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ANOMALOUS RECTIFICATION IN HORIZONTAL CELLS*

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SUMMARY

1. The electrical properties of horizontal cells in the mudpuppy in light and dark were measured with a pair of micropipettes separated by about 1 μm with low coupling resistance so that no bridge circuitry was required.

2. All horizontal cells studied showed significant anomalous rectification: the current–voltage characteristic for about 60 per cent of the cells studied had a slope resistance of about 20–30 MΩ at the dark potential level; the slope resistance increased by about 15 % for each 10 mV depolarization and decreased by about 15 % for each 10 mV hyperpolarization. The remaining 40 % of the horizontal cells showed a higher input resistance at corresponding potential levels but had similar rectifying properties.

3. The increase in resistance with depolarization developed with a time course of about 1/2 sec when steady steps of outward current were passed across the membrane, but the time course for resistance decrease with hyperpolarization was much shorter for steady inward current steps. In about half the horizontal cells there was a transient decrease in resistance lasting about 100 msec immediately following the outward current steps superimposed upon the slower sustained resistance increase.

4. The normal 20–30 mV hyperpolarizing light response was associated with little or no change in input resistance. However, if the membrane potential was held at the dark potential level with extrinsic current, thereby eliminating the potential-dependent resistance change, a light-elicited resistance increase of about 10 MΩ was measured.

5. The time-dependent change in membrane resistance elicited by polarizing steps of current obscured the reversal potential for the

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response. However, when the reversal potential was measured at short times following polarization of the membrane, before the time-dependent resistance change developed, it was estimated at between +15 and +50 mV.

6. The results suggest that the horizontal cell response is mediated by a light-elicited resistance *increase* at the synaptic membrane which is obscured by a potential- and time-dependent resistance *decrease* at another part of the membrane.

INTRODUCTION

Although horizontal cell activity has been studied for nearly two decades the membrane mechanism which generates the light response still remains obscure. Evidence for a transmitter-mediated permeability change in the subsynaptic membrane has been contradictory, since resistance changes and reversal potentials for the response have not been consistently observed.

The resistance during the light response has been shown to increase (Tomita, 1965; Tomita & Kaneko, 1965; Toyoda, Nosaki & Tomita, 1969), to decrease (Maksimova & Maksimov, 1971), or to show no measurable change (Tasaki, 1960; Trifonov & Utina, 1966).

In most cases, a reversal potential within physiological bounds for the response has not been found. When the horizontal cell membrane was depolarized, the response magnitude has been measured to increase (Gouras, 1960), decrease slightly (Nelson, 1973), or remain unchanged (MacNichol & Svaetichin, 1958; Watanabe, Tosaka & Yokota, 1960). In a series of experiments using extracellular current to polarize the horizontal cell membrane (Trifonov, Chailakhyan, Byzov, 1971; Byzov, Trifonov & Chailakhyan, 1972; Byzov & Trifonov, 1973) horizontal cells of turtle and fish were shown to have a non-linear current–voltage characteristic, and a reversal potential for the response was measured.

In an attempt to resolve some of these contradictory observations, horizontal cells in the mudpuppy, *Necturus maculosus*, were examined with a newly developed pair of micro-electrodes, with very low coupling resistance (Werblin, 1975). This allowed quantitative estimates of the electrical properties of the horizontal cells without bridge circuits, in which the membrane was polarized by currents passed through an intracellular electrode. It is shown that the electrical changes measured during the response are sufficient to support the contention that the hyperpolarizing response of horizontal cells is mediated by a resistance *increase* at the subsynaptic membrane which is often obscured by a potential-dependent resistance *decrease* in another region of the membrane.

METHODS

These experiments were performed concurrently with a study of the electrical properties of the rod membrane (Werblin, 1975), and the methods described there apply here as well.

Electrodes

Briefly, a double-barrelled electrode was pulled from 1 mm o.d. Pyrex capillary tubing on a Livingston-type micropipette puller. The pipettes were filled with 4 M potassium acetate and had tip resistances, measured with an a.c. bridge in Ringer solution, of about 500 MΩ. The double tip was bevelled at an angle on a rotating wheel surfaced with 0·3 μm aluminium oxide in such a way that one of the pipette tips was displaced from the other after bevelling by about 1 μm. The displaced bevelled tip had a resistance of about 50 MΩ.

Penetrating and stimulating

The pipette pair penetrated the retina by use of a 'home made' hydraulic advancer, but the eyecup was mounted upon a small speaker cone which was displaced or 'jolted' (Tomita, 1965) every few seconds by about 1–2 μm less than 1 msec. This procedure greatly facilitated penetration of the cells, and eliminated an otherwise sizeable increase in coupling resistance between the pipettes as the retina was penetrated (Gouras, 1960). Horizontal cells were easily identified by their greater response magnitude (30–40 mV), rather long latency (100–200 msec) to moderate flashes, and by their appearance in the sequence of responses obtained during a typical penetration (Werblin & Dowling, 1969).

Full-field test flashes, formed upon the P 11 phosphor of a cathode ray tube and focused upon the retina, were adjusted in intensity to elicit half-maximal responses in the horizontal cells; these were used throughout this study to elicit a 'standard' response (Normann & Werblin, 1974; Werblin, 1974).

Measurement of electrical characteristics

The current–voltage characteristic for the horizontal cells was evaluated either by presenting current steps of about 1–2 sec duration and monitoring the membrane potential, or by depolarizing the horizontal cell membrane at the rate of 20 mV/sec after first hyperpolarizing it to 100 mV. For the current–voltage curves presented in the Figures, the continuous curves were generated by the continuous polarization method, and the points were generated by the application of steady steps of current. The negative-resistance characteristic shown in Fig. 3*C* was generated by a conventional voltage-clamp circuit described previously (Werblin, 1975).

Horizontal cells are particularly easy to study in the retina of the mudpuppy. More than 100 units were evaluated by the continuous current–voltage curve method. Unless otherwise noted, all other experiments were repeated at least 10 times.

Simulating a fixed series resistance between the electrode and the membrane

No bridge circuit was used when the current–voltage characteristic for the horizontal cell membrane was measured. After reviewing the data it became evident that the electrical characteristics of some cells showing a relatively high slope resistance at all potential levels might have been influenced by the existence of a series resistance between the electrode and the membrane. To test this hypothesis, tape recordings of voltage–current data were displayed on an $X-Y$ plotter by use of the circuit shown in Fig. 1. This allowed the effect of a fixed resistance to be added or subtracted from

the current–voltage characteristic of each horizontal cell. Fig. 5 shows that the current–voltage curve of each horizontal cell can be related to a common characteristic by including a fixed simulated resistance of appropriate magnitude. The circuit satisfies the equations:

$$V_{out} = V_{in} \pm R.I_{in},$$
$$I_{out} = I_{in},$$

where V_{in} and I_{in} are the recorded membrane voltage and current, R is an adjustable constant which simulates the series resistance, and V_{out} and I_{out} the quantities plotted. This provided a convenient way to process the large quantity of data derived from the horizontal cells studied for this report.

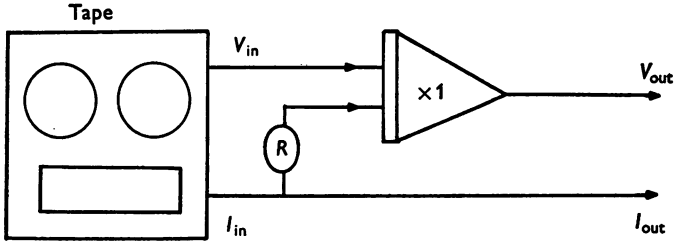

Fig. 1. Analogue computer circuit for adding and subtracting series resistance from taped data. For details see text.

RESULTS

Current–voltage relation in the dark

The current–voltage relation for a typical horizontal cell, evaluated with 2 sec current steps of different magnitudes, is shown in Fig. 2. Fig. 2*A* shows the superposition of ten traces; for each trace in this and subsequent experiments the current pulse was increased in magnitude by 0·5 nA or the polarity of the step was reversed, following the sequence 0·5 nA, −0·5 nA, 1·0 nA, and so on. The membrane potential in response to the steps is relatively constant in time for hyperpolarizations, but increases slowly with a time constant of about 0·5 sec for large depolarizing steps. The slow increase in polarization with depolarizing steps was characteristic of all horizontal cells studied.

Fig. 2*B* shows three current–voltage curves for the horizontal cell in Fig. 2*A*. The open circles plot the relation when the potential measurement was made 50 msec after the onset of the current step; the filled circles plot the potential levels for measurements taken 2 sec after the onset of the step. The current–voltage relation is roughly linear for measurements at 50 msec but is highly rectifying for measurements at 2 sec.

In addition to these two curves, the current–voltage relation was measured by depolarizing the membrane from −100 mV at the rate of 20 mV/sec. The resulting continuous curve in the Figure corresponds

roughly with the points measured 2 sec after presentation of the current steps.

In about 50 per cent of the horizontal cells studied, the slow increase in potential level following the onset of large depolarizing current steps was preceded by a transient hyperpolarization from the initial depolarized level. Response curves illustrating this phenomenon are shown in Fig. 3*A*.

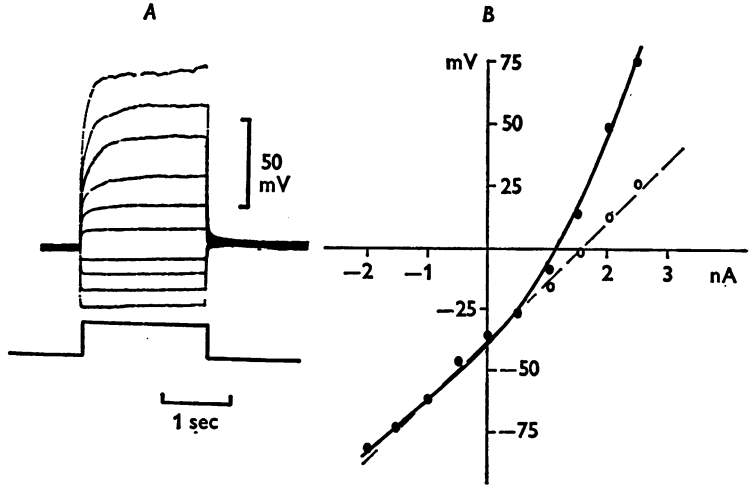

Fig. 2. Current–voltage characteristics in dark. *A*, superimposed traces of nine potentials elicited by current steps. Steps presented in the order 0·5, −0·5, 1·0, −1·0 nA, etc. *B*, membrane potential *vs.* current measured at 50 msec (open circles) and 2 sec (filled circles) after onset of step. Continuous curve generated by continuous depolarization by gradually increasing outward current (see text). In this and subsequent Figures, potential is with respect to an indifferent electrode, so the intersection with the ordinate is the dark potential level.

In this experiment a square wave of current about 0·2 nA in magnitude was superimposed upon the polarizing steps; the magnitude of the resulting square wave of voltage in Fig. 3*A* is a measure of the instantaneous membrane resistance. The curves show that the transient hyperpolarization elicited during the depolarizing steps is associated with a transient resistance decrease, and that the subsequent rise in potential is associated with a resistance increase with respect to the membrane resistance at the dark potential level.

The current–voltage curve measured during the transient decrease in resistance is plotted in Fig. 3*B* as the open circles (1). The curve determined with the potential measured at the end of the 2 sec step is plotted as the filled circles (2). The two curves deviate from linearity in opposite directions, and the deviation occurs mainly for large depolarizations, well

outside the physiological range of response for the cell. The two opposing resistance changes may not be significant in the normal response, but they help to explain another common finding in these experiments. When the membrane was depolarized continuously under voltage clamp (Werblin, 1975) a negative resistance region was often encountered at depolarizations above +35 mV. An example is given by the continuous curve in Fig. 3*C*. This apparent regenerative phenomenon resembles the depolarizing inactivation response described for gymnotid electroplaques (Bennett & Grundfest, 1966). It is accentuated in horizontal cells because the resistance increase is preceded by a transient resistance decrease as the membrane is depolarized.

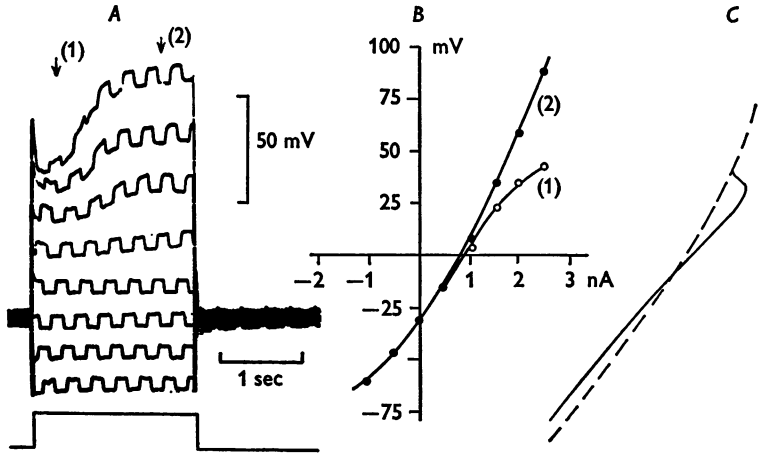

Fig. 3. Current–voltage relation and instantaneous resistance in dark. *A*, magnitude of current pulses same as in Fig. 1, but superimposed upon a 4 Hz 0·1 nA square wave. Resulting square wave of potential is a measure of the instantaneous membrane resistance. *B*, current voltage curves measured at 200 msec (open circles) and 2 sec (filled circles) following onset of current pulses. *C*, continuous curve, current–voltage relations determined by continuously depolarizing the membrane at 20 mV/sec while under voltage clamp. Dashed curve, current–voltage curve derived during continuous hyperpolarization at 20 mV/sec. Curves are shifted to the right and axes are inverted in *C* (voltage is the independent variable) for comparison with curves where current is the independent variable.

The activation–inactivation sequence measured in these horizontal cells resembles the sequence measured in the giant neurones in the sub-oesophageal ganglion of the snail by Gola & Romey (1971). These cells also show anomalous rectification.

These results confirm the measurements of Trifonov *et al.* (1971) in fish and Byzov *et al.* (1972) in turtle who showed a non-linear resistance characteristic and a regenerative effect in horizontal cells when polarizing

the membrane with *extracellular* electrodes. The results of this study in mudpuppy, derived by the more conventional method of polarizing the membrane with current passed through an intracellular electrode, help to confirm the validity of the earlier studies in which extracellular electrodes were used. The finding of anomalous rectification in fish, turtle and mudpuppy suggests that it may be a general characteristic of horizontal cells.

Current–voltage relations in dark and light

The responses elicited by a constant-intensity test flash taken at different initial membrane potential levels are shown in Fig. 4*A*. Each response is represented by a solid vertical bar in Fig. 4*B*. The vertical bars are positioned so that their upper ends correspond to the initial potential level and the lower ends correspond to the peak potential of the response. To record the responses in Fig. 4*A*, the membrane was first polarized to the

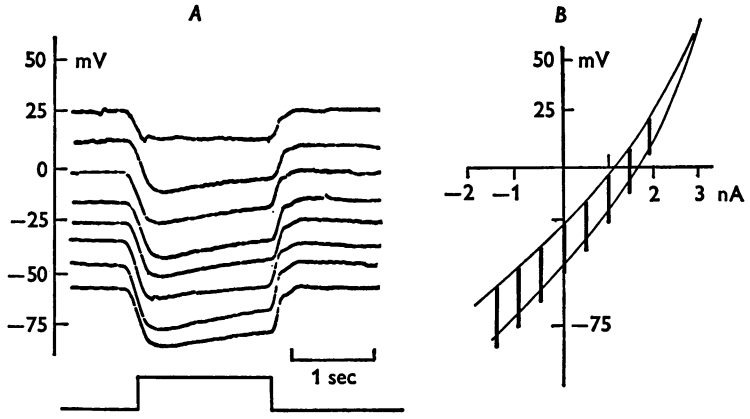

Fig. 4. Response magnitude versus membrane potential. *A*, membrane was displaced to potential levels indicated by scale at left for 5 sec. Then a 1·5 sec test flash was presented. *B*, vertical bars represent the initial-to-peak displacement of the response. Continuous curves were generated by depolarizing the membrane at 20 mV/sec in the dark (upper curve) and light (lower curve). The continuous curve in light intersects the vertical bars at about 75 per cent of the peak response.

initial potential level for 5 sec; then the test flash was presented. The response magnitude decreased monotonically from about 25 to 15 mV as the membrane was depolarized at fixed potentials from −57 to +25 mV. It was not possible to reverse the polarity of the response in any cell studied by this method, even when the membrane was depolarized past +100 mV.

The solid current–voltage curves in Fig. 4*B* were derived by depolarizing the membrane with the retina in steady dark or steady light from

– 100 at 20 mV per second. The upper curve is similar to the dark curves shown in Figs. 2 and 3. The lower curve, representing the current–voltage curve for steady-state light response, crosses the vertical bars corresponding to the light response at about 75 per cent of the peak value. The steady-state values of the response curves of Fig. 4*A* are also about 75 per cent of the peak values so that the curve can be interpreted as a measure of the steady-state light response. As shown below, these curves converge but never cross at depolarized potential levels.

Range of resistance values in horizontal cells

The experiment illustrated in Fig. 4 shows that the current–voltage curves derived by the continuous depolarization method in dark and light give a good estimate of the curves derived by the more conventional method of measuring the response while displacing the membrane to different fixed potential levels. It has the advantage that a complete pair of curves can be generated in 20 sec. Throughout these experiments the current–voltage relations for most horizontal cells were evaluated in dark

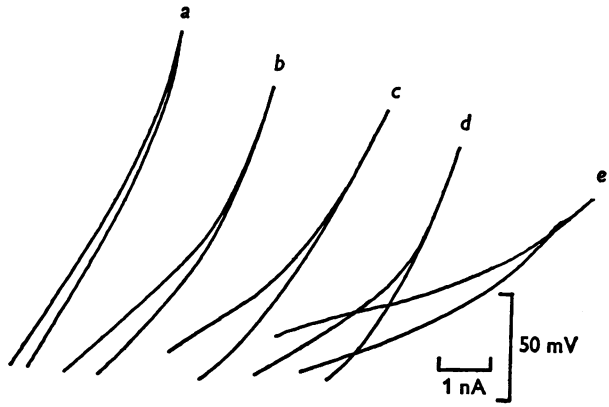

Fig. 5. Range of recorded current–voltage curves. 60 per cent of the recordings resembled curve *d*. About 10 per cent of the recordings are represented by each of the curves *a*, *b*, *c* and *e*. All curves were measured by depolarizing the membrane from – 100 mV at 20 mV/sec.

and light by this method immediately upon penetration. The results were compiled for more than eighty cells by displaying the pairs of curves on an *X*–*Y* plotter. Fig. 5 shows five curve pairs, which span the range of different characteristics found in all cells studied by these methods. About 60 per cent of the curve pairs could be superimposed with curves 5*d*. The remaining measurements were equally divided amongst curves 5*a*, *b*, *c* and *e*.

All of the curves have a similar form, showing a higher slope resistance

as the membrane was depolarized. This suggests that all may be derived from a single membrane current–voltage curve, but recorded through a resistance between the electrodes and the cell membrane which varied from one recording to another. To test this hypothesis, the analogue computer circuit shown in Fig. 1 and described in Methods was used to simulate the addition or subtraction of a fixed, series resistance while displaying the tape-recorded data with the X–Y plotter. Using this technique, the curves of Fig. 5*d* were modified by adding a simulated fixed resistance of $\pm$ 10 and $\pm$ 20 MΩ, as shown in Fig. 6. Each of these simulated curves can be aligned with one of the recorded curves of Fig. 5, supporting the contention that horizontal cells have a common current–voltage characteristic which is often recorded with a fixed resistance in series with the electrodes and the membrane.

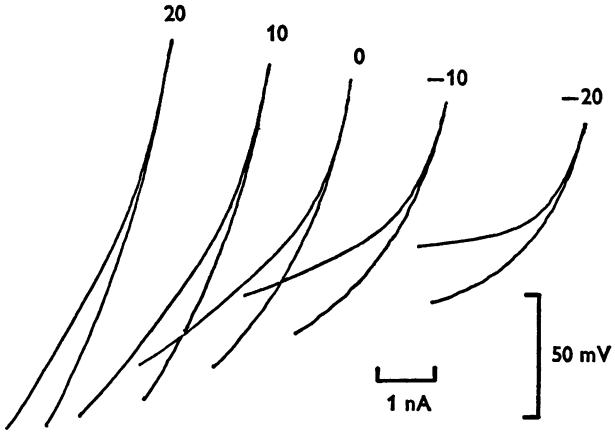

Fig. 6. Current–voltage curves derived from Fig. 5*d* by use of the analogue computer circuit described in Methods (see text).

The curves of Fig. 5*d* have been selected as a characteristic of the horizontal cell. They are the most commonly recorded, and most others could be modified to resemble Fig. 5*d* by the subtraction of a fixed resistance.

The slope resistance for the curves in Fig. 5*d* was evaluated at 10 mV increments from −65 to +45 mV, and plotted against membrane potential in Fig. 7 with dark resistance as open circles and light resistance as filled circles. The slope resistance at the dark potential was about 20 MΩ. It changed by about 15 per cent for each 10 mV polarization.

The difference in slope resistance in dark and light at each membrane potential level is plotted as the X's in Fig. 7. This difference curve applies to all curve pairs in Fig. 5 because each pair is related to the others through the addition of a fixed resistance to both members of the pair (see Fig. 6). The difference in slope resistances between the dark and light curves at

each potential level is about 10 MΩ, and remains constant over a considerable range of membrane potential levels, finally falling to zero above +45 mV as shown by the convergence of the dark and light curves in Fig. 5.

Resistance change during the light response

Fig. 7 shows that there is an increase in slope resistance of about 10 MΩ between the light and dark current–voltage curves at each fixed potential level, but that hyperpolarization of the membrane is associated with a *decrease* in resistance by about 15 per cent per 10 mV. As a result of these two opposing factors, the measured resistance change during the hyperpolarizing light response is considerably less than 10 MΩ. The separate components of (1) illumination resistance increase due to and (2) resistance decrease due to hyperpolarization, can be dissected as shown in the experiment illustrated in Fig. 8.

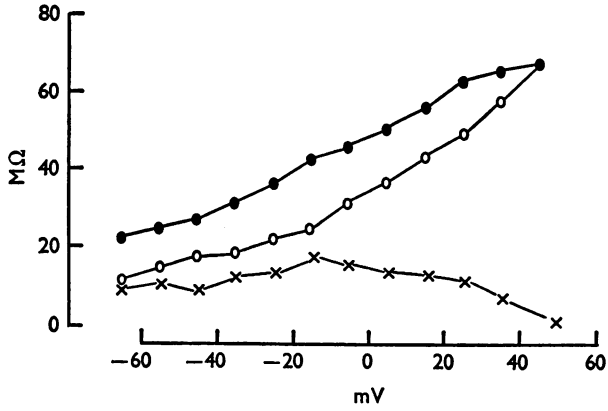

Fig. 7. Slope resistance in dark and light *vs.* membrane potential. Measurements taken from curves in Fig. 5*d*. Filled circles, light resistance; open circles, dark resistance; ×, resistance difference between light and dark at each potential level.

A horizontal cell was hyperpolarized by light while a small, 4 Hz square wave of current was passed across the membrane to monitor membrane resistance. The resulting square wave of voltage was 'balanced' by a conventional bridge circuit at the dark potential level, so the square wave is not discernible before the test flash. The test flash brought the horizontal cell from level (1) to level (2), and caused a 3 MΩ increase in resistance as seen in the bridge unbalance at (2). When the membrane was repolarized to the dark potential level in the presence of illumination by a steady outward current, a 12 MΩ resistance change was monitored (3).

These resistance changes can be explained by following the trajectory for the response and repolarization in the current–voltage plane shown in Fig. 8*B*. The normal light response brings the membrane from point (1) to point (2). There is little change in resistance between these points because the light-elicited increase in resistance is obscured by the potential-dependent decrease (compare slope resistances in inset). However, when the potential-dependent resistance decrease is removed by polarizing the cell back to the dark potential level (point 3), the light-elicited resistance increase alone can be measured.

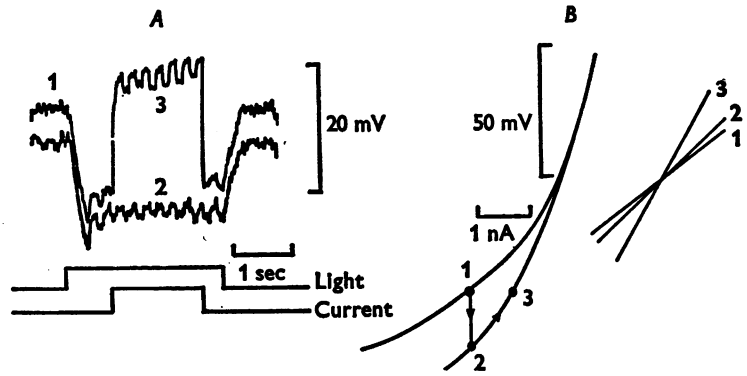

Fig. 8. Isolation of the light-elicited resistance change. *A*, lower recording normal response recorded with initially balanced bridge circuit showing small (3 MΩ) resistance change. Upper recording, membrane potential was driven back to the dark level during the light response by 1·0 nA current pulse. At the dark level, the resistance change during the light response was about 12 MΩ. *B*, current–voltage relation for the cell showing the trajectory followed by the experiment in *A*. Light brings the response from (1) to (2); current brings the membrane from (2) to (3). Slope resistance at (3) is greater than at (2) as predicted by *A* and shown in inset.

A reversal potential for the response

Fig. 2*B* shows that when the membrane potential is measured soon after the onset of the current step, the current–voltage relationship for the membrane is linear; the non-linearity develops later with a time constant of about 0·5 sec. On some occasions, when care was taken to measure the membrane characteristics in both dark and light at short times following the onset of a current pulse (before the non-linearity developed) a reversal potential for the response could be measured.

Fig. 9*A* shows eight superimposed recordings of a horizontal cell response. A pair of 100 msec current pulses was used to displace the membrane potential first before, then during the light response in each trace. The magnitude of the pulse pair was increased by 0·5 nA in subsequent traces.

The membrane potential just before the end of the pulses before and during illumination is plotted in the dark (filled circles) and light (open circles) in Fig. 9*B*. The curves are relatively linear; the dark resistance is about 30 MΩ and the light resistance is about 41 MΩ. In all cells examined by this method the current–voltage curves were linear in both dark and light, and the resistance difference was about 10 MΩ but the point of intersection fell often above +50 mV. The curves for this experiment intersect at about +15 mV, the lowest value for a reversal potential found in any of the horizontal cells studied.

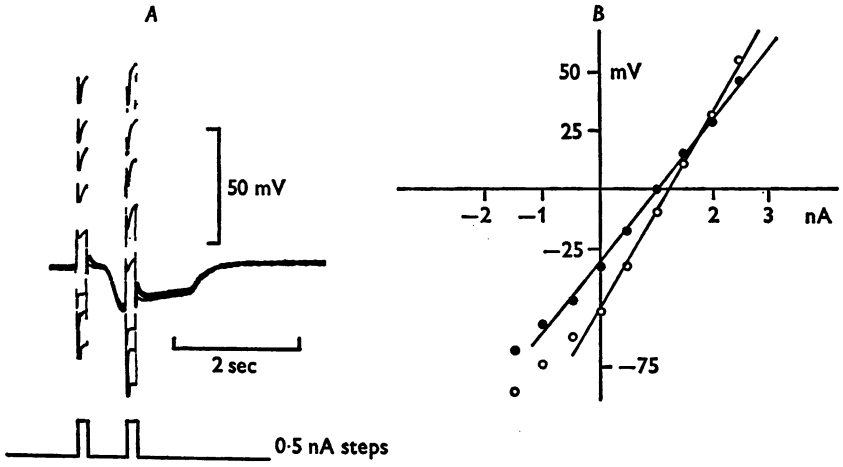

Fig. 9. Reversal potential for the response. *A*, in each of the eight superimposed traces, brief current pulses were presented before and during the light response. *B*, magnitude of the potential changes generated just before the termination of the pulses in the dark (filled circles) and light (open circles) versus current. Curves are linear with 15 mV intersection.

Although this cell showed an atypically low reversal potential, it may be the best estimate. Any series resistance between the electrodes and the synaptic membrane, either electrode coupling or cytoplasmic resistance, would tend to cause an overestimate of the reversal potential. However, a resistance shunt caused perhaps by damage to the membrane during penetration would not cause an underestimate of the reversal potential; only an overestimate of membrane current. Therefore each measured reversal potential is an upper but not a lower bound for the true potential level; the lowest measured value may be the most valid estimate.

DISCUSSION

Is horizontal cell activity mediated by a permeability change?

The measurements above suggest that the depolarizing effect of receptor transmitter, believed to be released continuously in darkness (Dowling & Ripps, 1973; Cervetto & Piccolino, 1974), is mediated by a resistance decrease at the subsynaptic membrane; light reduces the level of transmitter, leading to a resistance increase at the subsynaptic sites causing the membrane to hyperpolarize. These resistance changes are obscured by opposing, potential-dependent resistance changes at other regions of the horizontal cell membrane. A quantitative electrical model of the horizontal cell membrane has been constructed based upon the precedent established at other chemical synapses (Fatt & Katz, 1951; Fatt & Katz, 1953; Takeuchi & Takeuchi, 1960; Eccles, 1961; Kandel & Tauc, 1966). When values derived from measurements in the dark are associated with the elements in the model and the model is perturbed only by the measured light-elicited *resistance* change, the model generates the appropriate response potential over a broad range of membrane potential levels and shows no apparent reversal potential.

Framework for the model

Fig. 10 shows the form of electrical model for the horizontal cell membrane. R_s and R_n represent the resistances, and E_s and E_n represent the e.m.f.s of the synaptic and non-synaptic parts of the membrane. The switch in the synaptic channel is closed in the dark and open in light to simulate the resistance increase with illumination. Values are assigned to the elements on the basis of measurements taken from the dark and light current–voltage curves of the horizontal cell shown in Fig. 5*d*. This was the most commonly measured relationship, and can be derived from all other relationships by adding a fixed resistance as discussed in the text. The slope resistances in dark and light for 10 mV increments from -65 to $+45$ mV membrane potential are given as R_d and R_L in Table 1.

The two-channel representation of membrane resistance shown in Fig. 10 is an over-simplification of the true nature of the membrane in three significant ways. (1) The current–voltage curves of Fig. 5*d* were selected as representative but there is no guarantee that these too were not recorded in series with some fixed resistance between the electrodes and either the synaptic or non-synaptic membranes, in fact; (2) measurements at the soma, the probable site of the recording electrodes, give only the weighted contributions from synaptic and non-synaptic elements that extend from the soma by more than 400 μm in some cases

(Dowling & Werblin, 1969). The membrane potential, series resistance and resistance changes at the synaptic membrane are undoubtedly distributed along these processes; (3) the test flash used here elicited only a half-maximal response, so a certain part of the synaptic membrane was not 'turned off' by illumination. This inactive portion of the synaptic membrane has been combined with the *non-synaptic* channel in the model. Thus, R_s and R_n are representative of the equivalent synaptic and non-synaptic membrane at the soma.

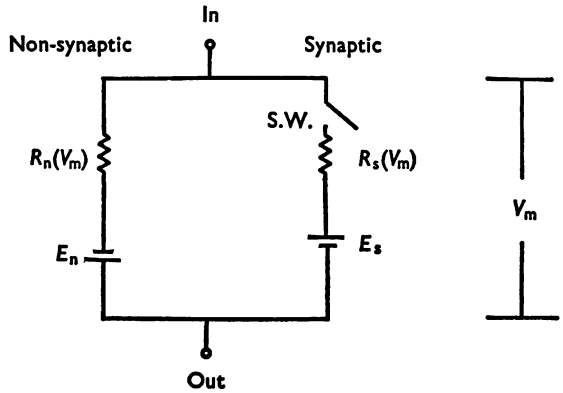

Fig. 10. Electrical model for the membrane. Synaptic membrane represented by R_s and E_s. The switch (Sw.) is open in light and closed in dark. Non-synaptic membrane is represented by R_n and E_n. Both resistances are functions of V_m membrane potential given in Table 1. $E_n = -65$ mV; $E_s = +15$ mV as derived from the data (see text).

Relationship between model and measurements

The resistance of the non-synaptic membrane R_n is the slope resistance of the curves in the light, because the switch in the synaptic channel is open in light. The slope resistance in the dark, R_d, when the switch is closed, is the parallel combination of R_n and R_s. The calculated values of R_s are shown also in Table 1. When no extrinsic current is passed across the membrane, the light potential, which gives the value of E_n in the model, is -65 mV. Knowing the values of E_n, R_n and R_s for the membrane at the dark potential level, the value of E_s is calculated to be $+15$ mV. This is close to the measured reversal potential for the response shown in Fig. 9. Assuming that the values of E_n and E_s are fixed, the dark level, magnitude of the response at different potential levels, extrinsic current and response magnitude can be calculated from the dark and light slope resistances. These calculated response magnitudes are also shown in Table 1 as V_r. They correspond closely to the form of the measured responses elicited at each potential level, as shown in Fig. 11, supporting the contention that the potential change measured in the horizontal cell can be explained

entirely in terms of the measured increase in resistance during light assuming the model in Fig. 10. The method of derivation of responses from the model and anticipated errors are outlined in the Appendix.

TABLE 1. Electrical measurements and predicted responses versus horizontal cell membrane potential

Membrane potential (mV) V_m	Measured resistances (Ω) R_d	R_L	Calculated resistances (MΩ) R_s	Measured response (mV) V_r	Predicted response (mV) V_r	Extrinsic current (nA) I_m
−65	12	23	25	30	—	—
−55	15	25	37	30	—	—
−45	17	28	43	30	—	—
−35	19	31	50	30	30	0
−25	22	37	54	29	31·6	0·336
−15	26	42	68	27	29	0·74
−5	31	46	95	25	26	1·09
5	37	50	142	23	20·8	1·32
15	43	56	185	22	21	1·4
25	50	62	258	22	21	1·49
35	58	65	538	15	21	1·57
45	66	66	∞	0	0	

Membrane potential, V_m; dark slope resistance, R_d; light slope resistance, R_L; calculated synaptic resistance in model, R_s; response magnitude, V_r; current required to displace membrane, I_m.

Resolution of the paradox

If the discrete electrical elements in the model of Fig. 10 can be taken as representative of the equivalent membrane resistances and e.m.f.s measured at the soma, then the model suggests a solution to the paradoxical findings discussed in the Introduction. The relatively small or non-existent resistance change, and the unusually large or non-existent reversal potential measured or extrapolated for the response can result from the anomalous rectification properties of the horizontal cell membrane. Similar membrane properties have been shown to obscure resistance changes and reversal potentials in other systems (Kandel & Tauc, 1966; Eccles, 1961; Nelson & Frank, 1967). It has been shown theoretically that a concurrent resistance increase and decrease associated with the response can obscure or eliminate a reversal potential (Brown, Muller & Murray, 1971).

Relation between the measurements and the membranes

It is almost certain that the horizontal cell membrane potential is not uniformly altered by extrinsic currents in these experiments. Horizontal

cell processes extend from the soma by more than 400 μm in mudpuppy (Dowling & Werblin, 1969), and some decrement potential due to electrotonic conduction would be expected particularly at the finer branches near the termination of the processes at the receptor terminals. Therefore, the measurements of resistance must be taken as a weighted contribution from many synaptic regions, each at a different membrane potential level, and each coupled to the soma through a different cytoplasmic resistance. This problem is accentuated by the findings of Byzov, Trifonov & Chailakhyan (1972), who show that the space constant for the horizontal cell is itself a function of membrane potential, decreasing with hyperpolarization. Therefore the measurements are only qualitative estimates for the properties of the synaptic and non-synaptic membranes.

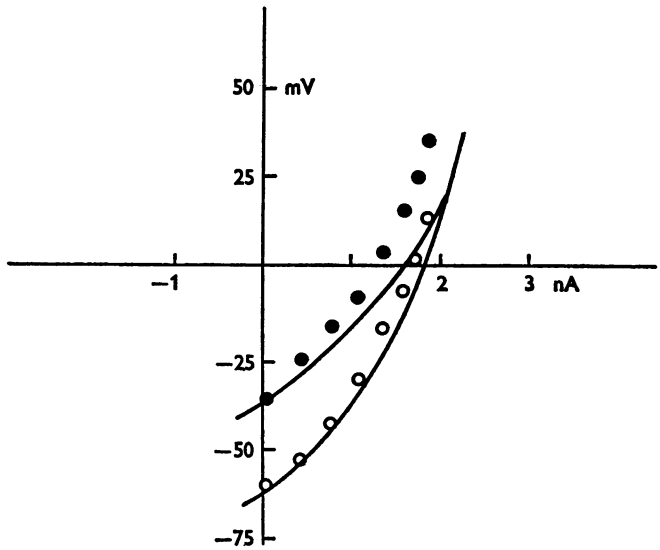

Fig. 11. Comparison of measured and predicted response curves based upon the model. Filled circles are predicted dark potentials and open circles are predicted light potential levels derived from the model and data in Table 1 by procedures in the Appendix. The curves have the general characteristics of those in Fig. 5*d*, shown as continuous curves, but are skewed to slightly higher resistance value due to the approximation procedure for extrinsic current discussed in the Appendix.

It is particularly difficult to interpret the voltage-dependent character of the synaptic resistance, R_s, in the model. From Table 1 it appears that the conductance change associated with the synaptic membrane during the light response is itself a function of membrane potential: the conductance change decreases as the membrane is depolarized. This is unlike the behaviour of a more conventional subsynaptic membrane, the neuro-

muscular junction, where conductance change is independent of membrane potential level (Takeuchi & Takeuchi, 1960).

The voltage-dependent properties of both the membrane resistances and space constant may be manifestations of activity at the reciprocal synapse between horizontal cells and receptors. Polarization of the horizontal cell could alter synaptic input to the horizontal cell itself by modifying receptor activity. If, as suggested above, receptor transmitter modified horizontal-cell membrane resistance, the space constant of the horizontal cell could be decreased with increasing receptor input. The role of the reciprocal synapse in the anomalous rectification of horizontal cells can best be studied by blocking synaptic transmission (Dowling & Ripps, 1973; Cervetto & Piccolino, 1974). The relative contributions to the membrane non-linearities from the membrane and reciprocal synapse with receptors and neighbouring horizontal cells will be considered in a subsequent study (L. Marshall & F. S. Werblin, in preparation).

APPENDIX

Estimation of the response potential using the model

The response potential generated by the model in Fig. 10 at each potential level plotted in Fig. 11 was estimated by the following procedure.

1. The current required to displace the membrane from the dark potential level V_d to each potential level V_m was determined using the fixed batteries and the value of R_n and R_s at each V_m,

$$I_m = \frac{V_m - \left[\dfrac{-R_n \cdot 65 + R_s \cdot 15}{R_s \cdot R_n}\right]}{R_d}.$$

This current will always be an underestimate of the current to depolarize the membrane because the slope resistance for the membrane increases monotonically. I_m for each potential level is given in Table 1.

2. In the presence of the polarizing current I_m, the 'response' was generated by opening the switch in the subsynaptic channel of the model. This served two functions: (i) R_s was eliminated from the circuit, so the membrane 'hyperpolarized', (ii) the value of R_n was modified because of the hyperpolarization. Responses were typically about 20 mV in magnitude, so the value of R_n at V_m -20 mV was substituted in the model, and the membrane potential, in the presence of I_m, but with R_n at V_m -20 mV was calculated. The response potential, V_r is given by

$$V_r = I_m [R_n (V_m - 20)] - V_m.$$

The substitution of a fixed R_n is again an approximation. R_n at V_m -20 is about 30 per cent less than R_n at V_m. This substitution is the essential

step that reduces the change in resistance with light response and obscures the reversal potential for the response. The slope resistance during the normal response changes very little. The curves of Fig. 11 are skewed to higher resistance values than those of Fig. 5*d* because the slope resistances at each potential level were used in step 1 above to approximate the membrane current levels.

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