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EFFECTS OF ADAPTING LIGHTS ON THE TIME COURSE OF THE RECEPTOR POTENTIAL OF THE ANURAN RETINAL ROD

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SUMMARY

1. The intracellular receptor potential of the retinal rod cell was recorded in the unperfused, isolated retina of *Rana catesbiana* and in the perfused, isolated retina of *Bufo marinus*. Qualitatively, the responses from the two preparations were similar.

2. The rate at which the receptor potential returned to the dark level at the termination of a pulse of light ($\dot{V}_{off}$) was measured at a fixed potential chosen to be about 0·6 of the way from the dark level to the peak of the response.

3. When the light intensity was such that less than about 10^{-5} of the photopigment was bleached per second, $\dot{V}_{off}$ increased as the duration of the pulse was increased, reaching a maximum in 50–100 s.

4. When a brief test flash was presented at various intervals after an adapting pulse lasting about 50 s, $\dot{V}_{off}$ for the test flash was greater than the value in the dark adapted state for times up to about 80 s after the adapting pulse.

5. It has been hypothesized that in the vertebrate rod light causes release from the disk sacs of particles which block conducting channels in the surface membrane (Yoshikami & Hagins, 1971, 1973). A modification is proposed in which the rate at which the blocking particles are converted to an inactive state can be increased by light adaptation.

6. This modified hypothesis will account qualitatively for the further observations that (*a*) during the response to illumination lasting several seconds the membrane potential recovers part of the way to the dark level and (*b*) if a second light pulse is superimposed on this background illumination then after the superimposed pulse the depolarization is increased.

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INTRODUCTION

Intracellular recordings from anuran photoreceptors have been described by Toyoda, Hashimoto, Anno & Tomita (1970) and by Brown & Pinto (1974). The response to light is a hyperpolarization which, when the stimulus is sufficiently bright, has the rapid undershoot (or spike) and plateau common to many other photoreceptor potentials. Brown & Pinto (1974) produced responses with no rapid undershoot by increasing the K^+ concentration of the bathing solution and, in *Gekko*, Kleinschmidt (1973) obtained the same result by applying a high (50 mM) concentration of aspartate. In most of the experiments reported here the aim has been to study responses not made more complicated by the presence of the rapid undershoot. This was done either by using rather low light intensities or by looking only at the later parts of the response.

A widely propagated hypothesis to account for the greater part of the response of the vertebrate rod is that the photoisomerization of a photopigment molecule initiates a chain of events which leads to release of diffusible particles from the disks. A current, carried by Na^+ ions, flows in the dark inward through the surface membrane of the outer segment (Hagins, Penn & Yoshikami, 1970). When the diffusible particles reach the surface membrane they block sodium channels and cause the intracellular potential to become more negative. Some experiments have suggested that the diffusible particles may be calcium ions (Yoshikami & Hagins, 1971, 1973; Korenbrot & Cone, 1972) but in the absence of direct evidence it appears prudent to use a non-committal name. During the course of the present work we learned of three papers describing an extensive study of the kinetics of the responses of turtle cones by Baylor & Hodgkin (1974) and Baylor, Hodgkin & Lamb (1974*a*, *b*) (which the authors kindly allowed us to see before publication). In these papers the name 'blocking particles' is used for particles which, the authors suggest, play a parallel role in cones and as it is applicable to both cones and rods we use this name.

We report that the rate at which the membrane potential returns to the dark level after illumination can be increased by light adaptation, and have studied this dependence in a qualitative way. The results fit readily into the framework of the hypothesis involving blocking particles and this interpretation has influenced the design of the experiments.

METHODS

The methods were unoriginal except in details. At Keio, a piece of the isolated retina of *Rana catesbiana* was placed receptor side up in a moist chamber mounted on a jolter as described by Tomita, Kaneko, Murakami & Pautler (1967). Microelectrodes were made as described by Kaneko (1973) and were filled with either

4M-KAc or a nearly saturated mixture of eight of the more soluble histological dyes (Colour Indices of the Society of Dyers and Colourists (1956): 23850, 24410, 42053, 42085, 45350, 45380, 45400, 45440). Normally no artificial bathing solutions were used: to prevent contamination of the intracellular recording by extracellular potentials the indifferent electrode was connected to the upper surface of the retina by a strip of lens tissue soaked in vitreous humour. The retina was stimulated at a wavelength of 500 nm irrespective of whether the recording was from the rhodopsin or the porphyropsin field of the retina (Reuter, White & Wald, 1971). In thirty cases it was confirmed that the recording was from a photoreceptor by applying a mist of 1 M-Na aspartate (pH 7·1) using a simple version of the apparatus described by Murakami, Ohtsu & Ohtsuka (1972). This procedure abolishes responses in second order cells but not in photoreceptors.

At Vanderbilt experiments were made on the isolated, perfused retina of *Bufo marinus* using a system based on that of Brown & Pinto (1974). The perfusion chamber was mounted on the stage of a microscope and the piece of retina was inspected with a ×32, 0·3 N.A. objective using transmitted infra-red light and an image converter. The micro-electrode was advanced towards an area in which the tips of the rod outer segments appeared bright and tidily arrayed: presumably the axes of these outer segments were aligned within about 30° of the vertical. The composition of the perfusate was: NaCl, 108 mM; Na_2SO_4, 0·6 mM; $NaHCO_3$, 0·3 mM; KCl, 2·5 mM; $MgSO_4$, 1·2 mM; $CaCl_2$, 1·6 mM; HEPES (N-2 hydroxyethylpiperazine N^1-2 ethanesulphonic acid), 3 mM; glucose, 5·6 mM. The pH was adjusted to 7·8 with NaOH. The perfusate was saturated with O_2 and flowed about 1 mm deep over the retina with a mean velocity of 1–2 cm s^{-1}. The temperature was 20·4–21·2° C.

Except where otherwise stated the data in the Figures are from *Bufo marinus* stimulated with a light spot 1·8 mm in diameter and of wave-length 480 nm and considered to be dark adapted. By this is meant that the animal was kept in the dark for at least two hours before dissection, dissected under deep red light and the rod showed little tendency to increase in sensitivity during recording.

Estimation of the fraction of photopigment photoisomerized. When the isolated retina is illuminated from the vitreous side and observed from the photoreceptor side it appears that nearly all the light emerges from the rod outer segments rather than the spaces between them (see also Snyder & Hamer, 1972). The light capture area of one rod is therefore taken to include its share of the space surrounding it and is 80 μm^2 or less. The axial optical density of a rod outer segment of *Rana pipiens* is not more than about 0·75 (Liebman & Entine, 1968). The quantum efficiency for bleaching is about 0·67 (Dartnall, 1968). Hence an incident flux at 500 nm of 1 photon μm^{-2} s^{-1} probably causes not more than about 44 photoisomerizations per sec in a rod with λ_{max} at 500 nm. This value is in agreement with the quantization of the rod response at very low intensities tentatively identified with single photoisomerizations by Toyoda & Coles (1973). The reduced effectiveness of light of 480 nm compared to light of 500 nm is negligible at the order of approximation used here.

The concentration of chromophores in the outer segment is about 2·4 mM (see Coles, 1972; Falk & Fatt, 1973). For a rod 50 μm long and 8 μm in diameter this gives $3·6 \times 10^9$ chromophores. Hence a bombardment by 10^{11} photons mm^{-2}, which is more than the greatest delivered in any one stimulus, should bleach only about $1·2 \times 10^{-3}$ of the pigment.

RESULTS

Identity of the cells producing recordings

Recordings were from cells penetrated within about 50 μm of the receptor surface of the tissue: in many cases penetration occurred as soon as the electrode touched the tissue and often when the electrode spontaneously came out of the cell it simultaneously lost contact with the tissue. Since the outer-segment layer is about 50 μm thick, and since rods contribute nearly all its volume (some 98 % in *R. pipiens* according to Liebman & Entine, 1968), it is concluded that most if not all of the recordings were from rod outer segments. This agrees with the conclusion of Toyoda *et al.* (1970) and Toyoda & Coles (1973).

Changes in the rate of recovery to the dark potential

Fig. 1*a* shows responses of a *Bufo* rod to long and short pulses of the same illuminance. The retina was initially in a steady state of dark adaptation and each pulse was preceded by at least 80 s of darkness. Increasing the intervals beyond this length appeared to have very little effect on the responses. In Fig. 1*b* the return of the potential to the dark level at the termination of the pulse is shown on an expanded time scale: tracings from the five responses in Fig. 1*a* are superimposed. It is clear that the time course of the recovery of the potential to the dark level is affected by the duration of the pulse. We have chosen to study one aspect of this change in time course, namely the rate of change of potential ($\dot{V}_{off}$) measured during the recovery at a fixed value of the membrane potential. The procedure is illustrated in Fig. 1*c* where tangents have been drawn to tracings from the third and second responses at a potential 0·6 of the way from the dark level to the peak of the long response. In this particular experiment the rate ($\dot{V}_{off}$) for the long pulse was 2·5 times that for the short one. The rationale behind this approach is that in the model described in the Introduction the membrane potential is supposed to depend on the fraction of channels in the surface membrane of the outer segment which are blocked by the blocking particles. Consequently the rate of change of potential at a given potential should be proportional to the rate at which the channels were becoming unblocked: Fig. 1*c* suggests that this net rate can alter, depending on the history of stimulation. The value of 0·6 times the peak excursion proved convenient for measurement. A large value was usually precluded by the fact that during a long pulse the membrane potential recovered part of the way to the dark level, while at smaller values (closer to the dark level) there appeared to be a markedly slower component of the recovery. Such an increase of $\dot{V}_{off}$ caused by weak light adaptation was observed to occur

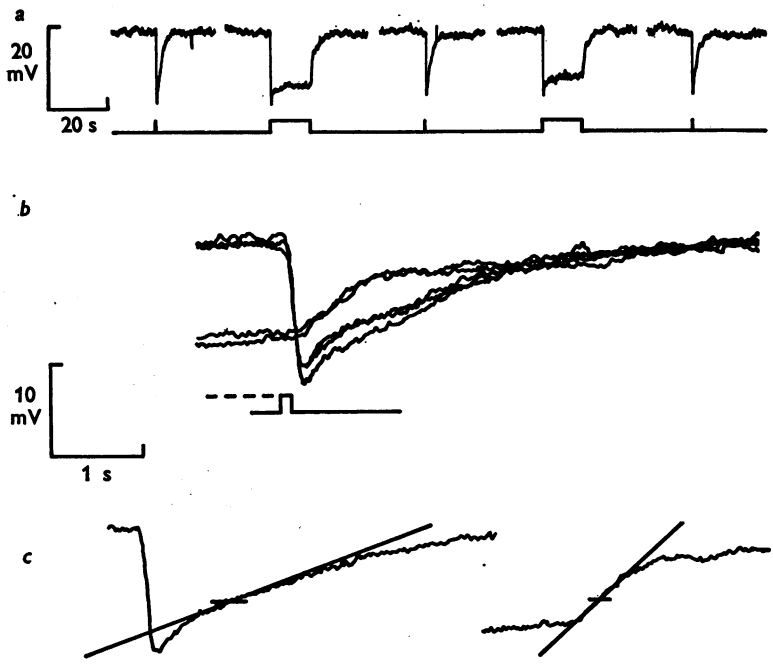

Fig. 1. Intracellular voltage recordings from a *Bufo* rod.

a, Illumination for the times shown by the lower line caused hyperpolarizing responses. The stimuli had an illuminance of 10^9 photons mm^{-2} s^{-1} and durations 0·15 and 26·5 s. The noise in this and subsequent records is dominated by that arising in the electrode. It is estimated that each long stimulus bleached less than 10^{-3} of the photopigment.

b, Tracings from the records in *a* on an expanded time scale are superimposed to show the differing time courses of the return to the dark level after long and sort pulses.

c, Tracings from the third and second records in *a* on the same scale as in *b*, showing how $\dot{V}_{off}$ was measured. The point on the recovery phase of the short response 0·6 of the way from the dark level to the peak excursion of the long response is marked and a tangent has been drawn giving a value for the slope of 3·7 mV s^{-1}. The point at the same potential on the recovery phase of the long response is also marked. The tangent there has a slope of 9·5 mV s^{-1}.

In most experiments, as here, the duration of the shortest stimuli was sufficiently long for all responses to have approximately the same peak amplitudes, but in some cases, where a short stimulus followed closely after a long one, the amplitude was depressed somewhat and the potential at which $\dot{V}_{off}$ was measured was calculated from the response to a long stimulus.

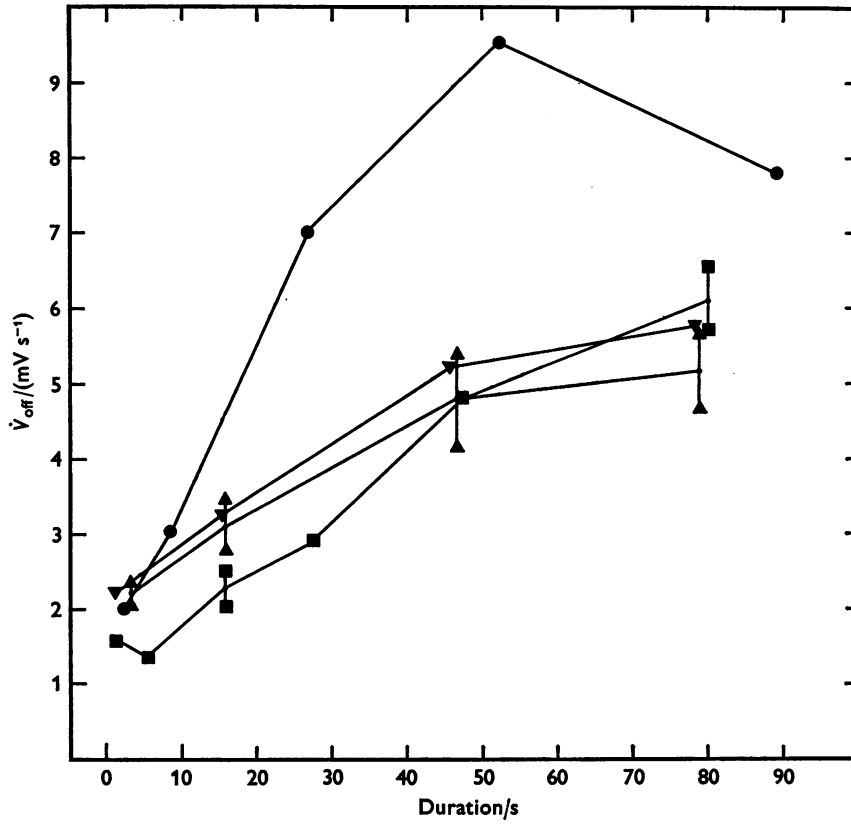

Fig. 2. Development of enhancement of the recovery process. Pulses of light of various durations were presented and the rate of change of potential during recovery ($\dot{V}_{off}$) was measured at a fixed potential for each cell as described in connexion with Fig. 1*c*. The abscissa shows the duration of the pulse and the ordinate shows $\dot{V}_{off}$. The pulses were separated by intervals such that the end of one pulse came at least 100 s after the end of the preceding pulse.

The values of $\log_{10}$ (illuminance/(photons mm^{-2} s^{-1})) were: ▼, 6·1; ■, 6·3; ▲, 6·3; ●, 9·1.

with complete or partial reversibility in all the more than 80 rods for which appropriate tests were made.

Dependence of the rate of recovery on the duration of the illumination

Fig. 2 shows $\dot{V}_{off}$ plotted as a function of pulse duration for the four cells for which the data extend to the longest times. In all experiments shorter and longer pulses were presented alternately. Results from these and thirteen other cells (not shown) indicate that as the pulse duration was

increased from about 1 s to about 30 s $\dot{V}_{off}$ increased, apparently smoothly and monotonically. At longer times $\dot{V}_{off}$ reached a maximum and in a few cases, particularly when the illumination was comparatively intense, it then declined somewhat (e.g. ● in Fig. 2). This decline appeared to be more marked the longer recording continued from one cell. Its cause will be called fatigue of the recovery process.

Of the six cells for which $\dot{V}_{off}$ was measured for pulses lasting over 75 s, in two $\dot{V}_{off}$ declined for the longest times and in the remaining four the time taken for the enhancement of $\dot{V}_{off}$ to come within $\frac{1}{2}$ of its maximum ranged from 30 to 45 s. Over the thousandfold range of intensities used, there appeared to be no marked change in the time constant of the early part of the curve of $\dot{V}_{off}$ *vs* pulse duration although the peak values of $\dot{V}_{off}$ tended to be higher at the higher light intensities.

The dependence of $\dot{V}_{off}$ on the number of photons absorbed

Fig. 3*a* shows responses to 10 ms flashes of increasing intensities. As has been described by many authors for rods of anurans and rods and cones of other vertebrates (e.g. Brown & Pinto, 1974) as the intensity is increased the response develops a rapid transient undershoot and the onset of recovery to the dark level is delayed. Fig. 3*b* shows the effect of bombarding a rod with similar numbers of photons spread out over tens of seconds. In this case the recovery was not significantly delayed except at the highest intensity. Moreover, there was a range of intensities, as shown by the third and fourth records, over which the rate of recovery, $\dot{V}_{off}$, was enhanced. This is illustrated by the graph of filled circles in Fig. 4.

It is apparent that the enhancement of $\dot{V}_{off}$ in Fig. 1 was not due only to the fact that during a long pulse the rod absorbed more photons: time is also required. The interrelationship of intensity and time in producing such enhancement has not been studied systematically. We simply observe that $\dot{V}_{off}$ is most markedly enhanced after long, comparatively weak pulses rather than brief, intense ones.

To make further experiments on enhancement of $\dot{V}_{off}$ a duration of about 50 s was chosen for the adapting pulse in the expectation that the enhancement would be near to its maximum but that the recovery process would not be unduly fatigued.

The persistence of enhancement of the recovery process in the dark. The record in Fig. 5*a* is of responses to the following sequence of lights: a flash, a control flash of a tenfold higher intensity, a 47 s adapting pulse which bleached an insignificant fraction of the photopigment (less than 4×10^{-4}), a test flash of the same intensity as the control, and, after 90 s of darkness, a second control flash. It is seen that the rate of recovery ($\dot{V}_{off}$) after the test flash was markedly higher than that after the control flashes,

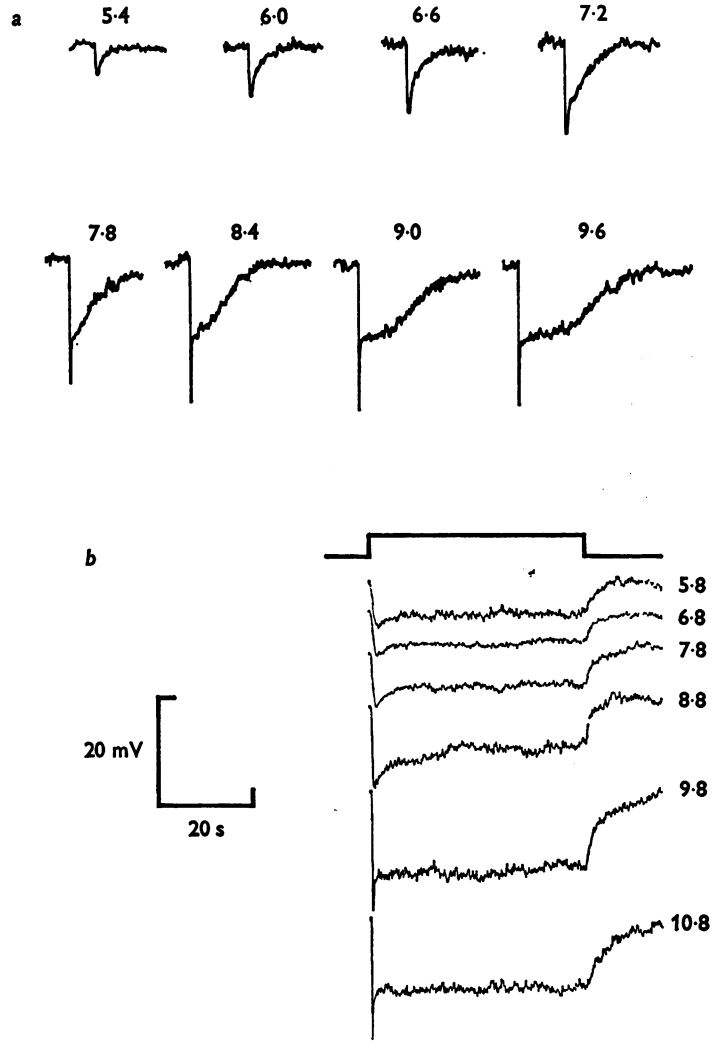

Fig. 3. The form of the response as a function of light intensity. *a* and *b* show responses from two different cells on the same scale. The stimulus in *a* was a flash lasting 10 ms and in *b* a pulse lasting 47 s. The number by each trace is a measure of the *amount* of light delivered by the stimulus and is $\log_{10}$ (number of photons incident on a square mm).

The stimuli were presented to the retina dark adapted for at least 80 s after all but the weakest preceding stimuli. Different intensities were used in a quasi-random sequence. The responses in *b* were obtained over a period of one hour and there was some change in amplitude during this time (mainly an increase) so accurate comparisons cannot be made even though most of the traces are computer averages of two or more responses.

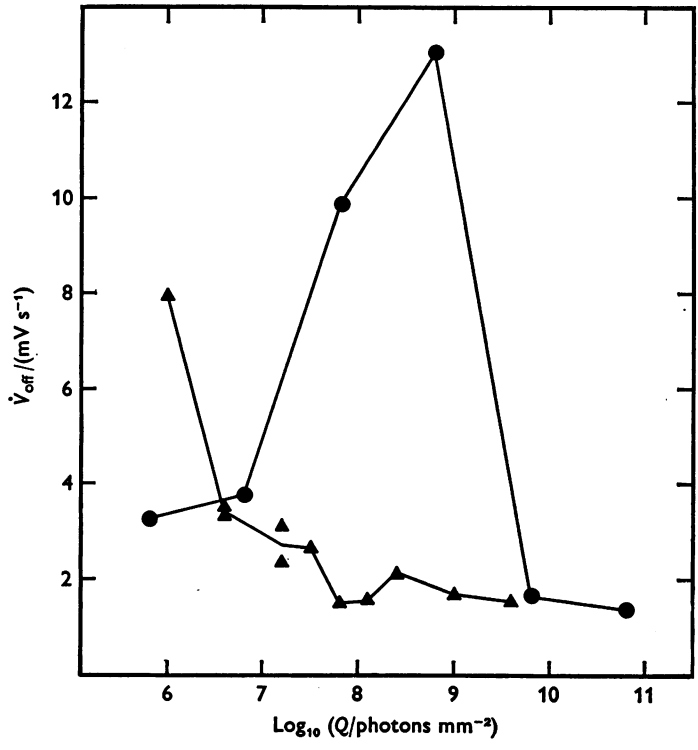

Fig. 4. $\dot{V}_{off}$ as a function of the amount of light in the stimulus. Ordinate: $\dot{V}_{off}$ measured at 5·2 mV from the dark potential. Abscissa: $\log_{10}$ (total number of photons incident on a square mm). The data were obtained from the cells which gave the responses shown in Fig. 3. ▲ :10 ms flash (sample responses in Fig. 3*a*). ● : 47 s pulse (Fig. 3*b*). $\dot{V}_{off}$ could not be measured for the weakest flash because the amplitude of the response was less than 5·2 mV and the value obtained for the second weakest is questionable because $\dot{V}_{off}$ was measured very close to the peak.

i.e., a form of light adaptation persisted after the long pulse. The amplitude of the response was slightly less than that of the controls but comparison of the wave form with the response to the first, weaker flash makes it clear that the adaptation was not simply equivalent to reducing the number of photons in the flash which were absorbed by the rod.

Fig. 5*b* shows the same experiment made using lower light intensities and in Fig. 5*c* superimposed tracings of the test response and the first control response are shown on an expanded time scale. Although the recovery phases differ markedly the excursion phases of the responses differ only near the peak and this small difference might readily be explained by the enhanced recovery process of the test response.

The time course of the decay in the dark of this form of light adaptation

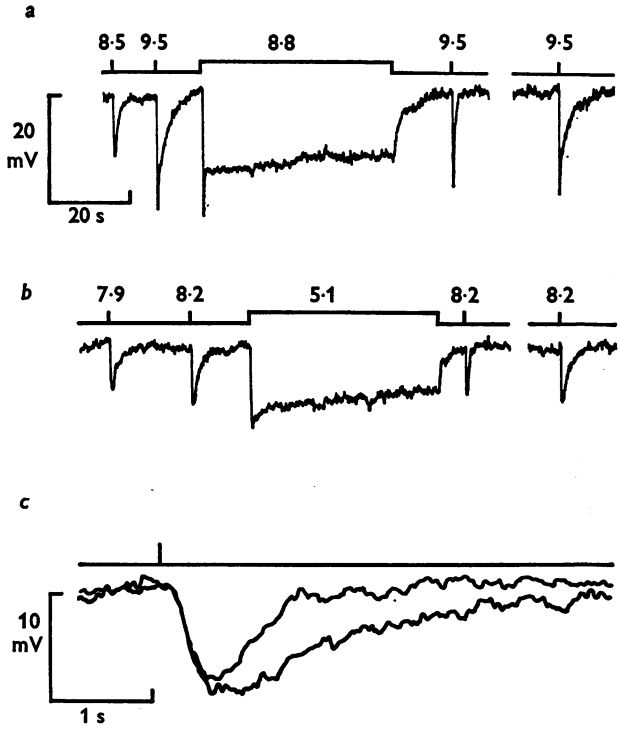

Fig. 5. *a*, Responses to the following sequence of stimuli: two 10 ms flashes, a 47 s pulse and a 10 ms flash and, after an interval of 90 s, another 10 ms flash. The number over each stimulus is a measure of its intensity given by $\log_{10}$ (I/(photons mm^{-2} s^{-1})).

b, An experiment similar to *a* in another cell and with lower light intensities. The scale is the same as for *a*.

c, Tracings of the responses to the second and third flashes in *b* on an expanded time scale.

was measured by presenting adapting and test lights separated by various intervals. Results from ten cells are shown in Fig. 6. For clarity, the results have been plotted on two graphs. In general each set of points was obtained from a different cell: those which suggest a time course with a negative second derivative have been plotted in Fig. 6*b*. In most cases $\hat{V}_{off}$ fell to close to the dark-adapted level within 20 s but was still falling slowly at 100 s.

The adaptation appears not to depend on the value of the membrane potential. The shift from the dark level 15 s after a pulse of some tens of seconds was measured for 33 responses. The mean value of this shift was less than the standard error which was 0·17 mV, about 1% of the peak amplitude of the response.

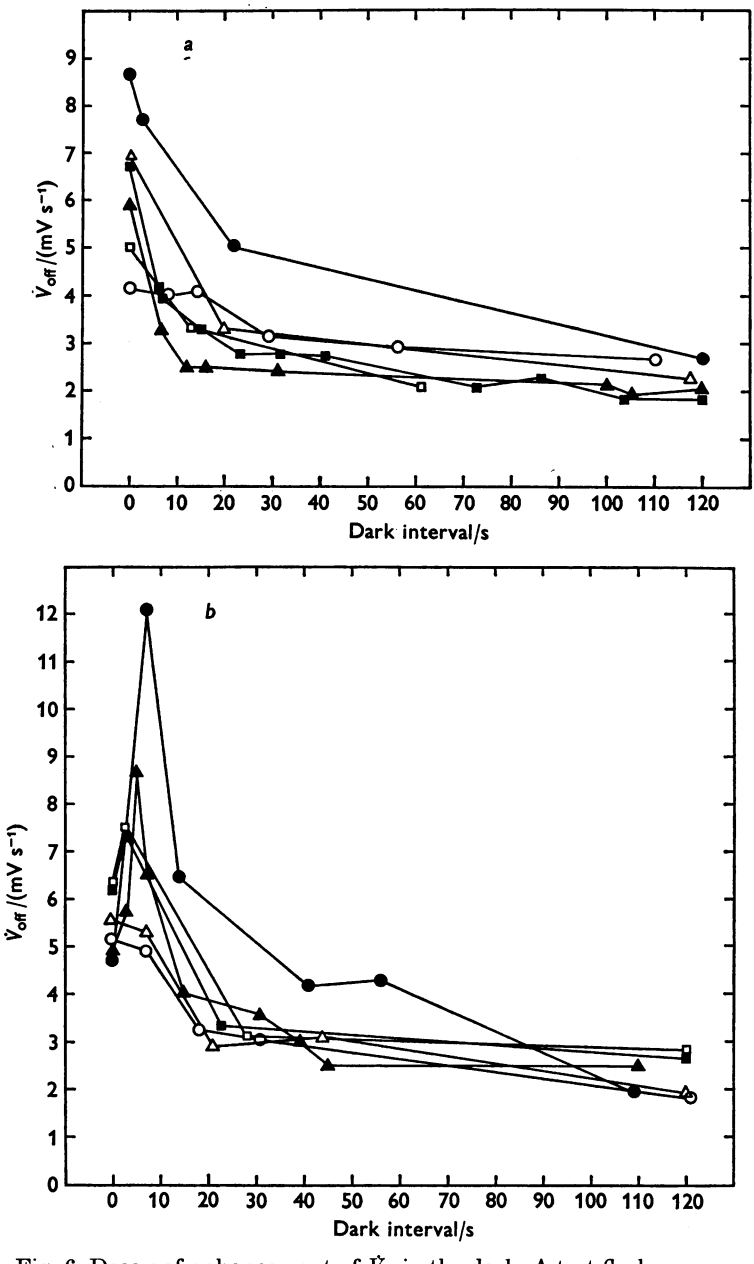

Fig. 6. Decay of enhancement of $\dot{V}_{off}$ in the dark. A test flash was presented at an interval after a 47 s adapting light and the cell was then allowed to dark adapt again. $\dot{V}_{off}$ of the test flash is plotted as a function of the dark interval between the adapting light and the flash. Results from twelve runs on ten cells have been distributed between two graphs for clarity.

Log_{10} (I/(photons mm^{-2} s^{-1})) of the adapting lights were *a*: ○, 5·3; ▲, 6·3; ■, 6·3; ●, 6·9; △, 7·9; □, 8·1. *b*: □, 5·9; ■, 6·2; ▲, 6·2; △, 7·1; ○, 7·3; ●, 9·0.

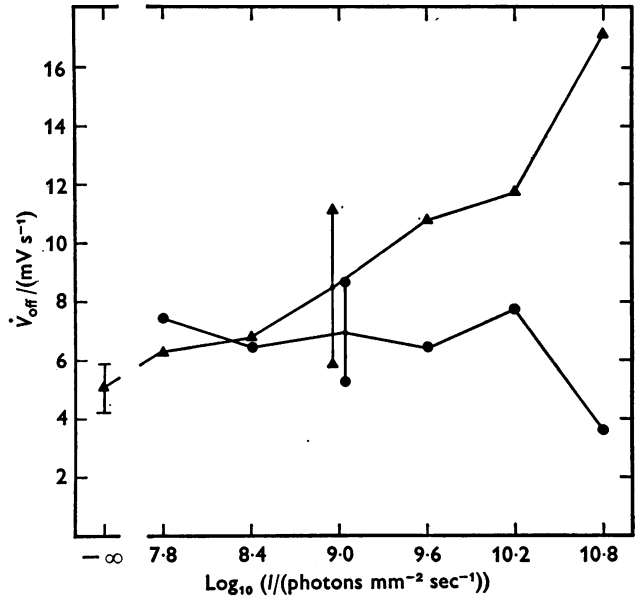

Fig. 7. The dependence of persisting enhancement of $\dot{V}_{off}$ on the intensity of the adapting light. The data were obtained from one cell stimulated according to the following pattern: dark adaptation for at least 80 s, a control flash of fixed intensity, a 47 s adapting light of variable intensity, a 15 s interval and a test flash of the same intensity as the control. The abscissa shows the illuminance of the adapting light.

▲: $\dot{V}_{off}$ for the test flash.

●: $\dot{V}_{off}$ for the corresponding adapting light itself.

The error bar on the point for $\dot{V}_{off}$ for a flash after no light adaptation shows the S.D. for seven measurements. The two points for $\log_{10}$ (I/(photons mm^{-2} s^{-1})) = 9·0 were obtained from the first and last measurements of the series.

The effect of increasing the adapting intensity on the enhancement of $\dot{V}_{off}$ for a subsequent test flash

For two cells a test flash of fixed intensity was presented 15 s after the end of an adapting pulse and $\dot{V}_{off}$ of the test response was measured; this procedure was repeated with various adapting intensities. At the highest of these intensities, $\dot{V}_{off}$ of the test response was continuing to increase although $\dot{V}_{off}$ of the long response itself decreased (Fig. 7). This appears to support the interpretation that the decline in $\dot{V}_{off}$ sometimes seen after very long pulses (e.g. ● in Fig. 2) and always seen after very intense stimuli (e.g. Fig. 4) is caused by some other process (which is here called fatigue) which masks an underlying state of adaptation.

Partial recovery of the membrane potential towards the base line during illumination

During steady illumination sufficiently weak that the plateau phase of the response is not initially strongly saturated the 'plateau' recovers, over tens of seconds, part of the way towards the dark level. Examples of this are shown in Fig. 10. Kleinschmidt (1973) has demonstrated a similar

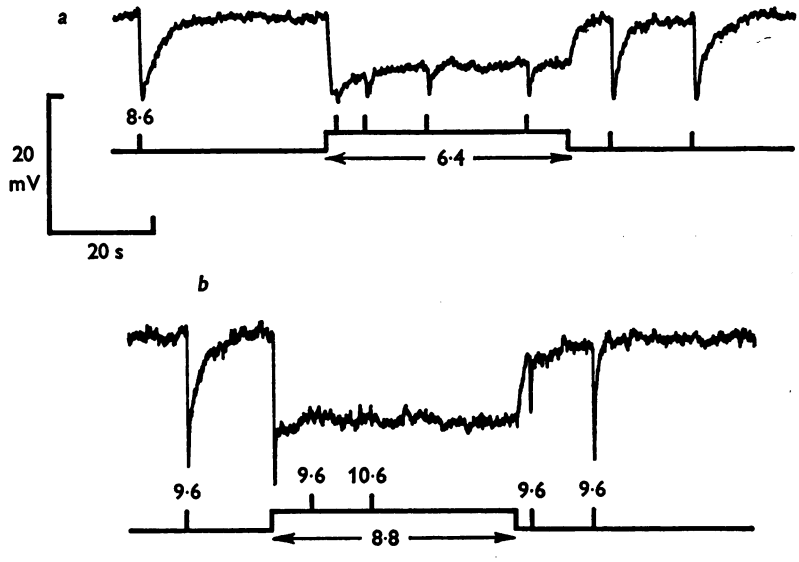

Fig. 8. Flashes superimposed on a background.

a, *Non-saturating intensities.* The duration of each flash was 10 ms and its illuminance is given by $\log_0 (I/(\text{photons mm}^{-2}\ \text{s}^{-1})) = 8{\cdot}6$. The illuminance of the background pulse is given by $\log_{10} (I/(\text{photons mm}^{-2}\ \text{s}^{-1})) = 6{\cdot}4$.

b, *Saturating intensities.* The procedure was similar to that for *a*. In the same units the illuminance of the background pulse was 8·8 and of the flashes 9·6 except for the third for which it was 10·6. During the pulse even a very intense flash evoked no detectable response.

phenomenon in *Gekko* rods and pointed out that it enables the rod to respond with a greater amplitude to an incremental stimulus superimposed on a background. The same phenomenon can be seen in anuran rods, is as shown in Fig. 8*a*, where each of the seven test flashes presented had the same intensity. The amplitude of the response to the flash presented about two seconds after the onset of the background was much smaller than that to a later flash although the peak hyperpolarization was slightly greater.

Is this partial recovery during a pulse related to the enhancement of

$\dot{V}_{off}$? There is some suggestion that this might be so. (1) The time course of development of the partial recovery and of $\dot{V}_{off}$ (Fig. 2) are of the same order. (2) Comparison of responses to pulses of increasing intensity (e.g. in Fig. 3*b*) shows that $\dot{V}_{off}$ begins to increase roughly in parallel with the appearance of the partial recovery. (3) The process which tends to drive the membrane potential towards the dark level, like the enhancement of $\dot{V}_{off}$ of a test flash, persists after the termination of adapting illumination. To demonstrate this it was necessary to shift the membrane potential away from the dark level by applying a weak background light on which the test pulse was superimposed. Records of two such experiments are shown in Fig. 9. The background was sufficiently weak that, as for the weakest pulses in Fig. 3*b*, the response to it alone showed little partial recovery, but after the stronger superimposed pulse the membrane potential was shifted towards the dark level for some tens of seconds.

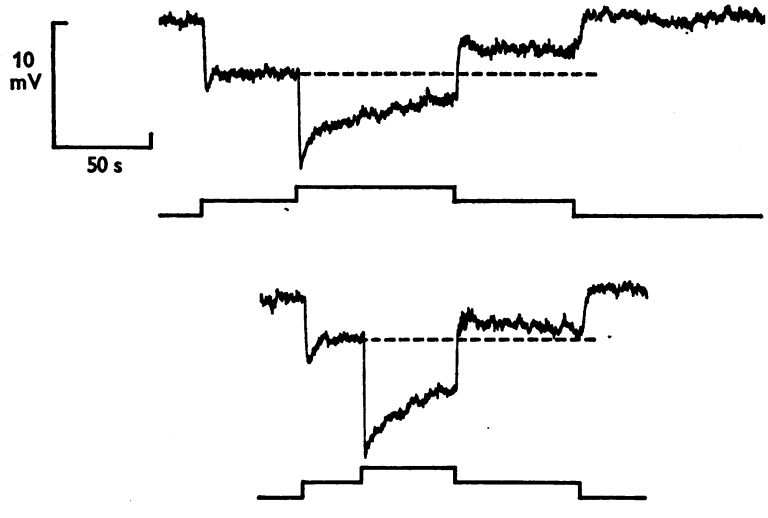

Fig. 9. A long pulse superimposed on a weak background. Two records are shown to give an indication of the amount of drift. The background intensity was produced by placing an I.R.-passing filter in series with a green interference filter and neutral density filters. Its value could not readily be measured but, by comparison with Fig. 3*b*, $\log_{10}$ (I/(photons mm^{-2} s^{-1})) $\simeq 4$. The superimposed pulse had an illuminance given by $\log_{10}$ I/(photons mm^{-2} s^{-1})) = 6·1.

Other causes of partial recovery are unlikely

Unlike the experiments on $\dot{V}_{off}$ the observations on the partial recovery are associated with shifts in the membrane potential. It is conceivable, therefore, that it is caused by electrical changes outside the outer segment. Experiments were made which suggest that such a cause is unlikely.

A first possibility that may be excluded is that there is feed-back from second order cells such as horizontal cells. Brown & Pinto (1974) have shown that in the *Bufo* retina perfused in a manner almost identical to that used here the replacement of 2 mM of the NaCl in the perfusate by 2 mM-Na aspartate caused the responses of the horizontal cells to be completely abolished within 15 s. (They also found that this treatment did not affect photoreceptor responses to stimuli lasting 2 s.) Perfusion with 2 mM aspartate for a much longer period (more than 2 min) did not prevent the partial recovery of the rod response to a long pulse (six cells, Fig. 10). Further, the partial recovery was observed unchanged when the diameter of the stimulating light before scattering was reduced from 3 mm to 100 μm. Matsumoto & Naka (1972) found that *R. catesbiana* horizontal cells showed marked spatial summation when the diameter of the light was increased from 0·4 mm to 3 mm.

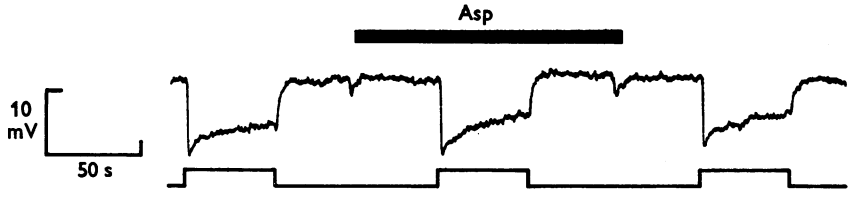

Fig. 10. Responses to long pulses. The illuminance is given by $\log_{10}$ (I/(photons mm^{-2} s^{-1})) = 6·1.

During the period marked by the bar the perfusate contained 2 mM aspartate (Asp). The small hyperpolarizations at the beginning and end of this period were caused by stray light entering the experiment cage.

A second possibility is that the e.m.f. producing the membrane potential is reduced. But, as shown in Fig. 8*a*, the peak hyperpolarization reached by test flashes superimposed on a background is not necessarily changed during the partial recovery, contrary to what would be expected on this hypothesis.

The rapid undershoot

Brown & Pinto (1974) showed that the rapid undershoot, which becomes prominent at high stimulus intensities, is selectively reduced by a raising of the concentration of K^+ in the perfusate. Further evidence for its having an origin different from that of the plateau is shown in Fig. 8*b*. If a pulse of light is of high intensity and saturates the plateau level then even very intense flashes evoke no response. Baylor *et al.* (1974*b*) have observed a similar phenomenon in turtle cones.

DISCUSSION

The results show that after stimulation of an anuran rod by a flash of light, for most of the range of illuminances used, the membrane potential returns towards the dark level at a rate which is little affected by the intensity of the illumination but which can be increased by light adaptation. The records obtained from *Gekko* by Kleinschmidt (1974) of responses to long pulses of increasing illuminance show the same phenomenon. If, as suggested, this enhancement of the recovery process also produces the partial recovery of the membrane potential during a long response then this mechanism contributes to two aspects of light adaptation, namely that the range of background intensities on which incremental stimuli can be detected is increased and the response can follow fluctuating light intensities to higher frequencies of fluctuation when the mean intensity is increased. This latter phenomenon has been demonstrated for frog rods by Toyoda & Coles (1973).

Adaptation probably occurs internal to the surface membrane. A change in any of the electrical properties of the rod, for example, the resistance of part of the surface membrane or the e.m.f. driving the dark current, would, in general, cause a change in the membrane potential of the outer segment. The fact that after an adapting light this was not observed would preclude such a change from involvement in the enhancement of $\hat{V}_{off}$ unless there were some further, compensating change which accurately restored the membrane potential to its dark-adapted value. $\hat{V}_{off}$ is also too slow by three orders of magnitude to be affected significantly by changes in the electrical time constant of the surface membrane. It is concluded that the simplest necessary modification of the basic hypothesis is that after light adaptation the net rate at which blocking particles are removed from the channels in the surface membrane is increased. This might happen either because blocking particles continue to be released from the disks after illumination for long enough for them to affect the time course of the recovery (2–10 s) and this time becomes shorter after light adaptation, or because the particles are inactivated more rapidly. The former hypothesis seems the less likely because it requires that there be a change in the later phases of the release of particles after a flash without any change in the earlier phases (see Fig. 5*c*) and we consider only the latter hypothesis.

The process limiting the rate of recovery of the membrane potential. A scheme representing part of the blocking particle hypothesis proposed by Baylor *et al.* (1974*b*) is shown in Fig. 11. The absorption of light by rhodopsin causes generation of the blocking particle Z_1 which can either bind to a channel B in the surface membrane and block it or be inactivated by way of a series of states Z_2, Z_3, ..., Z_n. Baylor & Hodgkin (1974) were

able to account for the speeding up of the rate of recovery of the responses of turtle cones by supposing that Z_1 was in equilibrium with BZ_1 and that, over a range of intensities, the rate limiting step was the conversion of Z_1 to Z_2 by a reaction which is reversible and autocatalytic. However, the increased rate of this reaction can persist only when the concentration of Z_2 is high and, therefore, since Z_2 and Z_1 tend to equilibrium, only when Z_1 is finite and the membrane potential is shifted from the dark level. In

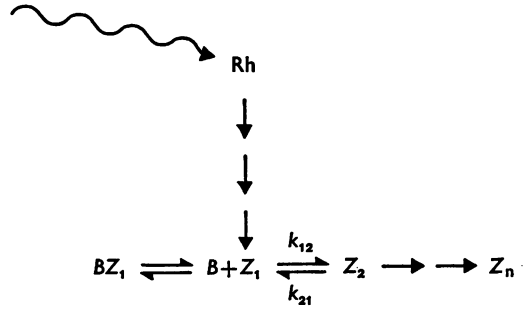

Fig. 11. A scheme for the blocking hypothesis, very similar to that suggested by Baylor *et al.* (1974*b*). When a photon is absorbed by a rhodopsin molecule a series of reactions leads to generation of blocking particles Z_1 in the intracellular space. A particle of Z_1 may either bind reversibly to a site B at a channel in the surface membrane, and block it, or be inactivated through a series of states $Z_2 \ldots Z_n$ where n is an unknown number. The interpretation of the present results is that k_{12} or the rate constant of a subsequent step is increased by light adaptation.

contrast, in anuran rods, since $\dot{V}_{off}$ can be enhanced in the absence of measurable membrane hyperpolarization, the increased rate of the rate-limiting step must persist when Z_1 is close to zero. The rate-limiting step could still be $Z_1 \leftrightharpoons Z_2$ with autocatalysis if the equilibrium between Z_1 and Z_2 were far to the right but then there would be no evidence to support the idea of autocatalysis. Since the morphology of the rod outer segment differs from that of the cone and since the time constant of the main component of the recovery of the potential to the dark level is an order of magnitude slower than that for moderate stimuli in the cone it seems reasonable to consider whether the results suggest other hypotheses. The delay in recovery after a strong flash (see e.g. Fig. 3*a*) is most readily interpreted as due to a persisting excess of free Z_1 so, under these conditions at least, it must be one of the reactions in the path $Z_1 \ldots Z_n$ which is rate limiting and not the dissociation of the BZ_1 complex. Taking this as generally true we propose the following speculative hypothesis which will also account for the observation that after the more prolonged and intense stimuli $\dot{V}_{off}$ was often observed to fall off from its peak value. Each

photoisomerization probably causes the release of at least some tens of blocking particles (Yoshikami & Hagins, 1973; Cone, 1973). The restoration of these to their original location or state will be an energy-requiring process and our suggestion is that weak light adaptation modulates this process so as to increase its rate. However, at least under the conditions of the present experiments, the absorption of much light (and the release of many blocking particles) causes the process to slow down, perhaps because the supply of energy (probably in the form of ATP) is temporarily depleted. It is not known whether the blocking particles are returned directly to the disks, in which case Z_2 would represent the particles in the state of being in the disks and $Z_n \equiv Z_2$, or whether some or all of them are sequestered temporarily elsewhere, for example, in the mitochondria in the inner segment.

A comparison of adaptation in rods and cones

The experiments described here overlap some of those on turtle photoreceptors (mainly the cones) described by Baylor & Hodgkin (1974) and Baylor *et al.* (1974*a*, *b*). Some comparisons between turtle cones and anuran rods are summarized below.

Similarities. (1) The rate of recovery to the dark level ($\dot{V}_{off}$) is increased by weak light adaptation. (2) During illumination lasting tens of seconds the membrane potential recovers part of the way to the dark level. We have pointed out that these two features increase the usefulness of the photoreceptor. (3) After the onset of strong illumination the response has a rapid undershoot. If the illumination is sufficiently intense, a flash, no matter how intense, presented during the subsequent plateau will produce no response.

Differences. (1) In cones there is a marked increase in the rate at which the potential recovers to the dark level after a flash as the intensity of the flash is increased. In the records from rods presented here this is not apparent: the enhancement of $\dot{V}_{off}$ requires seconds for its development. (2) In cones this speeding up is associated with a change in the dark potential and is interpreted by Baylor & Hodgkin (1974) as due to an autocatalytic effect of Z_2. In rods there is no evidence for such a change in dark potential and hence no evidence for autocatalysis. (3) After illumination lasting tens of seconds there is a depolarization ('rebound') in cones (Baylor & Hodgkin, 1974) but not in rods.

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