters of two cells, the rhabdomeral lobes of the cells are located where the distal ends of the cells touch, corresponding to the regions of maximum local sensitivity in Fig. 2.

We conclude that, as with other invertebrate and vertebrate photoreceptors, the cell body of ventral photoreceptors contains a special region through which photocurrent flows, corresponding to the region of greatest local sensitivity to light. Our result is consistent with a previous report that used suction electrodes to demonstrate that the photocurrent is localized to the area of the cell illuminated (Fein and Charlton, 1975*a*).

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