Light Adaptation in the Ventral Photoreceptor of *Limulus*

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**Abstract** Light adaptation in both the ventral photoreceptor and the lateral eye photoreceptor is a complex process consisting of at least two phases. One phase, which we call the rapid phase of adaptation, occurs whenever there is temporal overlap of the discrete waves that compose a light response. The recovery from the rapid phase of adaptation follows an exponential time-course with a time constant of approximately 75 ms at 21°C. The rapid phase of adaptation occurs at light intensities barely above discrete wave threshold as well as at substantially higher light intensities with the same recovery time-course at all intensities. It occurs in voltage-clamped and unclamped photoreceptors. The kinetics of the rapid phase of adaptation is closely correlated to the photocurrent which appears to initiate it after a short delay. The rapid phase of adaptation is probably identical to what is called the “adapting bump” process. At light intensities greater than about 10 times discrete wave threshold another phase of light adaptation occurs. It develops slowly over a period of $\frac{1}{2}$ s or so, and decays even more slowly over a period of several seconds. It is graded with light intensity and occurs in both voltage-clamped and unclamped photoreceptors. We call this the slow phase of light adaptation.

**Introduction**

Light adaptation may be defined as a reduction in the sensitivity of the visual system, or any of its component cells, that is determined by the past history of its exposure to light. Light adaptation in photoreceptors of the horseshoe crab, *Limulus*, has been studied in some detail (Fuoretes and Hodgkin, 1964; Dodge et al., 1968; Lisman and Brown, 1971, 1972a, b; Srebro and Behbehani, 1972a; Fein and De Voe, 1973), and although it is possible to carry out extensive experiments in this preparation the process is still not clearly understood.

If a bright flash of light (a conditioning flash) is presented to the ventral photoreceptor one can determine how long it takes the cell to recover its full sensitivity. Such experiments show that it requires several minutes, and this amount of time is far in excess of the time required for the visual pigment to completely regenerate (Fein and De Voe, 1973; Hillman et al., 1973). The
reduced sensitivity that follows the conditioning flash occurs without either a sustained depolarization of the photoreceptor, or a sustained change in its conductance.

It is a much more difficult task to describe the alterations of the receptor sensitivity that occur during ongoing activity. Dodge et al. (1968) approached this problem by using steady light stimuli in the lateral eye photoreceptor. In both the lateral eye and ventral photoreceptor low energy light stimuli produce discrete depolarizations of the cell membrane called discrete waves. Many discrete waves combine to form a response to a high energy light stimulus. A steady light produces a fluctuating level of depolarization whose statistical properties can be used to understand the rules by which discrete waves combine. The combination process is not a simple linear summation. The average discrete wave size is reduced as the frequency of discrete waves increases, and this finding has come to be called the “adapting bump” process. (The term “bump” is frequently used as a nickname for discrete wave.) However, it is difficult to obtain estimates of the time-course of light adaptation under steady light conditions that are comparable to those obtained using a conditioning flash.

The adapting bump phenomena is amenable to two different interpretations. Either the occurrence of a discrete wave per se initiates events which reduce the size of a subsequent discrete wave (discrete wave interaction) or the absorption of many photons initiates events which both produce many discrete waves and result in their reduced average size. As an example of the latter mechanism, one could imagine that some material which controls discrete wave size is used up in the production of discrete waves and the reduced amount of material renders subsequent discrete waves smaller. The two interpretations lead to different expectations about the nature of light adaptation. For example, they have implications with regard to the validity of the not infrequently made assumption that background lights and conditioning flashes are equivalent with regard to light adaptation. If discrete wave interaction is the root cause of the adapting bump process then it is possible that the ongoing discrete wave activity during the presentation of a background light imposes different characteristics on the cell than does the presentation of a conditioning flash. Discrete wave interaction might be operative only during a time period comparable with the duration of a discrete wave. (This could occur if some change such as the depolarization of the cell during a discrete wave mediated the adapting bump process.) Then the light adaptation produced by a conditioning flash would require a separate explanation from the process which causes the adapting bump phenomenon. On the other hand, the exhaustion of the cellular material which controls discrete wave size could recover slowly, and account for both the light adaptation observed in the adapting bump process, and that after a conditioning flash.

The two interpretations of the adapting bump process also have important
implications which concern its relationship to light intensity. If the adapting bump process is due to the exhaustion of the supply of some cellular material that controls discrete wave size, it is not likely that it would be manifest at very low light intensities, for if this were true it would imply that the material was present in very short supply. This would make it very difficult to explain the large transient responses of the photoreceptor to bright lights, as such transients may be many times the size of a discrete wave. The discrete wave interaction interpretation on the other hand suggests that the adapting bump process should be manifest at low light intensities. Large transient responses to bright lights could reflect some delay between the onset of discrete waves, which occur early and synchronously in response to a flash of high energy, and the adaptation.

The experiments we report here were designed to provide a description of the process of light adaptation that occurs during ongoing receptor activity, to examine its relationship to light intensity, and to determine its kinetics. We show that the adapting bump process occurs at very low light intensities and depends on the temporal overlap of discrete waves. It is a separate process from the light adaptation that follows a conditioning flash. These findings raised the important question of whether the adapting bump process depends on changes in cell conductance for it is well known that the cell conductance increases during the time-course of a discrete wave (Millecchia and Mauro, 1969). The increase is due in part to the absorption of light, and in part to depolarization of the cell membrane. Thus, even if the photocurrent produced per discrete wave were constant, one would expect that the temporal overlap of two discrete waves would result in the second one being smaller than the first. Using the voltage-clamp technique, we show that the adapting bump process is inherent in the mechanism that produces photocurrent, and we establish that the kinetics of the process is closely correlated to the kinetics of the photocurrent. This suggests that the inward movement of sodium ions which mediates the photocurrent is also responsible for initiating the process of light adaptation that occurs during on-going receptor activity.

MATERIALS AND METHODS

_**Limulus**_ obtained from Florida were used in these experiments. The ventral nerves were removed, desheathed, and treated with 1/4% Pronase in buffered seawater for 2-4 min. The nerves were pinned on a flat piece of Sylgard, a transparent rubbery material, and placed in the bottom of a shallow glass chamber. Artificial seawater made from Rila Marine Mix and buffered at pH 7.6-7.8 with Tris was used to bathe the nerve. Recordings were made using glass microelectrodes filled with 2 M KCl. Voltage clamping was accomplished using two microelectrodes bonded together. The double electrode assembly was made in the following way. An ordinary glass microelectrode was filled and constituted one member of the electrode pair. Another microelectrode was bent at a point 3-4 mm from its tip using a microforge so as to
produce a bend angle of about $150^\circ$. The bent electrode was then filled and the two electrodes were placed in a small double electrode holder. The electrode holder provided three degrees of movement for each electrode: vertical tilt, horizontal rotation, and linear advancement and withdrawal. A drop of Pyseal was placed on the blunt end of the bent electrode so that it could be rotated around its long axis with a forceps. The double electrode assembly was mounted on a shaft so that it could be rotated under a microscope and viewed in any perspective. The electrodes were brought into alignment under a microscope. When the terminal 3–4 mm of the electrodes were in good alignment it was possible to gently press them together without overriding. This separated the electrode tips by 10–20 µm. The electrode pair was cemented together using a solution of Lucite powder in trichlorethylene. Two glass crossbars were cemented across the electrode pair at 10–15 mm from their tips with Pyseal. The electrodes used had tip resistances between 5 and 10 MΩ measured in sea-water. Current passed through one electrode did not cause a voltage drop in the second electrode. The coupling capacitance between the two members of the electrode pair was low because they were in contact for only 3–4 mm. We used three methods to estimate the coupling capacitance: (a) applying a step of voltage to one of the electrodes and measuring the transient decay time at the second electrode, (b) applying a sinusoidal signal to one of the electrodes and measuring the signal at the second electrode as a function of the frequency of the applied sine wave, and finally, (c) using a double beat oscillator inductance resistance capacitance bridge. All three methods gave similar results, namely, that the coupling capacitance was usually $0.1–0.2 \times 10^{-12} \text{F}$.

The voltage clamp was conventional in design. Clamp current was measured by a current to voltage transconverter which provided a very low impedance shunt in the path that returned current to ground. The response time of the clamp was much less than 3 ms and thus much faster than any component of the photocurrent that we observed. Fig. 1 shows a record which indicates the fidelity of the voltage clamp. The lower record is the photocurrent and the upper record is the measured membrane potential at high gain and wide bandwidth. It shows that clamp control was maintained to within 10 µV (or better). Equally good control was observed for photo-

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Performance of voltage clamp. Upper trace, membrane potential at high gain and wide bandwidth during voltage clamp of discrete waves. Lower trace, photocurrent discrete waves due to low level steady illumination.
current responses of much larger magnitude and faster rise time. Thus the use of bonded pairs of electrodes was compatible with good clamp control and the small coupling capacitance between the two members of the pair was not troublesome. This agrees with work by Faber and Klee (1972) who used similar electrodes to voltage clamp Aplysia neurons whose responses are much larger and faster than those of the Limulus photoreceptor.

The experiments we report here consist of presenting 10-ms light flashes, pairs of identical 10-ms light flashes, or steady light to ventral and lateral eye photoreceptors. In the case of the ventral photoreceptor experiments the light spot covered the entire photoreceptor. Single flashes were used to examine the relationship of the failure rate (the proportion of trials on which no discrete waves occurred) and the average discrete wave size to the energy of the flash. Randomly intermingled flashes of several different energies were presented and the responses collected separately using a computer. Usually several hundred flashes were used and it required about 1/5 h to complete an experiment. Steady light was used to examine the relationship of interdiscrete wave interval to average discrete wave size. Light stimuli were applied for several minutes at various different intensities and the responses were analyzed using a computer. The initial 5 s of each record were discarded since they usually contained a large transient response. Pairs of identical flashes were used to examine the kinetics of the adapting bump process. These experiments consisted of presenting pairs of identical light flashes, each 10 ms long, at intervals of 5-10 s. The interval between the flashes in the pair was adjustable from 15 to 1,600 ms. This interval was selected under control of a computer so that during a single run which lasted from 10 to 45 min, an array of different intervals was presented in random order. The photocurrent responses produced were then sorted by the computer and grouped to form the average photocurrent response separately for each interval. At low light intensities as many as 100 responses were averaged for each interval used. At higher light intensities as few as 20 responses were averaged for each interval used.

RESULTS

Nonlinearities at Very Low Light Intensities

The response of either the lateral eye or ventral photoreceptor to the presentation of a brief flash of light of very low energy is variable from trial to trial and consists of a variable number of discrete waves. In addition, the discrete waves vary in peak amplitude so that it is very difficult to decide if there is any nonlinearity at very low light intensities by simple perusal of records. We circumvented these difficulties in the following experiment on the unclamped ventral photoreceptor. A sequence of 10-ms light flashes of several different intensities intermingled randomly was presented. For each flash intensity we measured the failure rate, that is, the number of trials which resulted in no discrete wave at all, and averaged the responses separately for each flash energy. If discrete waves are single photon responses then the relationship between the failure rate, \( p(0) \) and the energy of the flash, \( E \), should be

\[
p(0) = \exp(-KE),
\]

(1)
where $K$ is the quantum efficiency (discrete waves per photon) and $E$ has the units photons per flash. The product, $KE$, is the average number of discrete waves per trial. We found that our data fit Eq. 1 with good precision in all cases, and this confirms previous work by Yeandle and Spiegler (1973). Since the constant $K$ in Eq. 1 was determined using data from all the different flash intensities simultaneously, we could make a reliable estimate of the average number of discrete waves per flash for each intensity without actually having to count individual discrete waves. This was important because it was often difficult to make such counts reliably. Fig. 2 shows a plot of the average peak depolarization per discrete wave as a function of the average number of discrete waves per flash. The striking feature of this plot is that there is a substantial nonlinearity apparent at very low light intensities. For example, when on the average only two discrete waves per flash result, the average peak depolarization per discrete wave is only three-fourths the value produced by a light flash one-half as intense.

Fig. 2 also shows that when the temperature of the cell is decreased, the magnitude of the nonlinearity is reduced. Cooling the cell disperses the discrete waves in time. The discrete waves of the ventral photoreceptor, like those of the lateral eye photoreceptor, have a variable latency which at 21°C may range from 50 to 300 ms and averages about 120 ms. Thus, it seems that the temporal proximity of discrete waves is a critical factor in determining the nonlinearity. But as Fig. 2 shows, there is often a reduction in the average size of a discrete wave when the cell is cooled, and this could also be a factor.

Another way to approach the problem of nonlinearity at very low light intensity is to use steady illumination at various different intensities. We made
three measurements for each intensity used: the frequency of discrete waves, the variance of the fluctuating level of depolarization around the time-averaged mean, and the autocorrelation function (ACF). To accurately measure the frequency of discrete waves we used a new method of counting them that avoids errors due to their temporal overlap. (We have described this method elsewhere, Srebro and Behbehani, 1972b.) The autocorrelation function is defined as

\[ \text{ACF}(x) = \text{ave}[(v(t) - \overline{v(t)}) \cdot (v(t + x) - \overline{v(t)})], \]  

where \( x \) is called the lag and has the dimension time, \( v(t) \) is the cell voltage at time \( t \), and \( \overline{v(t)} \) is the time averaged cell voltage. The autocorrelation function changes as the adapting bump process becomes manifest and it was used by Dodge et al. (1968) to examine the process. The experiments were done on lateral eye photoreceptors and a small amount of tetrodotoxin was used to suppress nerve impulses.

We found that the discrete wave frequency is a linear function of the light intensity for frequencies as high as we could reliably measure, approximately 300 discrete waves per minute. Fig. 3 shows the relationship of the variance of the fluctuating depolarization to the frequency of discrete waves. The variance increases linearly with frequency up to a frequency of approximately 100 discrete waves per minute and then grows at a much slower rate. Fig. 4 shows that as the variance departs from its linear growth with discrete wave frequency, the autocorrelation function changes indicating that the adapting bump process is operative. Thus, it can be seen that a serious non-linearity arises when the discrete wave frequency is as low as 2–3/s. The results shown in Figs. 3 and 4 are typical of our findings in seven different cells that we studied in detail. The discrete wave frequency at which nonlinearities appeared ranged from 1.2 to 1.6 discrete waves per second and averaged 1.5 discrete waves per second. Since discrete waves have a duration of about 250 ms at 22°C, the temperature at which the experiments were done, there is bound to be an appreciable number that overlap in time at this frequency. Thus, by comparing the nonlinearity due to brief flashes of light with that found for steady illumination it is difficult to avoid the conclusion that temporal overlap of discrete waves is a critical factor in adapting bump process.

**Nonlinearity in Voltage-Clamped Ventral Photoreceptors**

Another way to examine the effects of light adaptation during ongoing receptor activity is to use pairs of identical flashes with an adjustable interval between the two flashes of the pair. We carried out these experiments on voltage-clamped ventral photoreceptors. If the energy of a flash is set at a level that is near discrete wave threshold, that is about one-half the presentations
Figure 3. The relationship of the variance of the depolarization caused by a steady applied light to the frequency of discrete waves. All the values shown (open circles) were determined for a single lateral eye photoreceptor. A small amount of tetrodotoxin was used to suppress nerve impulses. The variance was calculated around the time-averaged mean for a record of 2-min duration. The variance is given in arbitrary units proportional to its absolute value. The frequency of discrete waves was obtained using a method described previously. (See reference in text.) The straight line was drawn by eye through the origin and first four measured points.

Figure 4. Autocorrelation function (ACF) for the fluctuating depolarization due to a steady light. Lateral eye photoreceptor. A small amount of tetrodotoxin was used to suppress nerve impulses. The results shown are for the same cell as that shown in Fig. 2. The open circles correspond to a discrete wave frequency of 80/min. The closed circles correspond to a discrete wave frequency of 276/min. The definition of the ACF and the lag are given in the text (Eq. 2).

of the flash produced no response at all, then on the average, the response to two of these flashes separated by any interval is simply the linear summation of the average response to a single flash. This is hardly surprising, for at the discrete wave threshold, either the first or the second of the pair of flashes (but generally not both) produces a discrete wave. In order to examine discrete wave interaction it is necessary to increase the flash intensity to ensure that on most presentations of the pair of flashes each flash produces one or more discrete waves. We selected the appropriate flash intensity by adjustment to produce a failure rate of 0.2 which corresponds to approximately 1.6 discrete waves per flash or about 3 discrete waves per presentation of a pair of flashes, on the average, over a large number of flashes. Fig. 5 shows 12 randomly
selected trials from an experimental run. Three different interflash intervals were used and these were randomly intermingled with trials in which only a single flash was presented. The cell was voltage-clamped at a holding potential of \(-60\) mV which was its resting membrane potential in the dark. There is considerable variability in the latencies and amplitudes of the discrete waves that result. The discrete waves shown in Fig. 5 have a small late outward cur-
rent component. This corresponds to the late hyperpolarization seen in unclamped discrete waves and distinguishes the discrete waves of the ventral photoreceptor from those of the lateral eye photoreceptor which do not have a late hyperpolarization.

Fig. 6 shows tracings of the average responses to pairs of flashes at six different interflash intervals from 15 to 300 ms. (This is a different cell from that shown in Fig. 5.) The holding potential was $-40$ mV and no late outward current phase was visible. If the cell behaved as a linear device, then each of these responses would be the linear summation of the average response to a single flash. The expected response, that is the average response expected on the assumption of linear summation, was constructed by adding the average response to a single flash to itself after an appropriate shift on the time

![Figure 6. Photocurrent time-courses and calculated deficits for double flashes at different interflash intervals shown in milliseconds by the number to the right of each set of tracings. Heavy lines: observed photocurrents. Thin lines: photocurrent due to a single flash. In each case two curves are shown and the second curve is displaced to the right by an amount equal to the interflash interval. Filled circles: expected photocurrent equal to the sum of the two single flash curves in each set. Open circles: deficit time-course the difference between the observed and expected photocurrents. All values are averages over many presentations of the pairs of flashes. All the sets of curves and points are for a single voltage-clamped ventral photoreceptor. Holding potential $-40$ mV. Temperature $21^\circ$C. Tic marks beneath each set are 100 ms apart. The stimulus presentation began at a time corresponding to the first tic mark. Figure made from tracing of an X-Y plotter record.]
axis. The expected response is shown as filled circles in Fig. 6. The darker trace in each part of Fig. 6 is the average response actually observed. The observed average response is less than the expected one, and is systematically related to the interflash interval. The larger the interflash interval the more closely the observed response approaches the expected one. Also shown in Fig. 6 as open circles is the deficit, that is, expected minus observed average response. The most important feature of these responses is that for the early rising phase of the responses there are no appreciable deficits. This is especially important in the average responses corresponding to 15- and 25-ms interflash intervals, for it is clear that the two single flash responses overlap during the period of linear summation. The result is presented in a slightly different way in Fig. 7. In the top traces of this figure the deficit curves of the six different

![Figure 7](image_url)

**Figure 7.** Relationship of deficit time-course to the photocurrent time-course. In each of the two sets of records the curve with the largest peak is the photocurrent curve. The other curves are deficit curves found by using various different interflash intervals. (The method is described in the text.) The upper set of curves are a replot of the values shown in Fig. 5. The lower set of curves was determined on the same cell using a flash energy 10 times greater than that used to determine the upper set of curves. Tic marks occur at 100-ms intervals. Voltage-clamped ventral photoreceptor. Holding potential -40 mV. Temperature 21°C. The stimulus presentation began at the first tic mark.
delays are shown in a composite drawing and compared to the time-course of the average response to a single flash. The deficits form an envelope which outlines a reasonably well ordered deficit time-course. The deficit begins to appear about 50 ms after onset of the photocurrent and reaches a maximum slightly after the peak of the photocurrent response.

Another way of representing the adaptation effect is shown in Fig. 8. Here the ordinate, on a log scale, is the fractional deficit, that is the deficit at a time corresponding to the expected peak of the second flash of the pair of flashes divided by the magnitude of that peak. The figure shows results for three different voltage-clamped cells. While there is appreciable scatter, an exponential relationship is suggested by the approximately linear falloff of the fractional deficit on this semilog plot. The line was drawn "by eye" and corresponds to a time constant of about 75 ms. Similar results were obtained using unclamped photoreceptors.

All the results so far shown are for experiments in which the average number of discrete waves per flash was approximately 1.6. The lower traces of Fig. 7 show a composite of deficit curves for the same cell as shown in the upper part but at a flash intensity 10 times greater. The time-course of the photocurrent response is also shown. It has a slightly shorter latency, a more rapid rise, and peaks at a slightly shorter time than the corresponding trace for the lower intensity flash. The envelope of the deficit shows a slightly shorter onset, a more rapid rise, and an earlier peak than its counterpart at

![Figure 8. Recovery from light adaptation. Ordinate: fractional deficit at peak of expected second flash response. (See text for method of calculation.) Abscissa: delay between the two members of the pair of flashes (interflash interval) in milliseconds. (Semilog plot.) Results are for three different voltage-clamped ventral photoreceptors. Triangles: holding potential -40 mV. Filled circles, holding potential -60 mV. Open circles, holding potential -40 mV. The straight line was drawn by eye. The energy of each flash in the pair was adjusted to produce 1.6 discrete waves per presentation, on the average.](image-url)
lower intensity. There is a close relationship between the time-course of the deficit and the time-course of the photocurrent response at the two light intensities. This correlation was found in all of the cells we studied.

**Adaptation at Light Intensities Much above Discrete Wave Threshold**

The effect of increasing the light intensity of the pair of flashes is shown in Fig. 9 for an unclamped ventral photoreceptor. (Voltage-clamped cells behave in a similar way.) The fractional deficit at the expected peak of the second flash response is plotted against the interflash interval on a cartesian coordinate system. Three different intensities are shown. The lowest intensity corresponds to approximately 16 discrete waves per flash, and the highest to approximately 1,600 discrete waves per flash. The early decay of the fractional deficit for each of the three intensities follows the same exponential pattern. The time constant of the decay is not substantially different from that observed using less intense light flashes. However, there is a late phase of adaptation which is not seen with low intensity flashes. The late phase grows during the first 1/2 s or so and decays very slowly over a period of seconds. The magnitude of the late phase is graded with intensity. Fig. 10 shows the average response curves for selected interflash intervals from the run using

![Fractional Deficit vs Delay](image)

**Figure 9.** Recovery from light adaptation. Ordinate: fractional deficit at peak of expected second flash response. (See text for method of calculation.) Abscissa: delay between the two members of the pair of flashes (interflash interval). Results are for one ventral photoreceptor, not voltage-clamped. Dark resting membrane potential – 42 mV. Temperature 22°C. Filled circles: energy of each flash sufficient to produce 16 discrete waves per presentation on the average. Triangles: energy of each flash sufficient to produce 160 discrete waves per presentation, on the average. Open circles: energy of each flash sufficient to produce 1,600 discrete waves per presentation, on the average.
FIGURE 10. Late adaptation. Each curve is the average depolarization produced by single or double flashes. Curves are for the same cell as shown in Fig. 8 and for the flash energy sufficient to produce 1,600 discrete waves per presentation, on the average. Top trace, single flash. Middle trace, double flash, interflash interval 160 ms. Bottom trace, double flash, interflash interval 640 ms. Tic marks, 200 ms. Stimulus presentation begins at first tic mark in each case. X-Y plotter record.

the highest light intensity shown in Fig. 9. It can be seen that the average response corresponding to an interflash interval of 640 ms is smaller than the average response corresponding to an interflash interval of 160 ms. For the 160-ms interflash interval the peak of the response to the second flash occurs at a time when there is little residual effect of the first flash of the pair.

DISCUSSION

In the photoreceptors of Limulus light adaptation is a complex process that consists of at least two phases, a rapid phase and a slow phase. The rapid
phase of adaptation has characteristics that strongly suggest its identity with
the adapting bump process. This is most clearly revealed by the response of
the photoreceptor to low intensity steady illumination. Both the failure of the
response variance to increase linearly with light intensity and the accompanying
change in the autocorrelation function are very similar to the result of
Dodge et al. (1968). The most striking feature of our results is that these
changes, characteristic of the adapting bump process, occur at discrete wave
frequencies of only 2 per second. The data that Dodge et al. (1968) show be-
gins at an estimated frequency of over 100 per second. It is well known that
the intervals between discrete waves are exponentially distributed (Fuortes
and Yeandle, 1964; Yeandle and Spiegler, 1973). The duration of a discrete
wave is about 250 ms at 21°C. It is therefore a simple matter to calculate
that significant nonlinearities appear when the chances of temporal overlap
of discrete waves is approximately 0.4, and thus, not at all uncommon.

The experiments using brief flashes of low energy support the idea that dis-
crete wave temporal overlap is an important factor in establishing the adapt-
ing bump process. Again, response nonlinearities appear at or near discrete
wave threshold. Brief flashes produce discrete waves which tend to crowd
together in time and this results in a more dramatic adapting bump effect.
It is possible to calculate from the latency distribution that when two discrete
waves result from a single flash they are separated in time by about 50 ms
on the average at 21°C. If the number of discrete waves that follow a brief
flash were to follow the Poisson expectation about 15% of the trials would
result in 2 or more discrete waves even at discrete wave threshold (about 0.69
discrete waves per flash on the average) and about 41% of the trials would
result in 2 or more discrete waves at twice the threshold flash energy. It is
easy to see why serious nonlinearities could appear even at such low flash
energies as shown in Fig. 2.

We actually did check the validity of the Poisson expectation in the ventral
photoreceptor. In preliminary experiments we found that there was an appar-
tent discrepancy, such that there were too many single discrete waves and too
few multiple discrete waves. However, a careful examination of our records
revealed that a significant fraction of allegedly single discrete waves had sus-
picious irregularities of their time-courses, especially notches or brief plateaus
on the trailing edges of the waveform. We think that these represent the
remnants of discrete waves which are substantially reduced in size and thus
difficult to detect. When the temperature of the cell was decreased, the dis-
crete waves became dispersed in time, the numbers of discrete waves actually
found approached the Poisson expectation, and the irregularities in the dis-
crete wave time-course disappeared. This gave us more confidence that our
interpretation is correct.

Since the adapting bump process occurs at very low light intensities and is
mediated by temporal overlap of discrete waves, we think that it is unlikely that the adapting bump process represents the depletion of some cellular material which controls the size of discrete waves. Fig. 2 suggests that flashes which produce only 5 discrete waves would use up about half the alleged material. But while we have observed discrete waves in voltage-clamped ventral photoreceptors that involve as much as 5 nA of photocurrent, a bright flash of light can produce a transient photocurrent of 200 nA. This is very difficult to reconcile with the idea that a cellular material is depleted in the adapting bump process.

Our experiments using double flashes of light just above discrete wave threshold in voltage-clamped ventral photoreceptors not only provide estimates of the kinetics of the adapting bump process, but establish that this process is inherent in the photocurrent generating mechanism. The experiments show that there is a close correlation between the time-course of the photocurrent and the time-course of the adapting bump process. But the two processes are not synchronous. The adapting bump process always lags the photocurrent. We will discuss the possible origin of the lag later. However, regardless of the mechanism of the lag, its existence may explain why large transient photocurrent responses occur with bright flashes. A flash of high energy results in the production of many discrete waves, and the latencies of the ones that occur early are synchronous. The early synchronous discrete waves start before the adapting bump process can act and this probably causes the large transient response. For clarity, we point out that Fig. 8 which shows a significant fractional deficit at near zero delay is not in conflict with the above interpretation since the fractional deficit was measured at the peak of the expected second response, and thus approximately 215 ms or more after the first flash was presented. Extrapolation of the line in Fig. 8 back to zero delay is not justified by our data.

While the lag between the photocurrent time-course and the adapting bump process suggests an explanation for the large early transient photocurrent due to bright flashes, it does not imply linear summation at the peak of the photocurrent response. As Fig. 7 (top) shows, even at a light flash energy just above discrete wave threshold, there is a photocurrent deficit of about 25% at a time corresponding to the peak of the photocurrent response. Thus we are surprised by Lisman and Brown's (1970) brief report that the average peak photocurrent in the voltage-clamped ventral photoreceptor of *Limulus* is linear from near discrete wave threshold to about 100 times this light intensity. Nevertheless, we are in essential agreement with their statement that linear summation occurs during the early rising phase of the photocurrent response over a wide range of light intensities. It appears that the major disagreement concerns only the length of time after the onset of the response during which linear summation holds. We think that linear summation is
restricted to a period of about 50 ms from response onset. Lisman and Brown (1970) conclude that this period extends at least to 100 ms from response onset.

Our results also differ from results reported by Lisman and Brown in 1972 b. In this study, the authors used a 10-ms test flash and compared the photocurrent response to this test flash when it was presented before, during, and after the response to a 2-s adapting flash. The authors claim that the increment of photocurrent produced by the test flash when it was applied during the transient phase of the response to the adapting flash was the same as that produced by the test flash presented in the dark. However, a careful examination of their published records shows that the test response actually increased by more than 30% at peak when presented during the transient phase of the adapting response. The authors used an average of eight response patterns and the test flash produced only 2.5 nA of photocurrent. It seems likely that their test response contained only a few discrete waves and was highly variable so that the measurement was not very accurate. The actual delay between the presentation of the adapting flash and the test flash was not stated so it is difficult to estimate the magnitude of the fractional deficit we would expect. We would guess from the published record that the delay was about 75 ms and the percentage reduction we would expect would be about 35%.

Our finding that there is significant response nonlinearity at low light intensity in the ventral photoreceptor stands in contrast to the behavior of several other photoreceptors. Hagins (1965) described response linearity in the squid photoreceptor up to about 500 photons incident per second per cell. Penn and Hagins (1972) reported response linearity up to approximately 25 photon absorptions per receptor in the rat rod. Baylor and Hodgkin (1973) reported that in the turtle cone the responses evoked by very weak light flashes obey the rule of linear superposition. On the other hand, Fuortes and Hodgkin (1964) showed that response linearity in the ommatidial eye of Limulus, which contains 8–12 photoreceptors, held only over a very restricted range of less than 1 log unit from threshold. We do not see a compelling reason to assume that all photoreceptors should behave as linear devices at low intensity.

The correlation between the photocurrent time-course and the time-course of the adapting bump process suggests that the photocurrent itself may initiate the process. To discuss this in more detail it is necessary to consider the question of the origin of the lag between the photocurrent and the rapid phase of adaptation. If a discrete wave represents a sink of photocurrent localized to a small active membrane patch, one might expect that the rapid phase of adaptation would remain localized to that patch. However, the data shown in Figs. 2 and 3 imply that there must be some spatial spread. If this were not true, it would be virtually impossible to explain the rapid adaptation
seen at very low light intensities, for in this case the active membrane patches would be separated by considerable distances, probably tens of microns, in the cell membrane. It is therefore necessary to consider the possibility that the lag in the rapid phase of light adaptation represents time required to spread the effect over the length of the cell. However, as Fig. 7 shows there is still a considerable lag when the flash intensity is large enough to produce 16 discrete waves per flash, on the average, or 32 discrete waves per presentation of a pair of flashes. Under these conditions it is not likely that more than a few microns separate the presumed active patches of membrane. Thus, the lag probably does not reflect time required for spatial spread but rather the kinetics of some intermediary process that links the photocurrent to rapid adaptation. (These considerations imply that the rapid phase of adaptation is not spatially localized.)

The photocurrent represents an inward movement of sodium ions into the cell (Millecchia and Mauro, 1969). However, it is unlikely that a sufficient number of sodium ions enter the cell to reduce the sodium equilibrium potential especially at very low light intensity. Lisman and Brown (1972a) have shown that the intracellular concentration of ionized calcium has an important controlling effect on the magnitude of the photocurrent. There are several ways in which the photocurrent could lead to an increase in intracellular free calcium. The entry of sodium ions could initiate an exchange of sodium ions for calcium ions either across the cell membrane or with mitochondria. A membrane calcium conductance channel could be activated either by the entry of sodium ions or in a fixed relationship to the light-induced increase in sodium conductance. The lag between the photocurrent and the adapting bump process could therefore represent the kinetics of a process whereby the transient increase in intracellular sodium ion concentration leads to an increase in intracellular free calcium.

The decay of the rapid phase of adaptation is exponential with a time constant of approximately 75 ms at 21°C. Moreover, the rapid phase of adaptation decays with the same time constant at substantially higher light intensities. This provides further evidence that the adapting bump process observed at high light intensities is essentially the same as the process we have examined in some detail at low light intensities. It is interesting that the decay of the adapting bump process is similar in time-course to the regeneration of visual pigment in the ventral photoreceptor (Fein and De Voe, 1973; Hillman et al., 1973). However, as Fig. 7 shows, the rapid phase of adaptation is not likely to reflect the presence of a photochemical intermediate. The peak of the rapid adaptation process at light intensities just above discrete wave threshold is longer than 200 ms from the onset of the flash. By this time about seven of eight bleached visual pigment molecules would have already regenerated. In the barnacle photoreceptor where a photochemical intermediate does
appear to play an important role in controlling the adaptational state of the cell (Hochstein et al., 1973) that intermediate is long-lived and results in a prolonged depolarization of the photoreceptor. While some cells of the median eye of *Limulus* may behave this way (Nolte et al., 1968), the ventral photoreceptor does not. Thus we think it most unlikely that a photochemical intermediate is involved in regulating the adapting bump process.

The late phase of adaptation requires flash intensities that correspond to 100 or more discrete waves per flash and it is graded with light intensity. It builds up slowly over a period of several hundred milliseconds and decays even more slowly over a period of seconds. Its mechanism is obscure and may relate to active membrane and cellular processes. Lisman and Brown (1972) reported that in the ventral photoreceptor a 2-s adapting stimulus reduced the photocurrent response to a standard brief test flash. There was no recovery for the first 400 ms after the extinguishing of the adapting light. This result can be explained by the existence of the slow phase of adaptation.

The existence of at least two phases of adaptation with widely different time-courses suggests that it is dangerous to equate adaptation produced by background illumination with that caused by a conditioning light stimulus. A background light would in general bring into play both the rapid and late phases of adaptation, while in most experiments using a conditioning stimulus only the late phase of adaptation would operate. We have shown that in the lateral eye photoreceptor the late phase of adaptation produces no change at all in the time-course of the response to a flash of fixed intensity (Srebro and Behbehani, 1972a). We have found this to be true for the ventral photoreceptor as well. Fein and De Voe (1973) claim that equal energy flashes which produce approximately the same peak amplitude response with background illumination, and during the recovery from a previous bright flash, result in responses with different time-courses. During a background light the response has a shorter latency and peak time. This experiment needs to be repeated using voltage-clamp techniques for it suggests that the rapid phase of adaptation may shorten the latency of the discrete waves that compose the response.

Our results indicate that the rapid phase of adaptation has little or no spatial localization within the cell. On the other hand, some degree of local adaptation has been reported for the ventral photoreceptor (Yeandle and Spiegel, 1972), the squid outer segment (Hagins et al., 1962), and the photoreceptor of *Eledone* (Hamdorf, 1970). In all of these experiments a conditioning flash was used to elicit light adaptation, and this suggests that the late phase of adaptation may be spatially localized.
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