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5 August 1966

Simple Photoreceptors in Limulus polyphemus

Abstract. *The "olfactory nerve," the endoparietal eye, and the rudimentary lateral eyes of* Limulus (polyphemus) *contain simple photoreceptor cells that duplicate many of the electrical responses of the retinular cells of the lateral eye; the responses are a receptor potential consisting of an initial transient phase and a subsequent steady phase, low-amplitude fluctuations, and a small locally regenerative response to pulses of both light and current. Photic stimulation does not induce conducted action potentials, but does increase the membrane conductance. The receptor potential requires the presence of sodium ions in the external medium. Measurements of action and absorption spectra indicate a photopigment whose maximum absorption is of light with wavelength of 535 nanometers. The functional significance of these cells has not been ascertained.*

Gwilliam and Fahrenbach (*1*) have demonstrated that the eyes of barnacles are simple photoreceptors that are not organized into ommatidia. We now describe three analogous structures, the so-called olfactory nerves, the rudimentary lateral eyes, and the endoparietal eye, in *Limulus polyphemus*, and summarize our initial studies on their physiology.

The "olfactory nerves" in *Limulus* (*2*) consist of three bundles—two lateral and one medial—which run forward and ventrally from the protocerebrum to an integumentary structure of unknown function, variously called a ventral eye or olfactory organ (*3*). Large cell bodies (100 to 300 μ) are scattered along the desheathed lateral nerves. Our electron-microscopic studies show the presence of microvilli on the margins of the cell bodies.

The rudimentary lateral and endoparietal "eyes" are structurally very similar to each other (*3*). There are two of the former, one behind and internal to each compound lateral eye, and one of the latter, just behind and beneath the two external median eyes. These organs are easily recognized by the glistening white masses of connective tissue in which the photoreceptor cells are enmeshed. In each case, a tough sheath surrounds the whole organ. The axons are large and unbranched, those of each rudimentary eye running in two bundles into the nerve of the lateral eye and those of the endoparietal eye forming two bundles in the nerve of the median eye.

Impalement of these cells with microelectrodes is difficult unless the tough sheaths are removed or softened with trypsin. The former method is used on the olfactory nerves; the latter is used on the rudimentary and endoparietal eyes. Resting potentials in cells which give stable responses vary from −25 to −53 mv. A typical receptor potential is seen in Fig. 1*a*. There are two components: (i) an initial, transient depolarization which sometimes overshoots zero by as much as 15 mv; and (ii) a relatively flat steady phase which is always negative. Both the time-course and the amplitude of these components are affected by the degree of dark adaptation. For a light stimulus of constant intensity, the amplitude of the steady phase rapidly increases during the first 15 seconds of adaptation to dark and subsequently becomes time invariant. The amplitude of the transient phase rapidly increases for the first 60 seconds and continues to increase, although at a lower rate, for as much as 15 minutes. Cells fully adapted to the dark have a long transient phase (about 500 msec) while light-adapted ones show a sharper, faster transient phase (200 to 300 msec). Cells adapted to light usually exhibit a small repolarization between the end of the transient phase and the beginning of the steady phase.

Low-amplitude fluctuations, similar to those seen in the lateral eye of *Limulus* (*4*), appear when we allow the cells to adapt to the dark for 5 to 10 minutes (Fig. 1*b*). The amplitude of the fluctuation ranges from 1 mv to as high as 20 mv. Flashes of low intensity (large enough to give a receptor potential) reduce the fluctuations to zero.

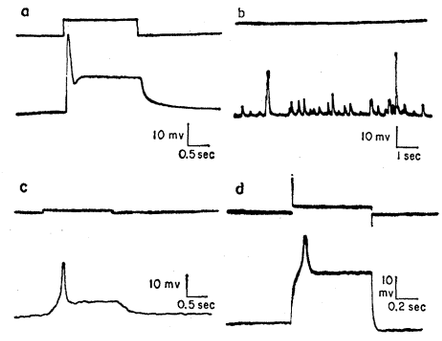

Fig. 1. Intracellular records from olfactory nerve cells. Upper trace monitors stimulus; lower trace monitors response. *a*, Receptor potential from light-adapted cell; *b*, low-amplitude fluctuations in dark-adapted cell; *c*, small spike-like response on steady phase of receptor potential with low-intensity light pulse; and *d*, spike-like response to depolarizing current pulse (approximately 10^{-8} amp). Experiments conducted at 20°C. The transient component of the receptor potential (*a*) should not be confused with the spike-like responses (*c* and *d*). Zero voltage for the lower trace (the response) is the base line of the upper trace (the stimulus monitor).

In cells adapted to the dark for about 60 seconds, the amplitude of the steady phase is a linear function of the logarithm of the light intensity throughout the range tested (Fig. 2). However, the amplitude of the transient phase is logarithmic only over the lower portion of the range and seems to become "saturated" in the upper portion.

The spectral sensitivity of the olfactory nerve cells, as determined by intracellular electrodes, is given in Fig. 3

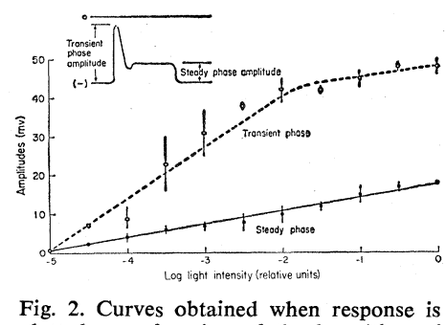

Fig. 2. Curves obtained when response is plotted as a function of the logarithm of the intensity for transient- and steady-phase components of receptor potential from olfactory nerve cells.

as the reciprocal of the relative number of quanta needed at each wavelength to evoke a 2-mv depolarization. The maximum sensitivity is at about 535 to 545 nm. The spectral sensitivity of these cells is similar to that of the lateral and median eyes of *Limulus* (*5*). The absorption spectra of the individual cells, measured in vivo with a microspectrophotometer (*6*), indicate a maximum bleaching at about 530 nm. It should be noted that maximum absorption at 520 nm has been reported for extracted pigment of the lateral eye of *Limulus* (*7*).

With light flashes of low intensity we often see a spike-like component on the leading edge of the receptor potential transient. If the time between light pulses is reduced, the receptor potential transient can be "adapted away," leaving the response, which is similar to an action potential, superimposed on the steady phase (Fig. 1*c*). This spike-like response has a duration of 150 to 300 msec and a maximum amplitude of about 25 mv. It is similar in shape and time-course to the "regenerative response" of the lateral eye as described by Benolken and others (*8*). The same response can be elicited with depolarizing pulses of current (Fig. 1*d*) or at the termination of a hyperpolarizing pulse. The response does not have a sharp threshold, and, up to a maximum of 25 mv, its amplitude depends upon the strength of the stimulus. We have never seen this response occur repetitively. In several experiments, we have seen the electrically evoked "regenerative response" in cells that were completely unresponsive to light. This finding suggests that there are two independent systems—one that gives rise to the receptor potential and another, the regenerative response.

With photic stimulation, in all three preparations we see electrotonically spread receptor potentials, but no evidence of propagated impulses. With electrical stimulation, action potentials are not recorded in nerve bundles running into the endoparietal and the rudimentary lateral eyes; however, in the lateral olfactory nerve, potentials of a single unit have been evoked with current in a few preparations. In each case this unit was not excited by light, although a sizable receptor potential could be recorded. It remains to be seen whether the axon involved is connected to a photoreceptor cell or to one of the "sensory" cells in the olfactory organ (*3*).

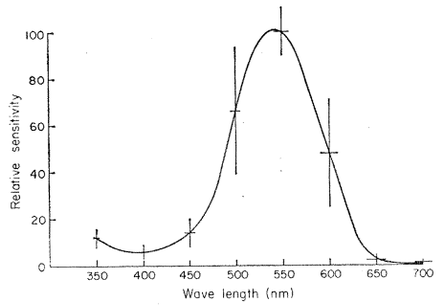

Fig. 3. Action spectrum of olfactory nerve cells (average of five experiments). Vertical bar indicates standard error in the mean.

That spikes are not produced upon photic stimulation implies that the regenerative response is not capable of leaving the cell body without marked decrement. These data are consistent with the reported failure to record propagated action potentials in the axons of arthropod retinular cells (*9*). There are several reports in which a second elevation of the compound action potential from the *Limulus* lateral eye nerve has been recorded and attributed to the population of retinular cell axons (*10*).

We altered the amplitudes of the transient and steady phases of the receptor potential by passing constant currents into the cell. In general, hyperpolarizing currents increase both amplitudes, and depolarizing currents decrease them. With sufficiently strong depolarizing currents, the receptor potential is abolished and in some cases inverted at a positive membrane potential. Using current pulses, we found that light produces a conductance increase in the cell, and, at least in the steady state, the greater the light intensity, the greater the magnitude of the conductance change. For a cell with a time constant of 20 msec, this can mean as much as 40 percent increase in the membrane conductance. The membrane capacitance is unaffected by exposure to light.

We varied the concentrations of the ions in the ambient medium to determine whether the change in conductance is ion specific. In artificial sea waters in which the sodium ions are replaced by sucrose or by lithium, choline, or tris(hydroxymethyl) aminomethane ions, the receptor potential is greatly reduced or often completely abolished, though reversibly. (The degree of reduction did not seem to depend on the substituent used.) Resting potentials under these conditions did not vary from the normal levels by more than 5 mv. Replacing sodium ions with potassium or ammonium ions depolarizes the cells to zero and abolishes the receptor potentials. Repolarizing the cell to −53 mv with currents through the electrode does not restore the receptor potential. However, if a small amount of sodium is present in such a medium with a high concentration of K (10 m*M* with respect to Na, 425 m*M* with respect to K), repolarizing the cells to −53 mv restores the receptor potential to about 15 percent of its normal amplitude. All media with reduced concentrations of sodium reduce or abolish the low-amplitude fluctuations and the nonpropagated action potential.

Sea water with little or no calcium greatly increases the amplitude of the steady phase and slightly increases that of the transient phase of the receptor potential. Sea water with a low concentration of magnesium has no effect. When nitrate is used instead of chloride, the cells become slightly hyperpolarized. The amplitude of the receptor potential increases, but the voltage to which the cell depolarizes upon photic stimulation remains constant.

Tetrodotoxin, a specific poison for the sodium component of action potentials, does not affect either the transient or the steady component of the receptor potential or the low-amplitude fluctuations even at concentrations of 10^{-2} m*M*. We do not have definitive evidence for an effect on the spike-like response. A 20-m*M* solution of the local anesthetic Procaine increases the amplitude of the transient phase by 25 percent and that of the steady phase by 75 percent; it decreases the fluctuations slightly. The effects of Procaine at this concentration are not easily reversed.

The function of these simple eyes is unknown. They may be neurosecretory organs and, in fact, Waterman and Enami (*11*) have presented cytological evidence for cyclic neurosecretory activities in the rudimentary eye cells. On the other hand, these cells are like sensory receptors in their ability to adapt rapidly and to respond to very small changes in light intensity. If they are receptors, the lack of propagated action potentials in their axons is perplexing. The space constants for the axons range from 2 to 4 mm—high values, but not large enough for meaningful electrotonic signals to reach the nearest synapses at least 6 cm away. These eyes may be used by the animal in its larval stages when the distances

between the receptors and the ganglia are much shorter. If this is the case, then perhaps the adult structures we have studied are simply vestigial organs.

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8 September 1966

Visual Spatial Aftereffect from Prolonged Head-Tilt

Abstract. Subjects with head upright were required to adjust a lighted bar in a dark room until the bar appeared vertical; the task was performed before and after 2 and 3 minutes of lateral head-tilt with their eyes closed. A visual spatial aftereffect was observed which varied as a function of the angle of head-tilt and which was opposite in direction to head-tilt.

The spatial judgments of a subject after he is exposed to visual, kinesthetic, tactile, or auditory stimulation differ from those judgments made before stimulation. Such modifications in judgments of size, shape, orientation, and direction (*1*) are referred to as figural, negative, or spatial aftereffects; these are well established for those sensory modalities which discriminate spatially. However, after one modality is stimulated (vision or kinesthesis) a spatial aftereffect does not occur when judgments are made with the other modality (*2*). Our experiments were concerned with judgments of visual orientation made after the head was tilted; we found that a visual aftereffect occurred when the head was returned to an upright position. Although changes in visual judgments of orientation occur during lateral head- or body-tilt (*3*), changes after prolonged tilt have not been reported. We have conducted two experiments confirming the occurrence of this spatial aftereffect and have shown that the magnitude of the effect is a function of the degree of head-tilt.

In the first experiment there were 30 subjects divided into two equal groups, one group serving as a control for the effects of head movement. Each subject was required to adjust a pivoted bar of light (152 by 0.9 mm) so that it appeared vertical. The bar, which was dimly lighted (2.08 mlam), was 183 cm from the eyes; there was no other source of light in the darkened room. The orientation of the bar could be read to the nearest 0.25 degree by means of a protractor scale. The angle of lateral head-tilt for the seated subject was controlled by a projecting rectangular frame pivoted on heavy uprights. An individual dental-composition bite-board was attached to the frame and was clenched between the subject's teeth throughout each trial. For the experimental group a trial consisted (i) of making, with head upright, five adjustments of the lighted bar to the vertical from each of five random starting positions (vertical and 5 and 10 degrees clockwise and counterclockwise); (ii) a 3-minute period with eyes closed and head tilted 30 degrees right or left; and (iii) a single adjustment of the bar to the vertical with the head again upright from the first of the five starting positions used in the pretilt series (*4*). The difference between the mean of the five adjustments before tilt and the single adjustment after tilt was a measure of the aftereffect. Before and after the tilt, the head was always upright. Each subject underwent one trial with head tilted left and one with head tilted right, the order of these alternating from one subject to another with a 5-minute interval between trials. For the control group the procedure was the same before and after the tilt, but in the intervening period the head was tilted 30 degrees left or right and then immediately returned to an upright position. This control was necessary since differences between adjustments before and after tilt could have been due to movement of the head from a slanted to an upright position with consequent stimulation of the semicircular canal system.

The first group (Fig. 1) showed a visual aftereffect of about 2 degrees in a direction opposite to head-tilt (*5*). Although there was no significant difference in the magnitude of the effect between left and right tilt ($p > .05$), the effect was significant for both when each direction was taken singly ($p < .05$) (*6*). There was no significant effect for the control group ($p > .05$).

In the second experiment eight subjects underwent nine trials similar to those of the first but with head tilted

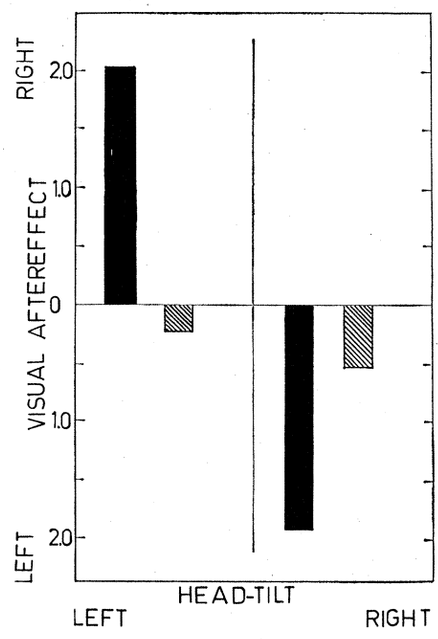

Fig. 1. Magnitude and direction of visual aftereffect resulting from 3-minute head-tilt to the left and to the right, together with control data.