Z. vergl. Physiologie 71, 315—325 (1971)
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Graded Illumination Potentials from Retinula Cell Axons in the Bug *Lethocerus*

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Received December 3, 1970

Summary. Intracellular responses to illumination have been recorded separately from the retinula cells and from their axons in the compound eyes of the giant water bug *Lethocerus*. The basic response in both places consists of an initial transient depolarisation followed by a plateau (Fig. 2). No action potentials were seen in either axons or retinula cells.

The responses are graded according to the intensity of the stimulus, to its position within the visual field of the cells and to the plane of polarization of the light (Figs. 3, 4). The angle of acceptance (dark-adapted eyes) measured in either retinula cells or axons is 9°. Similarly, the average value of the sensitivity ratio to light polarised at orthogonal planes is 3:1 in both places.

Experiments designed to reveal a presumed spike initiation region of the cells by reducing damage to the eye failed to reveal impulses. It is concluded that the receptor potential spreads electrotonically in the axon to the first synaptic region which lies up to 2 mm away. The values of membrane constants which would be required for conduction without severe decrement over such a distance are within the range measured in other systems.

Introduction

Except in the case of the honeybee drone (Naka and Eguchi, 1962), regenerative action potentials never appear in insect retinula cells when the recordings are made from the region of the microvillar rhabdom, the presumed site of generation of the receptor potential (Hagins *et al.*, 1962; Lasansky and Fuortes, 1969). It has been a matter of some speculation (e.g. Baumann, 1968) whether the graded receptor potential spreads electrotonically along the retinula cell axons to the first synaptic region. A good deal of circumstantial evidence suggests that this might be the case. Burtt and Catton (1956, 1959), using extracellular electrodes advanced through the eye of intact locusts from the corneal surface, discovered that it was not until an electrode was in the second synaptic layer (the medulla) that spikes were recorded. In the retina and the lamina they could detect only slow "illumination potentials". More recently Shaw (1968) and Scholes (1969) recorded slow potentials with the electrode placed intracellularly in the laminae of locust and housefly

respectively. In each case the responses were assumed to be from second-order neurons and the time course of the potentials followed closely that of the respective receptor potentials. Baumann (1968) has also looked at the response of an unidentified structure in the drone bee lamina. Both the single spike, characteristic of the onset of the receptor potential, and the subsequent slow response reached the lamina and maintained their typical relationship and time course.

The eye of the giant water bug *Lethocerus* affords an ideal preparation to record intracellularly the responses of retinula cell axons. The lamina is set back in the optic lobe near the middle of the head and the axons which emerge from the proximal surface of the basement membrane of the retina travel 1–2 mm to reach the optic lobe. The axons are 8–12 μm in diameter and easy to penetrate with a microelectrode. It is the purpose of the present report to show that the axons give graded responses, in every respect similar to the retinular receptor potential and in so doing to describe some of the basic functional characteristics of the eye. A more rigorous demonstration of the electrotonic nature of the conduction will be the subject of a subsequent communication.

Methods

Three species of giant water bug were used: *Lethocerus insulanus, Benacus griseus* and *Lethocerus americanus.* The latter two species were caught in Florida and were air mailed to Canberra in moist bags. The journey lasted seven days and the mortality rate was less than 20%. The Australian species, *L. insulanus* was collected in Northern New South Wales. All animals were kept in individual glass jars and occasionally fed on locusts. Under these conditions the animals survived for many months. Apart from external markings no morphological or physiological differences were observed in the eyes of the three species.

All experiments were performed at room temperature (20–25° C) on animals which had been adapted in total darkness for at least two hours. In order to eliminate diurnal variations, the same experimental time was adopted throughout, viz. middle to late afternoon. The eye was prepared for recording by bisecting the excised head sagittally. The lateral part of the eye was then sliced off with a razor blade in a plane parallel to the median plane. The half head was placed on a piece of filter paper moistened with Belastomid saline (Lockwood, 1961) buffered to pH 7.1 with HCO_3^-, and in contact with the indifferent silver wire electrode. By this arrangement the mediolateral axis of the eye was oriented vertically (Fig. 1). All these operations were carried out in dim white light and the eye was never brightly illuminated except for periods of less than one minute during the positioning of the electrode. The preparation was kept moist with saline and responses could be obtained for several hours.

Best results were obtained with microelectrodes filled with 2.8 M KCl and having resistances of 50–100 MΩ measured in physiological saline. The electrodes were advanced vertically through the eye either in the retina itself or in a zone 100–200 μm proximal to the basement membrane (Fig. 1). In this region histological examination revealed that the only structures present are tracheae, fat bodies and bundles of retinula cell axons which are discernible with a dissecting microscope.

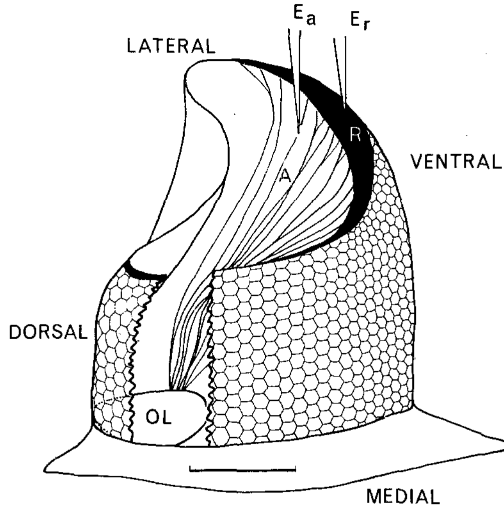

Fig. 1. Schematic representation of the preparation. *R* retina, *A* retinula cell axons, *OL* optic lobe (incorporating lamina), E_r position of electrode for retinula cell recordings, E_a position for axonal recordings. Scale about 500 μm

Successful and stable penetrations were characterized in all instances by a resting potential in excess of 30 mV. The recording method was conventional using a high impedance capacity-compensated input stage. Sometimes for the sake of baseline stability and ease of measurement the preamplifier was capacitatively coupled to the CRO.

The preparation was mounted on a firm platform with the eye at the centre of a perimeter device carrying the stimulating lamp. The stimulus was a pinhole exposed to light from a D.C. operated tungsten filament by means of an electromechanical shutter. The pinhole could be moved in spherical co-ordinates about the eye and could be placed at any point in the greatest part of the visual field with an accuracy of better than 0.5°. The pinhole subtended an angle of 0.8° at the cornea and was effectively a point source. The lamp was furnished with facilities for attenuation of the beam with neutral density filters (Kodak "Wratten") and with a polaroid plastic which could be rotated axially in 10° steps. The light emitted from the filament was found to be polarized and had to be demodulated by means of a pile of coverslips set at an angle to the beam.

Results

Basic Response

Graded slow depolarizations are produced in response to illumination. The responses of retinula cells are indistinguishable from those of their axons (Fig. 2A, B). The responses to high light intensities conform to the familiar sequence of a transient phase followed by a steady state plateau. A small, faster wave rides on the initial part of the transient

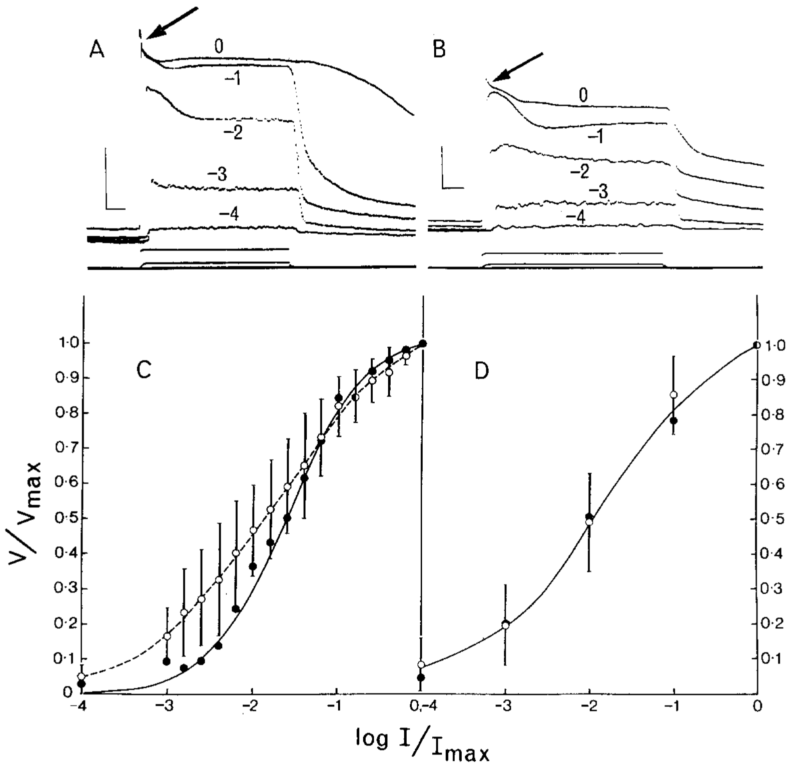

Fig. 2A–D. Superimposed sweeps of retinula cell responses to flashes of light of different intensities (in log units). Stimulus interval, about 10 sec, starting with the weakest. Lower trace, photocell record. Calibrations, 10 mV, 200 msec. B As in A but records taken from an axon. C and D Response-intensity relations for retinula cells (filled circles) and for axons (open circles). C Averages for response transients from 7 retinula cells and 15 axons. Solid curve, the function $I/I + k$ (see text). Spread is similar for both but ± 1 S.D. shown only for axons. D Averages from steady state plateau from 8 retinula cells and averages $\pm$ 1 S.D. from 12 axons. In contrast to the case of the transients there is, here, a high level of coincidence between axons and retinula cells

with the stronger stimuli (arrow). The responses to lower intensities are, again typically, uniform, maintained depolarizations often decorated with discrete bumps. The maximum depolarizations elicited in the retinula cells by the present light sources are 35–40 mV at the peak of the transient. The extent of attenuation which one expects to find in the axons if the conduction is decremental depends on the space constant of the fibre and the distance of the electrode from the rhabdom. The axonal response shown in Fig. 2B is just over 30 mV and it is the largest ever obtained (normal range 15–25 mV).

A relationship between response transient height and light intensity is easily derived from an electrical analogue of a photoreceptor membrane incorporating one light dependant conductance channel. Such a model has been applied to *Limulus* photoreceptors (Fuortes, 1959; Rushton, 1959; Benolken, 1961) and to vertebrate cones (Baylor and Fuortes, 1970):

$$\frac{V}{V_{\max}} = \frac{I}{I+k}$$

where V = response height to light intensity I, $V_{\max}$ = response height to maximum light intensity and k is a constant for the cell and is equal to the light intensity required to give a response equal to $0.5\,V_{\max}$.

It is evident from Fig. 2C that the response—intensity relation in the retinula cells measured on the visual axis of the cell fits this theoretical model. The validity of the equation rests on the assumption that at $V_{\max}$ the cell is short-circuited to the outside, i.e., the light-dependent conductance has reached an infinite value and the response is saturated. Shaw (1969) gives adequate evidence that this condition is approximated in the retinula cells of drone bee and locust. In the present experiment the stimulating sources were too weak to effect full saturation but the approximation is obviously good.

The fulfilment of the above condition is also suggested by the results of voltage clamp studies of Brown *et al.* (1969) on barnacle photoreceptor cells and also those of Millecchia and Mauro (1969) on the ventral eye of *Limulus*. The latter authors have measured currents of up to 300 nA during the transient driven by an emf of 15 mV. This corresponds to a cell input resistance of 50 KΩ. They have also shown that the "light-dependent" conductance is in fact composed of two parts, a voltage-time dependant "dark conductance" and a light-voltage-time dependent "light conductance". In the unclamped membrane the two components are functionally inseparable during the physiological response and for the transient peak they can be treated, together with their respective emf's, as one, lumped photosensitive channel. This is justifiable because the indication is that they both reach their maximum values at about the time of the peak of the transient.

The response transient-intensity curve taken from the axons, however, deviates considerably from the above relationship. It takes a shallower course and the spread indicated in Fig. 2C suggests that the difference may be significant. In order to test this point the gradients of the near linear parts of the curves taken from each individual cell (usually between $\log I = -1$ and -3) were measured graphically and used as indicators of the shape of the curves:

Average gradient of 7 retinula cells: 50.2 (in arbitrary units).

Average gradient of 15 axons: 40.4.

The difference in the means is significant at the 2% level ($t_{20} = 2.65$). Zettler and Järvilehto (1970) have reported a qualitatively similar difference between proximal and distal retina in the blowfly.

The reason for this difference between the retinula cells and axons is not clear but it may be partly due to the selective attenuation by the axonal cable of the higher waveform frequencies inherent in the rising phase of the transients generated by the higher light intensities. Nothing is known of the membrane constants involved and it would be futile to pursue this point further.

Visual Fields

Additional evidence for the complete equivalence of retinula cells and their axons comes from determinations of the visual fields. The centre of the field was first located. The extent of the field was then measured by presenting the ommatidium with stimuli of 50 msec duration once every 4 sec. Between flashes the pinhole was moved 1° at a time through the centre of the field either in a mediolateral or in a dorsoventral direction. The intensity of the light was attenuated so that the cell could function in its best dynamic range and also in order to prevent progressive light adaptation. The response heights were converted to equivalent light intensities by reference to the response—intensity curve for the same cell and thence to relative sensitivities on a linear scale. The results are summarized in Fig. 3. It is clear from Fig. 3C that the receptive fields measured in the retina are the same as those measured in the axons. The points are fitted reasonably by a Gaussian function with bandwidth at half height equal to 9°. There is a suggestion that above the 50% level the Gaussian is broader than the experimental results. Tunstall and Horridge (1967) reached a similar conclusion on the basis of optokinetic studies in the locust.

The measurements described here were, of course, taken with the eye in air. When the bug is in water the acceptance angle for light arising within the water is reduced by a factor equal to the refractive index of the water/cornea interphase (for small angles $\sin \Theta = \Theta$ rad). If the figure 1.5 is taken as an approximation to the refractive index of air/cornea (Kuiper, 1966—1.499 in blowfly, Varela and Wiitanen, 1970—1.490 in worker bee) and assuming the refractive index of water to be 1.33 the index for water/cornea works out at 1.13, which reduces the acceptance angle to 8°. For light arriving at the eye from air through water the angle is still 9°, the overall refractive index in this case being equal to that for cornea in air.

Polarized Light Sensitivity

The sensitivity of each cell to polarized light shone at the centre of its visual field was also measured, by a procedure analogous to that used for measuring the angle of acceptance. Fig. 4 shows that in this respect also the axons and the retinula cells are equivalent. The solid line in Fig. 4C is the function $2 \cos^2 \psi + 1$, where ψ denotes the angular orientation of the plane of polarization. This is essentially the intensity

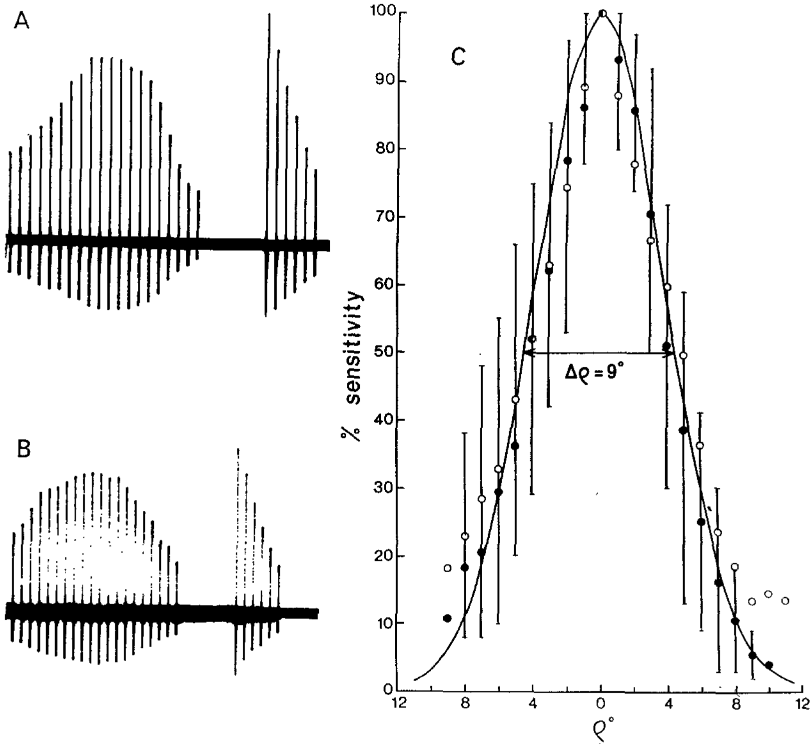

Fig. 3A–C. Responses to flashes at 1° intervals through the receptive field centres of a retinula cell (A) and an axon (B) recorded on a very slow time base (AC coupled). On the right of each sequence is a record of the responses of the cell to a series of flashes delivered at the centre of the field and each 0.3 log units (50%) dimmer than the previous. Response—intensity relations as those of Fig. 1C are plotted from such series. Angular or polarized light sensitivities for each cell are calculated by reference to its own response-intensity "calibration" curve. C Average angular sensitivities from 7 retinula cell fields (filled circles) and 8 axonal fields (open circles). No difference was found between mediolateral and dorsoventral extents. The solid curve is a Gaussian function arbitrarily chosen to fit the experimental points. For the sake of clarity 1 S.D. bars are shown for the axons only to the left of the peak and for the retinula cells only to the right of the peak. The variability is mainly attributable to irregularities in individual fields

of light transmitted or absorbed by an analyser as a polarizer is rotated in front of it, scaled to fit the sensitivity ratio measured experimentally (Shaw, 1969). The same range of values is evident in the retinula cells and in the axons. There are two classes of cells with polarized light sensitivity peaks 90° apart but results from only one class are included in Fig. 4. Cells of the orthogonal class are very rarely impaled.

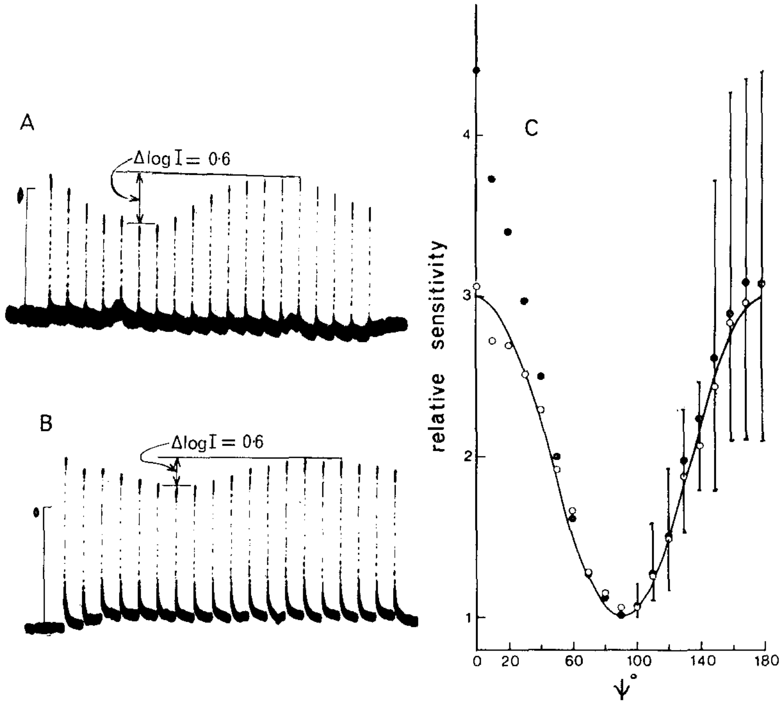

Fig. 4 A–C. Responses from a retinula cell (A) and an axon (B) to flashes of polarized light (delivered on axis), with the plane of polarization rotated by 10° between each flash. Voltage calibration, 10 mV. C Average results from 5 retinula cells (filled circles) and from 5 axons (open circles). Total spread similar for both but shown only for the axons. Solid curve, the function $2 \cos^2 \psi + 1$ (see text). The results from the retinula cells are asymmetrical about 90° because of some progressive adaptation to the stimulus. The effect in terms of the decrease in the absolute sensitivity of the cells is small and does not invalidate the applicability of the response—intensity relation to the second half of the polarised light response sequences. Note that the starting orientation of the plane of polarization is different from that in A and B

Discussion

Kennedy (1964) has attributed the absence of impulses from the responses recorded from most insect photoreceptors to experimental damage. Anoxia and electrode penetration damage are blamed for interfering with the function of a presumed spike initiation site which, in the unique case of the drone has been located at the base of the receptors by Naka and Eguchi (1962), although Shaw (1969) maintains that the action potential may invade the receptor itself. Part of Kennedy's criticism has been answered by Autrum and von Zwehl (1962, 1964)

who probed intracellularly in whole animals which were fed and maintained at body temperature, so that they survived under experimental conditions for two days. The characteristic initial spike was seen in drone but not in worker retinula cells. In order to prevent penetration damage Baumann (1968) recorded extracellularly in the drone and even so he failed to see spike trains superimposed on the receptor potential.

In the hope of avoiding penetration damage to a spike initiation centre some recordings were taken from the axons of the bug at locations several hundred μm proximal to the basement membrane, near the optic lobe. These responses, although reduced in amplitude and suffering from some temporal smoothing, still failed to reveal spikes. This left open the question of interruption of the blood and air supply to the head and so, experiments were performed on the intact bug, with the legs immobilized with insect wax. As small a cut as possible was made in the eye, but in order to facilitate penetration and to hold the cells for a reasonable time respiratory movements had to be dampened by the application of 2% agar in physiological saline to the cut end of the eye. The responses recorded 100–200 μm from the basement membrane were in every respect similar to those shown in Fig. 2B.

The weight of all these lines of evidence taken along with that derived from the work of Burtt and Catton (1956, 1959) on perfectly intact locusts (although slightly anaesthetized with urethane and kept moist with mammalian Ringer) and also the recordings taken from the first synaptic region (Baumann, 1968; Shaw, 1968; Scholes, 1969) seems to favour the hypothesis that insect photoreceptors do not generate trains of impulses but rely on electrotonic spread of slow potentials for the transmission of information to their terminals. The same is true of a crab muscle receptor which has been described by Ripley *et al.* (1968) and which can evoke reflexes without itself generating all-or-none impulses (Bush and Roberts, 1968). In this case the receptor potential has to be effective at a synapse 3–4 mm from the point of origin. This would require a long space constant and the large diameter of the fibre (50–60 μm) would be helpful in this respect. If one takes the membrane resistance (R_m) as 5000 Ω-cm^2 and the internal resistivity (R_i) as 60 Ω-cm, the length constant for a fibre 60 μm in diameter is 3.5 mm. The slowest signals would be reduced to approximately $^1/_3$ near the first synapses. The same problem arises in the insect eye, particularly in the water bug where distances of 1–2 mm have to be covered. The same figures for the bug retinular axons (diameter 10 μm) give a length constant of 1.4 mm. R_m varies widely even between animals of the same class (see for example Katz, 1966, pp. 46–47) and Boistel's (1959) estimate of 610 Ω-cm^2 for the giant axon in the cockroach ventral cord need not be universally applicable to insects.

Therefore, it may not be essential to look for or invoke new principles in the understanding of transmission without impulses. In spite of this, the mechanism of transmission at the first synapse is still enigmatic, especially in cases where optomotor responses can be obtained to visual stimuli of intensities so low as to elicit nothing but 1 mV quantum bumps in the primary photoreceptors (Scholes and Reichardt, 1969).

Note added in proof. Järvilehto and Zettler (1970) have now identified the responses obtained by Scholes (1969) from the lamina of the fly as electrotonically conducted receptor potentials in the retinula cell axons.

Järvilehto, M., Zettler, F.: Micro-localisation of lamina-located visual cell activities in the compound eye of the blowfly *Calliphora*. Z. vergl. Physiol. **69**, 134–138 (1970).

References

Autrum, H., Zwehl, V. von: Zur spektralen Empfindlichkeit einzelner Sehzellen der Drohne (*Apis mellifica* ♂). Z. vergl. Physiol. **46**, 8–12 (1962).
— — Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z. vergl. Physiol. **48**, 357–384 (1964).
Baumann, F.: Slow and spike potentials recorded from retinula cells of the honeybee drone in response to light. J. gen. Physiol. **52**, 855–875 (1968).
Baylor, D. A., Fuortes, M. G. F.: Electrical responses of single cones in the retina of the turtle. J. Physiol. (Lond.) **207**, 77–92 (1970).
Benolken, R. M.: Reversal of photoreceptor polarity recorded during the graded receptor potential response to light in the eye of *Limulus*. Biophys. J. **1**, 551–564 (1961).
Boistel, J.: Quelques caractéristiques électriques de la membrane de la fibre nerveuse au repos d'un insecte (*Periplaneta americana*). C.R. Soc. Biol. (Paris) **153**, 1009–1013 (1959).
Brown, H. M., Meech, R. W., Koike, H., Hagiwara, S.: Current-voltage relations during illumination: photoreceptor membrane of a barnacle. Science **166**, 240–243 (1969).
Burtt, E. T., Catton, W. T.: Electrical responses to visual stimulation in the optic lobes of the locust and certain other insects. J. Physiol. (Lond.) **133**, 68–88 (1956).
— — Transmission of visual responses in the nervous system of the locust. J. Physiol. (Lond.) **146**, 492–515 (1959).
Bush, B. M. H., Roberts, A.: Resistance reflexes from crab muscle receptors without impulses. Nature (Lond.) **218**, 1171–1173 (1968).
Fuortes, M. G. F.: Initiation of impules in visual cells of *Limulus*. J. Physiol. (Lond.) **148**, 14–28 (1959).
Hagins, W.A., Zonana, H. V., Adams, R. G.: Local membrane current in the outer segments of squid photoreceptors. Nature (Lond.) **194**, 844–847 (1962).
Katz, B.: Nerve, muscle and synapse. New York: McGraw Hill 1966.
Kennedy, D.: The photoreceptor process in lower animals. In: Photophysiology (A. C. Giese, ed.), Vol. II, p. 79–123. New York and London: Academic Press 1964.
Kuiper, J. W.: On the image formation in a single ommatidium of the compound eye of diptera. Symp. Functional Organization of the Compound Eye 35–50 (C. G. Bernhard, ed.). Oxford: Pergamon Press 1966.

Lasansky, A., Fuortes, M. G. F.: The site of origin of electrical responses in visual cells of the leech *Hirudo medicinalis*. J. Cell Biol. **42**, 241–252 (1969).
Lockwood, A. P. M.: "Ringer solutions" and some notes on the physiological basis of their ionic composition. Comp. Biochem. Physiol. **2**, 241–289 (1961).
Millecchia, R., Mauro, A.: The ventral photoreceptor cells of *Limulus*. III. A voltage clamp study. J. gen. Physiol. **54**, 331–352 (1969).
Naka, K. I., Eguchi, E.: Spike potentials recorded from the insect photoreceptor. J. gen. Physiol. **45**, 663–680 (1962).
Ripley, S. H., Bush, B. M. H., Roberts, A.: Crab muscle receptor which responds without impulses. Nature (Lond.) **218**, 1170–1171 (1968).
Rushton, W. A. H.: A theoretical treatment of Fuortes's observations upon eccentric cell activity in *Limulus*. J. Physiol. (Lond.) **148**, 29–38 (1959).
Scholes, J.: The electrical responses of the retinal receptors and the lamina in the visual system of the fly *Musca*. Kybernetik **6**, 149–161 (1969).
— Reichardt, W.: The quantal content of optomotor stimuli and the electrical responses of receptors in the compound eye of the fly *Musca*. Kybernetik **6**, 74–80 (1969).
Shaw, S. R.: Organisation of the locust retina. Symp. Zool. Soc. Lond. **23**, 135–163 (1968).
— Inter-receptor coupling in ommatidia of drone honey bee and locust compound eyes. Vision Res. **9**, 999–1029 (1969).
Tunstall, J., Horridge, G. A.: Electrophysiological investigation of the optics of the locust retina. Z. vergl. Physiol. **55**, 167–182 (1967).
Varela, F. G., Wiitanen, W.: The optics of the compound eye of the honey bee (*Apis mellifera*). J. gen. Physiol. **55**, 336–358 (1970).
Zettler, F., Järvilehto, M.: Histologische Lokalisation der Ableitelektrode. Belichtungspotentiale aus Retina und Lamina bei *Calliphora*. Z. vergl. Physiol. **68**, 202–210 (1970).

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