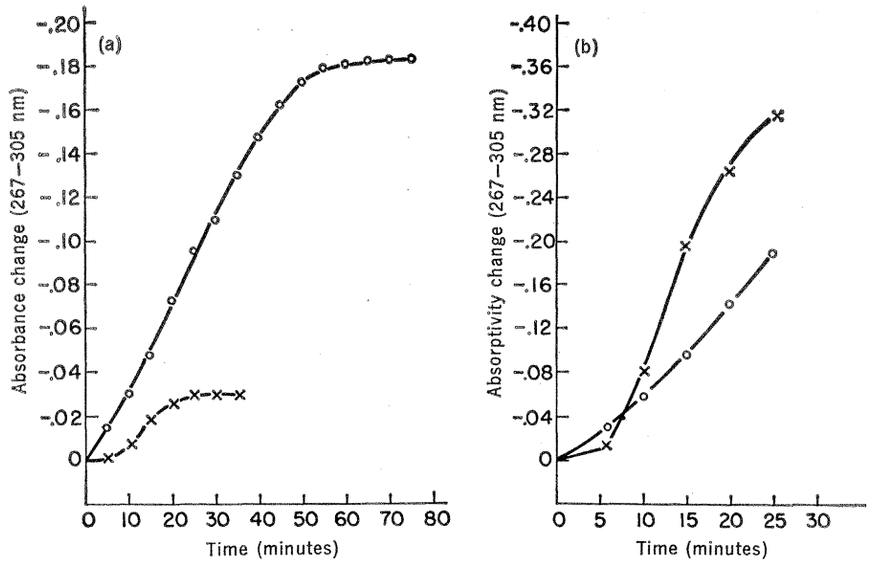

Fig. 2. Effects of the concentration of olfactory preparation on the chemically induced activity measured as change in absorbance (a) and as change in absorptivity (b). The protein concentration of the olfactory preparation was 0.50 mg/ml for the curves labeled O—O. After dilution the protein concentration of the preparation was 0.10 mg/ml (curves labeled X—X). At each concentration the preparation was stimulated with 250 μl of an aqueous solution that was 0.01M for linalool and $<1 \times 10^{-5}M$ for linalyl isobutyrate.

brane permeability needed for chemical sensing by biological systems. The possibility of complex involvement in the olfactory receptor mechanisms has previously been suggested by Dravnieks (*6*), who investigated threshold variations, and more recently by Rosenberg, Misra, and Switzer (*7*).

The activity that was monitored at 267 nm was not unique to the olfactory preparations but was found in varying degrees in preparations from brain and liver, and it is probably present in other biological-chemosensing tissues. The olfactory preparations exhibit the highest activity. In a representative experiment, brain had 41 percent and liver only 7 percent of the specific activity of an olfactory preparation. No chemically induced activity at 267 nm was observed with bovine serum albumin or with preparations of muscle.

The fact that the chemically induced activity was found in preparations of brain and liver does not exclude the possibility that an activity associated with olfactory molecular mechanisms was being monitored. The distinguishing characteristics of the olfactory mechanism might be the structure and location of the bipolar sensing cells rather than molecular mechanisms that are unique to the sensing cells. The olfactory bipolar cells, with their terminal swellings extending into the mucous lining of the nasal cavity and their axons terminating in the olfactory bulb, are ideally suited to facilitate communication between the odor-containing atmosphere and the brain. This unique anatomical arrangement is undoubtedly an important part of the overall mechanisms of olfaction. However, at the molecular level the mechanisms of the olfactory receptors could have much in common with the many other biological chemical sensors.

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21 August 1968

Conductance Changes Associated with Receptor Potentials in Limulus Photoreceptors

Abstract. *The receptor potential in* Limulus *photoreceptors appears to be a consequence not of permeability changes in the cell membrane but of alterations in a light-sensitive constant-current generator.*

The transduction of photic stimuli into neuronal activity in photoreceptor cells results usually in a graded decrease in transmembrane potential, or depolarization, known as the receptor potential (RP). The basic mechanisms underlying its generation are unknown. One hypothesis is that light causes an increase in conductance or permeability of the membrane to one or more ions, particularly sodium (*1, 2*). This conductance increase mechanism (CIM) is essentially the same as that proposed to account for the end-plate potential of the neuromuscular junction (*3*); it has been discussed elsewhere (*1*).

To test the validity of this hypothesis, we have examined a number of electrophysiological properties of photoreceptor cells in the ventral eye of *Limulus polyphemus* (*4*) by means of intracellular recording and electrical stimulation through micropipettes filled with 3M KCl. The organ was deprived of its blood vessel sheath and was mounted in a perfusion chamber, in which the temperature could be varied between 0° and 25°C and the composition of the extracellular fluid could be changed rapidly. All solutions were isosmotic with seawater, buffered at *p*H 7.8, and, where possible, of the same total ionic strength as seawater. Both steady current and up to three independently variable pulses of constant current could be passed through one intracellular microelectrode while changes in potential were monitored through that or a second microelectrode. We determined relations of current and voltage (*I-V* curves) by passing slowly varying currents (about 0.1 cycle/sec) and displaying these and the resulting transmembrane voltages directly on the X and Y axes, respectively, of the oscilloscope. Two independently variable steady or pulsed light beams, passed through heat-absorbing and variable neutral-density filters, were focused onto a single photoreceptor cell.

Three types of electrical activity can be recorded from *Limulus* ventral-eye

photoreceptors (*7*). First, long pulses of light evoke an RP whose waveform is like that evoked in other *Limulus* photoreceptors (*1*, *5*). Second, dark-adapted cells often have spontaneous small-potential fluctuations or "quantum bumps." Third, depolarizing current applied through a microelectrode evokes a spike. In addition, we find that the *I-V* curves of these cells are characteristic and vary with their functional state. Figure 1 shows typical *I-V* curves recorded in the dark and in different intensities of steady light. Light changes the curves from multi-rectifying to singly rectifying.

The fundamental idea underlying the hypothesis of the CIM is that light causes an increase in the permeability of the photoreceptor membrane to ions, particularly sodium. This permeability increase permits ions to flow down their electrochemical gradients, thereby generating the depolarizing potential known as the receptor potential. One crucial datum in support of the CIM is that the membrane resistance measured in the light is less than that found in the dark (Fig. 2A). In the experiment illustrated in Fig. 2A, brief constant-current pulses were passed through one microelectrode, and the membrane potential changes, which are directly related to membrane resistance, were monitored via another microelectrode. During the steady-state part of the RP, the potential changes evoked by the current pulses were less than those evoked in the dark (Fig. 2A); hence the receptor potential is associated with a decrease in membrane resistance.

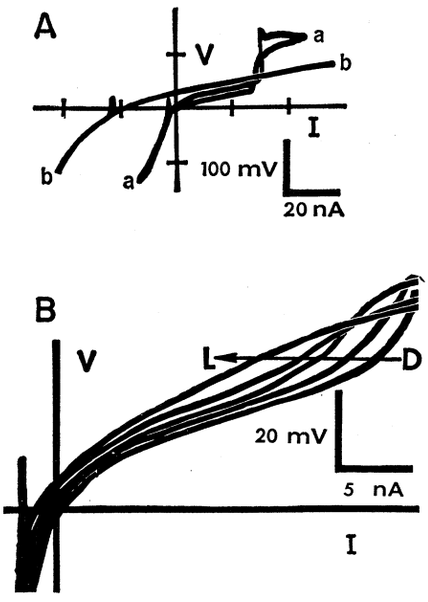

Fig. 1. Typical current-voltage (*I-V*) characteristics from a single *Limulus* ventral-eye photoreceptor cell in dark and in light. (A) *I-V* curves in complete darkness (*a*) and in steady light above saturation intensity (*b*). Origin drawn at resting membrane potential in the dark. (B) *I-V* curves in physiological range as a function of light intensity. Curve at extreme right was obtained near threshold. Progression of curves from dark to light ($D \rightarrow L$) was obtained by increasing light intensity in steps of 1.2 log units. Origin drawn at resting membrane potential in the dark. Curves were shifted along *I*-axis to facilitate comparison of slopes (resistances).

One of the corollaries of the CIM is that, at any given level of depolarization evoked by light or by current, the membrane resistance should be less in the light than in the dark. When, however, we depolarized the cell with current in the dark to the same steady membrane potential as that reached during illumination, we found the resistance equal to or less than that during illumination (Fig. 2B). Moreover, if in the presence of a steady light we repolarized the membrane with current to its dark-adapted resting potential, then the membrane resistance was the same or greater at that potential in the light than in the dark. Finally, steady light of moderate intensity left unchanged or actually increased the overall slope of the *I-V* curves in the physiological range (that is, about −70 to −15 mv) (Fig. 1B). Only with light intensities sufficient to saturate completely the photoreceptor's response to light did the slope resistance become less in light than in the dark (Fig. 1A).

These results show that steady depolarization in the physiological range of membrane potential is itself accompanied by an increase in conductance, the magnitude of which is relatively independent of whether it is evoked by current or light. That is to say, depolarization produced by any means leads to an increase in conductance because of the fundamental nonlinearity of the *I-V* characteristic of the photoreceptor membrane. The observation that the magnitude of the conductance change is nearly the same for a given level of depolarization, whether evoked by light or current, is inconsistent with the CIM model, which predicts a primary increase in conductance in addition to that resulting from any nonlinearity of the *I-V* relation.

Further evidence against the CIM model came from experiments in which the ionic environment was altered. If the RP were caused by an increase in the membrane's permeability to one or more ions, then selective removal of an ion involved in the RP from the extracellular fluid should alter the component of the RP contributed by that ion. We found that replacement of chloride with sulfate or propionate ions produced no significant change in the RP.

In confirmation of previous results (*4*), replacement of sodium ions with lithium or Tris ions completely but reversibly abolished the RP, although we found that the abolition of the RP was only transient. Specifically, the reduction of the RP began within a minute after the onset of sodium replacement and abolition occurred within 5 minutes. Some 5 to 10 minutes later, the RP began to recover; within 15 minutes it reached a steady-state level. During this steady-state in sodium-free solutions, the sensitivity of the photoreceptor to light was reduced by two or more log units (*6*). We have found that replacement of sodium with ammonium, hydrazinium, or quanidinium ions also reversibly abolishes the RP. This inability of small univalent cations to substitute effectively for sodium ions in the transducer mechanisms in *Limulus* photoreceptors contrasts sharply to the

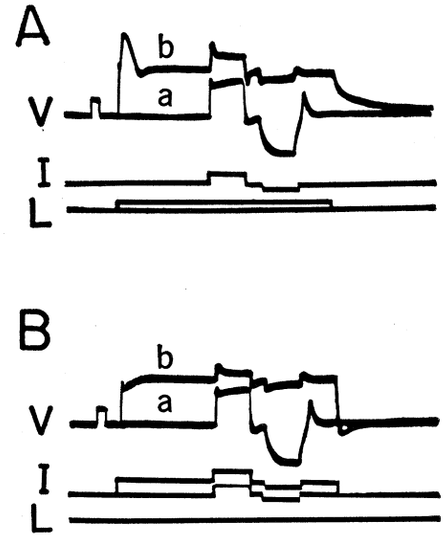

Fig. 2. Effects of depolarization by light (*L*) and current (*I*) on membrane resistance of *Limulus* ventral-eye photoreceptor cells. (A) Response to current pulses of same strength in dark (*a*) and in light (*b*). (B) Response to current pulses in dark, at resting membrane potential (*a*) and during depolarization by current (*b*) to same potential reached by light in 2(A). Calibration in *V* equals 10 mv, 200 msec.

ability of these ions to substitute for sodium in the generation of many bioelectric potentials that normally are a consequence of an increase in sodium permeability (*7*, but compare *8*). We have found also that continuous perfusion with potassium-free seawater could reduce the RP and that increasing the concentration of extracellular calcium ions fivefold over that in normal seawater could also significantly reduce the RP. Furthermore, each of these procedures (that is, replacement of sodium or potassium or an increase in calcium) usually depolarized the cell by some 10 to 20 mv, altered the *I-V* curves similarly to bright light, that is, from multirectifying to singly rectifying and abolished the spontaneous "bumps." When lithium or Tris was substituted for sodium, these latter changes occurred simultaneously with the abolition of the RP and were similarly transient. That is, during the period when the RP was abolished, the *I-V* curve was similar to that of a highly light-adapted cell, while during the phase of partial recovery of the RP in sodium-free solutions, the *I-V* curve resembled that of a partially light-adapted cell.

Our observations show that the CIM hypothesis is inadequate to explain the generation of receptor potentials in *Limulus* photoreceptors. Within the physiological range of membrane potential, light and current are essentially equivalent in these cells, in that membrane conductance is highly dependent on membrane potential but relatively independent of whether that potential is evoked by light or extrinsic current. It cannot be true both that there is such an identity in the action of light and current and that the RP is the result simply of a permeability (conductance) increase. Also, removal of either sodium or potassium ions from the fluid bathing these cells has qualitatively similar effects on their response and membrane properties. It is highly unlikely that the RP is caused by an increase in permeability to both of these ions, since it is abolished by removing either sodium or potassium ions from the external medium.

It is conceivable that conductance changes of a more complex nature could generate the RP directly. For example, illumination might lead to an increase in sodium conductance and a decrease in potassium conductance of exactly the right magnitude to duplicate the underlying nonlinearity of the cell membrane (Fig. 2). Such a mechanism, however, would require a number of *ad hoc* hypotheses. Among these would be that the increased sodium permeability requires the presence of extracellular potassium and the decrease in potassium permeability requires the presence of sodium in the medium (*8*). While we cannot categorically reject such a complex mechanism, we believe there is a more parsimonious way to account for our results (*6*).

We conclude, therefore, that the response of photoreceptor cells in *Limulus* is not generated by a conductance increase mechanism as has been generally supposed. Because of the basic similarity of the action of light and current in the physiological range of membrane potential, the cell membrane appears to possess a source of current which has the properties of a high source-impedance, constant-current generator. Moreover, light appears to control this source of transmembrane current. Because of the underlying nonlinearity of the cell membrane, a light-evoked depolarization of the cell is accompanied by a secondary increase in conductance. The identity and properties of the primary, light-dependent current-generator are considered elsewhere (*6*).

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A Role for the Sodium Pump in Photoreception in Limulus

Abstract. *The membranes of photoreceptor cells in* Limulus *have an electrogenic sodium pump which contributes directly to membrane potential and whose activity is changed by light. These light-induced changes in pump activity underlie the receptor potential.*

In the accompanying paper we presented evidence that the receptor potential (RP) in *Limulus* ventral-eye photoreceptors is not generated by a conductance increase mechanism; rather, the conductance changes associated with the RP appear to be secondary and a consequence of the basic nonlinearity of the current-voltage (*I-V*) characteristic of the photoreceptor membrane (*1*). We noted that some complicated conductance change mechanism might explain our results, but that they can be accounted for by a simpler hypothesis. We were led to our hypothesis, in part, by the basic similarity of action of light and current in the physiological range of steady-state membrane potential; and we suggested that the photoreceptors have a current generator in their membranes which is altered by the absorption of light. Our hypothesis is that the ventral-eye photoreceptors have an electrogenic sodium pump which contributes directly to steady-state membrane potential and which is the current generator altered by light.

Recently, a number of excitable cells have been found to have an electrogenic sodium pump (*2–8*). By definition, a pump is electrogenic if the ratio (β) of the potassium ions actively transported into the cell to the sodium ions actively transported out of the cell is other than one (*3, 4, 9*). This imbalance of ions moving through the pump constitutes a source of current which either hyperpolarizes or depolarizes the cell membrane directly, depending upon whether β is, respectively, less or greater than one. In any steady-state, of course, the current must be counterbalanced by an equal current moving in the opposite direction through the passive ionic pathways lying in parallel with the pump across the cell membrane.

An apparently obligatory component of the sodium pump mechanism is a Na^+,K^+-dependent adenosine triphosphatase, which, by catalyzing the hydrolysis of adenosine triphosphate (ATP),