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INITIATION OF IMPULSES IN VISUAL CELLS OF *LIMULUS*

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Some features of the responses recorded from visual cells of *Limulus* with intracellular electrodes have been described in a previous article (Fuortes, 1958*b*). Cells producing spikes of more than 40 mV were tentatively identified with eccentric cells. The response of these cells to prolonged illumination was found to consist of a sustained depolarization (generator potential) with superimposed spikes. It was observed that, in steady-state conditions, both amplitude of the generator potential and frequency of firing are approximately linear functions of the logarithm of light intensity, frequency being therefore a linear function of generator potential amplitude. Impulse firing could also be elicited by means of depolarizing currents applied through the impaling micro-electrode and frequency of firing was then found to be a linear function of current intensity.

In the present article some properties of responses elicited by illumination will be compared to those of responses evoked by currents and the results of interaction of light and currents will be described. The evidence obtained in this way indicates that membrane resistance decreases during illumination and suggests that a change of membrane permeability may be responsible for production of the generator potential.

METHODS

The eye was dissected from the animal and cut in two along its longest diameter. One half of the eye was immersed in artificial sea water and could be observed through a dissecting microscope ($\times 100$) under whose field the micro-electrode and the light beam were made to converge.

The electronic arrangement used for recording consisted of a 'negative capacitance' feedback input stage (Bak, 1958), a direct-coupled amplifier and a cathode-ray oscilloscope. Care was taken to maintain grid current to less than 10^{-12} A and it was usually possible to adjust feedback to a value giving a frequency response of over 10 kc/s without increasing noise to unbearable values.

When currents were passed through the intracellular micro-electrode a bridge circuit was used in order to balance the potential drop occurring at the input as a consequence of current flow. This

circuit was essentially similar to that described in a previous article (Frank & Fuortes, 1956) and is reproduced in Fig. 1. For the sake of simplicity the current through the micro-electrode was not monitored but was calculated from the values of the stimulating voltage and of the resistances R_3 and R_e. In order to reduce changes of current intensity due to changes of micro-electrode resistance, a value of 1000 MΩ was selected for R_3. If micro-electrode resistance changed considerably during current flow, a potential change due to bridge unbalance was recorded (see Frank & Fuortes, 1956, Fig. 11). Records revealing excessive changes of bridge balance during flow of current were therefore discarded. Compensation of steady potentials between micro-electrode and ground and calibration of electrode resistance were made as described by Frank & Fuortes (1955, p. 629; 1956, p. 452).

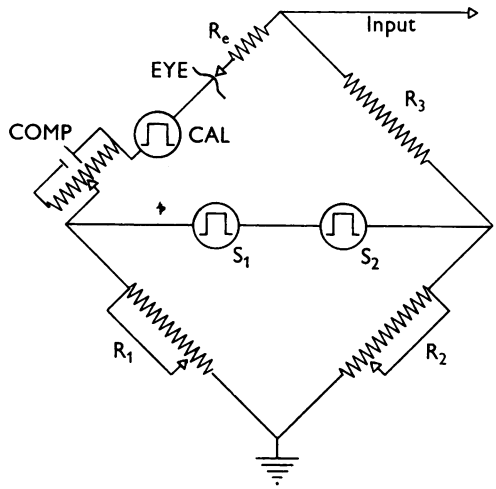

Fig. 1. Diagram of arrangement used for electrical stimulation and recording. R_e represents the micro-electrode, which usually had a resistance of 20–100 MΩ. The values of the resistors used are as follows: R_1, 1 kΩ; R_2, 30 kΩ; R_3, 1000 MΩ. The resistance of the pulse generator CAL is 100 Ω and the resistance COMP has the same value. Polarity of the compensation battery can be reversed by a switch not shown in the diagram. Electrical stimuli are delivered by S_1 and S_2. Stimulating and calibrating pulses are applied through radio-frequency stimulus isolation units. R_3 can be replaced by a 44 MΩ resistance by means of a switch not shown in the diagram. Comparison of the magnitude of a given calibrating signal recorded with a 44 MΩ shunting resistance and without shunt permits convenient measurement of electrode resistance (Frank & Fuortes, 1955). The preparation (EYE) is described in the text.

The light source was a 100 W concentrated arc lamp (Sylvania C 100) which emitted a bright white light, and the eye was stimulated by the image of a variable diaphragm. The diameter of this image varied between 50 and 500 μ in different experiments. Calibrated neutral filters were used to reduce light intensity as desired. The light was switched on and off by a mechanical shutter which was usually triggered by an electric pulse synchronized with the sweep of the cathode-ray oscilloscope.

The micro-electrodes were KCl-filled pipettes constructed and mounted as described in a previous article (Frank & Fuortes, 1955). It was observed that better results were obtained when high resistance (50–100 MΩ in Ringer's solution) electrodes were selected.

RESULTS

Responses evoked by light or current

The features of the responses recorded following stimulation with light or with depolarizing current through the micro-electrode have been described in a previous article (Fuortes, 1958*b*). Typical responses are illustrated in Fig. 2.

In Fig. 3 frequency of firing is plotted as a function of the current intensity in the dark (●) and of the generator potential amplitude in the absence of

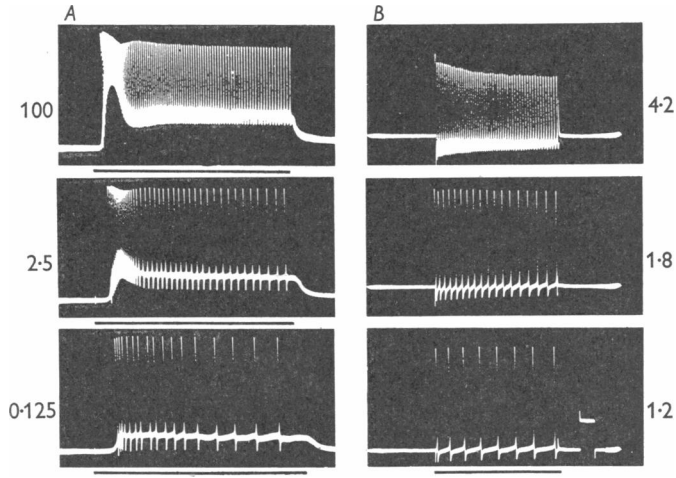

Fig. 2. Responses to light and to depolarizing currents. *A*. Responses to illumination at different intensities. Duration of illumination is slightly different in the three records, as is indicated by the black lines below. Figures on left give relative intensity of the light used. Opening of the shutter is indicated by small artifact before each response. Note decrease of latency with increasing intensity of light, *B*. Responses to depolarizing currents. Same unit as in Fig. 2*A*. Duration of depolarizing current was identical in all cases, as indicated by black line below. Figures on right give intensity of depolarizing current in nA. Square wave at bottom right is a 20 mV calibrating pulse; time line at bottom right, 1 sec.

current in the light (○). All measurements were made in the steady state and the amplitude of generator potentials was measured as the difference between membrane potential during illumination and membrane potential in darkness (see Fuortes, 1958*b*, p. 213). In this plot the slope of the straight line relating frequency of firing to generator potential amplitude is 0·77 $\frac{\text{imp/sec}}{\text{mV}}$ and the slope of the line relating frequency to current intensity is 4·44 $\frac{\text{imp/sec}}{\text{nA}}$. The ratio of the two slopes is 5·74 MΩ. In five other units in which responses to lights and to currents could be compared with some accuracy, values ranging

from 4 to 9 MΩ were obtained. If one assumes that currents producing firing at a certain frequency evoke in the cell a membrane depolarization identical with that elicited by light producing firing at the same frequency, then the ratio mentioned above measures the resistance of the cell's membrane. This assumption appears reasonable in first approximation but there is no assurance that it is strictly correct. For this reason the values obtained by the indirect method used in this study should be considered only approximate estimates of the membrane resistance of the impaled cells.

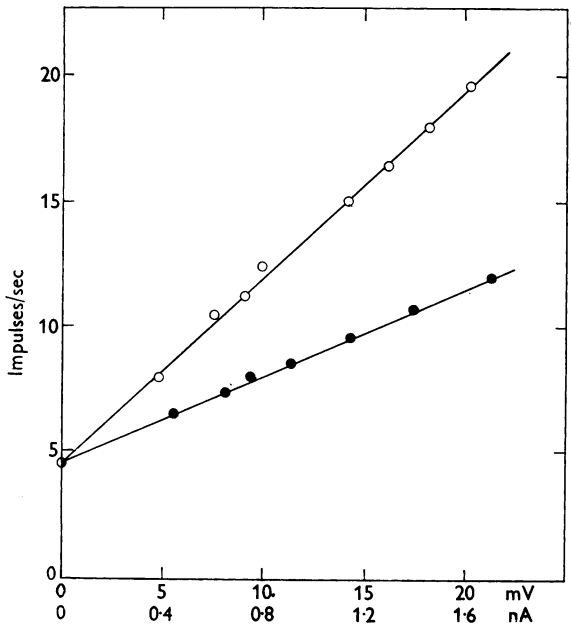

Fig. 3. Frequency of firing as a function of generator potential amplitude and of current intensity. Data from the same unit of Fig. 2 but in steady-state conditions (15–20 sec after start of stimulus). Abscissa, generator potential amplitude or depolarizing current intensity; ordinate, impulses/sec. The unit was firing continuously at this time, owing to some background illumination. ○, frequency as a function of generator potential amplitude; ●, frequency as a function of current intensity.

It is seen in Fig. 2*A* that the bottoms of the spikes are raised while the tops of the spikes are lowered when depolarization and frequency increase (cf. Hartline, Wagner & Tomita, 1953). The records obtained with stronger intensities of light show, in addition, that spike size and top potential decrease progressively with time during a prolonged train of impulses, even if frequency does not change during that time. When firing is evoked by depolarizing currents, potentials cannot be measured relative to the initial membrane potential, but it can be seen (Fig. 2*B*) that peak-to-peak spike amplitude

decreases with increasing frequency of firing and with time, as happens also with illumination.

Peak-to-peak spike amplitude is plotted as a function of frequency of firing in Fig. 4. The measurements taken when firing was induced by light are indicated by open circles, while the filled circles indicate measurements performed on the same unit when firing was elicited by depolarizing currents. In both cases spike amplitude was measured 5 sec after initiation of firing, and it is reasonable to assume that the difference in the early phases of the discharges evoked in the two ways (see Fig. 2) had little influence on the measurements

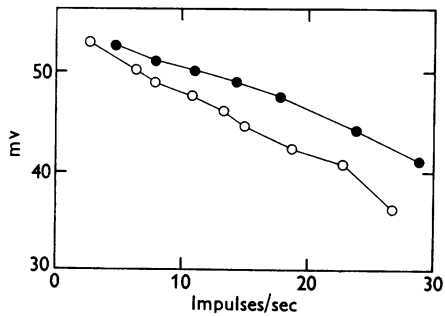

Fig. 4. Peak-to-peak spike amplitude as function of frequency. ○, firing at different frequency was evoked by illuminations of different intensity; ●, firing of the same unit was elicited by depolarizing currents through the micro-electrode. Peak-to-peak spike size was measured 5 sec after onset of light stimulation. Note that the spike was small in this unit (about 53 mV for slow firing frequency). In units producing larger spikes there was less difference in size of spikes evoked by light or by depolarizing current.

taken at this time. Comparison of the two plots shows that, for any given frequency of firing, spike size is smaller when firing is induced by illumination. Results to be presented later in this paper suggest that this difference may be the consequence of a decrease of resistance of the cell's membrane during illumination (see Fatt & Katz, 1951, for comparable results on the motor end-plate).

Combined action of light and current

Hartline, Coulter & Wagner (1952) found that frequency of firing elicited by a given light is increased by currents flowing through the eye in one direction and decreased by currents in the opposite direction. Similar experiments dealing with interaction between light and current were performed in the present study. In the experiment illustrated in Fig. 5, a steady current (0·9 nA in the top record) was passed through the impaling micro-electrode, and responses were evoked on this background by lights of different intensities (0·4, 6·25, 100).The steady current was then changed (−0·9, −2·3, −4·3 nA) and the same light stimulations were applied. The results show that depolarizing currents (+) decrease the drop in potential recorded through the bridge

and increase frequency of firing, whereas the reverse happens with hyperpolarizing currents (−).

As is shown in the plot of Fig. 6, the relation between recorded potential and intensity of current through the electrode is approximately linear for any one

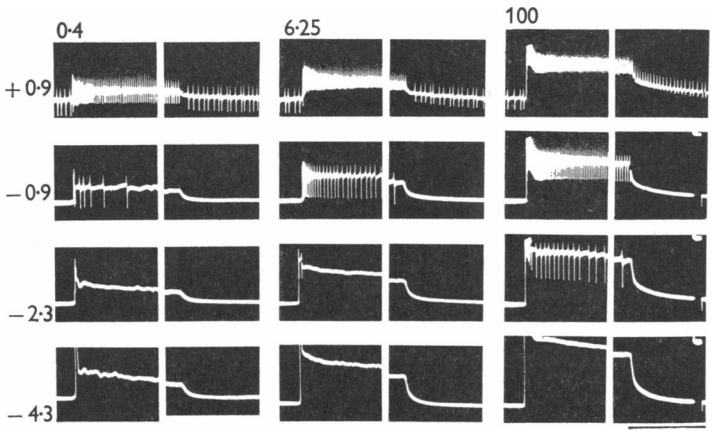

Fig. 5. Changes induced by currents in responses to light. Hyperpolarizing (−) or depolarizing currents (+) of intensity indicated by the figures on left were applied, and responses were produced by illumination at different intensities (0·4, 6·25, 100). Each pair of records shows beginning and end of a response to illumination lasting 15 sec. Since records were taken at high gain only a small portion of the spikes is visible. Square wave at extreme right is a 20 mV calibration pulse. Time line at bottom is 1 sec.

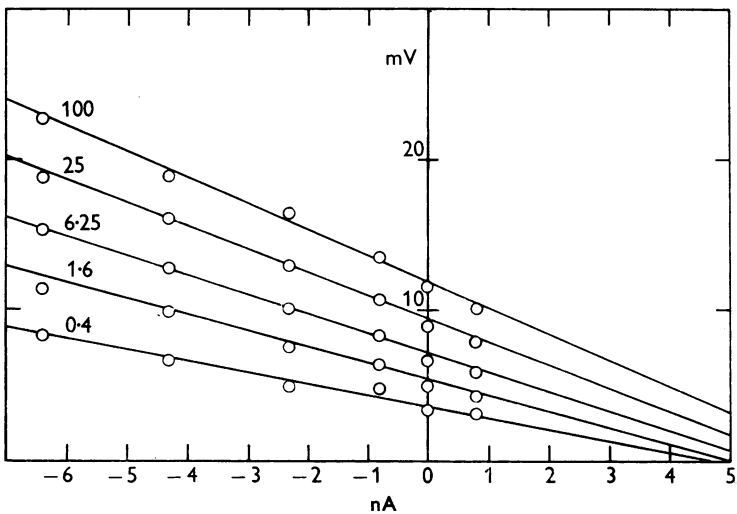

Fig. 6. Amplitude of potential change recorded following illumination, as a function of current. Data from same cell as Fig. 5. Abscissa, intensity of hyperpolarizing (−) or depolarizing (+) current through the micro-electrode; ordinate, generator potential amplitude. Each line joins points obtained with a given light intensity, as indicated by the figures on left. Note that the lines intersect the abscissa at different points.

of the light intensities used, and the lines obtained with different light intensities converge towards the right-hand side of the plot. It should be noted that the ordinate in this plot measures the change of membrane potential following illumination, but the drop in potential evoked upon the membrane by currents alone is disregarded.

If one extends the assumption proposed at the beginning (p. 17), postulating that frequency of firing is uniquely determined by membrane potential, then the frequency of firing evoked by any combination of light and current should bear the same relation to membrane potential as is found following stimulation with light only. If this postulate is valid, it should be possible to combine potential and frequency measurements in a single plot, in which the ordinate measures either membrane potential or frequency of firing.

In the unit of Fig. 6, the relation between frequency and membrane potential was about 5 $\frac{\text{imp/sec}}{\text{mV}}$ in the absence of currents, and the relation between frequency and current intensity was about 23·5 $\frac{\text{imp/sec}}{\text{nA}}$ in the absence of light. Membrane resistance in darkness was therefore taken to be 4·7 MΩ.

The data of Fig. 6 were then replotted, taking into account the drop in potential evoked by currents applied in darkness across the cell's membrane. This drop in potential is shown by the interrupted line in Fig. 7. The circles in this figure measure the height of the potential changes evoked by illumination, starting from the interrupted line, and the solid lines joining the experimental points show the membrane potential resulting from the combined action of light and current, relative to the membrane potential of the cell in the absence of both light and current. The dots near the circles measure the corresponding frequencies of firing, while those by the interrupted line measure the frequencies evoked by currents alone. A small correction was introduced in order to fit to the interrupted line in this plot the frequencies evoked by depolarizing current in darkness (see legend of Fig. 7).

Rushton (1959) has shown that a somewhat similar reconstruction of the experimental findings described in this paper can be deduced from two postulates only. Following a different line of argument, it will be pointed out here that the slopes of interrupted and solid lines in the plot of Fig. 7 have the dimension of a resistance: under the assumptions mentioned above, the slope of the interrupted line measures membrane resistance in darkness, and the slopes of the solid lines measure membrane resistance during illumination at the various intensities used.

Membrane resistance during illumination

Two types of experiments were performed to test the conclusion that resistance of the nerve cell's membrane decreases during illumination. The

first type is still based on the assumption that frequency of firing depends only upon membrane potential but this experiment is easier to perform than those described in the previous section, so that more precise measurements can be made. Typical results are illustrated in Fig. 8: light was switched on soon after the start of one sweep and a pulse of depolarizing current lasting 1 sec was applied after 2 sec of illumination. In the next sweep light was turned off

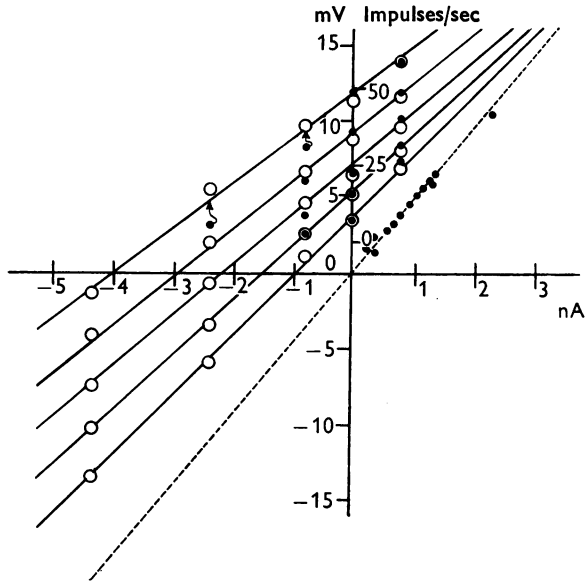

Fig. 7. Change of membrane potential and frequency of firing resulting from the combined action of light and currents. Data of Fig. 6 replotted, taking into account the change of membrane potential evoked by currents alone and combined with frequency measurements. Abscissa, current through the micro-electrode; ordinate measures both the change of membrane potential from resting potential and the frequency of firing. The circles measure the amplitude of the potential recorded following illumination, starting from the interrupted line. The dots measure frequency of firing. Those near the interrupted line are two sets of measurements taken some time after the others, following stimulation of the cell with currents alone. In order to fit these points to the plot the frequency scale has been shifted down with respect to the voltage scale, assuming that membrane potential had decreased by 1 mV when the later measurements were taken.

and the same current pulse was applied after 2 sec in darkness. Light intensity was constant throughout but current intensity was changed at each pair of sweeps, as is indicated by the figures at left.

Frequency of firing was plotted as a function of current intensity in Fig. 9. The filled circles indicate the measurements taken in darkness and the open circles those taken during illumination. In each case frequency was a linear function of current intensity, but the slopes of the two lines are different,

being 16·7 $\frac{\text{imp/sec}}{\text{nA}}$ in darkness and 12·2 $\frac{\text{imp/sec}}{\text{nA}}$ during illumination. Since the relation between frequency and generator potential amplitude had a slope of 2·2 $\frac{\text{imp/sec}}{\text{mV}}$ in this unit, the results show (subject to the assumption already mentioned) that membrane resistance dropped from 7·6 MΩ in darkness to 5·5 MΩ during illumination at the light intensity used.

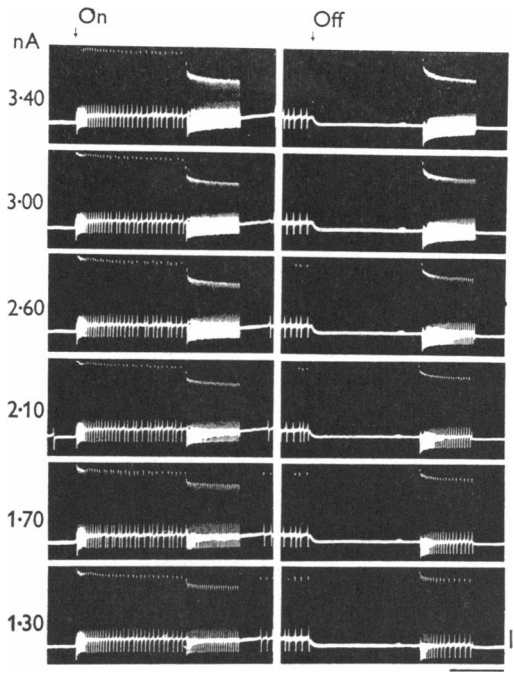

Fig. 8. Effects of depolarizing currents applied in darkness or during illumination. Light was applied soon after the start of a sweep and a pulse of depolarizing current lasting 1 sec was delivered after 2 sec of illumination (left-hand records). In the next sweep, taken after a 10 sec interval (right-hand records), the light was switched off and the same current pulse was delivered after 2 sec of darkness. Light intensity was constant throughout but current intensity was changed at each pair of sweeps as shown by figures on left. Vertical line at bottom right, 20 mV; horizontal line at bottom, 1 sec.

A different method for measuring changes in membrane resistance, which does not depend upon frequency measurements, was used in the second series of experiments. A sinusoidal current of 16 c/s and 10 nA was applied through the impaling micro-electrode, and the bridge was balanced so that only a small oscillation was recorded while the eye was kept in darkness. In order to prevent excessive firing during the depolarizing peaks of this alternating current, the cell was hyperpolarized by means of a steady current of 7·6 nA. It may be

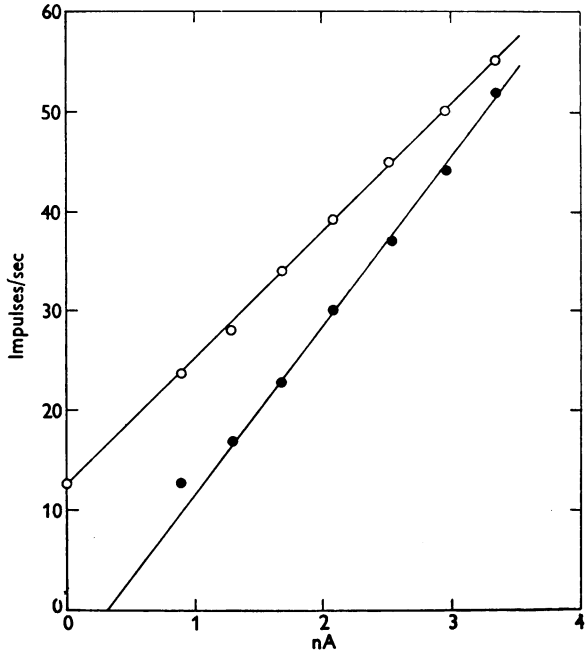

Fig. 9. Frequency of firing as a function of current intensity in darkness and during illumination. Data from the same cell as that of Fig. 8. Abscissa, current intensity; ordinate, frequency of firing. ○, frequency of firing evoked by depolarizing currents during illumination; ●, frequency of firing evoked by the same currents in darkness.

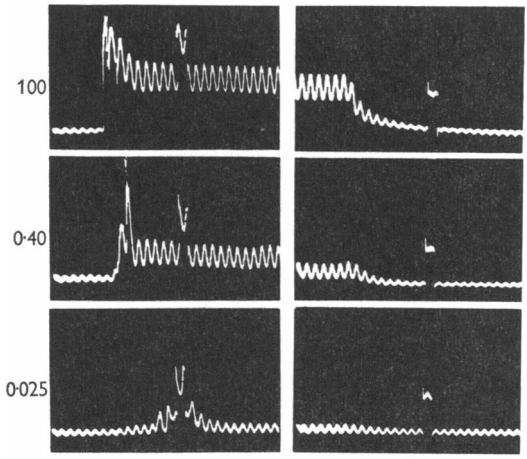

Fig. 10. Change of bridge balance during illumination. Alternating current at 16 c/s and 10 nA was applied through the micro-electrode and the bridge was balanced for minimum deflexion in darkness. Since alternating current of the intensity used would evoke intense firing, the unit was hyperpolarized by means of a steady current of 7·6 nA. With such current, generator potential amplitude is considerably increased (see Fig. 5). The records show responses to lights of different intensity, indicated by the figures at the left. The change of bridge balance during illumination reveals a change of impedance of the cell's membrane. The square pulse in each record is a 20 mV calibration; time line at bottom is 1 sec.

noted, incidentally, that bridge balance did not change as a consequence of the current, indicating that membrane resistance was not affected by the currents used.

Figure 10 shows that the potential oscillations evoked by the alternating current increased during illumination, being greater for stronger intensities of light. The results show therefore that membrane impedance changed during illumination. Assuming that membrane capacity was not altered, this impedance change should be ascribed to a change of membrane resistance.

DISCUSSION

The finding that generator potentials are accompanied by changes of membrane conductance suggests that the conductance change may be the cause of the generator potential. It is known that the concentrations of several ions are different in the fluids inside and outside nerve cells and it is thought that the resting membrane is highly permeable to some ion species but not to others. In these conditions a conductance increase would, in general, be expected to result in a potential displacement, because certain ions could then move across the membrane, following their concentration gradient, until their charges generate a potential gradient sufficient to oppose further movement.

According to this schema (see Fatt & Katz, 1951; Eccles, 1957, p. 60) the eccentric cell membrane can be represented by the circuit diagram of Fig. 11, in which E_r is the e.m.f. of the resting membrane, E_g is the e.m.f. giving rise to generator potentials and E_s is an external constant current generator.

If light decreases R_g, a potential drop will be elicited by illumination in the absence of currents, and in the presence of currents the potential drop evoked by a given light will be linearly related to current intensity, as is shown in Fig. 6, provided that E_r and E_g remain constant. Rushton (1959) has given a full account of the properties of this circuit showing that all experimental results can be quantitatively predicted if one assumes that frequency of firing is controlled only by membrane potential.

There is, however, an important complication. If generator potentials were due to a permeability change, then membrane potential during illumination would approach a fixed 'equilibrium potential' (represented by E_g in Fig. 11). If membrane potential before illumination were reversed with respect to this equilibrium potential, the potential change evoked by illumination would also reverse. This predicted reversal could not be produced by depolarizing currents in the experiments performed so far, and it is difficult at the present time to offer a satisfactory justification for this failure.

Assuming that unspecified complications arise when strong depolarizing currents are used, the equilibrium level of generator potentials could be determined by extrapolation of the results obtained with hyperpolarizing currents. In plots such as that of Fig. 7 the equilibrium potential would thus be defined by the intersectiou between interrupted and solid lines. Only two experiments of this type could be satisfactorily performed in the present study, but in both cases the intersection occurred at systematically different levels, when different light intensities were used. It is possible that the spread of the points of intersection is simply a consequence of experimental

errors (as suggested by Rushton, 1959), but other interpretations can be advanced. For instance, the equilibrium level could well be different for generator potentials evoked by dim or by bright lights, if light increased membrane permeability to more than one ion. Also, apparently different equilibrium levels would be found with the method used if generator potentials arose at different distances from the impaling micro-electrode when dim or bright lights are used, since they would then be affected to a different extent by electrotonically spreading currents from the micro-electrode.

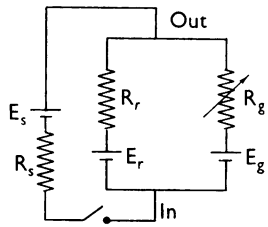

Fig. 11. Equivalent circuit of cell producing a generator potential. E_r and R_r are e.m.f. and resistance of the portions of the membrane producing a resting potential, and E_g and R_g are e.m.f. and resistance of the portions producing generator potentials. Polarity of E_g is represented in the same direction as E_r, in agreement with the experimental data. E_s is an external generator and R_s is a very high external resistance (1000 MΩ).

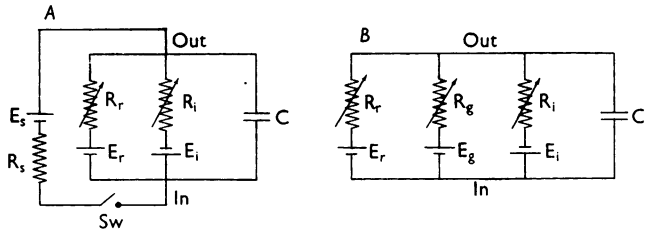

Fig. 12. Equivalent circuits of cells producing impulses. *A*. Diagram of cell activated by depolarizing currents. E_i and R_i are e.m.f. and resistance of the parts of the cell generating impulses. C is the cell's capacity, E_r, R_r, E_s, R_s as in Figure 11. *B*. Diagram of cell activated by light. All symbols as in Figs. 11 and 12*A*.

It may be appropriate to discuss at this point the great variability of spike size observed in intracellular records from visual cells of *Limulus*. It will be noted that the changes of spike size observed in this paper resemble those previously recorded by Eyzaguirre & Kuffler (1955) and the same conclusions probably apply to both findings.

It has been seen that (1) peak-to-peak spike amplitude is smaller when the cell is more depolarized and frequency of firing is higher; (2) for a given frequency of firing spike size is smaller when firing is evoked by light than when it is evoked by currents; (3) peak-to-peak spike size decreases with time during a train at constant frequency. These findings can be discussed with reference to the diagrams of Fig. 12, in which it is assumed that impulses are produced by the mechanisms described in other excitable tissues (see Hodgkin & Huxley, 1952*b*) and generator potentials originate as was suggested at the beginning of this discussion.

If membrane resistance were negligibly small, both at top and bottom of the spike, then peak-to-peak spike amplitude would not change owing either to external currents (Fig. 12*A*) or to changes of R_g (Fig. 12*B*). However, if membrane resistance were appreciable during impulse activity, being larger at the bottom than at the top of the spike, then

(*a*) Depolarizing currents would evoke a sizeable drop in potential at the foot of the spike and only a smaller drop in the same direction at the peak of the spike; peak-to-peak amplitude of spikes evoked by depolarizing currents would thus decrease with increasing current intensity;

(*b*) Decrease of R_g (Fig. 12*B*) would effectively shunt the potential changes evoked by impulse activity, both at top and bottom of the spike; amplitude of spikes evoked by illumination would therefore decrease with increasing light intensity;

(*c*) Peak-to-peak amplitude of spikes evoked by depolarization due to decrease of R_g would be smaller than that of spikes induced by equal depolarization resulting from external currents; the time constant of the circuit of Fig. 12*A* is longer than that of the circuit in Fig. 12*B*, but a change of a given magnitude and duration of R_1 will evoke a larger drop in potential in Fig. 12*A* than in Fig. 12*B*, because the initial rate of charge of the condenser C is not controlled by R_g, whereas the final value of the potential across C is decreased by the presence of this resistance.

The basic observations relating to size of spike can therefore be explained by assuming that the resistance between an intracellular electrode (presumably in the cell soma) and the outside does not decrease very markedly during spike activity. This suggests that impulses originate at some distance from the soma and is in agreement with previous conclusions (Tomita, 1956, 1957), as well as with other results to be described in a future article. In addition, 'sodium inactivation' (Hodgkin & Huxley, 1952*a*; cf. Eyzaguirre & Kuffler, 1955, p. 107) and changes of ionic concentrations following repetitive firing (Frankenhaeuser & Hodgkin, 1956) are probably important factors in controlling spike amplitude. It appears reasonable to conclude that generator potentials are a consequence of a change in permeability of some parts of the cell's membrane and that impulses originate at some distance from the soma, which they never invade. The finding that spikes are smaller when firing is induced by light than when it is evoked by currents can be ascribed to the decrease of membrane resistance during illumination.

Since the photoreceptor is supposed to be located around the dendrite (Miller, 1957) but not around the cell body of eccentric cells (T. Wanko, unpublished), it is reasonable to think that the generator potential originates in the dendrite and spreads from there to the soma. The functional organization of the eccentric cell appears therefore to be similar to that of the stretch receptors of lobsters, described by Eyzaguirre & Kuffler (1955).

The hypothesis that generator potentials are the result of a change in permeability provides a basis for speculation on the nature of the processes linking photochemical to neural activity in the eye. It has sometimes been assumed (Hartline, Wagner & MacNichol, 1952; Wald & Brown, 1952) that visual nerve cells are excited by electric currents originating in photoreceptors, and efforts have been made to record electrical activity from photoreceptors cells (Ottoson & Svaetichin, 1952; Svaetichin, 1953, 1956*a*, *b*). The findings described in this article are not consistent with the view that the generator potential originates as a drop in potential produced on the nerve cell membrane by currents generated by external elements, because such a drop in potential would not be a function of membrane potential (cf. Fatt & Katz, 1951). Also, the change in permeability supposed to be responsible for production of generator potentials could not be triggered by currents from photoreceptors, because no change in resistance is produced by currents through the membrane. For these reasons it is suggested that nerve cells in the *Limulus* eye are activated by a chemical product liberated by the photoreceptors during illumination (Grundfest, 1958*a*, *b*).

SUMMARY

1. Electrical properties of visual cells in the eye of *Limulus* have been studied by means of intracellular micro-electrodes.

2. A measurement of membrane resistance was obtained by comparing the responses elicited in the same unit by light or by depolarizing currents.

3. Peak-to-peak spike amplitude was found to be smaller when firing at a given frequency was evoked by light than when it was evoked by depolarizing currents.

4. The amplitude of the potential recorded following a given illumination is increased by hyperpolarizing currents and decreased by depolarizing currents. Conversely, frequency of firing evoked by a given light is decreased by hyperpolarizing currents and increased by depolarizing currents.

5. The increase of firing frequency evoked by a given depolarizing current is less during illumination than in darkness. If the drop in potential evoked by an alternating current is balanced by means of a bridge while the cell is in darkness, it can be seen that bridge balance changes during illumination. These results are ascribed to a decrease of membrane resistance during illumination.

6. It is suggested that generator potentials arise as a consequence of a change of permeability of the cell's membrane, and that this change of permeability originates by the action of a chemical substance liberated by the photoreceptor during illumination.

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