Discrete Waves and Phototransduction in Voltage-Clamped Ventral Photoreceptors

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ABSTRACT Discrete waves in the voltage-clamped photoreceptor of *Limulus* are remarkably similar in all essential properties to those found in an unclamped cell. The latency distribution of discrete waves is not affected by considerable changes in the holding potential in a voltage-clamped cell. Both large and small waves occur in voltage-clamped and unclamped cells and in approximately the same proportion. Large and small waves also share the same latency distributions and spectral sensitivity. We suggest that small waves may result from the activation of damaged membrane areas. Large waves have an average amplitude of approximately 5 nA in voltage-clamped photoreceptors. It probably requires several square microns of cell membrane to support this much photocurrent. Thus the amplification inherent in the discrete wave process may involve spatial spread of activation from unimolecular dimensions to several square microns of cell membrane surface. Neither local current flow, nor prepackaging of any transmitter substance appears to be involved in the amplification process. The possible mechanisms of the amplification are evaluated with relationship to the properties of discrete waves.

INTRODUCTION

About 15 years ago Yeandle (1958) discovered the existence of relatively small discrete depolarizations of the photoreceptor cell membrane in *Limulus* which he called "bumps." These events occur spontaneously and in response to low intensity light stimuli. They have been called by a variety of names including, more recently, "discrete waves," the term we will use. Their properties have attracted considerable interest for it is possible that each discrete wave represents a single photon absorption. The single photon hypothesis still remains unproven and controversial. (For a recent discussion of the problem see Yeandle and Spiegler, 1973.) Regardless of whether or not discrete waves are single photon responses, they are certainly electrical responses with extraordinary sensitivity to light, and for this reason alone, it seems

likely that their properties are closely related to the membrane and molecular mechanisms of phototransduction.

There are two properties of discrete waves that seem to us particularly important in this regard. The first is the highly variable and relatively long latency of the discrete waves that follow brief flashes of light. In both the lateral eye photoreceptor and the ventral photoreceptor the latency may range from 50 to 300 ms, and averages 120 ms at 21°C. The temperature of the cell has a marked effect on the latency distribution. The Q 10 of the latency is between 2 and 3 (Srebro and Behbehani, 1971) in the lateral eye photoreceptor. However, we have not been able to change the latency in any other way. Various changes in the ionic composition of the external bathing fluid such as lowering the calcium ion concentration or the sodium ion concentration, the application of drugs such as veratrine and D600, the application of neurotoxins such as DDT, batrachotoxin, and tetrodotoxin (TTX), the intracellular iontophoresis to tetraethylammonium ion (TEA), substantial changes in the dark resting membrane potential (as we show here), and light adaptation (Srebro and Behbehani, 1972 *b*) all have no effect on the latency dispersion. Any effects that these manipulations have on the behavior of discrete waves is restricted to changes in their size.

Several years ago Adolph (1964) reported that discrete waves come in two sizes, large and small. Large discrete waves are generally larger than 2 mV in peak amplitude. But since the average size of discrete waves varies from cell to cell it is often useful to construct a peak amplitude histogram, as such a histogram is usually bimodal. Small discrete waves may have a slower time-course than large ones (Adolph, 1964; Borsellino and Fuortes, 1968; Srebro and Behbehani, 1971). There is controversy about the latencies of the two types of discrete waves. Borsellino and Fuortes (1968) reported that the small discrete waves have a shorter and less dispersed latency than large ones. But Srebro and Yeandle (1970), and Srebro and Behbehani (1971) found no significant differences in their latencies. Yeandle and Spiegler (1973) showed that in the ventral photoreceptor small discrete waves are more likely to occur spontaneously while large ones are more likely to occur when a light stimulus is applied. The relative number of large and small waves is variable from cell to cell. Borsellino and Fuortes (1968) found that small discrete waves predominate in the lateral eye photoreceptor. On the other hand, Srebro and Yeandle (1970) and Srebro and Behbehani (1971) found that the large waves predominate in the lateral eye photoreceptor.

We do not understand the mechanism that produces large and small discrete waves. However, Yeandle and Spiegler (1973) showed that the relative number of large and small light-induced discrete waves is not changed by increasing the diameter of the stimulating light spot from 10 μm to a spot large enough to nearly cover one ventral photoreceptor cell. Thus the two classes

of waves do not reflect spatial properties of the cell. There are at least two mechanisms that could be responsible for the occurrence of two classes of discrete waves and that require further investigation. First, a larger discrete wave could be triggered by a smaller one due to a membrane voltage-dependent mechanism. It is known that the dark current-voltage curve in the ventral photoreceptor exhibits a zone of negative resistance near resting membrane potential (Lisman and Brown, 1971), and it is possible that some small discrete waves depolarize the cell membrane into the unstable zone and trigger sudden jumps to a more depolarized state. The larger discrete waves, according to this view, are "regenerative." The concept of a regenerative discrete wave has already entered into the thinking of several investigators interested in the problem of photo-transduction (Borsellino and Fuortes, 1968; Bass and Moore, 1970; Srebro and Behbehani, 1971). A second mechanism that could give rise to two size classes of discrete waves is the existence of more than one visual pigment. It is not uncommon to find a visual pigment with maximum absorption in the near ultraviolet in arthropod eyes. Another more remote possibility is the existence of significant amounts of the 9 cis isomer of rhodopsin (isorhodopsin). If two different visual pigments exist in the photoreceptor, it is possible that the transduction process could be different for each and result in two classes of discrete waves.

We have examined the properties of discrete waves in both voltage-clamped and unclamped ventral photoreceptors over a wide range of wavelengths of stimulating light from the near ultraviolet (340 nm) to the far red (greater than 700 nm). We find that both large and small discrete waves exist essentially in the same proportions in both voltage-clamped and unclamped cells. The wavelengths of the light have no effect on the proportion of large to small discrete waves. The latency of discrete waves is the same in voltage-clamped and unclamped cells, and is independent of the holding potential in a voltage-clamped cell. Finally, the central photoreceptor is an excellent cell in which to compare the latencies of large and small waves since they often occur in similar numbers and the small waves may be as large as 5 mV. We find no important difference in their latencies.

MATERIALS AND METHODS

We have already described the method of preparing ventral photoreceptors for electrode insertion, and the method of preparing bonded pairs of microelectrodes for voltage-clamp experiments in the previous paper. The experiments we report here consisted of presenting 10-ms flashes of light or steady light under voltage-clamped and unclamped conditions. Latency and peak amplitude measurements were made by hand from Grass penwriter records (Grass Instrument Co., Quincy, Mass.) and automatically using a voltage comparator and a computer. Different wavelengths of light were produced using interference filters and appropriate blocking filters where necessary. The light source was a Xenon arc (Xenon Corp., Medford, Mass.) and

all lenses in the optical system were either constructed of suprasil quartz or were reflecting optics. The mounting chamber, although made of Pyrex glass, was very thin walled. A transmission curve for the chamber and Sylgard mounting surface was obtained in a spectrophotometer and showed a transmission of about 40% at 340 nm.

RESULTS

Fig. 1 shows some randomly selected examples of discrete waves that result from the presentation of a 10-ms flash of light. The top line of the figure shows discrete waves from a cell in which we had inserted two electrodes but did

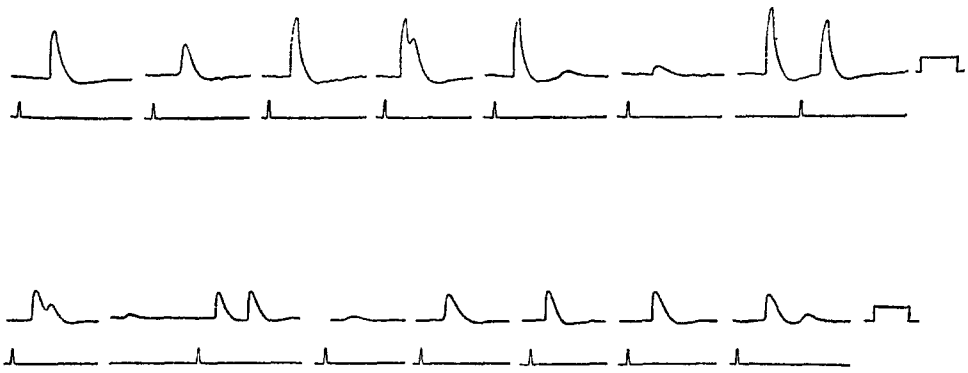

FIGURE 1. Discrete waves under voltage-clamped and unclamped conditions. All recordings are from the same ventral photoreceptor. In each line of records, the light flash is indicated just below the recording and was 10 ms long and of fixed energy. Top line of records: two electrodes were inserted into the photoreceptor but the voltage-clamp circuit was disenabled. Dark resting membrane potential, −69 mV. Calibration, 5 mV, 250 ms. Bottom line of records: voltage-clamp circuit enabled. Holding potential −60 mV. Calibrations 2.5 nA, 250 ms. Temperature 21°C.

not voltage clamp. The dark resting membrane potential was −69 mV. The bottom line shows discrete (current) waves from the same cell as the top line after voltage clamping was initiated. The holding potential was −60 mV. The records were taken on a curvilinear penwriter with frequency response up to about 40 Hz. There is remarkably little difference between the waveforms of the discrete waves under voltage-clamped and unclamped conditions. Careful examination revealed that the discrete waves seen in the voltage-clamped state had a slightly slower rise time. In some cells, the discrete waves seen in the unclamped state had a fast rising component followed by a somewhat slower rising component. Under voltage clamp the rise was always along a single monotonic curve. As Fig. 1 shows both large and small waves occur in both the voltage-clamped and unclamped state. The fifth and sixth stimuli in the top (unclamped) examples show small waves. The third and seventh stimuli in the bottom (voltage clamped) examples show small waves.

A spontaneous small wave also appears before the second stimulus in the bottom example.

Fig. 2 shows the latency distribution for discrete waves from the same cell as that shown in Fig. 1. The latency is the time from the onset of the flash to the first discrete wave. The latency distribution shown in the upper part of

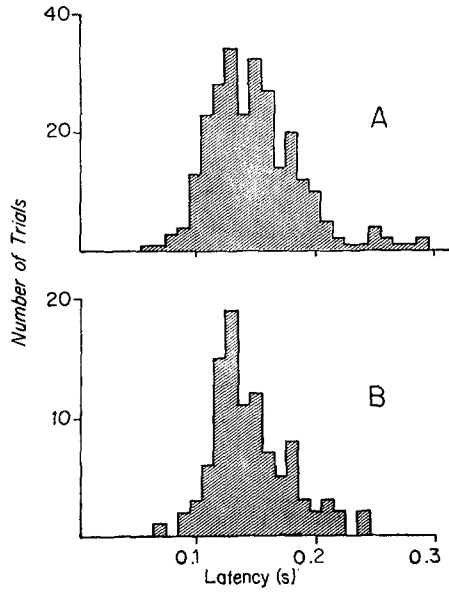

FIGURE 2

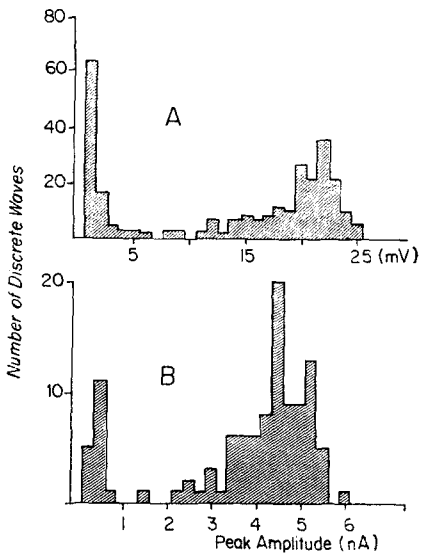

FIGURE 3

FIGURE 2. Latency distributions of discrete waves under voltage-clamped and unclamped conditions. The latency is the time between the onset of a 10-ms light flash and the start of the first discrete wave that follows it. The distributions shown are for the same cell as that in Fig. 1. (A) two electrodes inserted but voltage-clamp circuit disenabled. Dark resting membrane potential −69 mV. (B) Voltage clamped. Holding potential −60 mV. Temperature 21°C.

FIGURE 3. Peak amplitude distributions of discrete waves under voltage-clamped and unclamped conditions. The distributions shown are for the same cell as that in Fig. 1. (A) two electrodes inserted but voltage-clamp circuit disenabled. Dark resting membrane potential −69 mV. (B) Voltage clamped. Holding potential −60 mV. Temperature 21°C.

the figure was obtained while the double electrode was in place within the cell, but the cell was not voltage clamped. The dark resting membrane potential was −69 mV. The latency distribution shown in the lower part of the figure was measured when the cell was voltage clamped at −60 mV. The light flash was 10 ms long and was presented at time zero. It had the same intensity in both cases, and was adjusted to produce a failure rate of about one in three trials. It is apparent from this figure that there are no important

differences between the two latency histograms. Both show a sharper rise than fall and peak at approximately 120 ms. We studied nine cells in detail, and none of them showed a significant difference in the latency distribution of voltage-clamped and unclamped discrete waves. There was also no significant difference in the failure rate for voltage-clamped and unclamped discrete waves caused by flashes of the same intensity.

Fig. 3 shows the peak amplitude histograms for the same cell and the same experiment as shown in Fig. 2. It is clear that both large and small discrete waves occur in both the voltage-clamped and unclamped state. In this cell, the peak amplitude of the large discrete waves was, on the average, approximately 25 mV in the unclamped state. The peak amplitude of the large waves in the voltage-clamped state was about 5 nA. This corresponds to a cell resistance of about 5 MΩ, which is very nearly equal to the cell resistance measured by passing 5 nA of current through one microelectrode. In this cell there was a slightly higher proportion of large waves in the voltage-clamped cell, but this was not a consistent feature of the behavior of the nine cells we studied. The cell shown in Figs. 1, 2, and 3 was a particularly good one with large discrete waves in the unclamped state. Many cells had a smaller average discrete wave size, and also fewer large waves than found in this particular cell. Nevertheless, the photocurrent associated with small waves was usually less than 1 nA and averaged about ½ nA. The photocurrent associated with large waves usually averaged 4–5 nA. Thus the smaller average size of discrete waves which occurred in many unclamped cells appeared to be due to a lower cell resistance and not to a reduced photocurrent.

Since there is some controversy concerning the latencies of large and small waves, we examined the latency distributions separately. Fig. 4 shows latency distributions from the same experiment as shown in Figs. 1, 2, and 3 for small and large waves taken separately in the unclamped state. With reference to the peak amplitude distribution of the top part of Fig. 3, we considered any wave with a peak amplitude greater than 10 mV to be a large wave. Fig. 4 shows that there is only a very modest difference in the latency distribution. The latency distribution for the small waves has a peak which occurs about 20 ms later than that for the large waves. Although this difference proved to be significant by a chi-square test, it is most likely due to a trivial mechanism, namely, that it takes a bit longer to be sure that a discrete wave begins when it is small. The results shown in Fig. 4 are characteristic of all our results. In addition, correlation coefficients for peak amplitudes against latency were insignificant. There can be little doubt that large and small discrete waves share essentially the same latency distribution.

Fig. 5 shows the latency distribution found in another voltage-clamped cell at several different holding potentials from −71 to −40 mV. The same flash intensity was used throughout. There is no significant difference among these

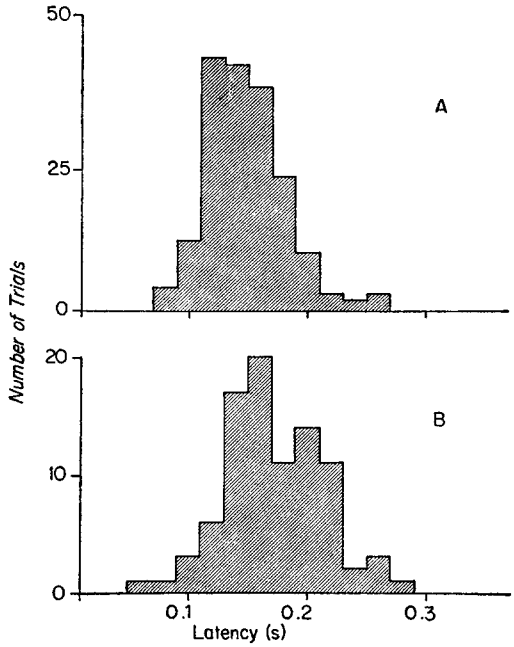

FIGURE 4. Latency distributions for large and small waves. Large waves are those with peak amplitudes greater than 10 mV. Same cell as that shown in Fig. 1 and peak amplitude histogram is shown in Fig. 3. (A) Latency distribution for large waves. (B) Latency distribution for small waves. Unclamped ventral photoreceptor. Dark resting membrane potential −69 mV. Temperature 21°C.

distributions. Thus the latency distribution is not affected by the holding potential at least within the range we could study. We often found it difficult to explore a wider range of holding potentials for several reasons. First, the discrete waves became smaller as the holding potential was moved toward 0. Second, the amount of current required to keep the cell at a holding potential much different from the dark resting membrane potential for the relatively long periods of time required to study discrete waves often cause the current passing electrode to become noisy. Finally, we found that cells kept at a holding potential more than 30 or 40 mV from the dark resting potential for periods of ½ or more, frequently stopped responding to light and required prolonged periods of time (½–1 h) to recover.

Fig. 6 shows three amplitude histograms from an unclamped cell. The middle histogram was constructed from the measurement of approximately 20 min of spontaneous discrete waves. The rate of spontaneous discrete waves was approximately 9 per minute. The top histogram was obtained by applying a steady light at 450 nm. The rate was approximately 21 discrete waves per minute. The lower histogram was obtained by applying a steady light at 545 nm. The rate was 18 discrete waves per minute. Two results are ob-

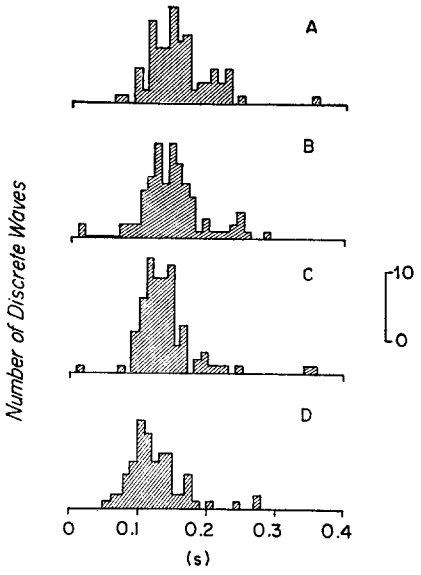

FIGURE 5

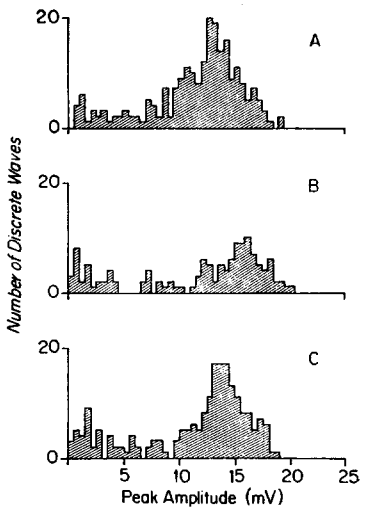

FIGURE 6

FIGURE 5. Latency distributions of discrete waves under voltage-clamped conditions for several different holding potentials. The light flash was 10 ms long and of equal energy in each case. Holding potentials: (A) −71 mV, (B) −60 mV, (C) −50 mV, (D) −40 mV. Temperature 22°C.

FIGURE 6. Peak amplitude histograms for discrete waves that occurred spontaneously and were induced by steady light at two different wavelengths. All the histograms are from a single unclamped photoreceptor. Dark resting membrane potential −56 mV (A) Steady light at 450 nm. Discrete wave frequency 21 per minute. (B) Spontaneous discrete waves. Frequency 9 per minute. (C) Steady light at 550 nm. Discrete wave frequency 18 per minute. Temperature 22°C.

vious from this figure. First, the peak amplitude distribution is essentially the same for the two different wavelengths. This finding held true as long as the discrete wave frequency was the same for wavelengths for 340 nm to greater than 700 nm. Secondly, there is a smaller proportion of large waves among the spontaneous discrete waves as compared to those induced by light. This finding confirms similar results reported by Yeandle and Spiegler (1973). The slight reduction in the average size of the large waves in the runs using light as compared to the spontaneous ones is not an infrequent finding, in our experience, and represents a modest but definite degree of light adaptation even at these very low discrete wave frequencies.

CONCLUSIONS AND DISCUSSION

Properties of Large and Small Discrete Waves

Our observations permit several conclusions about the properties of large and small waves. (*a*) Large discrete waves are not "regenerative," that is,

large waves do not result from membrane voltage changes initiated by small waves. (*b*) Large and small waves share the same spectral sensitivity. This suggests that there is only one visual pigment in the ventral photoreceptor. (*c*) Large and small waves share essentially the same latency distribution. (*d*) Both large and small waves occur spontaneously and are induced by light. But large waves occur less frequently as a spontaneous event than as a light-induced event. (*e*) The number of large waves is highly variable from cell to cell. If a single electrode is inserted within a cell, there is more likely to be a greater number of large waves than if two electrodes are inserted into the cell. (*f*) Large waves are associated with a peak photocurrent of about 5 nA on the average. Small waves, are associated with a photocurrent of about ½ nA, on the average. This is usually true regardless of the peak amplitudes of the waves in the unclamped photoreceptor.

These conclusions do not suggest a compelling explanation for the occurrence of large and small waves. We can rule out any possibility that they represent two different spectral mechanisms or that small waves cause large ones by a membrane voltage-dependent conductance change. We considered the possibility that large waves might represent coincident photon absorptions, but the relationship of the occurrence of a large wave (or a small wave) to the energy of the light flash that causes it follows the simple Poisson law and this implies that no coincidences are involved. Finally, we explored the possibility that the different proportion of large waves among spontaneous and light-induced ones is an artifact due to observer bias by automating the discrete wave detection process and using a computer to form peak amplitude histograms. These histograms verified that the difference was no artifact.

We are impressed with the great lability of the large wave process and its tendency to be suppressed by the insertion of two microelectrodes. We therefore suggest that small waves may arise from membrane patches which are damaged. Damaged membrane patches must have two characteristics in keeping with the above conclusions. First, the photocurrent that a damaged membrane patch can support is substantially less than the photocurrent that normal membrane can support. Second, the visual pigment associated with damaged membrane is thermally unstable as compared to the visual pigment of normal membrane. This may explain why spontaneous discrete waves are often small. If light is absorbed equally well by visual pigment molecules in damaged and undamaged membrane, then it follows that the proportion of large waves induced by light is different from the proportion found among spontaneous waves. Thus if there is more normal membrane than damaged membrane in a cell, the light-induced discrete waves are more often larger ones. It is curious that the photocurrent associated with large waves is usually, at least 10 times greater than the photocurrent associated with small waves. To state this in another way, it is curious that a bimodal peak ampli-

tude histogram exists. One might expect that damaged membrane areas would grade continuously into normal membrane with regard to the amount of photocurrent produced. The existence of a clearly bimodal peak photocurrent distribution suggests that the damage may result in the loss of some as yet unknown amplification process with a gain of at least 10.

Our hypothesis concerning the origin of large and small discrete waves is compatible with the idea that spontaneous discrete waves are due to thermal isomerizations of visual pigment molecules. In a previous study (Srebro and Behbehani, 1972 *a*) we presented the evidence for this conclusion based on experiments using the lateral eye photoreceptor. Yeandle and Spiegler (1973) refuted the conclusion because of the larger proportion of small waves among spontaneous discrete waves than among light-induced ones, in the ventral photoreceptor. But if we are correct in our guess that the small waves result from damaged membrane patches containing unstable visual pigment, then thermal isomerization may still be the root cause of spontaneous discrete waves. We also point out here, that in the lateral eye photoreceptor, we find that only a small fraction of the waves are small, usually much less than 10%, for both spontaneous and light-induced waves.

Amplification Inherent in Discrete Wave Process

A large wave in the ventral photoreceptor is associated with a photocurrent of approximately 5 nA (on the average). It is known that the photocurrent is carried largely by sodium ions (Millecchia and Mauro, 1969; Brown and Mote, 1971). The maximum photocurrent that a single sodium conductance channel may support is about 0.01 nA (Ehrenstein and Lecar, 1972). Thus it seems likely that there are at least 500 sodium channels involved in the production of a large wave. There may be considerably more, however, since we have no estimate of the lifetime of an open sodium channel. The density of sodium channels in the photoreceptor membrane is unknown. If we assume that there are as many as 170 per square micron, which is the channel density in a node of Ranvier, and the highest known density in neural membrane (Hille, 1968), then the area of membrane involved in the production of a large wave is greater than 3 μm^2. There are two ideas which have been proposed to explain the statistical behavior of discrete waves. One idea is that each discrete wave results from a single photon absorption. A second idea is that as many as 50 photons may be absorbed per discrete wave produced, (Yeandle and Spiegler, 1973), but that these absorbed photons cause the production of a substantial number of "messenger" molecules, and each messenger molecule has a small probability of initiating a discrete wave (Srebro and Yeandle, 1970; Yeandle and Spiegler, 1973). In either case, a single molecular event, either the absorption of a photon by a visual pigment molecule, or the reaction of a single messenger molecule with a membrane mole-

cule must initiate a discrete wave. This suggests that the amplification inherent in the discrete wave process involves the spread of excitation along the photoreceptor membrane from the molecular dimensions of the triggering event to an area of several square microns needed to support the photocurrent. One could argue that the sodium channels of the ventral photoreceptor could carry more current than 0.01 nA, and certainly there are differences between the ionic specificity of nerve membrane sodium channels and those found in the ventral photoreceptor (Millecchia and Mauro, 1969). But it seems unlikely that ventral photoreceptor sodium channels could be vastly different from those in nerve membrane. Both exhibit a high degree of selection of sodium ions over potassium ions for example, which probably implies a similar pore size and similar limits on current-carrying capacity. It is noteworthy also that Cone (1973) recently reached a similar conclusion concerning the necessity of substantial spatial spread of excitation in the ventral photoreceptor membrane based on different considerations.

Latency Distribution of Discrete Waves

Our observations permit several conclusions about the latency of discrete waves. (*a*) The latency distribution is the same in unclamped cells and in voltage-clamped cells at a holding potential comparable to the dark resting membrane potential. (*b*) The holding potential does not significantly affect the latency distribution in a voltage-clamped cell. (*c*) Large and small waves share essentially the same single latency distribution.

The last point is in disagreement with observations by Borsellino and Fuortes (1968) in the lateral eye photoreceptor and requires further comment. These authors claim that there is a synchronized depolarization that underlies the discrete wave process. After examining several hundred lateral eye and ventral photoreceptors, we have observed only a few cells that behave in this way. In these cells, the small synchronized depolarization lasted for a much greater time than the interval during which discrete waves were produced. After a pulse of light several log units more intense than that required to produce discrete waves, these cells showed a prolonged recovery period during which there was a sustained depolarization and the sensitivity to light was much reduced. We have considered them to be injured cells.

The latency distributions we observe all have a skewed shape. They rise with a power law relationship to time and decay exponentially. The order of the power law rise is high, and ranges from 8 to 12. In previous studies on the lateral eye photoreceptor we showed that the order of the power law rise was variable from cell to cell and increased with temperature in a single cell (Srebro and Behbehani, 1971). The power law relationship of the early portion of the latency distribution is reminiscent of the power law relationship of order 4 found for the latency dispersion of miniature end-plate potentials

(Katz, and Miledi, 1965; Eccles, 1972). In this case it has been postulated that it results from a requirement that four calcium ions cooperate in the release of a transmitter vesicle. There is no evidence that some unknown excitator molecules are prepackaged in the ventral or lateral eye photoreceptor from electron microscopic studies (Lasansky, 1967; Clark et al., 1969). If each alleged excitor molecule opened 1 sodium conductance channel, then our observations suggest that a prepackaged unit would contain more than 500 of them. Thus, if an anatomical structure contained the alleged excitor molecules it should have dimensions approximating a presynaptic vesicle and should be visible on electron micrographs.

Models to Explain Discrete Wave Properties

An adequate model to explain the properties of discrete waves is really a model for the molecular and membrane events of phototransduction. It should not only fit within the constraints dictated by the discrete wave properties, but should also explain the amplification process which we think represents spatial spread in the photoreceptor membrane. The most important result of the work we report here is that the discrete wave latency distribution, time-course, and amplitude distribution are only modestly or not at all affected by the dark resting membrane potential or by changes in membrane potential. These findings argue against the idea that phototransduction involves membrane processes similar to those found in neuronal membrane such as axon and presynaptic membrane. Our findings clearly quash our previously published hypothesis that a voltage-dependent membrane process is an important factor in the spread of excitation in photoreceptor membrane (Srebro and Behbehani, 1971).

In addition to the properties of discrete waves already discussed several other observations provide constraints on an adequate model. (*a*) In the ventral photoreceptor large discrete waves appear to arise de novo out of a quiet base line. This is in contrast to the lateral eye photoreceptor where prepotentials are not uncommon. (*b*) While the latency and peak amplitudes of large discrete waves in the ventral photoreceptor vary a great deal from trial to trial, the time intervening between the earliest detection of a large wave and its peak, about 100 ms at 21°C, is much less variable, being constant to within about 20 ms. (*c*) The latency dispersion of discrete waves in the ventral photoreceptor is the same when a 10-μm diameter spot of light is used to elicit them or a spot of light that nearly covers the photoreceptor is used to elicit them (Yeandle et al., 1972).

With these constraints in mind, it is worthwhile to return to the question of the nature of the amplification process and to the spatial spread of excitation that it suggests. The two ways that amplification is known to occur in the nervous system, the flow of electrical current (local current flow in axonal

membrane) and the use of transmitter vesicles (prepackaging) do not seem to be involved in the photoreceptor. This leaves two possibilities still open.

(*a*) There could be interaction between adjacent sodium conductance channels presumably by intermolecular energy transfer between the protein molecules of the channels. There are several arguments that speak against this idea. First, it would require an enormously dense packing of sodium channels. A ventral photoreceptor may produce as much as several hundred nanoamperes of photocurrent, which implies that the total number of sodium channels in the cell is less than about 10^6. If these channels were spread over the cell surface there would result a channel density of less than 100 per square micron. Even if the channels were only present in the microvillous membrane, the channel density would probably not exceed that in a node of Ranvier, and in this structure there is no evidence of channel interaction. Secondly, if channel interaction were the cause of the spatial spread of excitation, it would likely be a two-stage process. That is, first a few channels would open when a visual pigment molecule absorbed a photon, or when a messenger molecule arrived at the membrane, and then the excitation would spread. This implies that prepotentials should be a uniform feature of discrete waves, but in the ventral photoreceptor they do not occur very often.

(*b*) Since the molecules that open sodium channels are not prepackaged, it could be that they are formed by chemical amplification from a plentiful intracellular substrate. In this scheme the absorption of a photon, or the arrival of the messenger molecule at the membrane, would initiate the formation of a macromolecular complex that acted enzymatically to form the required new molecules which then diffused short distance to open sodium channels. If discrete waves are single photon responses the model might also explain the latency dispersion of discrete waves by the additional assumption that the macromolecular complex required the association of 8–12 component parts. Another possibility along similar lines is that the absorbtion of a photon opens a membrane channel for some species of molecule other than sodium ions, and that the collection of 8–12 of these molecules at nearby membrane sites brings about the opening of a large number of sodium channels. Thus the relatively long and variable latency of discrete waves could be an integral part of the amplification process.

But the latency dispersion could have several other origins, and since this phenomenon seems to us to be central to the question of phototransduction, it merits further consideration. If discrete waves are not single photon responses, the latency dispersion could reflect the kinetics of the production of the messenger molecules. But in this case the relatively long latency would have nothing to do with the amplification process. We find this an uncomfortable assumption, for at the very least it is an inefficient process. If discrete waves are single photon responses, then the latency could represent a photo-

chemical delay. For example, if we assume that a visual pigment molecule upon the absorption of a photon progresses through a sequence of molecular states to arrive at the "correct" state to initiate a discrete wave, then the variable latency could reflect this process. However, at 21°C the average latency of a discrete wave in the ventral photoreceptor is about 120 ms. It is known that the visual pigment of the ventral photoreceptor regenerates rapidly (Fein and De Voe, 1973; Hillman et al., 1973). At 120 ms approximately 7 of 10 visual pigment molecules that absorbed photons would have already regenerated. Thus it does not seem likely that the correct molecular state is a photochemical intermediate. A diffusional delay also seems unlikely in view of Yeandle's result (1972) that the latency dispersion of discrete waves does not change with the size of the stimulating light spot. At least these results suggest that there are no specialized centers for discrete wave production scattered at distances of tens of microns along the photoreceptor membrane. Short-range diffusion could still play some role in determining latency but if this is the case the diffusion rate of the excitatory molecule would have to be very slow (on the order of 0.1 μm/ms).

It is not our intention to propose a specific model of phototransduction. Instead, we emphasize that the last 15 years of collecting observations on discrete waves provide many pieces of an intriguing puzzle which is yet unsolved. The results we present here suggest that the question of the amplification process inherent in the production of discrete waves may be a useful phenomenon around which to organize the findings. Since the amplification does not appear to proceed by well described neurophysiological mechanisms such as the flow of electrical current or vesicle prepackaging, we think that more attention must be given to the intervention of a chemical mediator in the process.

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