Slow and Spike Potentials Recorded from Retinula Cells of the Honeybee Drone in Response to Light

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ABSTRACT Responses to light recorded by means of intracellular microelectrodes in isolated heads kept in oxygenated Ringer solution consist of a slow depolarization. Light adaptation increases the rates of depolarization and repolarization and decreases the amplitude of the response. Qualitatively these changes are similar to those observed in *Limulus* by Fuortes and Hodgkin. They are rapidly reversible during dark adaptation. In retinula cells of the drone eye a large single spike is recorded superimposed on the rising phase of the slow potential. The spike is a regenerative phenomenon; it can be triggered with electric current and is markedly reduced, sometimes abolished by tetrodotoxin. In rare cases cells were found which responded to light with a train of spikes. This behavior was only found under "unusual" experimental conditions; i.e., towards the end of a long experiment, during impalement, or at the beginning of responses to steps of strongly light-adapted preparations.

Experiments performed in different laboratories on invertebrate eyes have shown that responses to light of single visual cells recorded with intracellular microelectrodes have many similarities. In all eyes so far examined the response to a brief flash consists of a slow depolarization which starts with some delay and outlasts considerably the duration of the light pulse. Responses to longer stimuli are composed of two parts, one phasic (the transient), and the other maintained (the plateau). Fuortes and Hodgkin (1) have shown in *Limulus* that the shape of responses to weak lights or very soon after applying a strong light resembles the shape of responses of a low-pass filter containing several equal stages of exponential delay; furthermore, these authors have found that the modifications of time course and amplitude of visual responses observed in light-adapted eyes can be reproduced in the low-pass filter model by a decrease of the time constant which controls the rate of decay of the response.

The purpose of the experiments presented in the first part of the present paper was to describe visual responses of dark- and light-adapted retinula cells of the drone of the honeybee and to see whether the *Limulus* model of Fuortes and Hodgkin applies to the drone. The results show that there are qualitative similarities between the linear responses of the two preparations. Yet responses to strong lights are much more complicated in the drone. In addition, the simple relation observed in *Limulus* between time scale and sensitivity does not seem to apply to the bee.

A feature of visual responses of drone retinula cells are spikes which are regularly found superimposed upon the slow potential (2, 3). The presence of these spikes led to the suggestion that the function of retinula cells consists not only in the generation of slow receptor potentials in responses to light but also in the translation of the slow potential into spikes which propagate to more central structures of the visual system (2). The significance of these spikes and their properties is discussed in the second part of this paper.

METHODS

The head of the drone was separated from the body and divided into two halves by a section passing through the eyes parallel to the longitudinal axis of the ommatidia. The two halves were placed in a small Lucite chamber continuously perfused with oxygenated Ringer solution of the following composition (mM): NaCl 199.7; KCl 3.1; NaH_2PO_4 0.2; Na_2HPO_4 1.8; $CaCl_2$ 1.8; dextrose 5.6. This solution is similar to that used by Yamasaki and Narahashi (4) for the cockroach giant axon but osmotic pressure was reduced by slightly lowering the concentration of NaCl. This prevented the shrinkage of visual cells observed with the original solution. The experiments were performed at a room temperature of about 23°C. Satisfactory responses to light could be obtained for up to 12 hr from preparations kept under these conditions.

Some experiments were performed on preparations kept in a humid chamber. No difference was observed between the responses to light obtained with these two techniques. The perfusion method was preferred because impaling and maintaining the electrode inside the cell were easier. In good experiments, the electrode could be kept in a single cell for more than 30 min.

Stimulation The light emitted by a 6 v tungsten filament was passed through a heat-absorbing filter and focused on a diaphragm which could be occluded by a magnetic shutter. The light beam was then collimated and the image of the diaphragm focused on the eye with a microscope objective. The diameter of the illuminated area was 800 μ and covered the whole length of an ommatidium. The light was monitored by an RCA929 photocell or a selenium cell. The selenium cell had a slow response and gave some deformation of the light flash (Fig. 1). Light intensity was controlled by calibrated neutral density filters and was measured on a logarithmic scale, taking as unity the intensity of the unattenuated light. In order to study the effect of light adaptation, the beams of two light sources mounted at a 90° angle were reunited

FIGURE 1. Light flash of 25 msec duration recorded with a fast (a) and a slow photocell (b). The slow cell has been used in the experiment illustrated in Figs. 3 and 7.

through a double prism. The unattenuated intensities and the diameters of the two beams were adjusted so as to be equal.

Recording Intracellular potentials were recorded by means of glass electrodes filled with 3 M KCl solution. The DC resistance of the electrodes was between 8 and 30 MΩ. The electronic equipment consisted of a Bak (5) wide band electrometer with negative capacitance feedback and a cathode ray oscilloscope. In some experiments constant current was passed through the intracellular microelectrode and the resulting potential changes were recorded through a bridge circuit which could be adjusted to balance out the potential drop across the microelectrode. The positions of the light and of the electrode were controlled by observation through a stereomicroscope. Single ommatidia, but not individual cells, could be seen clearly.

RESULTS

Responses to Short Pulses of Light Responses of a visual cell to flashes of 25 msec duration and of increasing intensities applied every 6.3 sec are shown in Fig. 2. Responses to weak lights consist of a slow depolarization reaching its maximum after the end of the flash. The repolarization of the cell is slower than the depolarization and for small responses follows a smooth time course. These small responses are similar to those evoked by dim flashes in visual cells of *Limulus* and can be fitted by the model proposed by Fuortes and Hodgkin (1).

The responses to brighter flashes are considerably more complicated. With lights of medium intensity, the rising phase often includes a notch and the repolarization consists of an initial fast and late slow component. With strong lights the slow wave is preceded by a spike of almost constant amplitude and the repolarization is dominated by the slow component. The spikes slightly overshoot the outside potential, but no overshoot was ever observed for the peak of the slow wave.

Features similar to those just described can also be seen in Fig. 3. In this cell responses were evoked by flashes of increasing intensities and of three different durations: 8 msec in a, 25 msec in b, and 100 msec in c. It can be seen in a and b that the time-to-peak of the slow wave first decreases and then increases with increasing light intensity. The slow component of repolarization is less marked than in the previous cell (Fig. 2), but can be seen in the response

to the strongest 25 msec flash. One may observe in c (100 msec flashes) that the shape of the response changes markedly with increasing light intensity: with weak intensities, the depolarization increases throughout the duration and also for a short time after the end of the illumination; with stronger lights repolarization begins well before the end of the illumination. It is apparent from these records that very complicated nonlinearities occur in the responses to strong illumination.

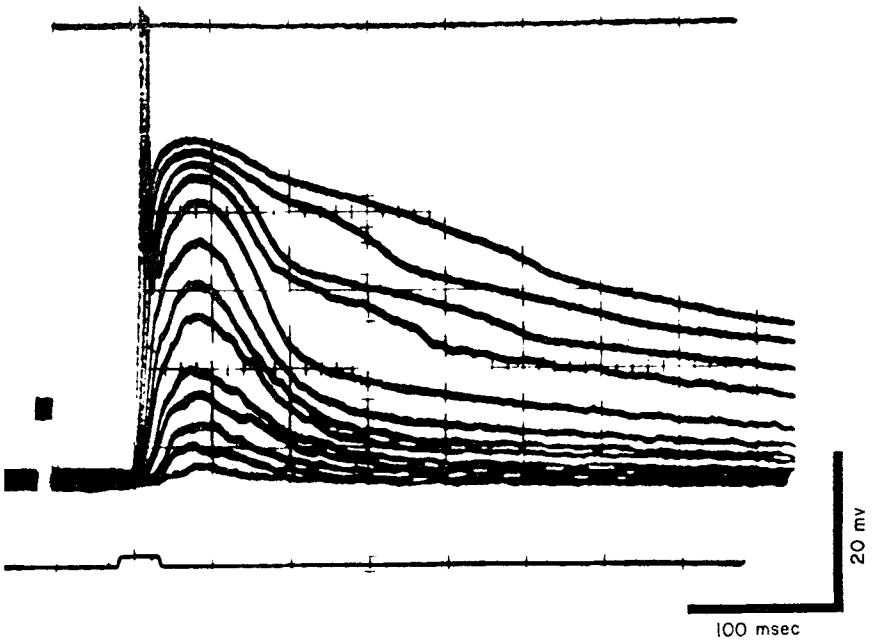

FIGURE 2. Responses of a cell to 25 msec flashes. Light intensity was doubled at each trial once every 6.3 sec. The light flash recorded with a fast photocell is shown on the bottom trace. The top trace corresponds to the potential recorded after withdrawing the electrode from the cell. The square wave at the beginning of the recording represents in this and in some following figures a calibration pulse (10 mv, 10 msec).

Responses to Long Pulses of Light Fig. 4 shows responses to lights of approximately 5 sec duration. Weak intensities evoke a depolarization at the onset of illumination. This depolarization is maintained throughout the illumination or may show a slight increase in amplitude. Small irregular waves similar to the "discrete waves" of *Limulus* (6) and locust (7) are superimposed on the light-induced depolarization. Above a certain intensity (-2.7 in this cell) the responses have two components, an early transient and a late plateau. With strong lights the amplitude of the discrete waves decreases, the transient component becomes more pronounced and is followed by a slow, strongly damped oscillation. During the plateau the potential remains approximately

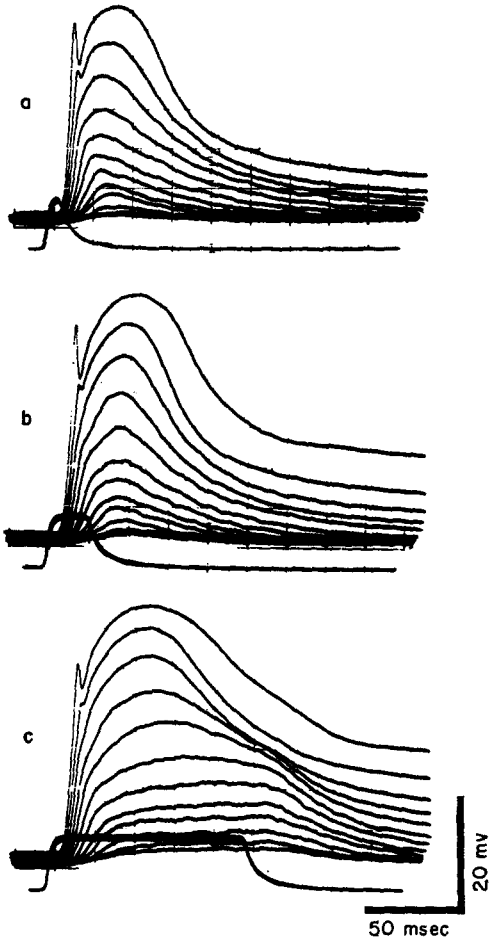

FIGURE 3. Responses to flashes of various intensities and three different durations. 8 msec in (a), 25 msec in (b), and 100 msec in (c). Starting with the dimmest light, the intensity was doubled for each successive stimulation.

constant. With the strongest intensities the duration of the transient component is increased and the amplitude of the oscillation decreased. Although the figure does not show this clearly because of the slow sweep speed, the decay from the transient to the plateau is quite similar to the decay of responses to short flashes, being fastest for lights of medium intensities.

The spike-like depolarization mentioned above occurs also in responses to long pulses and can be seen in the response −1.5. In the experiment illustrated it is masked by the fast-rising transient when stronger lights are used. The repolarization following illumination with dim light is approximately the mirror image of the depolarization at the onset of the stimulation. The return to the dark potential after strong illumination is more complex; immediately after the light is switched off, the cell repolarizes rapidly to a value smaller than the potential measured in darkness. This phase is followed by a slower repolarization which brings the potential below the dark potential.

After this transient hyperpolarization the potential returns slowly to its original value. This third phase is too slow to be seen in Fig. 4. As was the case for the flashes shown in Fig. 2, the fast phase of repolarization is most pronounced in responses to light of intermediate intensities (−1.5); with stronger lights the fast phase decreases in amplitude and the slow phase predominates.

The time course of repolarization depends not only on light intensity, but also on the duration of illumination. In the experiment illustrated in Fig. 5,

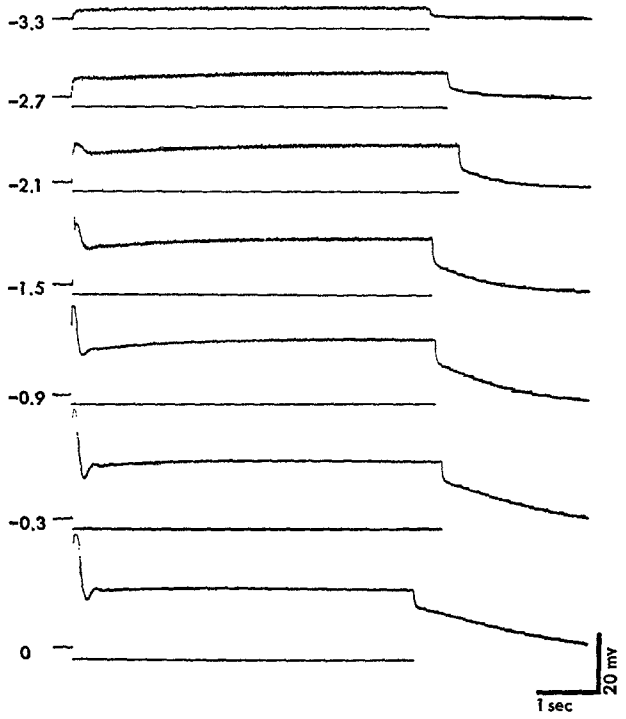

FIGURE 4. Responses to long pulses of light. The numbers at the left indicate relative light intensity in log scale. The stimuli were applied in order of increasing intensity and were separated by intervals of up to several minutes.

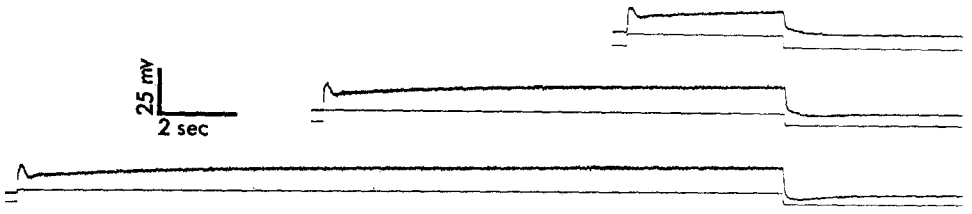

FIGURE 5. Influence of duration of illumination on the potential changes which follow a response to a pulse of light of moderate intensity (−2.4).

three pulses of equal light intensity and of 4, 12, and 20 sec duration were applied to the eye. As in Fig. 4, the repolarization of the cell following the 4 sec pulse can be divided into an early fast and a late slow component. With the longer periods of illumination the amplitude of the fast component is markedly increased and the potential undershoots the potential measured in darkness. The level to which the cell is depolarized during 12 and 20 sec of illumination is about the same. This indicates that the time course of repolarization at the end of the light pulse is determined by the duration of illumination rather than by the final level of depolarization.

Light Adaptation

It is well-known (1) that light adaptation occurs both as an aftereffect of illumination and as a result of the presence of a background of light. In the drone as in *Limulus* light adaptation changes the sensitivity of the photoreceptor and the time course of its response; the two changes are, however, not as intimately related as in *Limulus*.

AFTEREFFECTS OF ILLUMINATION

In the experiment illustrated by Fig. 6, the eye was kept in complete darkness for 6 min and then stimulated with bright flashes of 25 msec duration at the

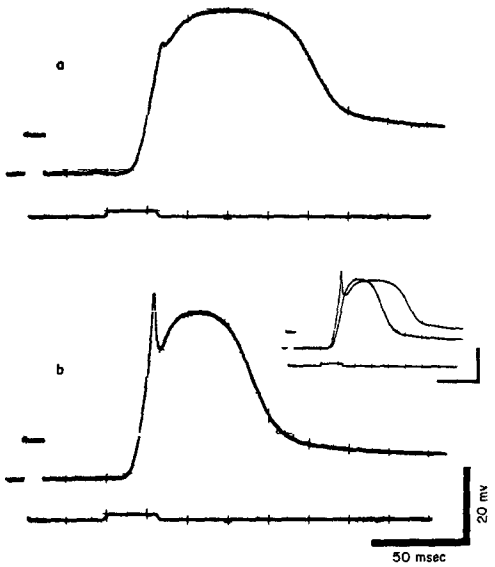

FIGURE 6. Aftereffects of illumination. In (a) response to a flash of 25 msec duration applied to a preparation kept in darkness for 6 min; in (b) steady-state response of the same cell when stimulated once every 6.3 sec. Flash duration and intensity in (a) and (b) are identical. In the inset, responses (a) and (b) are superimposed. Calibration pulse 10 mv, 10 msec.

rate of one flash every 6.3 sec. The figure shows isolated and superimposed responses to the first (a) and to a later flash (b). The resting potential and the amplitude of the responses are the same in both cases; in the response of the light-adapted preparation (b) the rates of depolarization and repolarization are increased, the duration of the response shortened and the amplitude of the spike increased.

RESPONSES TO FLASHES SUPERIMPOSED ON A BACKGROUND

Similar effects are observed when flashes are superimposed on a steady adapting light. Flashes of 8 msec duration were applied at intervals of 6.3 sec, doubling light intensity at each subsequent trial. The flashes were applied first without and then with background illuminations. Sufficient time was allowed after each increase of background intensity to permit stabilization of the membrane potential.

Fig. 7 illustrates the effects of four background lights, $\log_{10} i = -3.0, -2.4,$

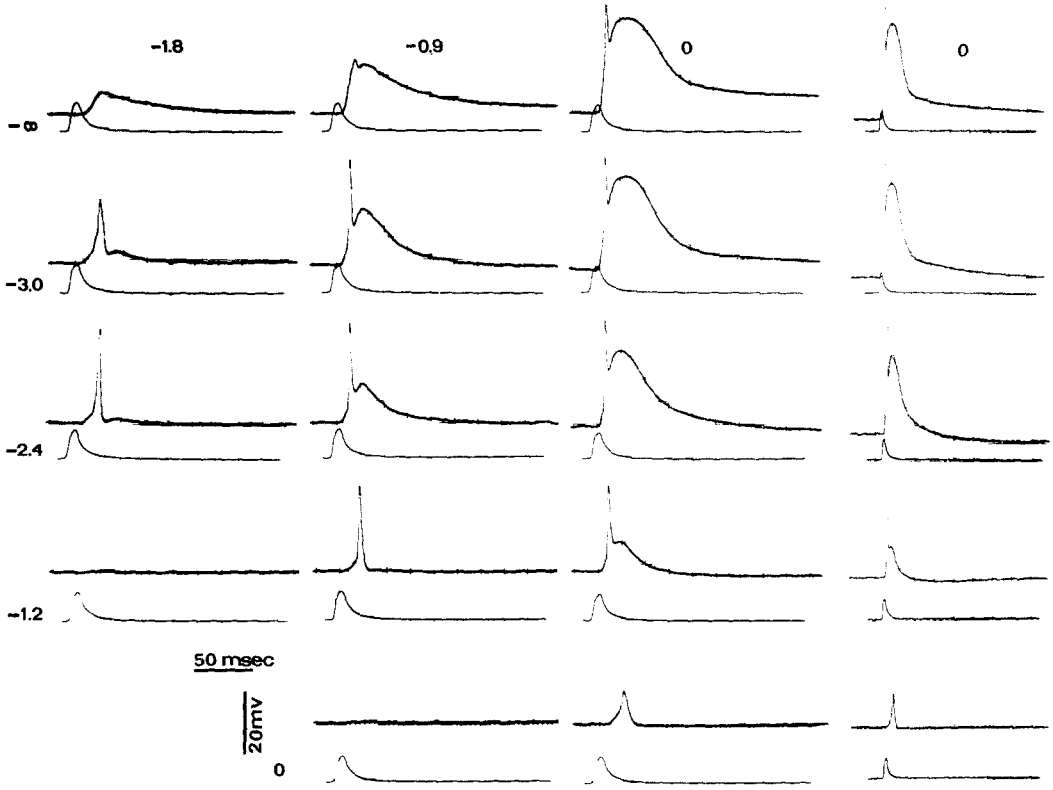

FIGURE 7. Responses to flashes superimposed on a background light. The numbers above each column are the $\log_{10}$ of the relative intensity of the flash. The numbers at the left of each row give the intensity of the background light. Responses of the top row were recorded without background light. In the fourth column the responses to the bright flash are recorded with sweep speed reduced by a factor of 2.5.

-1.2, and 0 on responses to flashes of three different intensities, $\log_{10} i = -1.8$, -0.9, and 0. The most striking effects of the background illumination are:

1. a reduction of the membrane potential, shown in the figure by an elevation of the base line relative to the light signal,
2. a decrease in the duration and amplitude of the slow potential,
3. an increase in the rate of repolarization (compare backgrounds $-\infty$ and -3.0; flash -0.9,
4. a transient hyperpolarization which follows the slow potential (see adapting light -1.2, flash 0).

The last phenomenon is better illustrated by the column at the right of the figure, where the responses to the bright flash are recorded with a slow sweep speed. In addition, moderate backgrounds tend to increase the size of the initial spike whereas stronger backgrounds tend to decrease it.

The amplitude of the slow response to flashes superimposed on backgrounds of different intensities was measured and plotted against light intensity in two different ways. In Fig. 8 a the peak amplitude of the slow wave, ΔV, is plotted against the $\log_{10}$ of flash intensity ($\log_{10} \Delta i$). The plot shows that dim adapting lights do not affect the amplitude of the responses to flashes while stronger backgrounds decrease it. All points in this plot can be fitted by appropriate lateral shifts of a curve of constant shape. This means that a given increase in background illumination is equivalent to diminishing the intensity of the flash by a constant factor.

In Fig. 8 b, the total potential change resulting from the effect of both the background and the flash (V) is plotted as a function of $\log_{10} (i + \Delta i)$. With this plot, the total light intensity corresponding to a given point on the abscissa is the same for all curves: the light was applied in a single flash for the curve $\log_{10} = -\infty$, while it was subdivided into a background i and a flash Δi for the other curves. This plot shows that for weak backgrounds the total depolarization evoked by a given intensity is larger when the light is in the form of a flash superimposed on a dim background than if it is applied in a single flash.

Modifications similar to those reported for responses to flashes were observed in responses to long pulses of light superimposed on a background. Fig. 9 a shows that the amplitude and the duration of the initial transient are decreased and that the rate of repolarization is increased. Fig. 9 b shows that the peak voltage attained with a bright pulse is, as with a flash, slightly larger in the presence of moderate backgrounds (-2.4, -1.8) than in their absence.

TIME COURSE OF LIGHT AND DARK ADAPTATION

In the experiment illustrated by Fig. 10 bright flashes of 25 msec duration were applied to the eye at a frequency of 1/6.3 sec. Flashes were applied first with-

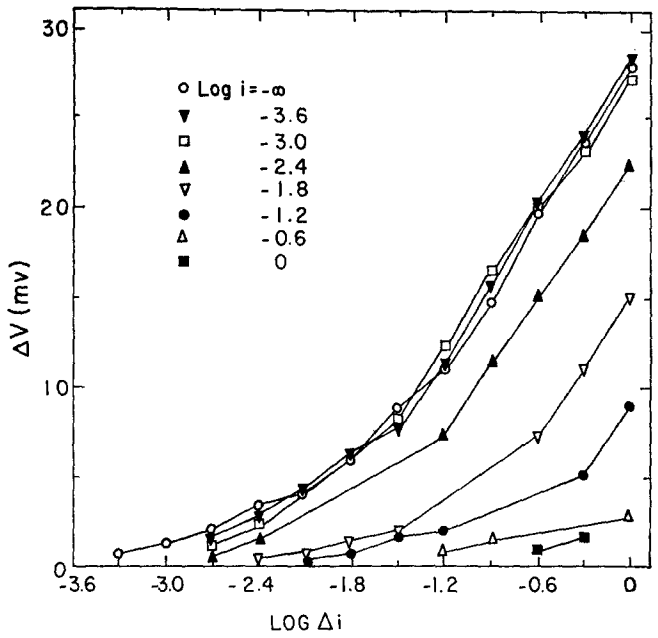

FIGURE 8 *a*

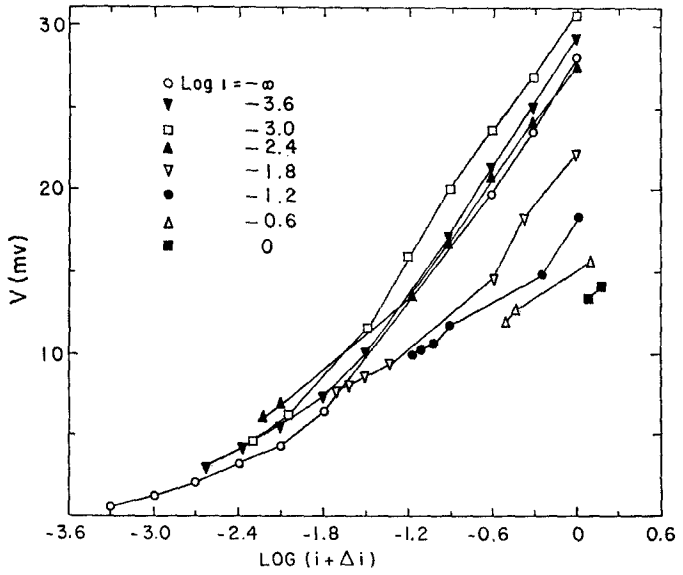

FIGURE 8 *b*

FIGURE 8. Effect of background lights on a stimulus-response curve. In (a) the amplitude (ΔV) of responses to flashes is plotted vs. the $\log_{10}$ of flash intensity (Δi). After determination of a control curve (open circles), the flashes were superimposed on background lights of increasing intensity each represented by a different symbol. In (b) the total potential change V evoked by the flash and background light is plotted vs. the $\log_{10}$ of the sum of the two light intensities ($i + \Delta i$).

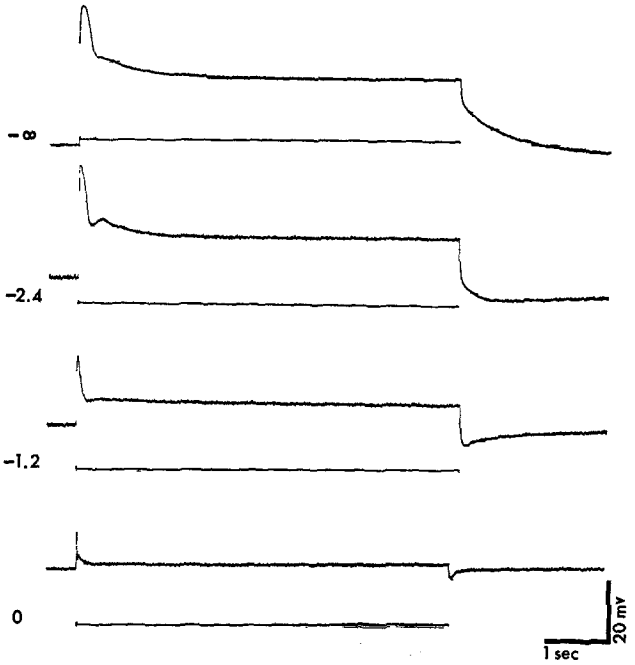

FIGURE 9 *a*

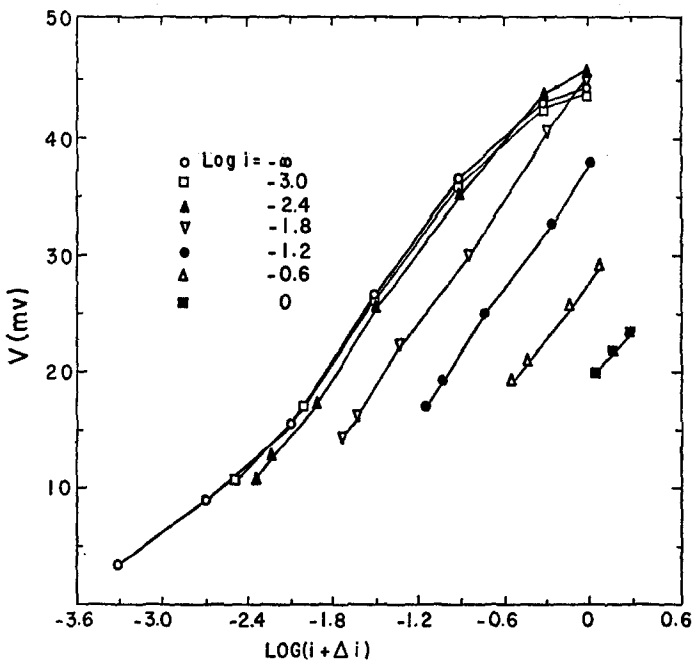

FIGURE 9 *b*

FIGURE 9. Action of background lights on responses to long pulses of light. In (a) action on a response to a bright pulse of light. The figures indicate the background intensity in log scale. In (b) action on the amplitude of the initial transient of responses to long pulses. The total potential V evoked by background and light pulse is plotted vs. the $\log_{10}$ of the sum of the two light intensities.

out an adapting light and then superimposed on background lights of increasing intensity. After application of the strongest intensity, the background light was turned off while the stimulation with bright flashes continued. The following parameters were measured: steady-state potential V_s, amplitude of the slow wave ΔV, and duration Δt of the response (measured 13 mv above

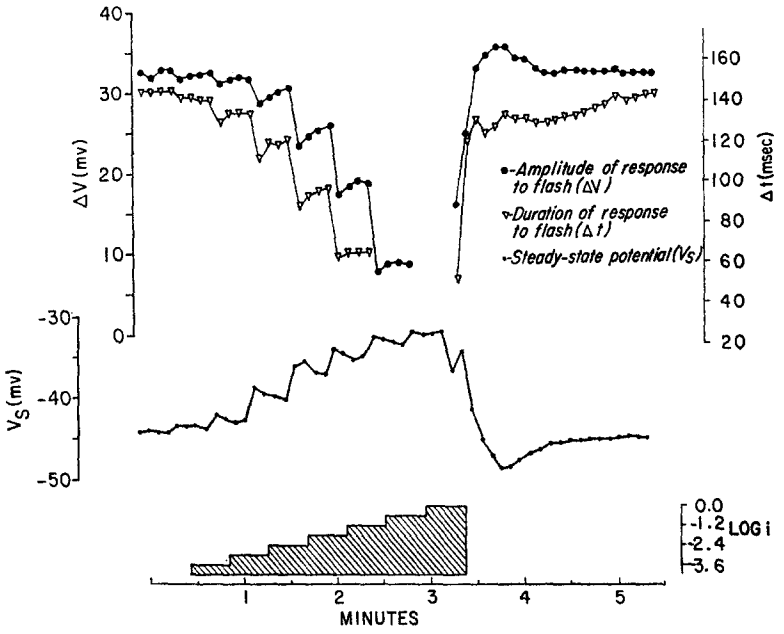

FIGURE 10. Effect of a stepwise increase of background illumination on responses to a bright flash of 25 msec duration. ΔV measures the amplitude of the response to the flash. V_s is the steady-state potential determined between two flashes (the polarization of the cell membrane decreases as the intensity of the background is increased). The duration (Δt) of the responses was measured 13 mv above the steady-state potential. Due to a too small amplitude and the impossibility of distinguishing between slow wave and spike, duration and amplitude of the responses could not be determined with the strongest adapting lights.

steady-state potential). At the beginning of each intensity increment of the adapting light, the amplitude and duration of the response and of the steady-state potential decreased; then all three parameters recovered partially.

When the adapting light was turned off, all parameters recovered. The amplitude of the response and the steady-state potential regained their initial values (after a transient overshoot) in approximately 90 sec. Recovery of the duration was rapid during the first few seconds, but required about 2 min to be complete. Similar short times for dark adaptation have been found in the honeybee worker by Goldsmith (8) who recorded the electroretinogram, and by Autrum and Seibt (9) in behavior experiments. Dark adaptation is

also rapid in *Calliphora* (10) and in *Limulus* (11). The latter preparation presents a transient overshoot of the amplitude of the response very similar to that observed in the drone.

Spike Potentials

SINGLE SPIKES Responses of the drone retinula cell to bright flashes are characterized by a large spike superimposed on the rising phase of slow potentials. This spike sometimes behaves as an all-or-nothing phenomenon (Fig. 2). In most cells, however, it has a graded amplitude over a certain range of light intensities (Fig. 7). The relation between the $\log_{10}$ of light

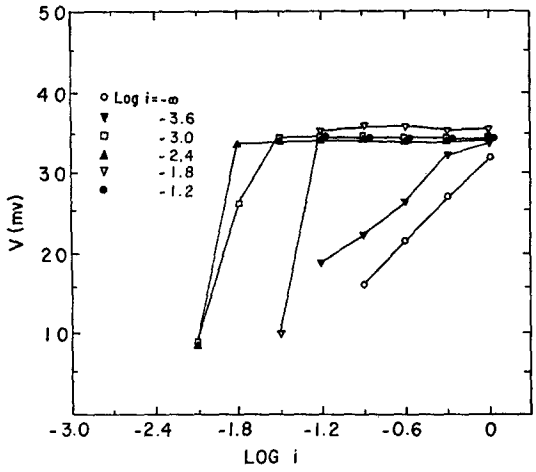

FIGURE 11. Relation between flash intensity and amplitude of the spike potential. The open circles were determined without background light, the other curves with background lights of increasing intensity. All amplitudes were measured between the membrane potential determined in darkness at the beginning of the experiment and the top of the spike.

intensity and the amplitude of the spike is shown in Fig. 11. This figure shows, in addition, the effect of background lights of five different intensities. The relation is linear without background, whereas with strong backgrounds the amplitude of the spike increases rapidly, and then reaches a value which is not further affected by the intensity of the flash. The figure does not show the decrease in amplitude usually found when very strong backgrounds were applied to the eye.

Single spikes were found in all cells examined, but in some cells they became evident only under special experimental conditions. In the cell illustrated in Fig. 9 a, for instance, the spike became visible only when a strong background light was applied to the cell.

REPETITIVE SPIKES In retinula cells of the drone the initial spike is sometimes followed by another of smaller amplitude. This spike disappears when light intensity is strong. Very exceptionally (in five cells out of more than a thousand examined) spikes were also found superimposed on the plateau. An example of this type of response is given in Fig. 12. The spikes

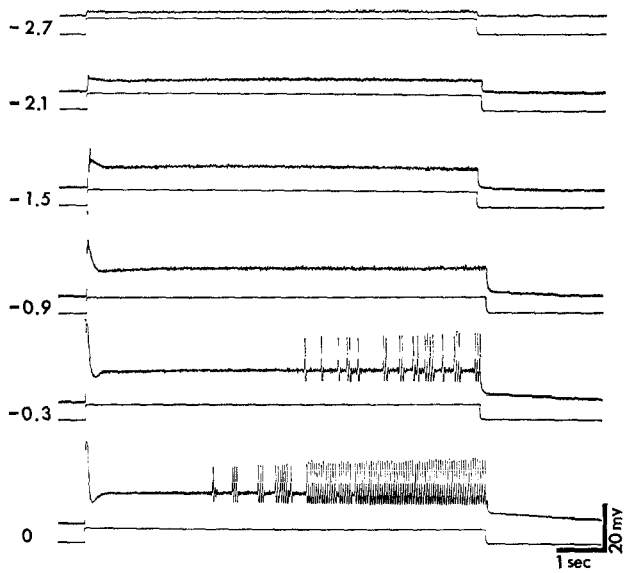

FIGURE 12. Responses to long pulses of light of a cell, which in addition to the early spike, discharges spikes during the steady state.

seem to arise from the peak of the discrete waves which are best seen in response −0.3. The response illustrated in Fig. 12 was recorded from a relatively old preparation (7 hr after the beginning of the experiment). Before this recording was made, the cell responded to pulses of light with a single spike, later the firing persisted also in darkness. In three cells repetitive firing was observed when a strong pulse was applied to a strongly light-adapted preparation. One cell started to fire during penetration of the cell membrane by the microelectrode and then ceased.

SPIKES EVOKED BY CURRENT Single spikes similar to those observed in response to light can be evoked by current applied through the impaling microelectrode. Fig. 13 a shows that a weak outward (depolarizing) current produces a potential change consisting of an initial hump followed by a steady state. With stronger currents the steady-state depolarization increases only moderately while the initial hump increases markedly and grows into a large single spike, followed by a hyperpolarizing afterpotential. A transient hyperpolarization is also present at the end of a response to the depolarizing current pulse. The behavior of the retinula cell in response to depolarizing current is strikingly similar to that observed in the giant axon of the cockroach by Yamasaki and Narahashi (12). The effects of hyperpolarizing currents also show similarities to those obtained with the giant axon membrane. When a pulse of inward (hyperpolarizing) current is applied to the retinula cell, the

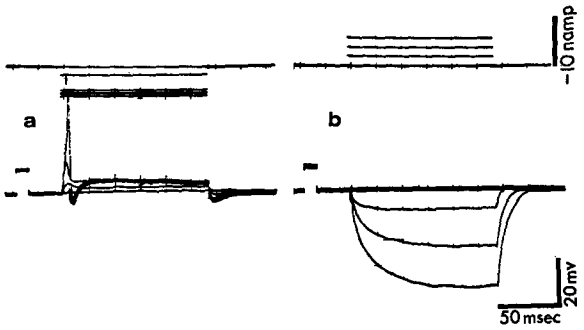

FIGURE 13. Action of constant current of different intensities on the membrane potential of a single visual cell, depolarizing current (a), and hyperpolarizing current (b). Calibration pulse 10 mv, 10 msec. 1 namp = 10^{-9} amp.

membrane potential increases slowly, and reaches a steady state whose amplitude is approximately proportional to current intensity (Fig. 13 b). Repolarization of the cell at the current "break" is faster than the depolarization and does not present an overshoot. The asymmetry between the effects of hyperpolarizing and depolarizing currents of equal intensity is clearly illustrated in Figs. 14 a and b. Note that the outward current produced only a depolarizing hump, in the case shown in Fig. 14 a, whereas when its intensity was slightly increased, it triggered a large spike (Fig. 14 b). In Fig. 15, the amplitude of the initial hump or spike, and of the steady-state voltage is plotted as a function of current intensity. The slope is 1.3 MΩ at the origin; it increases to 3.6 MΩ with inward currents of more than 2 namp and (in the steady state) decreases sharply with outward currents.

Spikes evoked by light and by current and recorded from the same cell are compared in Fig. 16. In parts a-c of the figure, a spike is triggered with light pulses of increasing intensity. In parts d-f another spike is triggered with

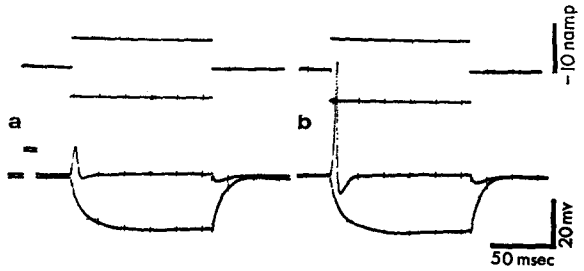

FIGURE 14. Action of constant depolarizing and hyperpolarizing current of equal intensity on a single visual cell. A slight increase of current intensity in (b) has little effect on the steady-state potentials but triggers a large spike. Calibration pulse 10 mv, 10 msec.

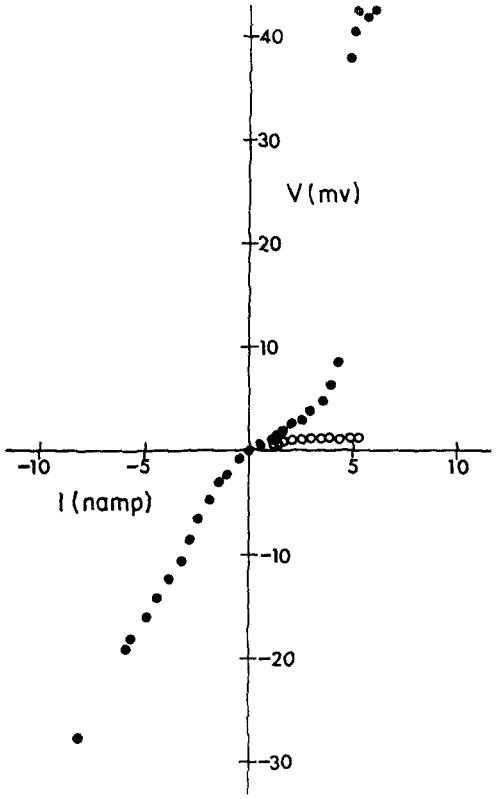

FIGURE 15. Relation between current intensity I and the potential change V of the membrane. Filled circles measure the maximum deflection; i.e., steady state for hyperpolarizing currents and early hump or spike for depolarizing currents. The open circles measure the amplitudes at the end of the depolarizing current pulse.

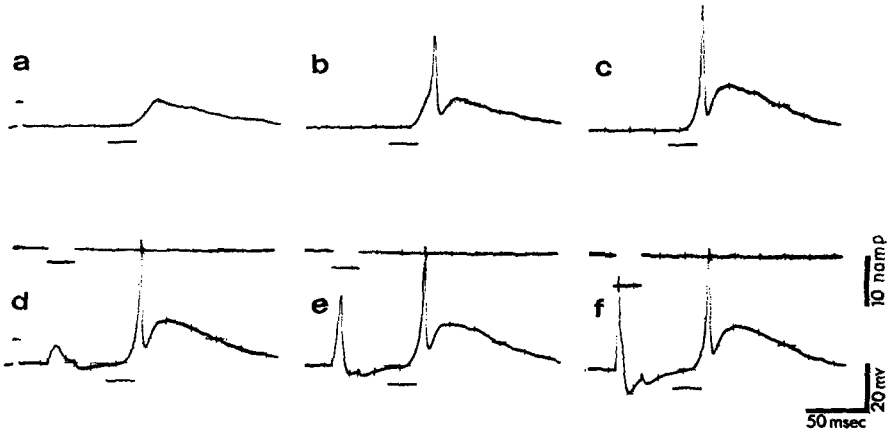

FIGURE 16. Spikes triggered witht light and current. In (a), (b), and (c) stimulation only with light of increasing intensity; in (d), (e), and (f) the light flash used in (c) is preceded by current pulses of increasing strength. Calibration pulse 10 mv, 10 msec.

current pulses of increasing strength. The spikes evoked by currents or by light are similar in shape. The difference in amplitude is most probably due to summation of the slow potential with the spike.

EFFECT OF TETRODOTOXIN Tetrodotoxin is known to block selectively the Na-carrying mechanism responsible for the spike in several excitable tissues. Experiments with this compound indicate that Na-movement participates in the production of the spike observed in the drone visual cell. In the experiment illustrated by Fig. 17, a first spike was triggered by a current

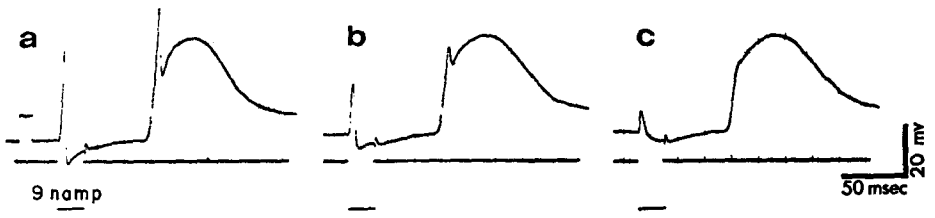

FIGURE 17. Action of tetrodotoxin (10^{-5} g/ml) on spikes evoked by current and light. (a) was recorded before application of the drug and shows successively calibration pulse (10 mv, 10 msec) a spike triggered by a constant current pulse of 9×10^{-9} amp, and a response to a bright flash. (b) was recorded 7 min after the beginning of the perfusion with the tetrodotoxin. (c) was obtained 3 min after (b).

pulse and a second with a bright flash of light. Tetrodotoxin added to the perfusion fluid at a final concentration of 10^{-5} g/ml markedly diminished the magnitude of both spikes (Fig. 17 c). A similar observation was made in seven other cells. In two cells the spike was abolished by this same concentration of tetrodotoxin. The effect of tetrodotoxin was reversible but much more time was needed for recovery than for suppression of the spike. In the experiment illustrated in Fig. 17, there was some loss of membrane potential accompanied by minor changes in the slow wave but these effects were variable and could not be attributed with certainty to the action of the drug.

DISCUSSION

Responses to light of single retinula cells of the drone recorded with intracellular microelectrodes consist of a slow potential change and a single spike superimposed on the rising phase of the slow wave. The results presented in this paper are in agreement with those previously reported by Naka and Eguchi (2) and by Autrum and v. Zwehl (3) in that they show that the shape of the slow potential and of the intensity-response curves observed in the drone are similar to those described for retinula cells of other invertebrates. Furthermore the results show that there are qualitative similarities between the effects

of light and dark adaptation in the eyes of the drone and other invertebrates.

Applicability of the Model of Fuortes and Hodgkin In *Limulus*, responses to dim flashes can be fitted by the linear equation:

$$V = kV_0\Delta t t^{n-1} \cdot \frac{e^{-t}/\tau_1}{\tau_2^n}$$

V is the change in membrane potential evoked by the flash of light; $V_0\Delta t$ is proportional to the quantity of light in the flash; t is the time after the beginning of the flash; τ_1 and τ_2 are constants, and n is a number (corresponding to the number of stages in the electrical analogue) which may be different for different cells but remains constant in individual cells, being approximately equal to 10 on the average. Nonlinearities observed in *Limulus* in response to strong lights are interpreted as being due to a dependence of τ_1 upon V. These nonlinearities appear with some delay so that the early phase of visual responses of *Limulus* remains linear with respect to light intensity even following bright illumination.

The linear equation was found suitable to reproduce responses to dim flashes in the drone; however, since the responses of the drone are faster than those of *Limulus*, τ_1 is smaller, but the average value of n is approximately the same as in *Limulus*. Linearity at early times also seems to apply in responses to strong flashes but it is often difficult to demonstrate due to the development of the initial spike.

Other qualitative similarities between the visual responses of the drone and of *Limulus* were observed in the experiments concerned with light adaptation. Responses to flashes superimposed on a background are apparently diphasic in both preparations, and this finding is in agreement with the predictions of the nonlinear model. However, it should be noted that the response of the model is in fact oscillatory and may appear to be simply diphasic due to strong damping (A. L. Hodgkin, personal communication). It is important to note, however, that number of features of the responses of drones are not reproduced by the model developed for *Limulus*. For instance the model predicts that the time-to-peak of the response to flashes decreases monotonically as light intensity is increased, but experimental responses of the drone show that the time-to-peak first decreases and then increases. For this reason the simple relation between sensitivity and time-to-peak expected in accordance with the model and observed in *Limulus* under different conditions of light adaptation, does not apply to the bee. Another reason for the failure of this relation is that in *Limulus* light adaptation changes only the rate of decay whereas it changes both rate of decay and rate of rise in the drone (Fig. 6). Apparently, light adaptation affects only the parameter τ_1 in *Limulus* but influences both τ_1 and τ_2 in the drone. It should be mentioned that in *Limulus* Fuortes and Hodgkin (1) sometimes found changes in rate of rise due to light

adaptation, but that these were so small that they could be neglected. In conclusion, it seems that the linear model may apply to the bee, but the nonlinear model fails due to greater complexity of the nonlinear processes in the bee.

Spike Potentials One component of the drone response is a large single spike which is found superimposed on the rising phase of responses to light and at the beginning of responses to a depolarizing current step. Such large spikes have not been observed in the eyes of other arthropods. A small notch similar to the one illustrated in Fig. 6 a has, however, often been recorded from retinula cells of the cockroach and the grasshopper (13) and of *Limulus* (1).

The results presented in this paper suggest that this spike is a regenerative phenomenon. Na movement seems to play an important role in its generation. In fact the spike is abolished by tetrodotoxin, a substance which selectively blocks the regenerative Na influx in many membranes (14). Light and current-evoked spikes are also abolished in Na-free solutions (15). Neither the action of tetrodotoxin nor that due to a lack of Na can be explained by an effect on the slow potential.

It seems, therefore, that the drone visual cell has the properties required for generation of graded potentials and also properties generally attributed to electrically excitable cells that are responsible for generation of a spike.

The question arises whether the retinula cell spike has a functional significance. In *Limulus* light regularly evokes repetitive spikes at a frequency which is a function of light intensity. According to Tomita (16) the repetitive spikes of *Limulus* originate from the eccentric cell axon where they are triggered by the slow potential which arises in the retinula cells and spreads passively to the eccentric cell. Although retinula cells of the drone usually respond to light with a single spike, and only exceptionally with a train, Naka and Eguchi (2) suggested that the single spike is an experimental artifact resulting from a complete abolition of all spike potentials except the first one. According to this hypothesis, the normal retinula cell of the drone transforms light into a slow potential and the slow potential into a train of spikes; it thus combines the functions which Tomita (16) ascribes respectively to retinula and eccentric cells in *Limulus*.

A similar interpretation was put forward by Kennedy (17), who suggests that slow potentials with a single spike, as observed in the drone, and slow waves without superimposed spikes, as found in most other insect retinula cells, are the responses of damaged cells.

Several points argue against such an interpretation and favor the view that retinula cells normally fire only a single spike.

1. Repetitive firing was found in the drone only under "unusual" experimental conditions, i.e. towards the end of a long experiment or during impalement or, if the preparation was exposed to air and allowed to dry

or, at the beginning of responses to steps of strongly light-adapted preparations.

2. Responses including only a single spike were found as a rule by Autrum and v. Zwehl (3) in experiments performed on intact drones fed and maintained at normal body temperature during the whole experiment. These experiments produced minimal damage to the animal but involved impalement of the visual cells. However, if impalement were the cause of absence of repetitive firing, one would expect to find regularly multiple spikes in extracellular recordings. This is not the case: repetitive firing is as infrequent in extracellular as in intracellular recordings (Baumann, unpublished data). If one admits that retinula cells do not fire repetitively, one has to ask at what point the slow potential is translated into a series of spikes necessary to signal intensity and duration of a light stimulus. A possible mechanism is suggested by the experiment illustrated in Fig. 18. The retinula cell layer of a drone eye was stimu-

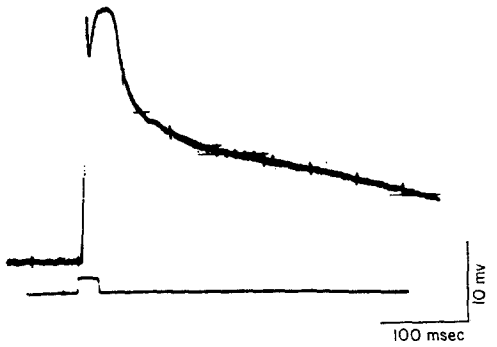

FIGURE 18. Potentials recorded from an unidentified structure in the lamina in response to a 25 msec flash applied to the retinula cell layer.

lated with a flash of light and the response recorded intracellularly in an unidentified structure in the lamina. It is seen that in the lamina the response to light still consists of a large slow potential and a single spike. Perrelet (personal communication) has found that retinula cell axons of drones have almost the same diameter as the cells themselves. It is therefore possible that the slow potential spreads passively to the proximal termination of the retinula cell axon with little decrement. In favor of a central origin of repetitive firing are experiments carried out by Burtt and Catton (18). Recording with extracellular electrodes at different depths in the locust eye, these authors observed spike activity, giving well-defined responses to stimulation with light, when the electrode tip reached a depth corresponding to the outer border of the second synaptic region. In the retinula cell layer they only recorded potentials in response to light.

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