

# Light Response of a Giant *Aplysia* Neuron

ARTHUR M. BROWN and H. MACK BROWN

From the Departments of Physiology and Medicine, College of Medicine, University of Utah, Salt Lake City, Utah 84112. A. M. Brown's present address is the University of Texas Medical Branch, Galveston, Texas 77550.

**ABSTRACT** Illumination of an *Aplysia* giant neuron evokes a membrane hyperpolarization which is associated with a membrane conductance increase of 15%. The light response is best elicited at 490 nm: the neuron also has an absorption peak at this wavelength. At the resting potential ( $-50$  to  $-60$  mV) illumination evokes an outward current in a voltage-clamped cell. This current reverses sign very close to  $E_K$  calculated from direct measurements of internal and external  $K^+$  activity. Increases in external  $K^+$  concentration shift the reversal potential of the light-evoked response by the same amount as the change in  $E_K$ . Decreases in external  $Na^+$  or  $Cl^-$  do not affect the response. Therefore, the response is attributed to an increase in  $K^+$  conductance. Pressure injection of  $Ca^{2+}$  into this neuron also hyperpolarizes the cell membrane. This effect is also due largely to an increase in  $K^+$  conductance. The light response after  $Ca^{2+}$  injection does not appear to be altered. Pressure injection of EGTA abolished or greatly reduced the light response. The effect was reversible. We suggest that light acts upon a single pigment in this neuron, releasing  $Ca^{2+}$  which in turn increases  $K^+$  conductance, thereby hyperpolarizing the neuronal membrane.

## INTRODUCTION

Phototransduction in excitable cells such as photoreceptors involves alteration of a pigment by light which leads to a change in the cell's membrane potential. A great deal is known about the first step in the sequence; namely, changes in a photopigment produced by light (Wald, 1968). A considerable amount is also known about the last step in the sequence; namely, the ionic mechanisms underlying the changes in membrane potential (Kikuchi et al., 1962; Bortoff and Norton, 1967; Toyoda et al., 1969; Millechia and Mauro, 1969; Baylor and Fuortes, 1970; Brown et al., 1970). Almost nothing is known about the intervening steps whereby the pigment change is followed by an alteration of membrane potential. Several years ago, Fuortes (1959) implicated a transmitter in the processes linking photochemical to neural activity,

and a role for  $\text{Ca}^{2+}$  has been suggested recently (Yoshikami and Hagins, 1971). One reason for this lack of information is that photoreceptors, even those in invertebrates, tend to be small cells less than  $100\ \mu\text{m}$  in diameter. This limits application of microtechniques, in particular microinjection techniques. However, illumination alters the membrane potential in certain large ( $>250\ \mu\text{m}$  diameter) neurons in the ganglia of invertebrates which contain light-sensitive pigments (Arvanitaki and Chalazonitis, 1961). We investigated the light-evoked hyperpolarization (LEH) in the giant neuron of the abdominal ganglion of *Aplysia californica* (R2, according to Frazier et al., 1967). We found that (a) it is best elicited at 490 nm and there is a peak in the absorption spectrum of R2 at this wavelength, (b) it is associated with an increase in membrane conductance, (c) the reversal potential of the LEH corresponds to the equilibrium potential of  $\text{K}^+$  ions indicating a  $\text{K}^+$  permeability increase, (d)  $\text{Ca}^{2+}$  increases  $\text{K}^+$  conductance in this neuron (Meech, 1972), and (e) the light response is reversibly blocked by the intracellular injection of EGTA. We suggest that light absorbed by a pigment ( $\lambda\ \text{max}\ 490$ ) may cause the release of  $\text{Ca}^{2+}$  which in turn increases  $\text{K}^+$  permeability, thereby hyperpolarizing the membrane. Preliminary results have been reported (Brown and Brown, 1972; Brown and Hughes, 1973).

#### METHODS

##### *Biological Preparation*

The abdominal ganglion of *Aplysia californica* was pinned to a Sylgard ring (Dow Corning Corp., Midland, Mich.) located on a quartz window which formed the bottom of a chamber (vol. 1.5 ml). R2 was freed from the rest of the ganglion cells by slitting the capsule. In some experiments, the soma was ligated at its junction with the axon, the position of the ligature being subsequently verified by histological examination. The cell was perfused with artificial seawater (ASW) (Brown and Berman, 1970) which was modified in certain experiments as discussed in the text. The osmolality of the solutions was checked with a freezing point depression osmometer and varied between 980 and 1020 mosmol. The pH was adjusted to 7.6. The preparation was maintained at room temperature ( $20^\circ\text{C}$ ) or at  $1^\circ\text{--}5^\circ\text{C}$ , by cooling the preparation chamber with a 40% vol/vol methanol/water solution circulated through a refrigeration unit by a constant-flow pump.

##### *Electrical Recording*

The methods of measuring membrane potential,  $E_m$ , and membrane current during voltage-clamp have been described (Brown et al., 1970).  $E_m$  was measured as the potential difference between a pair of 3-M KCl-filled micropipettes (resistance 3 to  $5 \times 10^6\ \Omega$ ); one electrode was inserted into the cell, and the other was used as the reference electrode. Another 3-M KCl-filled micropipette with a resistance of 0.5–1.5  $\text{M}\Omega$  was inserted into the cell and used as the current-injecting electrode in

voltage- or current-clamp experiments. Other solutions such as 0.6 M  $\text{Na}_2\text{SO}_4$  and 2 M K-acetate were used to fill the micropipettes in some of the experiments without affecting the results.

#### *Measurement of Internal Ionic Activities*

Techniques for measuring intracellular  $\text{K}^+$  and  $\text{Cl}^-$  activities ( $a_{\text{K}}^i$ ,  $a_{\text{Cl}}^i$ ) have been described (Russell and Brown, 1972 *a* and *b*). Intracellular  $\text{Na}^+$  activity ( $a_{\text{Na}}^i$ ) was measured with microelectrodes made from NAS 11-18 sodium glass (Corning Glass Works, Science Products Div., Corning, N. Y.) according to a miniaturization of the method of Hinke (1959; see Thomas, 1970) and an alternative method described by Thomas (1970). The drop-through electrodes had tips that projected 10–50  $\mu\text{m}$  beyond the tip of the insulating glass micropipette. The recessed-tip electrodes had tips recessed 10–20  $\mu\text{m}$  from the end of the insulating glass micropipette. The  $\text{Na}^+$ -sensitive microelectrodes had resistances of  $5 \times 10^{10}$  to  $10^{11} \Omega$ , slopes of 59–61 mV per 10-fold change in  $a_{\text{Na}}^i$ , and selectivities of between 30 and 60:1 over  $\text{K}^+$ . The selectivities were determined from the differences in voltage output in three solutions of differing  $\text{Na}^+:\text{K}^+$  composition at equal ionic strength. The response time of the drop-through electrodes was about 2–4 s; that of the recessed-tip electrodes ranged from 7 to 30 s. The electrodes were calibrated before and after use and only those giving the same slope and selectivity were considered to have given reliable measurements of  $a_{\text{Na}}^i$ .

#### *Intracellular Pressure Injections*

The technique has been described by Meech (1972). Micropipettes of 2.5  $\mu\text{m}$  diameter were filled with filtered salt solutions at concentrations of 0.5–1.0 M. Pressures of less than 5 lb/in<sup>2</sup> ejected fluid in a stream from the tips. The pipettes were inserted into the cell with or without pressure; tip negative-current pulses (10 nA) were passed through the  $\text{Ca}^{2+}$  pipettes continuously to help keep the pipette tips patent. Impalement was signalled by the voltage changes registered by a voltage-sensing micropipette already in the neuron. In order to rule out nonspecific effects of pressure injection, another pressure-injection pipette containing a different solution was always inserted into the same neuron.

Estimates of the volume ejected from a KCl-filled pipette were derived from measurements of changes in  $\text{Cl}^-$  activity in a known volume using a chloride liquid ion-exchanger microelectrode. About  $10^{-11}$  liters was ejected in 1–6 s.

#### *Light Stimulus*

Light was transmitted to the giant cell by a fiber optics bundle connected to either a tungsten-halide source or a Hg-Xenon source via a monochromator (QPM 30, Schoeffel Instrument Corp., Westwood, N. J.). The light path was interrupted by the vane of an electromagnetic shutter which was remotely driven so that the light could be applied as rectangular pulses. Intensity was reduced as necessary using neutral density filters.

The energy flux at the level of the preparation was measured with an YSI-65 radiometer.

#### *Light Absorption Studies*

The absorption spectrum for R2 was studied using a microspectrophotometer. The cell was dissected free from the ganglion, ligated at the junction of cell body and axon, and the ends of the ligature fixed to a wax ring formed on a quartz microscope slide. The light source was a tungsten-halide lamp operating from DC voltage supply. A motor-driven diffraction grating monochromator provided a continuous scan from 350 to 700 nm. The motor was coupled to the paper-advancing mechanism of a chart recorder. The monochromatic light was focused through the condenser of an inverted Reichert MEF2 microscope (American Optical Corp., Buffalo, N. Y.) onto the cell. The light was then collected by the objective of the microscope, and could be viewed through another ocular or transmitted through an aperture of variable slit width (10  $\mu\text{m}$  aperture) to a photomultiplier tube (EMI No. 9558 QB, Varian Associates, Plainview, N.Y.). The output of the photomultiplier tube was amplified by a lock-in amplifier (PAR 124) phase-locked to a light chopper.

The cell was dark-adapted for 60 min before scanning. In two experiments, the absorbance difference spectrum was studied. The cell was dark adapted as above, scanned, illuminated with intense white light for 10 min, and the scan repeated. The cell was then dark adapted for another 60 min and scanned once more. The difference spectrum was obtained by subtracting the two curves.

### RESULTS

#### *Effects of Light on Membrane Potential, Membrane Current, and Membrane Conductance*

Illumination of R2 with white light produced a slow hyperpolarization of 2–5 mV after a delay of about 400 ms. The amplitude was dependent upon the intervals between light stimuli. The potential change reached its most negative value in about 20 s (Fig. 1 A) and became less negative with sustained illumination. Termination of the light pulse was followed by repolarization which was usually complete within 1 min, although this was variable. The hyperpolarization was associated with about a 15% reduction in membrane resistance when the latter was measured with constant current pulses (Fig. 1 B). The conductance increase was on the order of  $10^{-7} \Omega^{-1}$ , and was independent of the light-evoked change in  $E_m$  since the same changes occurred during voltage-clamp (Fig. 1 C). In this experiment, the cell membrane was maintained at  $-64$  mV with the voltage-clamp circuit. Command pulses were applied to the voltage-clamp amplifier to change the membrane potential from  $-64$  to  $-75$  mV. Illumination was followed by an outward light-evoked current (LEC) which was  $3.7 \times 10^{-9}$  A at a holding potential,  $E_H$ , of  $-64$  mV, and at  $-75$  mV, LEC was  $2 \times 10^{-9}$  A. The LEC had the same

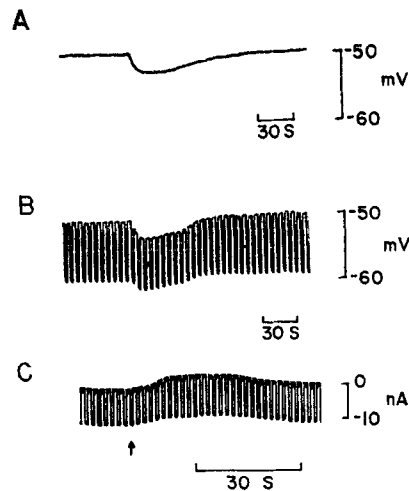


FIGURE 1. A. LEH of R2. B. 10-nA current pulses applied before, during, and after LEH. C. Different cell. LEC when membrane potential was held at  $-64$  mV by voltage-clamp circuit and 11 mV hyperpolarizing command pulses were superimposed. Arrow indicates onset of illumination. Duration of illumination was: A, 38 s; B, 53 s; and C, 25 s.

time-course as the change in membrane potential. This was to be expected since the membrane time constant of about 180 ms is much shorter than the time-course of the LEC. In this cell the conductance increase was also about 15% from  $9.0 \times 10^{-7} \Omega^{-1}$  in darkness to  $1.1 \times 10^{-6} \Omega^{-1}$  during illumination.

The possibility that the light response was due to synaptic action was ruled out in the following ways. (a) Brief exposure to low or zero  $\text{Ca}^{2+}$ , which abolishes or greatly reduces synaptic action, actually usually enhanced the response to light. The enhancement was best seen when membrane conductance was not increased by zero  $\text{Ca}^{2+}$ . (b) In three experiments, the cell was ligated between the soma and axon and removed from the ganglion. The light-evoked change in membrane potential was similar to that observed in nonligated cells.

Another possibility was that light enhanced the activity of the electrogenic  $\text{Na}^+$  pump thought to be present in these cells. Such a suggestion has been made for the ventral photoreceptors of *Limulus* (Smith et al., 1968). To test this, the cell was exposed to ouabain ( $2 \times 10^{-4}$  M) or  $\text{K}^+$ -free solutions. Each of these treatments caused immediate depolarization, presumably due to blockage of the electrogenic  $\text{Na}^+$  pump (Carpenter, 1970). At this time, the light-induced hyperpolarization was not appreciably changed despite a larger driving force on the  $\text{K}^+$  ion. We attribute this to the increased membrane conductance in the dark which occurs at such depolarized resting potentials. In this instance, the increment of LEC would be very small.

### *Spectral Studies*

The action spectrum of the light response was studied under both voltage- and current-clamp conditions; the results were similar in either case. Fig. 2 (triangles) shows that at equal energies of monochromatic light, the peak hyperpolarization was obtained at 490 nM. Conversely, using a minimal criterion response of  $3 \times 10^{-10}$  A during voltage-clamp, the wavelength requiring the least energy to elicit this LEC was also 490 nM. These results may be compared to those of Arvanitaki and Chalazonitis (1961), who showed that light at 490 nM and 579 nM had maximum effects on the membrane potential of *Aplysia* neurons. However, in their experiments the shorter wavelengths elicited a hyperpolarization of the neuronal membrane, whereas longer wavelengths evoked a depolarization.

The absorption spectrum revealed prominent peaks at 490, 535, 567, and 610 nM. This data does not differ greatly from that reported for neurons of *Aplysia depilans* (Chalazonitis and Arvanitaki, 1951). The 490 nM peak was attributed to carotenoid pigments, whereas the others were attributed to hemoproteins found in these cells. In two cells the absorbance difference spectrum after illumination showed a peak at 490 nM. A pigment has been extracted from freshwater snail neurons which also has an absorbance maximum after illumination near this wavelength (Benjamin and Walker, 1972).

### *Reversal Potential of LEH or LEC*

Both the LEH and the outward LEC under voltage-clamp became smaller as the membrane potential was shifted to more negative values. At about  $-76$  to  $-80$  mV in all cells, there was no potential change (Fig. 3), and LEH became positive at membrane potentials more negative than this ( $-79 \pm 3.5$  mV, 12 cells). LEC became inward at these potentials. In the current-clamp experiments, the membrane potential change produced by long current pulses changed slowly or crept from its initial value before light was applied. The direction of the creep depended upon which side of the LEH reversal potential the membrane was being maintained at by the current. At  $E_m$ 's less negative than the reversal potential,  $E_R$ , the membrane potential gradually became less negative as the current injection was maintained (Fig. 3). At  $E_m$ 's more negative than  $-80$  to  $-85$  mV, the membrane potential became more negative with time (Fig. 3). We found that the values of the reversal potentials for both LEH and the membrane potential "creep" were very close to the values of the potassium equilibrium potential,  $E_K$ , simultaneously determined from the directly measured values of  $a^i_K$  and  $a^o_K$ . We have no explanation for the increase in the time to peak response at more negative potentials.

A comparison of current-voltage ( $I-V$ ) relationships of R2 in the dark and during illumination is shown in Fig. 4. The  $I-V$  curves in the light or dark have the same general characteristics. The difference in current at each of several membrane potentials provides an estimate of the voltage dependence of LEC, shown as the open triangles. LEC was outward at voltages less nega-

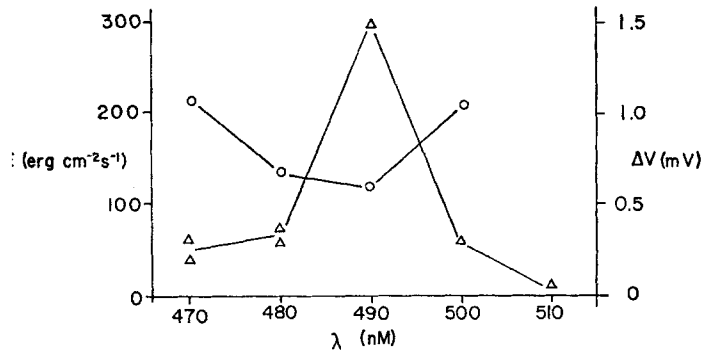


FIGURE 2

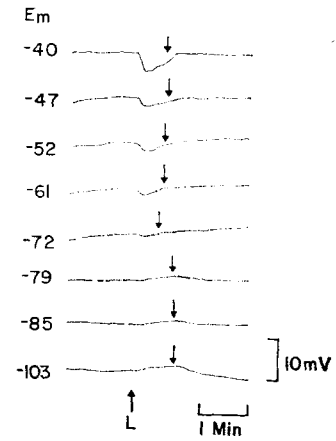


FIGURE 3

FIGURE 2. Circles, left ordinate: Energy flux—wavelength relation to elicit the same change in membrane current by light (0.3 nA). Triangles, right ordinate: Change in membrane potential elicited by flashes of light with the same energy at each wavelength. FIGURE 3. Sign and magnitude of LEH at different membrane potential levels produced by passing inward current across the membrane. Arrows indicate duration of illumination.

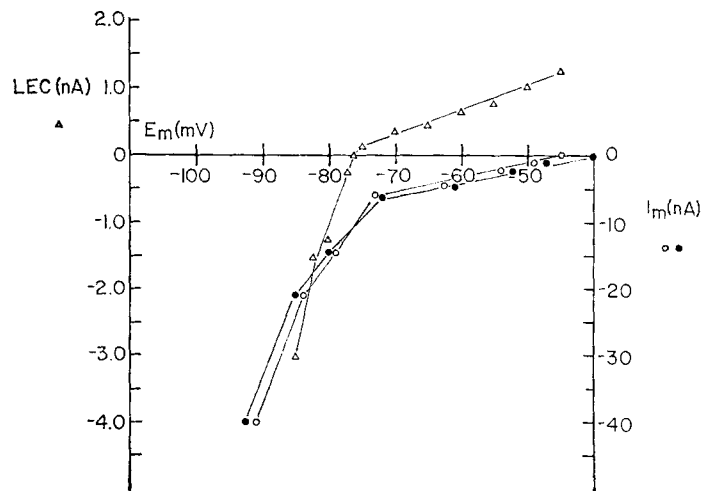


FIGURE 4. Current-voltage ( $I-V$ ) relationship of membrane in the absence (●) and presence (○) of illumination. Current values (LEH) are on the right ordinate. The LEC, which is the difference between these two curves, is represented by the triangles. Its calibration is on the left ordinate.

tive than  $-77$  mV, and this portion of the  $I$ - $V$  curve is linear. LEC was inward at membrane potentials more negative than  $-77$  mV. For a given increment in membrane potential, the current is greater in the inward than the outward direction. Thus, the voltage dependence of LEC resembles the anomalous rectification of the membrane in the absence of illumination (Tauc and Kandel, 1964).

*The Relationship of LEH to the Equilibrium Potentials of  $K^+$ ,  $Na^+$ , and  $Cl^-$*

Average values (millimolar) of  $a^i_K$  and  $a^i_{Cl}$  in R2 are  $165 \pm 3.4$  and  $37 \pm 1.6$ , respectively (Russell and Brown, 1972 *a* and *b*). Average values (millimolar) of  $a^i_{Na}$  are  $17 \pm 2.2$  (28 cells). Average  $a^i_K$  for 12 cells in the present series was  $177 \pm 4.4$  mM (Table I) which does not differ significantly from that of the larger series. The values (millimolar) of  $a^o_K$ ,  $a^o_{Na}$ , and  $a^o_{Cl}$  in ASW are 7,  $350 \pm 1.0$ , and  $341 \pm 3.5$ , respectively. The calculated values (millivolts) of  $E_K$ ,  $E_{Na}$ , and  $E_{Cl}$  are  $-80 \pm 1.2$ ,  $+77 \pm 3.1$ , and  $-56.4 \pm 0.5$ , respectively. The reversal potential for the light-evoked changes in membrane potential or membrane current during voltage-clamp were close to the average value of  $E_K$ . We checked this directly by making simultaneous measurements of the reversal potential of LEH or LEC and  $E_K$  in 12 cells. The values are shown in Table I. The correspondence is very good.

The dependence of  $E_R$  upon  $E_K$  was examined further by increasing  $a^o_K$  and measuring the change in reversal potential. When  $a^o_K$  was increased, the membrane conductance was greatly increased for inward current which made it

TABLE I  
COMPARISON OF  $E_K$  AND THE REVERSAL POTENTIAL  $E_R$  OF THE  
LIGHT-EVOKED HYPERPOLARIZATION IN R2

Cell	$a^i_K$	$a^o_K$	$E_K$	$E_R$
	mM	mM	mV	mV
1	168	7	-80	-78
2	155	7	-78	-77
3	189	7	-83	-83
4	155	7	-78	-74
5	189	7	-83	-85
6	204	7	-85	-84
7	168	7	-80	-75
8	204	7	-85	-83
9	182	7	-82	-79
10	161	7	-79	-74
11	168	7	-80	-76
12	182	7	-82	-81
$N = 12$	$\bar{X} = 177.0$ SEM = $\pm 4.97$		$\bar{X} = -81.2$ SEM = $\pm 0.7$	$\bar{X} = -79.0$ SEM = $\pm 1.15$



difficult to pass enough current through the current-injecting micropipette to reverse the light-evoked changes in membrane potential or current. Nevertheless, in four cells it was possible to determine changes in the reversal potential in a high  $K^+$  solution. The results from one experiment are shown in Fig. 5. Current-voltage relationships in darkness and during illumination were obtained in ASW with an  $a^{\circ}_K$  of 7 mM as shown by the open and closed circles. The LEH reversed sign at about  $-81$  mV.  $E_K$  determined from measurements of  $a^{\circ}_K$  at this time was  $-82$  mV. Then the  $K^+$  concentration was raised to 30 mM ( $a^{\circ}_K = 21$  mM) and the  $I-V$  relation again obtained as shown by the

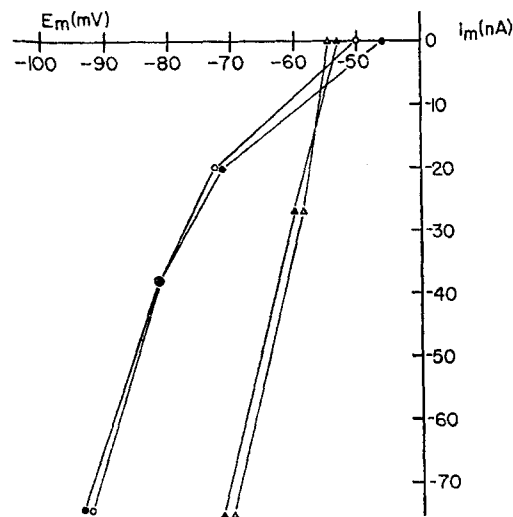


FIGURE 5. Current-voltage relationships of R2 membrane at external  $K^+$  activities of 7 mM (filled and open circles) and 21 mM (filled and open triangles). The filled and open symbols represent membrane  $I-V$  relations in the darkness and light, respectively. The simultaneously determined values of  $E_K$  in ASW were  $-82$  mV ( $a^{\circ}_K$ , 7 mM) and  $-54$  mV ( $a^{\circ}_K$ , 21 mM).

triangles. LEH reversed sign at  $-56$  mV.  $E_K$  at this time was  $-54$  mV. The close correspondence of  $E_K$  and the reversal potential of LEH,  $E_R$ , in this experiment and in those tabulated (Table II), suggests that LEH is produced almost exclusively by a permeability increase to  $K^+$  ions.

It was also possible to compare the light-evoked current LEC, measured directly (voltage-clamp) with the light-evoked current, LEC', calculated from  $E_m$ ,  $E_K$  and the light-evoked change in membrane conductance  $\Delta G_m$ . Hence,

$$LEC' = \Delta G_m (E_m - E_K), \quad (1)$$

where  $\Delta G_m$  is attributed entirely to an increase in  $K^+$  conductance. It should be noted that  $\Delta G_m$  is a steady-state measure of membrane conductance, but in several cells the  $I-V$  relationship of the membrane at the reversal potential

during light was sufficiently linear to allow the calculation to be made with small error. The results are shown in Table III. For cells 6 and 9, LEC was calculated using the  $\Delta G_M$  obtained while passing constant current pulses across the membrane; this result is compared with the LEC when the cell membrane was voltage clamped to the resting level.

For cell 12, step changes in inward membrane current for a small change in membrane potential (11 mV) were obtained before and during light, allowing

TABLE II  
EFFECTS OF CHANGING  $[K_o]$  ON  $E_K$  AND  $E_R$

Cell	$a_K^i$	$a_K^o$	$E_K$	$E_R$
2	155	7	-78	-77
		21	-50	-44
6	204	7	-85	-84
		55	-33	-36
11	168	7	-80	-76
		35	-40	-38
		21	-52	-52
12	182	7	-82	-81
		21	-54	-56

TABLE III  
COMPARISON OF LEC MEASURED DURING VOLTAGE-CLAMP WITH LEC' CALCULATED USING EQ. 1

Cell	$E_K$	$E_H$	LEC ( $10^{-9}$ A)	$E_m$	$\Delta G_M$ ( $10^{-7} \Omega^{-1}$ )	LEC' ( $10^{-9}$ A)
	mV	mV		mV		
6	-85	-46	2.7	-46	0.64	2.5
9	-82	-54	6.7	-55	2.3	6.4
12	-82	-64	3.7	-64	2.1	3.8
	-82	-75	2.0		2.1	1.5

$E_H$  is the holding potential of the membrane during voltage-clamp and was set at or close to  $E_m$ .

the conductance change and LEC to be determined simultaneously (Fig. 1 C). The outward current at  $E_H$  -64 mV was  $3.7 \times 10^{-9}$  A and at  $E_H$  -75 mV, it was  $2.0 \times 10^{-9}$  A. The values of LEC' calculated from Eq. 1 were  $3.8 \times 10^{-9}$  A and  $1.5 \times 10^{-9}$  A, respectively.

#### *Effects of Some Ionic Changes upon the Light Response*

Brief exposure to  $Na^+$ -free ASW using less permeable ions such as  $Li^+$  or Tris, did not appear to change the light-evoked membrane hyperpolarization. As noted earlier, brief exposure to zero or 0.1 mM  $Ca^{2+}$  solutions (substituted

with  $Mg^{2+}$ ) either did not change or actually enhanced LEH. A complete analysis of zero  $Ca^{2+}$  on the passive membrane of R2 and the light response, has not been made. Substitution of  $Rb^{+}$  for  $K^{+}$  slightly increased or did not alter the magnitude of the light-evoked hyperpolarization; the same was true of methane-sulfonate-substituted  $Cl^{-}$ -free ASW. By far the most intriguing results came from changes of internal  $Ca^{2+}$  activity. The evidence that the LEH of R2 was due to increased  $K^{+}$  permeability and that it was enhanced by low external  $Ca^{2+}$ , taken with Meech's report (1972) that injection of  $Ca^{2+}$  into *Aplysia* neurons increased  $K^{+}$  conductance, led us to test the effects of changes in internal  $Ca^{2+}$  upon both  $K^{+}$  conductance and the photoresponse of this neuron.

*Effects of Pressure Injection of  $Ca^{2+}$  upon  $K^{+}$  Conductance in R2*

Injection of small amounts of the 0.5 M  $CaCl_2$ , 0.5 M  $KCl$  mixture at pressures of 2–3 lb/in<sup>2</sup> for 5–10 s, produced a large increase in membrane conductance (Fig. 6 A). At  $E_m$ 's less negative than –65 to –80 mV, the membrane hyperpolarized; at  $E_m$ 's more negative than these values, the membrane depolarized. Injection of larger amounts was often accompanied by an initial depolarization, independent of  $E_m$ , followed by a hyperpolarization and a very slow recovery. The slow recovery is shown in Fig. 6 B. Repetition of such injections caused an irreversible reduction in membrane resistance. During voltage-clamp, injection caused large current changes which were outward for holding potentials less negative than –65 to –80 mV, and inward for more negative holding potentials (Fig. 6 C). By contrast, pressure injection of 0.5 M  $KCl$  alone either had no effect or produced an inward current or a small depolarization with little conductance change.

In three of the neurons, the reversal potential of the  $Ca^{2+}$ -evoked hyperpolarization was about –65 mV. An example is shown in Fig. 7 B. This was less negative than the simultaneously measured  $E_K$  of –75 mV in these cells. In another neuron, the reversal potential of the  $Ca^{2+}$ -evoked hyperpolarization was –75 mV, which was similar to the simultaneously measured  $E_K$  of –76 mV in this cell (Fig. 7 A). These discrepancies will be discussed.

*Effects of the Pressure Injection of EGTA upon LEH Response of R2*

The cell was illuminated for about 30 s at 4-min intervals to obtain four or five control responses. About 2 min before the next illumination, EGTA was injected. The pressure injection of 0.67 M EGTA in darkness was accompanied by a small depolarization or a transient inward current during voltage-clamp and a slight increase or no change in membrane conductance. The subsequent light-evoked outward current was abolished or greatly reduced after EGTA injection and the effect was reversible over the next 30–60 min. Subsequent injection of EGTA produced similar effects. However, repeated injections of

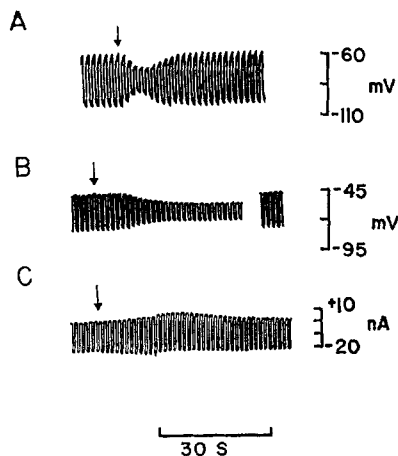


FIGURE 6

FIGURE 6. A and B. Membrane potential changes produced by  $\text{Ca}^{2+}$  injection in R2 (arrows).  $\text{Ca}^{2+}$  was injected from a pipette filled with 0.5 M  $\text{CaCl}_2$ -0.5 M KCl mixture. 10 nA inward current pulses were being passed across the membrane during the period of injection. In B, the break in the record represents 3.3 min. C. Membrane current changes during  $\text{Ca}^{2+}$  injection during voltage-clamp; membrane potential was being shifted from  $-65$  to  $-80$  mV by the voltage-clamp circuit.

FIGURE 7. Changes in membrane potential ( $\Delta V_{\text{Ca}}$ ) produced by small injections of  $\text{Ca}^{2+}$  at different  $E_m$ 's. In A,  $\Delta V_{\text{Ca}}$  reversed sign at  $-76$  mV, whereas in B,  $\Delta V_{\text{Ca}}$  reversed sign at  $-65$  mV.

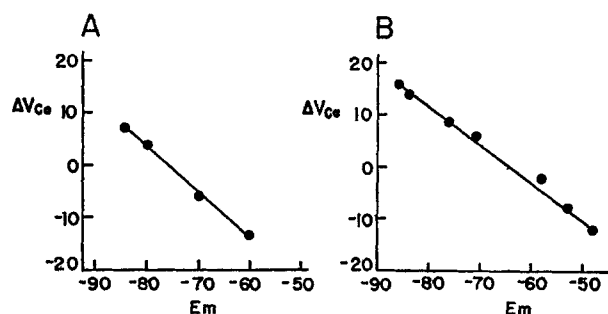


FIGURE 7

EGTA or any solution for that matter, often produced an irreversible depolarization and increase in membrane conductance. The effects of illumination were not tested when this occurred (Fig. 8). Similar results were obtained in another two experiments. Pressure injection of  $\text{Ca}^{2+}$  when the membrane potential was clamped to  $-50$  to  $-60$  mV did not seem to alter the light-evoked current. The light-evoked current was merely superimposed on the large increase in outward current produced by the  $\text{Ca}^{2+}$  injection. Pressure injection of 0.5 M KCl alone reduced the outward current produced by illumination. Pressure injection of 0.5 M  $\text{MgCl}_2$  at similar pressures and for similar durations as those used for 0.5 M  $\text{CaCl}_2$  cause a small reduction in LEC.

#### DISCUSSION

##### *Membrane Mechanism of LEH*

Light hyperpolarizes R2 because of an associated permeability increase to  $\text{K}^+$  producing an outward current across the cell membrane. The evidence for this conclusion is:

(a) The light-evoked current is outward and the membrane hyperpolarizes when  $E_m$  is less negative than  $E_{\text{K}}$ ; when  $E_m$  is more negative than  $E_{\text{K}}$ , the current is inward and the membrane is depolarized by light.

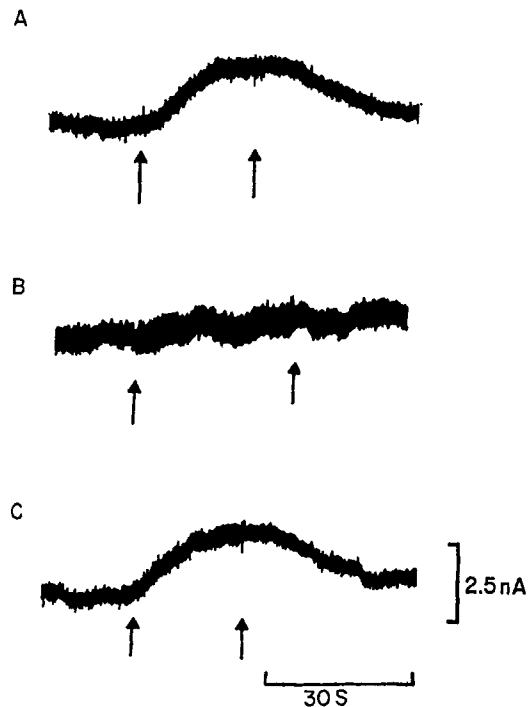


FIGURE 8. Effects of pressure injection of 0.67 M EGTA on light-evoked current of R2. A. Control response. B. 1 min after EGTA injection. C. 30 min after EGTA injection. In each case, light was applied between the arrows.

(b) The membrane current evoked by light at a given membrane potential closely approximates the current calculated from Eq. 1 which attributes the increase in  $G_M$  solely to an increase in  $G_K$ .

(c) The current appears to be unaffected by changes in external  $Cl^-$  or  $Na^+$ .

A reduction in external  $Ca^{2+}$  usually increased the light-evoked hyperpolarization. By contrast, a reduction in internal  $Ca^{2+}$  abolishes the light-evoked hyperpolarization. The permeability sequence in the dark for R2 is:  $K \geq Rb > Cs > Na = Li$  (Eaton, Russell, and Brown, manuscript in preparation). Therefore, it is reasonable that replacement of external  $K^+$  with  $Rb^+$  either slightly increases or does not change the light-evoked hyperpolarization.

The primitive chordate *Salpa* (Gorman et al., 1971) and the invertebrate *Pecten* (McReynolds and Gorman, 1970) possess photoreceptors that respond to illumination with a membrane hyperpolarization and an increase in membrane conductance. Recently, it has been shown that the *Pecten*-hyperpolarizing receptor potential is  $K^+$  dependent (Gorman and McReynolds, 1972). Vertebrate photoreceptors are also hyperpolarized by light but in those studied so far, membrane conductance is decreased, not increased (Bortoff and

Norton, 1967; Toyoda et al., 1969; Baylor and Fuortes, 1970). Photoreceptors in the eye of *Aplysia* respond to illumination with graded depolarizations (Jacklett, 1969); however, the ionic mechanism has not been investigated.

#### *Action Spectrum*

Monochromatic light most effectively evoked the LEH at 490 nM. The sign and wave form of LEH evoked at other wavelengths appeared to be similar to that elicited at 490 nM, the only difference being that more energy was required at other wavelengths to elicit a potential change of the same magnitude. Arvanitaki and Chalazonitis (1961) found that neurons of *Aplysia depilans* were hyperpolarized by 490 nM light, but they were depolarized by 520 nM light. This might represent a species difference, or alternatively, that the giant cell in *Aplysia depilans* receives synaptic input from another neuron that responds to 520 nM light.

We found that R2 has a peak in its absorption spectrum at 490 nM, suggesting that one pigment mediates the LEH. R2 also absorbs light strongly at several other wavelengths similar to those reported by Arvanitaki and Chalazonitis (1961) for other *Aplysia* neurons. They have attributed these peaks to the presence of carotenoproteins and hemoproteins within the neuronal cytoplasm. However, the electrophysiological action spectrum peak at 490 nM in our experiments suggests that these other pigments do not play a significant role in generation of LEH. The carotenoproteins which absorb maximally at about 490 nM are thought to be located in discrete lipochondria granules which are present in the cytoplasm (Arvanitaki and Chalazonitis, 1961).

The functional importance of this neuronal photosensitivity is not clear in *Aplysia* because of the deep-lying positions of the central ganglia. However, *Aplysia* demonstrate light-entrained behavior in the absence of eyes (Lickey et al., 1970) and light entrainment of neurons in the abdominal ganglion are well known (Strumwasser, 1965).

#### *Transduction Mechanism for the LEH*

Pressure injection of  $\text{Ca}^{2+}$  produced an outward current and increased membrane conductance in R2, mimicking the effects of light on R2. In one cell, the reversal potential for this effect was equal to  $E_{\text{K}}$ ; in three others it was less negative. The latter differences may be due to changes in  $\text{Na}^+$  or  $\text{Cl}^-$  permeability if too much  $\text{Ca}^{2+}$  is injected, or to an increase in  $a_{\text{Cl}^-}$ . In this regard Meech (1972) has found that the reversal potential of the hyperpolarization produced by  $\text{Ca}^{2+}$  injection varies about 50 mV per decade change of external  $\text{K}^+$  concentration, suggesting that the major change is a  $\text{K}^+$  permeability change. We have not examined this further, since a precise description of the ionic changes produced in R2 by the pressure injection of  $\text{Ca}^{2+}$  was not the primary purpose of the present experiments.

Pressure injection of EGTA reversibly abolished the light response, whereas pressure injections of  $MgCl_2$  or  $KCl$  reduced it. The mechanism whereby the EGTA effect is reversed is not known. Most likely, the effective EGTA concentration is reduced either by leakage from the soma across the cell membrane or down its axon, or the EGTA is inactivated in some way. Even though there appear to be some nonspecific effects due to injection alone, it seems that in R2 light and pressure injection of  $Ca^{2+}$  evokes an outward  $K^+$  current, and the photoresponse is blocked by EGTA. It therefore seems plausible that  $Ca^{2+}$  plays a role in the coupling between light capture by a photopigment and the ensuing changes in membrane current. Speculation about this possibility has been made for vertebrate photoreceptors (Yoshikami and Hagins, 1971). However, appropriate changes in  $a^{i}_{Ca^{2+}}$  during illumination have not yet been demonstrated in any photoreceptor.

We are grateful to Dr. R. Meech for help with the pressure-injection experiments.

This work was supported by United States Public Health Service, National Institutes of Health Grants NS 09545, HE 10977, and EY 00762.

Received for publication 30 March 1973.

#### REFERENCES

- ARVANITAKI, A., and N. CHALAZONITIS. 1961. Excitatory and Inhibitory Processes Initiated by Light and Infra-Red Radiations in Single, Identifiable Nerve Cells. F. Florey, editor. Pergamon Press, Inc., New York. 196.
- BAYLOR, D. A., and M. G. F. FUORTES. 1970. Electrical responses of single cones in the retina of the turtle. *J. Physiol. (Lond.)* **207**:77.
- BENJAMIN, P. R., and T. S. WALKER. 1972. Two pigments in the brain of a fresh-water pulmonate snail. *Comp. Biochem. Physiol.* **41B**:813.
- BORTOFF, A., and A. L. NORTON. 1967. An electrical model of the vertebrate photoreceptor cell. *Vision Res.* **7**:253.
- BROWN, A. M., and P. R. BERMAN. 1970. Mechanism of excitation of *Aplysia* neurons by carbon dioxide. *J. Gen. Physiol.* **56**:543.
- BROWN, A. M., and W. R. HUGHES. 1973. Effects of intracellular injection of EGTA on the photoresponse of an *Aplysia* giant neuron. Abstracts of the Biophysical Society 17 Annual Meeting, Columbus, Ohio. 129a.
- BROWN, H. M., and A. M. BROWN. 1972. Ionic basis of the photoresponse of *Aplysia* giant neuron:  $K^+$  permeability increase. *Science (Wash. D. C.)* **178**:755.
- BROWN, H. M., S. HAGIWARA, A. KOIKE, and R. MEECH. 1970. Membrane properties of a barnacle photoreceptor examined by the voltage-clamp technique. *J. Physiol. (Lond.)* **208**:385.
- CARPENTER, D. O. 1970. Membrane potential produced directly by the  $Na^+$  pump in *Aplysia* neurons. *Comp. Biochem. Physiol.* **35**:371.
- CHALAZONITIS, N., and A. ARVANITAKI. 1951. Identification et localisation de quelques catalyseurs respiratoires dans le Neurone d'*Aplysia*. *Bull. Inst. Oceanogr.* **47**:994.
- FRAZIER, W. T., E. P. KANDEL, I. KUPFERMAN, R. WAZIRI, and R. E. COGGESHALL. 1967. Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica*. *J. Neurophysiol.* **30**:1288.
- FUORTES, M. G. F. 1959. Initiation of impulse in visual cells of *Limulus*. *J. Physiol. (Lond.)* **148**:14.
- GORMAN, A. L. F., and J. S. McREYNOLDS. 1972. Potassium dependence of the hyperpolarizing receptor potential of the scallop photoreceptor. *Biol. Bull. (Woods Hole)* **143**:464.

- GORMAN, A. L. F., J. S. McREYNOLDS, and S. W. BARNES. 1971. Photoreceptors in primitive chordates: fine structure, hyperpolarizing receptor potentials and evolution. *Science (Wash. D. C.)*. **172**:1052.
- HINKE, J. A. M. 1959. Glass microelectrodes for measuring intracellular activities of sodium and potassium. *Nature (Lond.)*. **184**:1257.
- JACKLETT, J. W. 1969. Electrophysiological organization of the eye of *Aplysia*. *J. Gen. Physiol.* **53**:21.
- KIKUCHI, R., K. NAITO, and I. TANAKA. 1962. Effects of sodium and potassium ions on the electrical activity of single cells in the lateral eye of the horseshoe crab. *J. Physiol. (Lond.)*. **161**:319.
- LICKEY, M., S. ZACH, and P. BIRRELL. 1970. Circadian rhythm in neuron R15 of *Aplysia*: circannual modulation and extraoptic entrainment. *Fed. Proc.* **29**:325.
- McREYNOLDS, J. S., and A. L. F. GORMAN. 1970. Membrane conductances and spectral sensitivities of *Pecten* photoreceptors. *J. Gen. Physiol.* **56**:392.
- MEECH, R. W. 1972. Intracellular calcium injection causes increased potassium conductance in *Aplysia* nerve cells. *Comp. Biochem. Physiol.* **42A**:493.
- MILLECHIA, R., and A. MAURO. 1969. The ventral photoreceptor cells of *Limulus*. III. A voltage-clamp study. *J. Gen. Physiol.* **54**:331.
- RUSSELL, J. M., and A. M. BROWN. 1972 *a*. Active transport of chloride by the giant neuron of the *Aplysia* abdominal ganglion. *J. Gen. Physiol.* **60**:499.
- RUSSELL, J. M., and A. M. BROWN. 1972 *b*. Active transport of potassium by the giant neuron of the *Aplysia* abdominal ganglion. *J. Gen. Physiol.* **60**:519.
- SMITH, T. G., W. K. STELL, and J. E. BROWN. 1968. A role for the sodium pump in photoreception in *Limulus*. *Science (Wash. D. C.)*. **162**:456.
- STRUMWASSER, F. 1965. The demonstration and manipulation of a circadian rhythm in a single neuron. In *Circadian Clocks*. J. Aschoff, editor. North Holland-Publishing Co., Amsterdam. 442.
- TAUG, L., and E. R. KANDEL. 1964. An anomalous form of rectification in a molluscan central neurone. *Nature (Lond.)*. **202**:1340.
- THOMAS, R. C. 1970. New design for sodium-sensitive glass micro-electrode. *J. Physiol. (Lond.)*. **210**:82P.
- TOYODA, J., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Vision Res.* **9**:453.
- WALD, G. 1968. The molecular basis of visual excitation. In *Les Prix Nobel*. Elsevier Publishing Company, New York.
- YOSHIKAMI, S., and W. A. HAGINS. 1971. Light, calcium and the photocurrent of rods and cones. Abstracts of the Biophysical Society 15th Annual Meeting, New Orleans, La. 47a.