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The Site of Visual Adaptation

Recent experiments suggest that the main site of visual adaptation is in the bipolar-cell layer of the retina.

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One of the striking features of visual systems is their ability to adapt and function over an enormous span of light intensity. The human eye, for example, can visually discriminate over a luminance range of about 10 billion to 1. The possible mechanisms involved in visual adaptation have long puzzled and fascinated students of visual physiology.

One obvious mechanism to explain adaptation is regulation of the amount of light entering the eye by the pupil. In bright light the pupil closes down rapidly, but, in the human eye, the diameter of the pupil varies only between 2 and 8 millimeters, allowing a change in area of 16 times. This can account for adaptation only over a range of about one log unit. Thus, the major part of adaptation must be attributable to changes in sensitivity in the retina or elsewhere in the visual pathway.

Since the formulation of Hecht's classic photochemical theory in the 1920's, one persisting idea has been that changes in the sensitivity of the visual system are related to the bleaching and regeneration of the visual pigments (*1*). The primary evidence for this view came from observations that the time course of dark adaptation follows roughly the time course of regeneration of visual pigment in the eye (*1, 2*). However, numerous experiments (some of them dating as far back as the 1930's) have shown that factors other than loss and resynthesis of pigment must also be involved in visual adaptation. The evidence for this has come from experiments that showed that there may be large changes in visual sensitivity without significant changes in the concentration of visual pigment (*3*).

In recent years, several studies have clarified the contribution of the photochemical and non-photochemical factors in visual adaptation. Other experiments have related these factors and suggested mechanisms to explain adaptation. In this article, I shall review a number of these experiments and present some evidence and speculation regarding the site and the cells involved in visual adaptation.

The Process of Adaptation

Figure 1 illustrates adaptation by the rat eye to both light and dark over a considerable range of adapting (background light) luminances (*4*). Included are the concentrations of rhodopsin remaining in the retina after a 5-minute exposure to the various adapting intensities. The sensitivity of the rat eye was determined by measurement of the light necessary to evoke an electroretinogram of constant size (50-microvolt b-wave); concentrations of rhodopsin were determined by direct extraction. In the rat, the visual receptors are probably all rods; hence the relations described here are for a rod system. However, similar relations have been shown to hold also for the cones of the human eye (*5*).

When a background adapting-light is turned on, the sensitivity of the eye is quickly reduced. Except at the lowest intensities of background light, the increase in threshold (or the decrease in sensitivity) is linearly proportional over a wide range of intensities to the adapting luminance, the slope of the line being almost 1. This is the well-known Weber-Fechner relation in which $\Delta I/I = C$, where ΔI is the increment intensity, I is the background intensity, and C is a constant value. Increment (or contrast) thresholds, which are the terms given to thresholds measured against background illumination, are established exceedingly quickly when the light is first turned on—too quickly, in fact, to be accurately measured by the usual electroretinographic techniques. However, with human subjects, numerous experiments in which psychophysical techniques are used for estimation of threshold have shown that most of the change in light adaptation is completed within 0.1 second (*5, 6*), although it takes several seconds for the threshold to settle to its final value. Thus, the first point to be emphasized is that the main process of adaptation to light is very fast, requiring only the time needed for a neural (or synaptic) process to occur.

This leads to the next observation, that the loss of sensitivity during adaptation to light is virtually unrelated to the amount of bleaching of visual pigment (Fig. 1). In fact, no measurable bleaching of pigment occurs in the eye until the background light is 5 to 6 log units above the threshold of the electroretinogram. This happens because the eye responds readily to very low intensities of light (1 quantum per 200 rods to elicit a measurable b-wave, for example); but at the same time it contains enormous amounts of visual pigment (about 30,000,000 molecules per rod) (*7*). At high adapting intensities that substantially bleach pigment, the increment threshold rises only at about the same rate as it does with lower, nonbleaching intensities (Fig. 1). Thus, the second important point is that the eleva-

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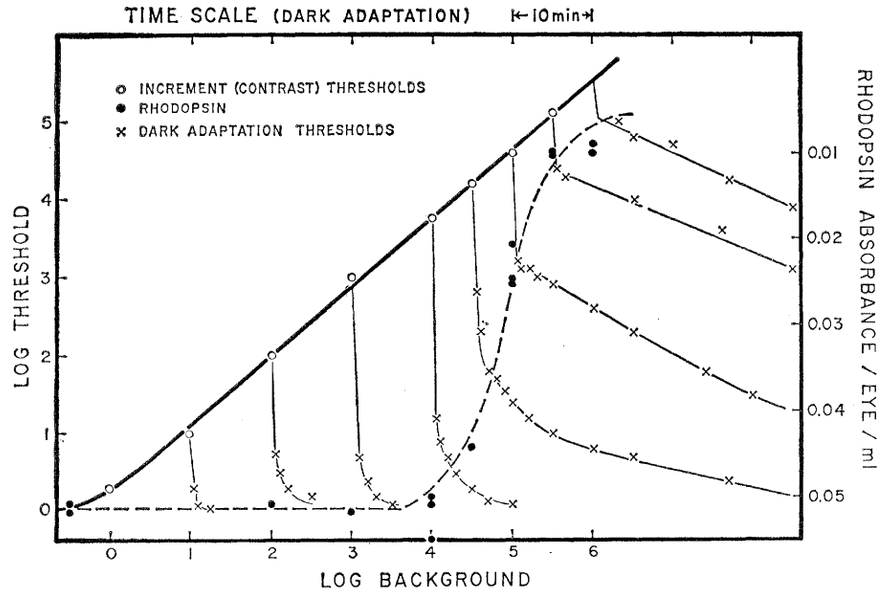


Fig. 1. Visual adaptation in the rat eye as determined by sensitivity of the b-wave of the electroretinogram. During light adaptation (open circles, heavy line; increment thresholds) the increase in the logarithm of the threshold is linearly proportional to the logarithm of the background luminance, except at the dimmest background luminances. Dark adaptation (crosses, thin lines) is rapid until the eye is adapted to background luminances bright enough to bleach significant quantities of rhodopsin (filled circles, dotted line) in the 5-minute adaptation period. With bright background luminances, a slow component of dark adaptation is observed, the extent of which depends on the amount of rhodopsin bleached (*4*).

tion of threshold during adaptation to light depends almost entirely on the intensity of the background or adapting light, not on the amount of visual pigment bleached. It is true that if a substantial fraction of the pigment is bleached, fewer quanta can be absorbed and the threshold will correspondingly increase; but this increase in threshold is small compared to the total change of threshold during light adaptation. For example, with half the pigment gone the threshold would be doubled, but this is a change of only about 0.3 log unit, compared to a total increase of some 4 to 5 log units induced by that adapting intensity.

Adaptation to the dark, on the other hand, is somewhat more complicated; two distinct phases of recovery of sensitivity can ordinarily be distinguished (Fig. 1). With dim adapting intensities that do not measurably bleach visual pigment, dark adaptation is extremely rapid, being mostly completed within seconds (*6*) (Fig. 1). This rapid rate of recovery seems comparable to the rapid loss of sensitivity during light adaptation. At adapting intensities bright enough to measurably bleach visual pigment, a slow phase of dark adaptation is also observed. The extent of the slow phase of adaptation is related to the amount of pigment bleached.

When virtually all the pigment is bleached, the slow component accounts for almost all of dark adaptation, and complete dark-adaptation requires 2 to 3 hours in the rat. Parallel measurements of the recovery of visual pigment and of the logarithm of the threshold during slow dark-adaptation show they are closely correlated (*4*). In the human eye during dark adaptation, a similar relation between the recovery of the log threshold (measured psychophysically) and the regeneration of visual pigment has been demonstrated by Rushton (*8, 9*) to hold for both the rods and cones after they have been exposed to light bright enough to bleach most of the visual pigments.

Thus, during dark adaptation we can distinguish both fast and slow processes. Slow dark-adaptation, which is related to regeneration of visual pigment, is frequently termed photochemical or bleaching adaptation; fast adaptation, not related to pigment regeneration, is often termed neural or field adaptation (*4, 8*). Fast dark-adaptation seems the converse of the rapid course of light adaptation, and thus we can simply say that the eye possesses rapid neural mechanisms for changing sensitivity (or gain) of the visual system, these mechanisms being independent of pigment concentrations.

Although it may appear that the two types of adaptation (neural and photochemical) are vastly different, there is reason to suppose that they may be related and perhaps may even have a common mechanism. Crawford (*5*) was the first to point out the similarity of raised thresholds during slow (photochemical) dark-adaptation and raised thresholds when dim background light is shining on the eye. Barlow (*10*) has argued that the elevation of threshold during photochemical adaptation is caused by an increase in "noise" or "dark light" (maintained activity) in the receptors and that bleached rhodopsin gives rise to this increased activity. Thus, it is suggested that the outer segments of the receptors may signal during photochemical dark-adaptation, perhaps as they do when dim light is falling on the eye, and this may account for a possible equivalence of the two situations.

Impressive evidence supporting these ideas has been presented. First, it is well known that after monocular light-adaptation, the pupil of the contralateral (dark-adapted) eye is constricted like the pupil of the light-adapted eye. Recently, it has been shown that subsequent dilatation of the pupil of the dark-adapted eye follows the course of slow dark-adaptation of the eye that was light-adapted, even if the light-adapted eye had been temporarily blinded during the period of light adaptation by firm digital pressure on the eyeball (*11*). Since slow dark-adaptation is related to the concentration of visual pigment, a signal indicating the amount of bleached rhodopsin must arise in the retina of the light-adapted eye, and it seems most likely that this comes from the outer segments.

Second, after adaptation to a bright light, a positive afterimage can be seen for several minutes during dark adaptation, and it has been suggested often that the afterimage is the sensation associated with the signal from the bleached pigment. Recently, Barlow and Sparrock (*12*) presented evidence that the signal giving rise to the afterimage is also the one that raises the visual threshold during photochemical dark-adaptation, by demonstrating that the decrease in threshold during dark adaptation exactly matches the fading of the stabilized afterimage on the

retina. That is, the elevated threshold during photochemical dark adaptation is simulated in a dark-adapted eye when presented a light that matches in luminance the brightness of the stabilized afterimage.

Finally, Cone (*13*) has shown an equivalence of electroretinographic responses during light adaptation, fast (neural) dark-adaptation, and slow (photochemical) dark-adaptation; so it would seem reasonable to suggest that one mechanism accounts for all visual adaptation. Recently, however, Rushton (*14*) has described a complex experiment which suggests that there may be a slightly different mechanism for neural and photochemical adaptation; thus, at the present time, this point remains uncertain.

One might suppose that the site of the mechanism that decreases sensitivity (or gain) in the visual system is in the receptor cells themselves, perhaps close to the visual-pigment molecule in the outer segments. Recent experiments, however, suggest that this is probably not the case. Lipetz (*15*) first demonstrated that, when one part of the receptive field of a frog ganglion-cell was adapted to light, the sensitivity also diminished in other parts of the field. That is, parts of the ganglion-cell receptive field that did not receive light had, nevertheless, been adapted. This result suggested that mechanisms subserving adaptation are located more centrally than the receptor outer-segments, at a locus where at least lateral interaction in the retina can take place. To account for these and similar observations, Rushton (*16*) has proposed that the retina possesses "pools" onto which large numbers of receptors converge and which are responsible for adaptation. Rushton and his co-workers have performed a number of ingenious experiments providing evidence for the concept that adaptation in the visual system is primarily in a pool and not in the individual receptors. I wish to note only one here, and that is the experiment in which a portion of the retina was bleached with a striped pattern such that some receptors were strongly bleached while others were spared (*17*). When this was done and sensitivity was then tested over the entire area, the results were the same as if the retinal area had been bleached with a slightly dimmer light evenly spread. Subsequently, dark adaptation also proceeded evenly over the entire test field; this finding showed unequivocally that the sensitivity of an area of retina not directly illuminated is reduced almost as much as a nearby area that is strongly bleached.

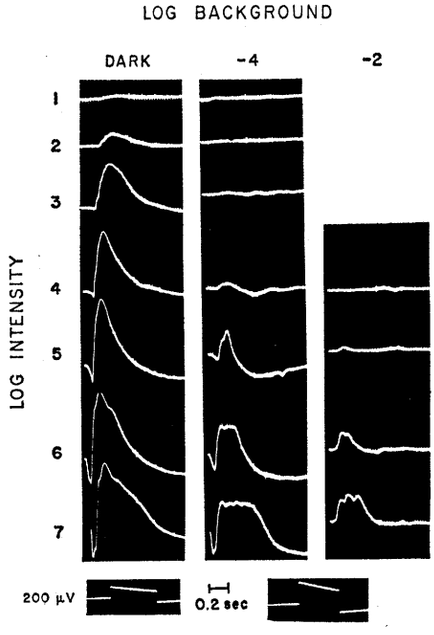


Fig. 2. Electroretinograms evoked from the rat eye with flashes of light (1/50th second) over a range of 7 log units of intensity, with no background (dark) and background light attenuated with a 4.0 or 2.0 neutral-density filter. At low light intensities, only the corneal positive b-wave is observed; higher intensities are required to elicit the corneal negative a-wave. With dim background light (logarithm of background luminance equal to −4), the b-wave shows more adaptation than the a-wave. With higher background light (log background of −2) the a-wave disappears and only the b-wave can be elicited from the eye. Oscillations of the peak of the b-wave are often seen when a background light is on and intense stimuli are used to elicit the electroretinogram.

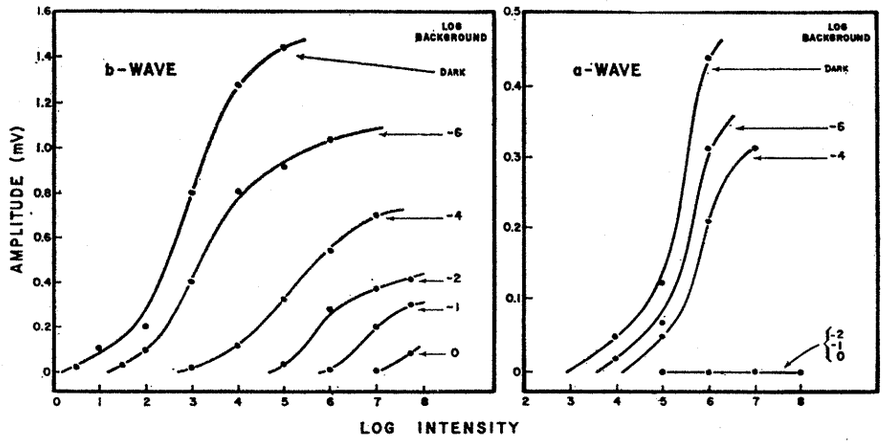


Fig. 3. Intensity-response curves of a- and b-waves of the electroretinogram with background light of graded intensities. With increasing background luminance, the b-wave intensity-response curves are shifted to the right in proportion to the added background intensity. The a-wave intensity-response curve shows only a small shift to the right with dim background luminances, but above a log background of −2, the a-wave saturates such that no further a-wave response can be elicited regardless of the stimulating intensity.

The problem before us is to identify the site of visual adaptation. Many years ago, Craik and Vernon (*18*) demonstrated that visual adaptation is retinal in origin, by showing that adaptation proceeds normally in a human subject even if the eye is temporarily blinded by pressure on the eyeball during the period of light adaptation. That the site of adaptation in the visual system is more peripheral than the ganglion cells of the retina also was indicated by numerous studies showing that the b-wave of the electroretinogram has adaptation properties comparable to those observed in psychophysical adaptation (*19, 4*). It is well known that one can record a normal electroretinogram in eyes from which the ganglion cells have been lost; thus the ganglion cells cannot contribute to the electroretinogram. Recently we have looked into this further and have found that adaptation, as measured by the b-wave of the electroretinogram, is also perfectly normal in eyes from which the optic nerve has been severed and in which most of the ganglion cells have degenerated (*20*). Thus, neither ganglion cells nor centrifugal fibers (if they exist in all vertebrates) appear involved in visual adaptation.

It now seems quite well established that the b-wave of the electroretinogram arises in cells of the inner nuclear layer; and although direct identification has not been made, it also

seems most likely that the b-wave arises from the bipolar cells (*21–23*). However, the a-wave of the electroretinogram appears to arise more peripherally in the retina than the b-wave, probably in the outer plexiform layer (*21–23*).

Does the a-wave also have adaptation properties comparable to those of the b-wave and of psycho-physical adaptation? Some recent experiments indicate that the a-wave does not adapt in the same way; but how it does adapt is not entirely clear. For example, Brown and Watanabe (*24*) have recently shown that during weak, repetitive stimulation with light the a-wave does not show much adaptation as compared with the b-wave. However, Cone and Ebrey (*25*) have found that the a-wave disappears when the background intensity is raised beyond a certain level, so that no a-wave can be elicited regardless of the stimulating intensity. In a more recent paper, Cone (*26*) has extended this work and has shown that the a-wave does not change much in amplitude with dim background illumination that significantly reduces the size of the b-wave, but that the a-wave is sharply attenuated with brighter background illumination, and it eventually falls below the b-wave amplitude. To clarify further this important point, I have measured adaptation of the a-wave over a wide range of background intensities and have compared its properties of adaptation with those of the b-wave.

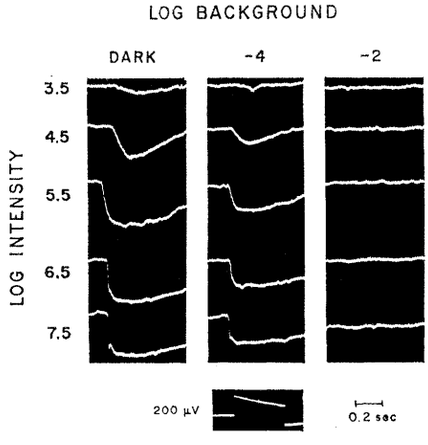


Fig. 4. The isolated a-wave of the rat electroretinogram elicited with a range of intensities, in the dark, and with a background light attenuated with a 4.0 or 2.0 neutral-density filter. The isolated a-wave was produced by clamping of the optic nerve with a small forceps which interrupted the retinal circulation. With dim background illumination (log background of —4) the a-wave shows little adaptation; with higher background illumination (log background of —2) no a-wave can be elicited regardless of stimulus intensity. Flash duration, 1/50 second.

Experimental Results

In our first experiment, a- and b-waves from the same animal (albino rat) were recorded and measured over a wide range of stimulus and background intensities (*4*). Figure 2 shows typical electroretinographic responses recorded from a rat in the dark, and in background light attenuated with either a 4.0 or 2.0 neutral-density filter. At low stimulating intensities in the dark-adapted eye, only the corneal-positive b-wave is seen. The corneal-negative a-wave is first detected in a gross recording when the stimulus intensity is about 2.5 log units above the threshold of the b-wave. For measurement of the size of the potential, the a-wave is measured from the base line to the start of the b-wave, and the b-wave is measured from the trough of the a-wave to the peak of the b-wave. Figure 3 shows curves (in which the size of the response is plotted as a function of the stimulus intensity) of the b-wave elicited from a dark-adapted retina, and from a retina adapted to light when graded neutral-density filters were interposed in the adapting beam. With background illumination, the intensity-response curves of the b-wave are shifted to the right; the amount of the shift is related to the intensity of the background light. At the higher background intensities the amount of the shift is about equal to the amount of the added background light. For b-waves with lower amplitudes of potential (0 to 200 microvolts), the intensity-response curves are quite parallel at all background luminances, but at higher amplitudes they are not parallel. As Cone has demonstrated (*13*), with increasing background illumination the intensity-response curves reach a maximum size more quickly.

Compared with the b-wave, the a-wave shows a distinctly different behavior in response to background illumination (Fig. 3). At lower background intensities (6.0 and 4.0 neutral-density filters in the adapting beam), the intensity-response curves of the a-wave are shifted slightly to the right, but they show much less shift than those of b-waves at comparable background luminances. At higher background luminances, however, no a-wave response can be elicited with any stimulus intensity. Thus, the a-wave shows little evidence of adaptation with low background luminances, but then it saturates and is lost with moderate background light. Figure 2 demonstrates clearly the difference, with repect to adaptation, between the a- and b-waves of the electroretinogram. With the background light attenuated with a 4.0 neutral-density filter, for example, the a-wave adapts much less than the b-wave; thus, the electroretinographic response shows a prominent a-wave potential. With higher background intensities (that is, a 2.0 neutral-density filter in the adapting beam) the a-wave of the electroretinogram disappears, so that no negative component of the electroretinogram is seen with any stimulus intensity.

One of the difficulties in measuring magnitudes of the a- and b-waves of the electroretinogram is that they are of different polarities; thus, it is difficult to determine the true amplitude of one wave when the other is present. However, it is possible by various techniques to isolate the a-wave and study it independent of the b-wave. In another experiment, I used Cone and Ebrey's technique (*25*) of isolating the rat a-wave by recording after clamping the optic nerve with a small forceps inserted behind the eyeball, to shut off retinal circulation. The b-wave originates in the nuclear layer, which depends on the intraretinal circulation accompanying the optic nerve, and the b-wave is lost when the nerve

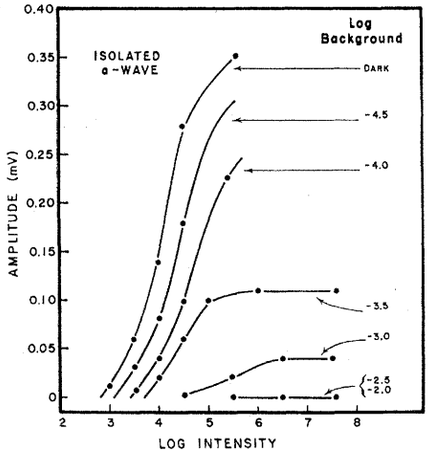
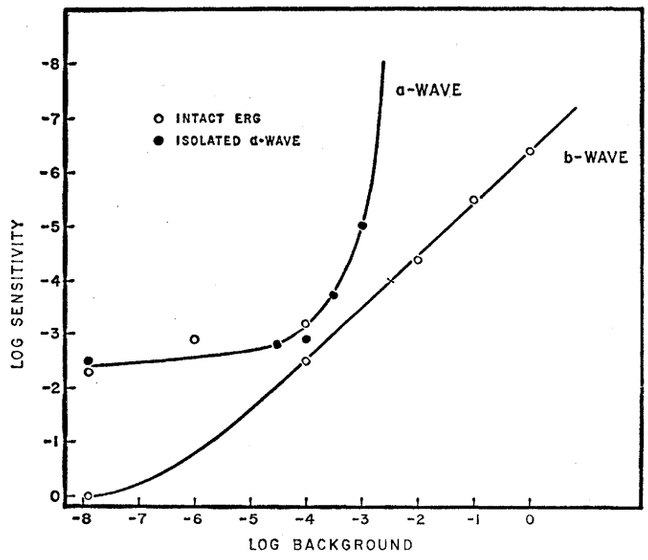


Fig. 5. Intensity-response curves of the isolated a-wave with background light of graded luminance. With dim background (that is, log background of —4), the a-wave shows little adaptation. At higher background luminances (that is, log background of —2.5), the a-wave saturates and no response can be elicited with any stimulus intensity.



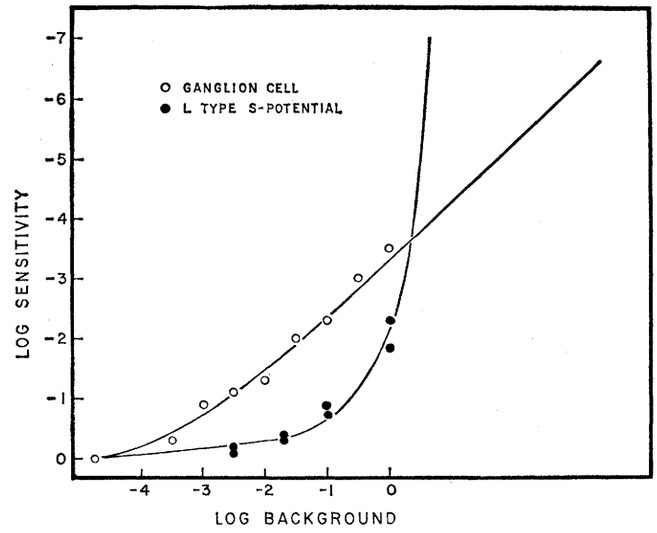


Fig. 6. (left). Adaptation of a- and b-waves of the electroretinogram to background light. Except at lowest background luminances, the decrease in the logarithm of the sensitivity of the b-wave is linearly related to increase in the logarithm of the background luminances. The a-wave shows little adaptation to dim background luminances, but sharply saturates with brighter background luminances (that is, greater than log background of −3). Fig. 7. (right). Adaptation (to background light) of ganglion cell (open circles) and L-type S-potential (closed circles) in the carp retina. The curves to which the points are fitted are from Fig. 6 of a- and b-wave adaptation to background light. Data from Witkovsky (*30*). Response criterion for ganglion-cell sensitivity was three action potentials; for L-type S-potential, 5 and 10 millivolts (lower and upper points respectively).

is clamped. The a-wave arises more distally in the retina, in cells apparently maintained by the choroidal circulation, which is not appreciably affected by this procedure. Figure 4 shows isolated a-waves elicited with a wide range of intensities from a preparation dark-adapted and then illuminated with background light attenuated with either a 4.0- or 2.0-neutral-density filter. (This response might be more properly termed Granit's P-III wave (*27*) rather than the a-wave, since only its leading edge is the a-wave of the electroretinogram of an intact retina.) The isolated a-wave of the rat is a sustained potential that lasts the duration of a prolonged stimulus and then slowly decays (*25*). The results of experiments on the effect of background light on the isolated a-wave confirm the effects of background light on the a-wave of the intact electroretinogram. With low intensities of background light the a-wave shows little adaptation, and then it saturates and is lost with moderate background luminance (Fig. 4 and 5). In the experiment described in Fig. 5, the background intensity was increased by half-log-unit steps to show the sharp saturation point of the a-wave (between 3.0 and 2.5-log attenuation of the background light).

A comparison of adaptation of the a- and b-waves to background light is shown in Fig. 6. Except at the lowest background intensities, the loss of log sensitivity of the b-wave is linearly related to the increase of the logarithm of background intensity. The a-wave, on the other hand, when measured externally from the eye, as here, has an apparent threshold higher than the b-wave by about 2.5 log units; but since the a-wave shows little adaptation to background lights of lower intensity, it approaches the sensitivity of the b-wave when the background light is attenuated by a 4.0 or 5.0 neutral-density filter. At higher background intensities the a-wave sharply saturates, and from then on only the b-wave can be elicited from the eye.

Conclusions

These experiments demonstrate that the a-wave of the electroretinogram shows properties of adaptation different from those of the b-wave or of psychophysical adaptation. The b-wave arises in the bipolar-cell layer (*21–23*), whereas the a-wave arises more peripherally in the retina, in the region of the outer plexiform layer (*21–23*). The precise locus of origin of the a-wave in the outer plexiform layer is not yet clear. Evidence has been presented that the a-wave arises in the receptor terminals (*23*), and thus, presumably, it would be related to the generation of the b-wave. However, this does not seem consistent with the observation that the a-wave saturates with moderate background illumination (*25*). The b-wave represents an event subsequent to receptor processes, and it is difficult to see how the a-wave can be related to the generation of the b-wave since the a-wave is lost with moderate background intensity but the b-wave is not. Thus, it seems to me that the a-wave may arise elsewhere in the outer plexiform layer, possibly in the horizontal cells (*22*). Intracellular responses have been recorded from cells in the outer plexiform layer in retinas of certain vertebrates, and evidence has been presented that some of these recordings are from the horizontal cells (*28*). These responses, the L-type S-potentials, have many properties similar to the a-wave (or P-III) of the electroretinogram (*23, 22*). For example, the S-potentials, like the a-wave, are sustained potentials in response to light (*28*) and, like the a-wave, remain when the retinal circulation is clamped (*23*).

For this discussion, the most pertinent similarity is that the response of the L-type S-potentials to background light is very much like that of the a-wave (*30*). The L-type S-potentials saturate with moderate background illumination, much as the a-wave does, and at an intensity at which one can still record responses of the ganglion cells. Figure 7 shows data sent me by Witkovsky (*30*) on the adaptation to background light of a ganglion cell and an L-type S-potential from the

carp retina. The curves to which the points are fitted are those (from Fig. 6) of the adaptation of the a- and b-waves to background light. The fit of the S-potential responses to the a-wave curve, and of the ganglion cell responses to the b-wave curve, is extraordinarily close.

Some arguments have been presented that there are differences between the a-wave and S-potentials, so it is not yet clear whether the two responses have the same origin (*31*). However, the fact that they show a similar response to background light leads me to conclude that the b-wave is the first response of the visual system to show typical adaptation to background light—that is, adaptation similar to psychophysical adaptation. Thus, it seems likely that the main site of adaptation in the visual system is located in the bipolar-cell layer (see *32*).

Speculations

The evidence outlined above suggests that the main site of visual adaptation in the vertebrate retina is associated with the bipolar-cell layer. Can we speculate about a possible mechanism of adaptation of the bipolar cells? One of the attractive postulates put forward to explain adaptation in the visual system is that of a feedback system such that the signal at one stage feeds back onto a previous stage, reducing the latter's sensitivity or gain. Fuortes and Hodgkin (*33*) have formulated such a feedback or gain-control system to account for responses in the eye of *Limulus*; Rushton (*14*), with the collaboration of Hodgkin, has shown how such a system can account for adaptation in man and other vertebrates.

Recently, Boycott and I (*34*) have examined with the electron microscope the synaptic organization of the primate retina. One of the striking findings was that there are reciprocal synapses between the terminals of the bipolar cells and the amacrine cell processes. Similar reciprocal contacts between amacrine and bipolar cells have now been seen in all other types of vertebrate retinas we have examined, including that of the rat. Figure 8 is a diagram of the typical complex synaptic arrangement of bipolar terminals, amacrine processes, and ganglion-cell dendrites in the inner plexiform layer. At presumed points of synaptic contact, synaptic ribbons that are directed between the amacrine process and ganglion-cell dendrite are found in the bipolar terminals. Associated with the ribbons are specializations consisting of some thickening and densification of the membranes; these specializations are similar to those seen at most synapses in the central nervous system. The membrane thickening on the amacrine process and ganglion-cell dendrite is greater than that of the bipolar cell, which suggests that at the ribbon the bipolar cell is making synaptic contact with both elements and that the polarity of the junction is from bipolar cell to amacrine process and ganglion-cell dendrite. A short distance from the ribbon, the amacrine process can often be seen making a clear synaptic contact back onto the bipolar terminal, thus completing the reciprocal junction. This reciprocal contact has the characteristics of most synapses in the central nervous system: a dense aggregation of synaptic vesicles on the presynaptic amacrine side, a widened extracellular synaptic cleft, and thickenings of both the pre- and post-synaptic membranes. It appears that these reciprocal synapses are present at all junctions of bipolar cells with amacrine cells, and thus this synaptic arrangement would seem to serve admirably for a feedback system on the bipolar cell. We might postulate that stimulation of the amacrine process by the bipolar terminal results in an inhibitory synaptic feedback on the bipolar terminal by the amacrine process; this would reduce the sensitivity or gain of the bipolar cell in proportion to the amount of excitation of that bipolar cell. This arrangement could perhaps provide a mechanism for the adaptation of bipolar cells.

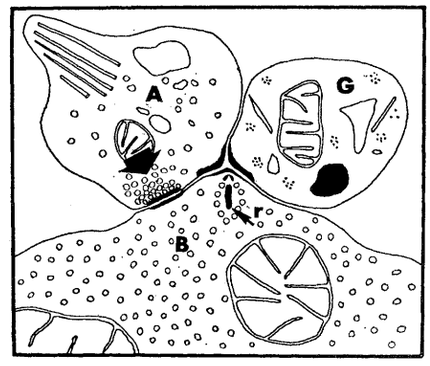


Fig. 8. A diagram of the reciprocal synaptic contacts between bipolar-cell terminals (*B*) and amacrine-cell processes (*A*) in the primate retina. At the synaptic ribbon (*r*), the bipolar terminal makes a dual synaptic contact with a ganglion-cell dendrite (*G*) and an amacrine-cell process (*A*). Just lateral to the ribbon (0.5 to 1 μ away), the amacrine cell makes a reciprocal synaptic contact back onto the bipolar cell (wide arrow) (*34*).

Amacrine cells extend processes throughout the inner plexiform layer and make reciprocal contacts with bipolar cells over wide areas (0.5 to 1 mm) (*34*). The amacrine cells also make synaptic contacts with each other. Thus, the amacrine cells would seem also to fit well the spatial requirements for Rushton's adaptation pools, for these cells apparently link bipolar terminals in the inner plexiform layer in a reciprocal fashion such that activity in one bipolar cell would affect adjacent bipolar cells via the amacrine cells. Thus, when sensitivity of one bipolar is reduced, sensitivity would be similarly reduced in adjoining bipolars by means of the amacrine contacts on the bipolar terminals.

It is worth noting that reciprocal synaptic contacts similar to those between bipolar and amacrine cells in the vertebrate retina have recently been noted in an invertebrate visual system (*35*) and in the olfactory bulb (*36*). Thus, reciprocal synaptic contacts appear to be a feature of afferent pathways and may account for adaptation in sensory systems in general.

Summary

In response to background illumination, the adaptation properties of the b-wave are similar to those observed in the human eye with psychophysical methods. With increasing background luminance the b-wave sensitivity is diminished; except at the lowest background intensity the elevation of the log threshold is linearly related to the increase of background intensity, the relation having a slope of almost 1. The a-wave, however, behaves quite differently. At low background luminances it shows little adaptation. With higher background luminances the a-wave saturates, and no a-wave potential can be elicited with any stimulus intensity. The L-type S-potentials respond to background light in much the same way as the a-wave does. Thus, the b-wave is the first of the

known responses in the visual system to show typical adaptation properties. This suggests that the site of visual adaptation may be in the bi-polar-cell layer, the presumed locus of b-wave generation. Recent electron microscopic studies have demonstrated reciprocal synapses between the bipolar terminals and amacrine processes, and it is suggested that such a synaptic arrangement could account for visual adaptation by a mechanism of inhibitory feedback on the bipolar cells.

References and Notes

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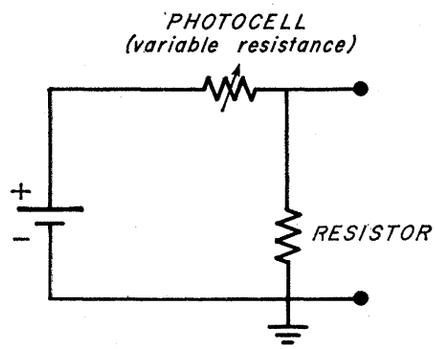
32. More recent experiments indicate that the a-wave may not show any "biological" adaptation, in the sense that the a-wave responds to background light very much like this simple photocell circuit whose output saturates at a set light intensity (illustration below).



Thus, like responses from such a circuit, the maximum a-wave potential that can be obtained while a background light is on is simply the difference between the potential evoked by the background light and the maximum a-wave response. When the background light is bright enough to give the maximum a-wave potential, no further a-wave response can be evoked with any stimulus intensity. The b-wave behaves quite differently, however. For example, with background lights bright enough to evoke the maximum b-wave, one can still elicit a b-wave with sufficiently bright stimuli. Thus, there is clearly some "adapting" mechanism associated with the b-wave that does not appear to operate on the a-wave.
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Construction of Phylogenetic Trees

A method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability.

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Biochemists have attempted to use quantitative estimates of variance between substances obtained from different species to construct phylogenetic trees. Examples of this approach include studies of the degree of interspecific hybridization of DNA (*1*), the degree of cross reactivity of antisera to purified proteins (*2*), the number of differences in the peptides from enzymic digests of purified homologous proteins, both as estimated by paper electrophoresis-chromatography or column chromatography and as estimated from the amino acid compositions of the proteins (*3*), and the number of amino acid replacements between homologous proteins whose complete primary structures had been determined (*4*). These methods have not been completely satisfactory because (i) the portion of the genome examined was often very restricted, (ii) the variable measured did not reflect with sufficient accuracy the mutation distance between the genes examined, and (iii) no adequate mathematical treatment for data from large numbers of species was available. In this paper we suggest several improvements under categories (ii) and (iii) and, using cytochrome *c*, for which much precise information on amino acid sequences is available, construct a tree which, despite our examining but a single gene, is remarkably like the classical phylogenetic tree that has been obtained from purely biological data (*5*). We also show that the analytical method employed has general applicability, as exemplified by the derivation of appropriate relationships among ethnic groups from data on their physical characteristics (*6*, *7*).

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