AN ELECTROCHEMICAL MODEL FOR DEPOLARIZATION OF A RETINULA CELL OF *LIMULUS* BY A SINGLE PHOTON

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ABSTRACT The response of a visual cell in the eye of *Limulus* is treated mathematically in terms of a model derived from the properties of excitable nerve membranes. Electron microscopic sections of the rhabdomere indicate that its structure is a close-packed array of cylindrical tubules, the interiors of which communicate with the retinula cell cytoplasm, while the external interstitial fluid is a conducting medium continuous with the extracellular space of the ommatidium. If a single highly conducting channel is opened in this membrane structure, it can be shown how the excitation can spread to depolarize the retinula cell by several millivolts. Intense activity of "sodium pumps" in the rhabdomal membrane would be required to maintain the ionic concentrations in the interstitial fluid.

INTRODUCTION

The compound lateral eye of *Limulus*, the horseshoe crab (*Xiphosura polyphemus*) has acquired a central role in studies of the visual process, primarily owing to the fact that quantitative electrical data can be recorded from individual cells of this preparation. A comprehensive critical view of work in this field was recently published (Wohlbarsht and Yeandle, 1967). A crucial question is how the absorption of a single quantum of light in the rhabdomere of an individual ommatidium can lead to an appreciable depolarization of a retinula cell (Fuortes and Yeandle, 1964). The overall "power amplification" of this process has been estimated to be from 10^5 to 10^6. Several mechanisms have been suggested to explain this result, including enzymatic reactions, release of special transmitters, and analogs of solid-state photomultipliers. We propose to demonstrate, however, that the structure of the ommatidium is such that electrochemical processes similar to those occurring in nerve conduction can account satisfactorily for most of the effects observed, the free energy required to amplify the original photochemical reaction being provided by the usual differences in electrochemical potential across neuronal membranes.

Slow Discrete Retinular Depolarizations

At low light intensities intracellular recording from retinula cells or eccentric cells reveals slow discrete depolarizations called "quantum bumps." These potential fluctuations persist in the dark at a frequency ν of about 0.5 sec^{-1} at 293°K. The characteristics of the quantum bumps can be summarized as follows (Yeandle, 1957).

1. Amplitude A is 3 to 8 mv.
2. Width at half-height is 0.08–0.16 sec.
3. Latency from onset of illumination is 0.15–0.5 sec, dependent on temperature. At about 295°K the latency varies inversely with light intensity, but at about 280°K the latency appears to be almost independent of intensity.
4. Average frequency ν varies with light intensity I at low I, but at higher I the bumps merge into a continuous depolarization with a superimposed spike depolarization at its onset.
5. Hyperpolarization of retinula cells decreases ν but increases A.
6. Statistics of the random firings best fit a one-quantum process as the origin of the excitations.

These are the basic properties of quantum bumps, which any quantitative theoretical model must try to explain.

STRUCTURE OF THE OMMATIDIA AND THEIR PHOTORECEPTORS

The structure of an ommatidium has been compared with that of an orange, in which the segments represent retinula cells, and the axis is occupied by the dendrite of the eccentric cell. The region surrounding the dendrite is the rhabdome, formed of folded and convoluted structures of large surface area, the microvilli. Viewed in section normal to the axis, the structure presents the appearance of a wheel, the dendrite and its surrounding microvilli forming the hub, with the microvilli between neighboring retinula cells forming radiating spokes.

The electron microscopic investigation of Lasansky (1967) has provided the most complete details of the subcellular organization. We have made further electron microscopic examinations in order to resolve a number of questions. Fig. 1 shows an electron micrograph of the central region of a rhabdome with the membrane structures of the microvilli demarcating the boundaries between retinula cells. The visual pigment, which is an invertebrate type of rhodopsin (520_1) (Hubbard and Wald, 1960) is believed to be dispersed in the membranes of the microvilli and constitutes about 10% of their structural proteins.

Under the electron microscope the microvilli appear to be constructed of typical plasma membranes, but no chemical work has yet been done to characterize them. In osmium-stained electron microscope sections, each membrane can be visualized as two electron-dense layers separated by a lighter region. Where two such mem-

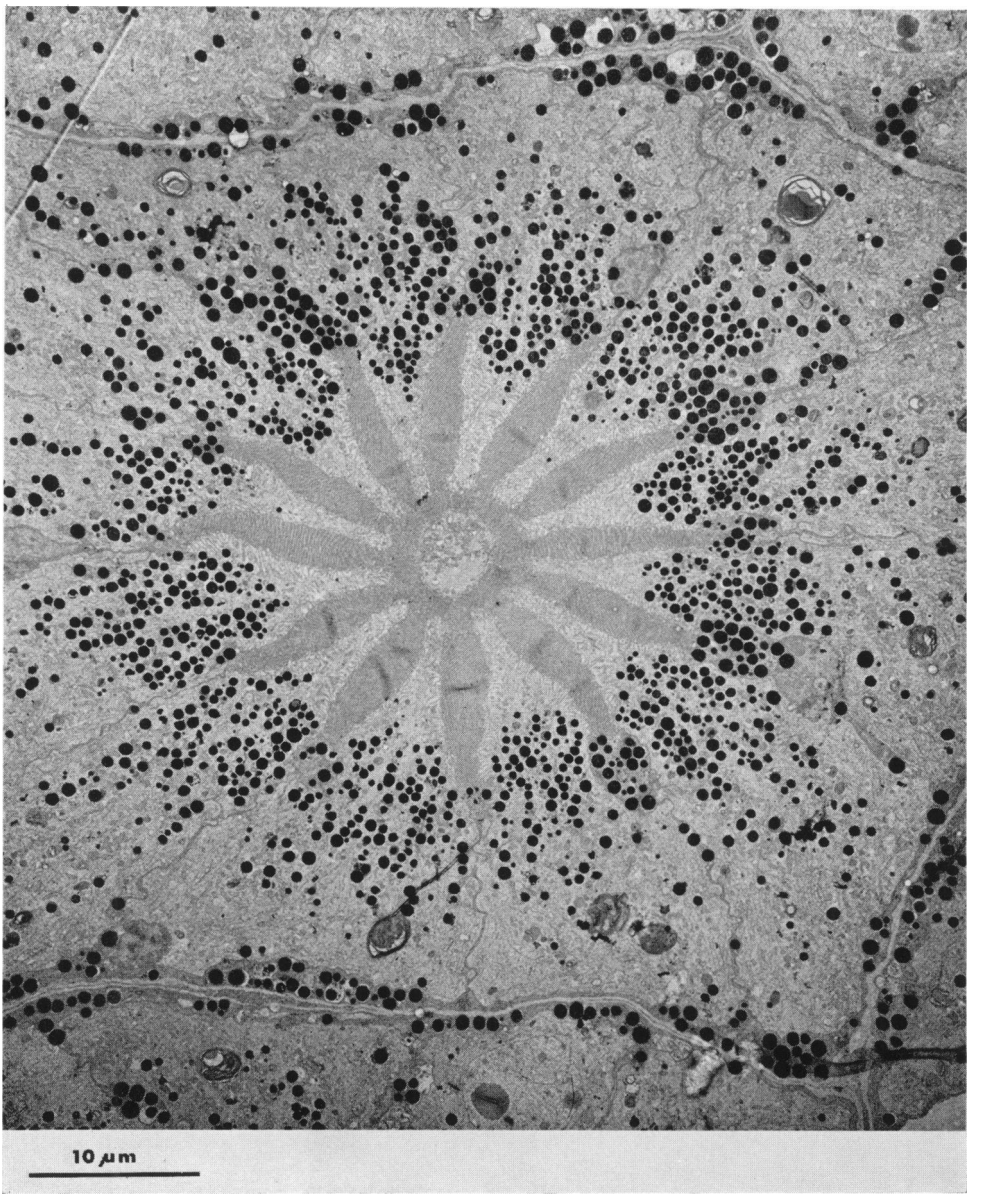

FIGURE 1 A horizontal section through an ommatidium in lateral eye of 12 cm *Limulus*. Fixed with glutaraldehyde and osmic acid, stained during dehydration with uranyl acetate; after sectioning, stained with lead citrate. Embedded in Epon 812 Resin (Fisher Scientific Company, Pittsburgh, Pa.). Siemens Elmiskop I at 80 kv.

branes come together there is little or no detectable spacing between them so that the structure appears, in the "five layer pattern" discussed by Lasansky, as if one electron-dense side was being shared by two contiguous membranes.

The structure of a rhabdomere thus appears to approach closely that of a close-

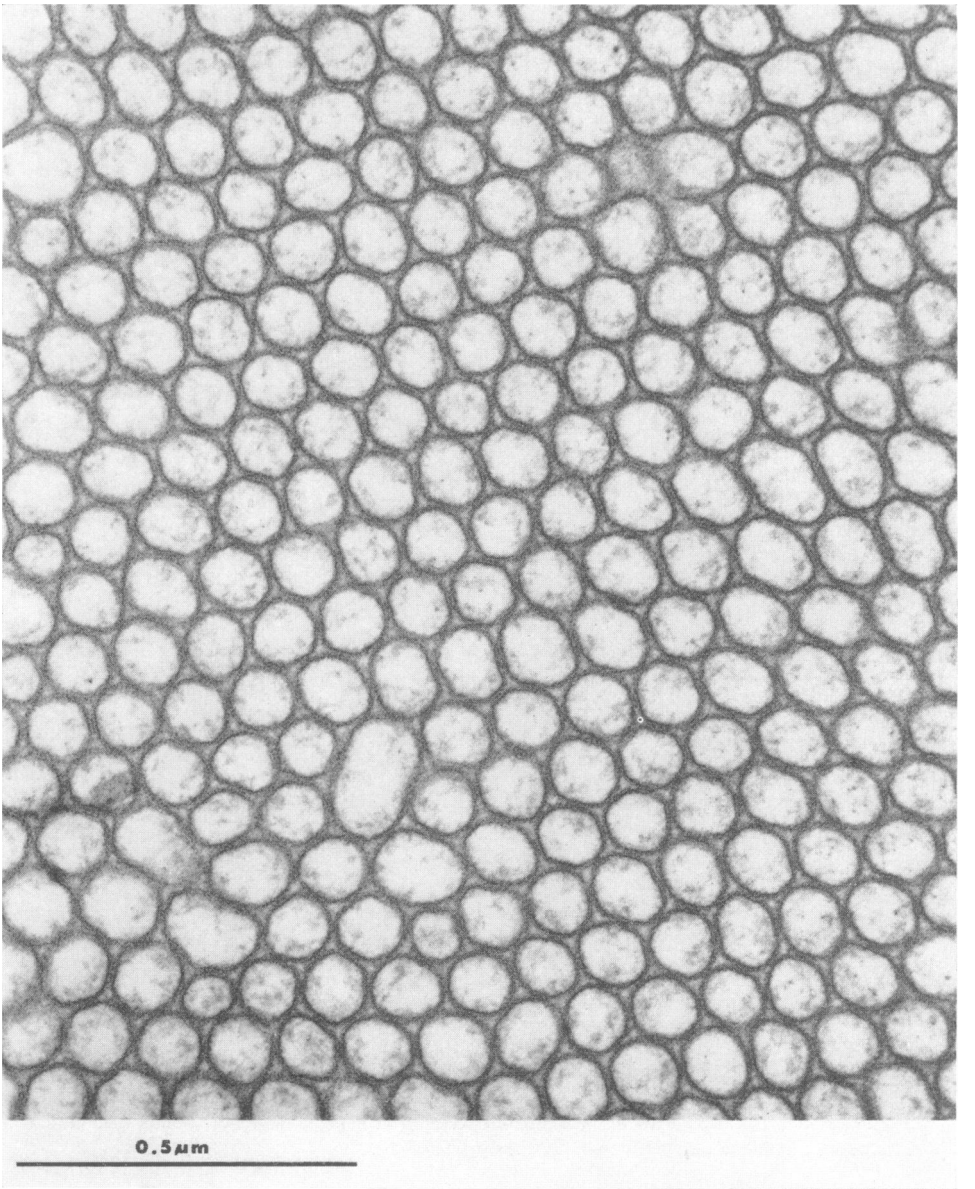

FIGURE 2 *a* Section through close packed microvilli of ommatidium shown in Fig. 1; about 0.05 μm section.

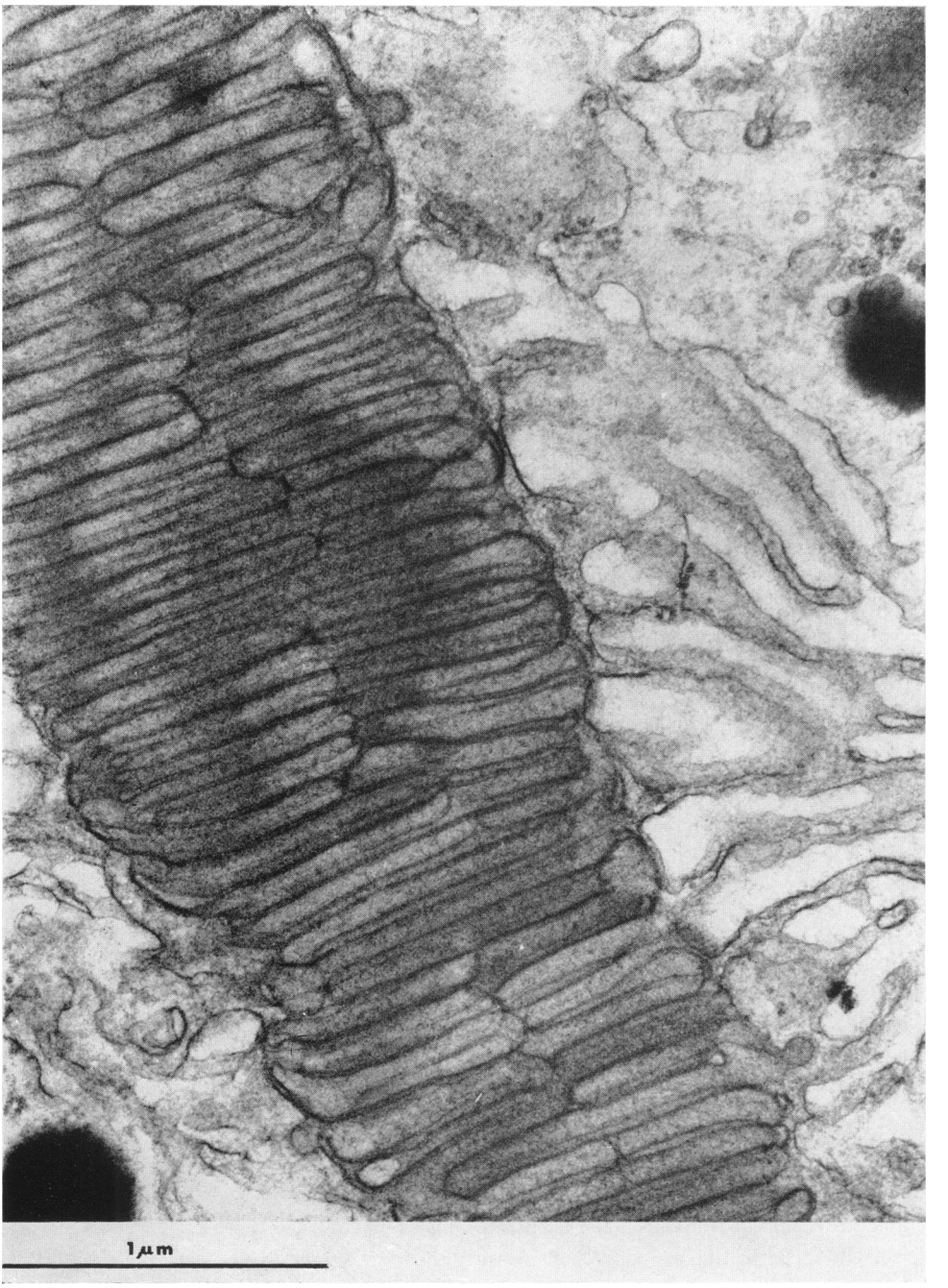

FIGURE 2 *b* Section in parallel orientation, about 0.13 μm thick.

packed array of cylindrical elements formed by the microvilli. In almost all the sections that we have examined, the axes of the cylinders are perpendicular to the "spokes" of the rhabdome, and either parallel to the axis formed by the dendrite of the eccentric cell or 90° from this orientation. In any given section parallel to the dendrite, the orientations of the microvillar cylinders are predominantly the same, although it seems reasonable that regional transitions should exist between the two orientations, and we have observed sections that could be interpreted as transition regions.

Fig. 2 *a* shows a section through the microvilli that are aligned parallel to the dendrite. We should note the hexagonal symmetry of the array, characteristic of close packing of the circular cross-sections. In ideal closest packing each circle would have six points of contact with its nearest neighbors. It appears, however, that there is a narrow spacing between most of the circles in this orientation. In addition, there is the interstitial open space wherever three cylinders meet. Fig. 2 *b* shows a section taken in the orientation perpendicular to that in Fig. 2 *a*. This was a section of greater than usual thickness (about 0.15 μm), which shows, therefore, parts of two layers of cylinders. We can distinguish the membranes of the two superimposed layers, and their positions demonstrate that the "upper" cylinder is placed over the cleft between two "lower" cylinders. This section thus confirms the fact that the cylinders are close packed in the second orientation as well as in the first.

The open spaces in the microvillar packing can provide a continuous interstitial fluid network that permeates the rhabdomere and communicates with the exterior of the retinula cell in the cleft beyond the rhabdomere. We shall assume in the subsequent analysis that this interstitial space is filled with an ionic solution that is high in sodium and low in potassium, and hence similar to the extracellular fluid surrounding somatic or axonal membranes in other tissues of marine invertebrates. The interstitial space thus provides an electrically conducting probe that allows any changes in membrane potentials within a rhabdomere to be transmitted to the extracellular fluid outside the retinula cells. We might think of the entire structure as being a volume of regularly packed electrically excitable membranes with a microstructure resembling that of a neuronal axon except that the higher resistance pathways are external to the membranes in the case of the rhabdomal structures. It is likely, furthermore, that the electrical conductivity of the interstitial medium is lower than that of the aqueous solution that bathes the axons in, for example, a squid axon preparation.

In any given electron microscopic cross-section through the boundary between two retinula cells and normal to the dendrite, many microvilli can be observed to open into the cytoplasm of the retinula cells. The microvilli from one retinula cell meet those from the adjacent cell along an irregular plane of demarcation that is clearly visible in most sections. The ends of the microvilli that face this plane almost invariably appear to be closed, so that there is a tight junction formed by the opposition of the cylinders from adjacent cells. On each side of the junction, about one

cylinder in every three appears to open into the retinula cells whereas the intervening pair of cylinders appear completely closed. Since the thickness of the section is about 0.05 μm and the diameter of the cylinder about 0.06 μm, it is likely this closed appearance is a correct representation of the three-dimensional structural arrangement.

The functional structure of the rhabdome thus appears to be based upon two principles.

1. It provides an extensive surface area and long optical path for efficient light absorption by rhodopsin bound in the membrane.
2. It provides a system of electrically excitable membranes and ionically conducting interstitial fluid which can pick up and amplify (by a mechanism to be discussed later) the voltage change across any membrane section, which is produced by the primary process of photoactivation.

Evidence from Conductance Data

The electron microscopic studies reveal three types of electrical contact in the rhabdomal structures.

(*a*) The electrical contacts between adjacent retinula cells form direct close contacts without apparent extracellular spacing (Lasansky's "five layer structures"). Such close contacts are seen at many areas between the ends of microvillar tubes belonging to one retinula cell and the corresponding structures in the adjacent cell. Similar close contacts have been observed to provide low resistance electrical contacts in other structures (Farquhar and Palade, 1963) and are in general not excitable (Bennett, 1966).

(*b*) The contacts between the interior of a retinula cell and the interior of the dendrite of the eccentric cell appear to be partly similar to those in (*a*) but partly to be transmembrane contacts in which a microvillus from the retinula cell contacts the eccentric cell. A definite boundary can often be observed around the central ring of the rhabdome, separating the distal process of the eccentric cell and the surrounding retinula cells.

(*c*) The contacts between the interior of a retinula cell and the extracellular interstitial space are transmembrane contacts differing from those in (*b*) in that the membrane involved should be the site of a considerable potential difference (50–70 mv), whereas the interiors of retinula and eccentric cells, in the resting state, would be at nearly the same potential.

The measurements of the effective conductances of the different contacts, and their response to illumination, provide some support for the description of the structure that has been outlined (Borsellino et al., 1965). Three different conductances have been distinguished: (α) between retinula (R) and eccentric (E) cells, increased by illumination, (β) between two R cells, not increased by illumination, but high at all times, and (γ) between R cell and external region, increased by illumination. The

fact that γ is markedly increased by illumination leads to two probable conclusions. (*a*) The photoactivation of the rhabdomal membrane provides a path of decreased resistance from the interior of a retinula cell to the extracellular fluid through the interstitial fluid space described above. (*b*) There is no excitable electrical connection between adjacent retinula cells through this interstitial fluid, i.e., the interstitial fluids of each member of a pair of adjacent retinula cell rhabdomeres are electrically connected mainly through the cleft beyond the rhabdomere. If this were not true, the photoactivation of the rhabdomal membrane would necessarily lower the resistance β as well as γ. Actually the region between two R cells beyond the rhabdomere is closely occupied by material from the accessory pigment cell, so that there is never a close approach of R cell membranes beyond the rhabdomere. In the present model the effect of light on γ would be especially pronounced because of the electrical excitability of the membrane involved. The absence of any photoeffect on β is impossible to explain on this simple model, since a considerable part of the β resistance should be due to the paths through the microvilli, which are markedly photosensitive in the present model. The conductance β was not directly measured, since the positioning of the electrodes in the R cells was unknown. Perhaps the method of indirect calculation masked the sensitivity to light, and further, more direct measurements of β would be needed to resolve this question.

THEORY OF PHOTOACTIVATION

The theoretical problem may now be conveniently divided into three parts. (*a*) How does the absorption of a single photon activate a channel in the rhabdomal membrane? (*b*) How does the active spot thus produced send a depolarizing pulse through the interstitial fluid of the rhabdomere? (*c*) How does such a spreading or propagated depolarization produce the quantum bump of 2–8 mv recorded by an intracellular electrode in the retinula cell?

The discussion of the first question must necessarily be rather speculative. Electrophysiological data alone cannot closely define the primary process of transduction that follows light absorption by the visual pigment, and neither the chemical kinetics of the photochemical reaction (Hubbard et al., 1965) nor the associated structural changes in the photoreceptor membranes are as yet completely understood. Therefore we shall postpone discussion of this question until the end of the paper, at which point it will be appropriate to consider also possible mechanisms for the thermal or spontaneous generation of depolarization pulses in the dark.

Thus for the present we shall simply *assume* that one channel is opened in the membrane by a photochemical process, and then show how the electrical effect so produced can spread sufficiently to effect a depolarization of the retinula cell by several millivolts. It is probable that the ring of microvilli around the distal process of the eccentric cell can also undergo a photoactivation, which would lead to generation of a pulse in the E cell, and would also act by lowering the resistance of the

region separating it from the retinula cell, thus facilitating the E cell response to retinular depolarizations.

At the present time there is no direct experimental evidence relative to the electrical excitability of the microvillar membranes of the *Limulus* rhabdome. Hagins and McGoughy (1968) have shown that the fast photovoltage (early receptor potential) in squid retina is localized in the photoreceptor membrane. In the analysis of Hagins (1965), the primary receptor current was localized in the region adjacent to absorption of a photon, but no electrical spread of excitation was postulated. Recent work by Fulpius and Baumann (1969) suggested that unless the slow potential in *Limulus* photoreceptor cells was generated at a site "which is not easily accessible," it would be necessary "to postulate that some unknown mechanism other than passive ionic movement through the membrane is responsible for the generation of the receptor potential." Owing to the high resistance of the interstitial extracellular fluid in the rhabdome, microelectrodes implanted in retinula cells cannot deliver a potential pulse across the photoreceptor membranes, but extremely small dipolar electrodes might provide suitable probes for direct electrical excitation.[1]

Let us assume that the rhodopsin molecules are closely associated with fast channels for ionic transport through the rhabdomal membrane, similar to the sodium channels in an axonal membrane. Absorption of a single quantum of visible light by a rhodopsin molecule is thus often followed by opening of a normally closed fast channel. Adjacent to the photochemically activated channel, we assume that there exist electrically excitable channels, with critical depolarization and accommodation properties analogous to those in an axonal membrane.

The probability of electrotonic spread of excitation from the open channel to an adjacent closed channel will depend on the surface density of channels, N. In the nodes of Ranvier of frog sciatic nerve, it was estimated (Bass and Moore, 1968) that $N = 8.9 \times 10^9$ cm^{-2}, corresponding to a mean distance between channels of about 0.1 μm. In lobster axon the number of channels as estimated from blocking by tetrodotoxin (Moore et al., 1967) is $N = 1.3 \times 10^9$. Such a figure would probably provide a lower limit for N in the rhabdomal membranes. An upper limit could be estimated from the density of closest packing of rhodopsin molecules on the membrane surface. With a molecular weight of 27,000, (Heller, 1969), a rhodopsin molecule should have a cross-sectional area of about 3700 A^2. This figure would correspond to a distance between channels of 61 A or a density $N = 2.72 \times 10^{12}$ cm^{-2}. Precise data on the concentration of rhodopsin in the *Limulus* photoreceptor are not available, but rhodopsin molecules appear to make up about 10% of the membrane protein. Hence an upper limit based on rhodopsin content would be $N = 2.7 \times 10^{11}$ cm^{-2}. Since the light activated and electrically activated channels

[1] This idea was suggested by Dr. W. A. Hagins.

need not be identical, we can choose a value of $N = 10^{10}$ cm^{-2} as a reasonable estimate of the density of channels in the rhabdomal photoreceptor.

The maximum sodium current through an activated channel in the node of Ranvier (frog) was estimated to be 6.7×10^{-12} amp (Bass and Moore, 1968). Hille (1968) obtained 40×10^{-12} amp for frog node channels, and 200×10^{-12} amp for lobster axons. When a current of such magnitude is drawn from one photochemically activated channel it will produce depolarization at the sites of adjacent channels. If these fast channels can be activated electrically, i.e. if the membranes of the microvilli behave electrically like axonal or excitable somatic membranes, this depolarization may be sufficient to trigger the excitation of fast channels at sites neighboring the original locus of photoactivation. Thus the original active spot would spread electrically to produce an activated patch in the surfaces of the rhabdomal membranes.

We shall now show that, given certain electrical and structural parameters for the system, a spread of activation from a single open channel can occur. Let $\bar{V}$ denote the uniform potential in the retinula cell body, which is assumed to be equipotential since the internal resistance of the cell is low compared to resistances of membranes and extracellular clefts. The zero of potential is taken in the extracellular fluid at some distance from the retinula cell. In the ordinary resting condition $\bar{V} = E_K$ where E_K is the Nernst potassium potential, about -70 mv at 25°C. The potential at any point in the cleft outside the retinula cell is denoted as V, and in the resting steady state $V = 0$ everywhere on the cell boundary. If for any reason all or part of the cell membrane becomes depolarized, then $V < 0$ and $\bar{V} > E_K$. The total potassium current over the cell walls will be given by

$$I_K = -\int_{S_1} \frac{E_K - (\bar{V} - V)}{\delta} \sigma_m^K \, dS, \tag{1}$$

where S_1 is the area of cell in which potassium channels are open, δ is the membrane thickness, and σ_m^K is the conductivity of the cell membrane to potassium ions. Similarly the total sodium current will be given by

$$I_{Na} = -\int_{S_2} \frac{E_{Na} - (\bar{V} - V)}{\delta} \sigma_m^{Na} \, dS, \tag{2}$$

where S_2 is the area with open sodium channels (we are neglecting the sodium current in the unexcited membrane). E_{Na} is about 50 mv for squid axon and Adams and Hagins (1960) showed for squid that the composition of retinal rod receptors was similar to that of nerve cells. When a new steady state is established

$$I_K + I_{Na} = 0 \tag{3}$$

and our problem is to find the value of $\bar{V}$ for which this occurs. The difficulty is that

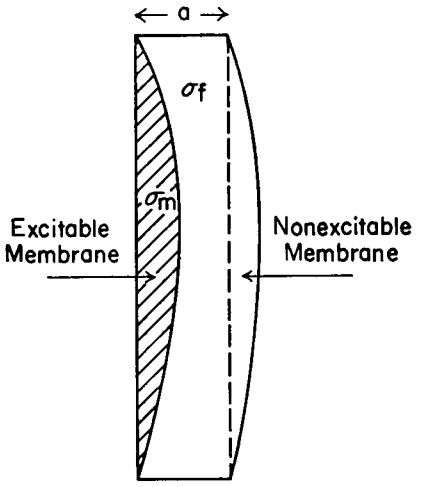

FIGURE 3 *a* Model for mathematical treatment of spreading excitation in rhabdomere between adjacent retinula cells. The interstitial medium in the cleft with conductivity σ_f is separated from the body of the retinula cell by the convoluted excitable membranes of the microvilli, which are replaced in the model by a planar membrane with transconductance σ_m. It is assumed that the light initiated excitation, in the membranes that are part of one retinula cell, is not able to spread to membranes of the adjacent cell.

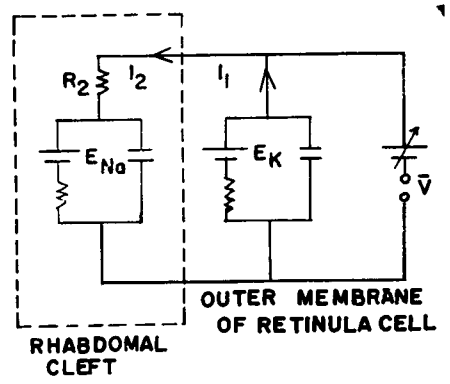

FIGURE 3 *b* Simplified equivalent circuit for the rhabdome model. An initial photochemical excitation of the convoluted microvillar membrane spreads electrically to produce an area of membrane through which (mainly) sodium current flows into the retinula cell. Current I_2 is drawn through the high resistance R_2 of the interstitial fluid and passed into the retinula cell, raising its potential $\bar{V}$.

V itself depends on $\bar{V}$ (as well as on position) so that we cannot simply integrate and solve for $\bar{V}$ until we have determined $V = V(\bar{V})$ for any particular configuration of active and inactive membranes that may be of interest.

The convoluted structure of the rhabdomere makes it virtually impossible to solve exactly for the spread of electrical depolarization from an initial site of activation. We shall therefore use a drastically simplified model for the rhabdomere and the surroundings of the retinula cell, as shown in Fig. 3 *a*. Fig. 3 *b* is a diagram of the model, in the form of a simplified equivalent circuit.

The half of a rhabdomere that is part of a given retinula cell will be represented by a semicylindrical section of conducting fluid bounded on one side by an excitable membrane and on the other side by an nonexcitable membrane. In order to take into consideration the convolutions of the microvilli, the excitable membrane will be assigned a *surface roughness factor s*, calculated so as to increase the geometrical area of the boundary (excitable) membrane to the actual interfacial area of the

microvilli between the retinula cell and the cleft. In the subsequent calculation, the effect of this factor will simply be to increase the density of excitable channels from N to sN. These channels will determine the transconductance σ_m of the excitable membrane, which will be either $\sigma_m{}^{K}$ if only slow (potassium) channels are open or $\sigma_m{}^{Na}$ if fast (sodium) channels are also open. The values of σ_m will be estimated from data on resting and activated axolemma of the squid. The resistance of the interstitial medium appears to be anisotropic in the actual rhabdomal structure, as a consequence of the preferred orientations of the microvillar cylinders. Such regular orientations of the microvilli probably imply similarly regular orientations of the molecules of visual pigment, and hence a receptivity of the eye to polarization of light. (Waterman, 1954). In any case, to simplify further the mathematical treatment, we shall ignore this anisotropy of resistance, and use a single conductivity σ_F to describe the interstitial fluid. The conductivity of 0.5 M sea water, which is similar in composition to the extracellular fluid in marine organisms, is $4 \times 10^{-2}\ \Omega^{-1}\ \text{cm}^{-1}$ at 25°C.

There is considerable evidence that in any densely packed structure of nervous tissue, such as neuropile, the effective extracellular conductivity is much less than that in an ordinary aqueous solution of the same composition (Adey et al., 1966). We shall thus consider that $\sigma_F = \xi\sigma_0$ where σ_0 is the conductivity of sea water and ξ is a factor which may be as low as 10^{-2} in the narrow interstitial passages of the rhabdomere.

We may note that our simplified model is essentially two-dimensional. The finite thickness a of the semicylindrical slot between the two retinula cells is not considered in the calculation of the electrical potential, except as it determines the electrical conductance of the "two-dimensional" medium.

We shall consider a stationary state solution for the potential V in this system to try to answer the question as to whether a single locus of photoactivated membrane can produce a sufficient depolarization at an adjacent *electrically excitable* channel to cause propagation of the activation.

Let us consider an annular element of the membrane centered on the origin at which a single channel has been photochemically activated. Then, in the steady state, the excess current entering this volume element in a radial direction is just balanced by the current through the membrane.

$$\Delta(2\pi r \cdot a \cdot i_r) + 2\pi r \Delta r \cdot i_m = 0, \tag{4}$$

where i is a current density. Hence,

$$\frac{a}{r}\frac{\partial}{\partial r}(r i_r)\Delta r + i_m \Delta r = 0. \tag{5}$$

Now

$$i_r = -\sigma_F \frac{\partial V}{\partial r}, \tag{6}$$

where σ_p is the conductivity of the interstitial medium outside the retinula cell. And

$$i_m = \frac{\sigma_m(E - \bar{V} + V)}{\delta}, \tag{7}$$

where σ_m is the transmembrane conductivity, δ is the membrane thickness, and $E = E_K$ or E_{Na} depending upon whether the region of membrane considered is activated or not. The membrane is thus assumed to be locally polarized exactly like a squid axonal membrane, although in the retinula cell itself, the average potential never exceeds $V = 0$. If we define

$$\alpha^2 = \sigma_p a\delta/\sigma_m \tag{8}$$

and

$$v = V - \bar{V} + E, \tag{9}$$

equation 5 reduces to

$$\frac{\alpha^2}{r}\frac{d}{dr}\left(r\frac{dv}{dr}\right) - v = 0. \tag{10}$$

SOLUTION OF EQUATION FOR SPREAD OF EXCITATION

The solutions of equation 10 are well known (McLachlan, 1955) to be

$$v = AI_0(r/\alpha) + BK_0(r/\alpha), \tag{11}$$

where $I_0(r/\alpha)$ and $K_0(r/\alpha)$ are the modified Bessel functions of first and second kinds, respectively. The first question we ask is whether the photochemical activation of a single channel can lead to sufficient depolarization over adjacent regions of the membrane to cause electrical activation of neighboring channels. In other words, can the initial photochemical event lead to an "action potential" in the membranes of the rhabdomere? If a single activated channel is placed at the origin, $\sigma_m = \sigma_m^K$ everywhere else in the membrane, and also we can set $\bar{V} = E_K$, so that in equation 11 $v = V$.

The boundary conditions are then specified as follows. We assume[2] that the conductance of the intercellular cleft beyond the rhabdomere, at $r > R$, is so much higher than that at $r < R$, that we can set $V = 0$ for $r > R$, or $V(R) = 0$. The other boundary condition is specified by the current I through the channel at the origin. If r_0 is the distance from the photoactivated channel to the nearest electrically excitable channel in the membrane,

$$I = -\sigma_p 2\pi r_0 a\left(\frac{\partial V}{\partial r}\right)_{r=r_0}. \tag{12}$$

[2] This symmetry of the model, while essential for a tractable estimate, cannot change the orders of magnitude of the results.

The two conditions determining the constants in equation 11 are, therefore,

$$AI_0(R/\alpha) + BK_0(R/\alpha) = 0$$

and

$$-2\pi r_0 \frac{a}{\alpha} \sigma_F [AI_1(r_0/\alpha) - BK_1(r_0/\alpha)] = I. \tag{13}$$

These yield

$$A = \frac{-I\alpha}{2\pi r_0 a \sigma_F} \frac{K_0(R/\alpha)}{I_1(r_0/\alpha)K_0(R/\alpha) + I_0(R/\alpha)K_1(r_0/\alpha)} \tag{14}$$

and

$$B = \frac{I\alpha}{2\pi r_0 a \sigma_F} \frac{I_0(R/\alpha)}{I_1(r_0/\alpha)K_0(R/\alpha) + I_0(R/\alpha)K_1(r_0/\alpha)} \tag{15}$$

Therefore, from equation 11 the potential at r_0 from the photoactivated center is

$$V(r_0) = \frac{I\alpha}{2\pi r_0 a \sigma_F} \frac{I_0(R/\alpha)K_0(r_0/\alpha) - K_0(R/\alpha)I_0(r_0/\alpha)}{I_1(r_0/\alpha)K_0(R/\alpha) + I_0(R/\alpha)K_1(r_0/\alpha)}. \tag{16}$$

We shall now evaluate the various parameters. The value of I is taken to be 2×10^{-11} amp as estimated on p. 10. The roughness factor s for the typical rhabdomere is calculated from the electronmicroscopic pictures to be somewhat less than 10, so that, since the normal membrane conductance is $5 \times 10^{-10}\ \Omega^{-1}\ cm^{-1}$, we use $\sigma_m = 5 \times 10^{-9}\ \Omega^{-1}\ cm^{-1}$. For the activated membrane we multiply σ_m by $\simeq 10^2$ (or α by 10^{-1}). For σ_F we estimate $4 \times 10^{-4}\ \Omega^{-1}\ cm^{-1}$ which is $\xi \simeq 10^{-2}$ times the value of 0.5 M sea water at 25°C. Although generally the resistivities of intercellular media are only three or four times those of the cellular environments (Cole, 1968), in this case the very close spacing of the membrane may raise this factor considerably. This assumption of a high interstitial resistivity is necessary in the present model, although if the estimated current per channel is increased, the estimated resistivity can be lowered proportionately. The value of a is obtained by multiplying the average total width of a rhabdomere, 4.7×10^{-5} cm by 0.093, the fraction of open area normal to axes of cylindrical microvilli, as obtained from the electron micrographs, to yield $a = 4.4 \times 10^{-6}$ cm. With these values we obtain $\alpha = 5.0 \times 10^{-4}$ cm. The value of $R = 10^{-3}$ cm.

It is generally reckoned that 10 to 30 mv depolarization is required to initiate an action potential. For instance the critical depolarization for the cat motoneuron is 30 mv for the soma membrane but only 10 mv for the membrane in the neighborhood or the axon hillock (Fuortes et al., 1957). The most likely explanation for such differences would appear to be a variation in N, the surface density of excitable channels, and hence in r_0, the mean distance between channels. The quantitative relation between V and r_0 is indeed expressed in our equation 16.

Let us therefore calculate from equation 16 the value of r_0 that would correspond, in the particular case of the idealized model rhabdomal membrane, to a depolarization of 12 mv. The result from equation 16 is $r_0 = 100$ A. Considering the roughness factor, $s = 10$, of the model membrane this figure is well within the experimental ranges estimated for the surface density N of available channels (p. 19).

As more channels are activated, the current density along the cleft *increases* while the current path is *shortened.* Thus an activated patch will grow from a single channel until, at its outer edge, the effect of the current density increase upon the ohmic drop is compensated by the path shortening. We do not include this straightforward but lengthy calculation (complicated further by local circulating currents) because it is too sensitive to the rough experimental values. We can conclude, therefore, that photoactivation of a single channel in the microvillar membrane can be followed by a purely electrical spread of the activation by a mechanism analogous to that responsible for action potentials in nerve conduction, provided, of course, that the rhabdomal membrane is electrically excitable as postulated in this model.

Spread of Activation to Generate a Quantum Bump

The next problem is whether the active patch in the rhabdomere can spread sufficiently to cause a total depolarization of a retinula cell equal to the 2 to 8 mv observed in the quantum bumps. The problem of the growth and decay of the active region would require the solution of the potential equation including the time to provide $V(r, t)$. It is still possible, however, to obtain considerable insight into the problem by considering a series of quasi-stationary states in which the active and inactive sections of membrane occupy fixed areas. One such pattern of active and inactive "patches" is illustrated in Fig. 3 *c*.

In order to see the order of magnitude of the depolarizations, let us consider a case in which an entire rhabdomere is activated. From equation 11 we evaluate the potential as a function of r, and hence determine $(\partial V/\partial r)$ at $r = R$, the edge of the model rhabdomere. From this value we can find the current that is drawn into the cleft from outside the retinula cell:

$$I = -\sigma_r \, 2\pi Ra(\partial V/\partial r)_R. \tag{17}$$

This current is returned through the external membranes of the retinula cell:

$$I = \sigma_m \frac{A}{\delta} (E_K - \bar{V}). \tag{18}$$

Here A is the area of the external membrane. Thus the part of the retinula cell outside the rhabdomere is treated as an equivalent membrane resistance drawing current I from the cleft. (In the quasi-stationary state the capacitative charging current is neglected.)

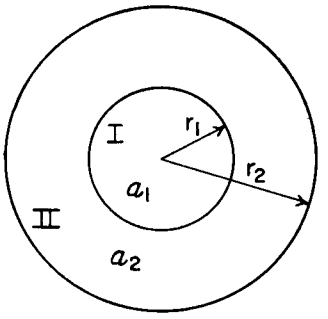

FIGURE 3 *c* An activated region I surrounded by an inactive region II in the rhabdomal membranes.

The constants in equation 11 are found from the requirement that $v < \infty$, so that $B = 0$, and $v(R) = E_{Na} - \bar{V}$, so that $A = (E_{Na} - \bar{V})/(I_0[R/\alpha])$. Hence

$$\left(\frac{\partial V}{\partial r}\right)_R = \frac{E_{Na} - \bar{V}}{\alpha} \frac{I_1(R/\alpha)}{I_0(R/\alpha)}. \tag{19}$$

We thus obtain from equations 17–19,

$$\frac{E_K - \bar{V}}{E_{Na} - \bar{V}} = \frac{-\sigma_F 2\pi R a \delta}{\alpha \sigma_m A} \frac{I_1(R/\alpha)}{I_0(R/\alpha)}. \tag{20}$$

The appropriate parameters are as follows (now α belongs to *activated* membrane, and σ_m to the smooth *external* membrane):

$$\alpha = 5.0 \times 10^{-5} \text{ cm} \qquad \sigma_m = 5 \times 10^{-10} \ \Omega^{-1} \text{ cm}^{-1}$$

$$R = 10^{-3} \text{ cm} \qquad \delta = 7 \times 10^{-7} \text{ cm}$$

$$R/\alpha = 40 \qquad A = 10^{-5} \text{ cm}^2$$

$$\sigma_F = 4 \times 10^{-4} \ \Omega^{-1} \text{ cm}^{-1} \qquad a = 4.4 \times 10^{-6} \text{ cm}$$

These yield $\bar{V} = -47.6$ mv or a depolarization of 22.4 mv. It is of course most unlikely that an entire rhabdomere of one retinula cell would ever be in a steady state of activation, but the calculation suffices to show that sufficient current can be drawn into the retinula cell by this mechanism to cause the observed depolarization pulses of 2–8 mv.

Maintenance of Ionic Concentrations in Interstitial Space

In the model described, the spread of activation through the microvilli and the appearance of depolarization in the retinula cell both demand a high electrical resistance in the interstitial fluid of the rhabdome. Furthermore, the total volume of this fluid is so small that its ionic composition can be markedly altered as a result of even

a few firings of the membranes.[3] Because of the high resistance of the interstitial fluid, diffusion of ions such as Na^+ and Ca^{2+} from the external medium cannot be sufficiently rapid to maintain the ionic composition of the interstitial fluid. (For an ion with a diffusion coefficient of 10^{-7} cm^2 sec^{-1} the mean diffusion time into a rhabdome 10 μm long would be about 5 sec.) Therefore, the restoration of the ionic compositions must require an intense activity of the sodium pumps in the membranes of the microvilli.

It is highly significant that the response of visual cells of *Limulus* eye to a light stimulus is rapidly (but reversibly) abolished by addition of 2,4-dinitrophenol to the external fluid (Borsellino and Fuortes, 1968). Smith and coworkers (1968) have used this result to support a model of the visual response that is based on a direct effect of the photoactivated membrane on the pump mechanism. It is difficult to see, however, how a single locus of photoactivation could be expected to alter the activity of the pump over a wide surrounding area. In our model the importance of the pump is based on the necessity to maintain the ionic concentrations in the relatively small interstitial volume in the rhabdome.

It is not possible at present to give any quantitative theory for the width of the depolarization pulses.[4] Slow action potentials have been observed in a variety of systems. According to one theory (Lettvin et al., 1964) the closing of the fast channel depends on diffusion of Ca^{2+}, displaced during the activation process, back to the membrane. In the interstitial fluid of low ionic conductance, the Ca^{2+} diffusion would probably be unusually slow, and this low Ca^{2+} mobility may be a factor causing the unusually wide depolarization pulses.[5] Both the small cross-section of the interstitial fluid between the microvilli and the (postulated) high resistivity of the interstitial fluid would decrease the speed of propagation of any excitation, but this effect would hardly be sufficient to account for the slowness of the depolarization pulses.

THERMAL GENERATION OF DISCRETE POTENTIALS

The frequency of spontaneous depolarization pulses in the retinula cell of *Limulus* is relatively high, as compared, for example, to a squid axon preparation. This fact suggests that some fluctuation mechanism can cause appreciable depolarization of the rhabdomal membranes at ordinary temperatures.[6] The theory of such thermal

[3] As in the case of the interstitial volume in brain tissue, the volume in vivo may be considerably greater than the apparent volume in electron microscopic sections prepared with the usual fixatives (Haareveld and Malhotra, 1967).

[4] Of course, we do not have a solution for the propagation equation for the complex geometry of the rhabdomere, and it may turn out to have unexpectedly long time constants.

[5] If the closing is indeed controlled by availability of Ca^{2+}, we would expect it to be delayed by $\xi^{-1} \simeq 10^3$ times the usual width of $\sim 10^{-3}$ sec, which gives the right order.

[6] Some authors have considered the spontaneous depolarization to be analogous to the miniature end plate potentials at neuromuscular junctions (Adolph, 1964). The principal consequence of light absorption is then considered to be release of some unknown transmitter substance which diffuses to the membranes of the retinula cell and produces depolarization by reacting there with specific ad-

fluctuations as outlined in an earlier paper (Bass and Moore, 1968) would indicate that the rhabdomal membranes in *Limulus* contain more available channels than a typical nerve preparation. In addition it is possible that each channel can be more readily activated.

Let us consider the fluctuation argument. If a local excitation leads with probability p to a discrete polarization pulse, the spontaneous frequency ν of the pulses should be proportional to the spontaneous excitation rate of individual channels in the rhabdomere:

$$\nu = p(Z/\tau) \exp(-A_{\min}/kT). \tag{21}$$

Here Z is the number of sites capable of spontaneous activation, τ is the rise time of a pulse, and $A_{\min}$ (the reversible work expended to open a channel) can be identified with $\Delta A^{\ddagger}$, the free energy of activation of transition-state theory. We can estimate that $pZ = NS$ where S, the total area of rhabdomal surface membrane in one retinula cell, is about 6×10^{-4} cm^2 from available electron micrographs. With $N = 10^{10}$ cm^{-2} and $\tau = 0.05$ sec, the observed $\nu = 0.5$ sec^{-1} at 20°C would correspond to $\Delta A^{\ddagger} = 0.53$ ev. The molecular process responsible for the spontaneous depolarizations remains to be identified.

An explanation of the latency of the first appearance of quantum bumps following a flash of low intensity light cannot readily be found. The latency with *Limulus* preparations appears to be from 150–500 msec, apparently too high to be controlled by the Metarhodopsin I → Metarhodopsin II reaction. [Ebrey, 1968]. The extensive measurements of Kirschfeld (1966) on eye of *Musca* show latencies of only 4–25 msec for discrete depolarization in individual retinula cells. Such latencies will require the addition of special features to our chemical-electrical model.[7] For instance they might be related to the rate of closing channels by calcium ions, which may have an especially low mobility in the interstitial space between microvilli. During dark adaptation the concentration of calcium ions may reach a high level, and one or two abortive firings may be necessary to reduce this level before excitation can spread. The latency time actually corresponds to about the mean time between quantum bumps.

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sorption sites. It is not easy to construct a working model of photoreceptive processes based on this mechanism. The observation that shrinkage of the rhabdome in hyperosmotic sucrose does not influence the visual cell responses is evidence against a diffusion of transmitter across the R cell from the rhabdomere (Borsellino and Fuortes, 1968). Diffusion of transmitter within the rhabdomere would require specialized release mechanisms and enzyme systems for which no evidence yet exists.

[7] The problem of latencies can be handled quite readily in a model based on diffusion of an active transmitter from the site of photoactivation to the membrane walls, if one postulates that absorption of one photon leads to release of some 10^6 transmitter molecules. Cone (1964) discusses this. Such a model predicts (correctly) that with increasing intensity the latency is reduced, but (incorrectly) that duration of quantum bumps is *increased.*

Dr. M. G. F. Fuortes of the National Institutes of Health has been especially helpful in providing data on the conductances and other useful comments.
The electron microscopy was performed by Mrs. Mary Sue Hertweck at Indiana University.

This work has been made possible by the support of the National Institutes of Health and the National Science Foundation.

Received for publication 17 May 1969 and in revised form 25 September 1969.

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