

First Events in Vision: The Generation of Responses in Vertebrate Rods

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Anyone who has glanced at a micrograph of a rod will be impressed by its unusual and specialized structure (preparations used to study the physiology of rods are shown in Figs. 1–3). At one end is the cylindrical photoreceptive organelle, the outer segment. Within it are stacked membrane discs that contain the integral protein rhodopsin. At the other end is the inner segment from which originates a synaptic process that is filled with vesicles and contains sites for transmitter release. Vision begins with the absorption of light in the outer segment. The subsequent events can be roughly sketched. Phototransduction ends in the outer segment with the closure of membrane channels through which sodium ions normally flow; the electrical signal produced by closing these channels spreads to the inner segment and through gap junctions into neighboring rods. As the signal spreads, it is modified by additional currents that flow through each inner segment. At the synaptic base, the modified signal closes channels through which calcium ions enter; a fall in the internal calcium concentration interrupts the continuous exocytosis of transmitter laden vesicles. A decrease in the rate at which transmitter molecules are liberated acts as a message that is communicated to adjacent neurons. In this review, I shall elaborate upon this sketch by adding in some of the interesting details.

SOLITARY RODS

The absorption of light by rhodopsin initiates biochemical events that are still not well understood (19). Their ultimate effect is to close channels in the outer-segment membrane and reduce a current that flows across this membrane (13). The alteration in current can be measured by either of two techniques. In the first, a rod attached to a small piece of retina is positioned so that its outer segment fits snugly into a large pipette and the inner segment is left protruding from the opening. The current flowing between the part drawn into the pipette and the part left outside is then measured (4). In the second, individual rods are isolated from the intact eye and maintained as solitary cells (Fig. 1). They are then penetrated by micropipettes and studied with the voltage-clamp technique

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(1, see below). The advantages of the voltage-clamp technique are that (a) it allows the membrane potential to be maintained at any value and (b) it can also be used to study currents that are not controlled by light (see below). As far as they can be compared, both techniques yield similar results.

To perform a voltage-clamp experiment, a current is continuously injected through an intracellular micropipette; the magnitude of the injected current is automatically adjusted moment by moment to maintain the membrane voltage at a constant level. Because a constant voltage is maintained, the sum of the injected and biological currents must be zero. Consequently, the injected current must exactly compensate for the alteration in biological current induced by light. An example is shown in Fig. 4. In the upper left is the normal voltage response to a flash. Initially the membrane potential was ~ -45 mV. After a flash of light, the voltage increased to a peak and then declined to a less polarized level before slowly returning to the original membrane potential. The record in the lower left shows that no current was injected during the response. At the right are records from the same cell produced by an identical stimulus during voltage-clamp conditions. In this situation, a constant voltage was maintained (upper right) by injecting a time-varying current (lower right).

Analysis of the currents observed during a voltage-clamp experiment allows insight into the phototransduction mechanism. In the upper part of Fig. 5 are superimposed the currents produced by a series of light flashes of increasing intensity. Each flash produced a graded response that reached a peak and then declined. The time at which each response reached its peak value was approximately constant (vertical line in figure). The relationship between light intensity and the amplitude of each response at this fixed time is shown in Fig. 6. As the intensity was increased, the current change became larger until a maximum value was obtained. Brighter lights did not produce a larger response. The line is the best fit of a Michaelis equation to the observed values. This equation describes the relationship between light intensity and the amplitude of each response at the moment it reaches its peak. The same equation can also be used to approximately describe the amplitudes at any other time following a flash (1). Therefore, the amplitude at each moment during the response to a flash can be described by assuming the transient activation of a

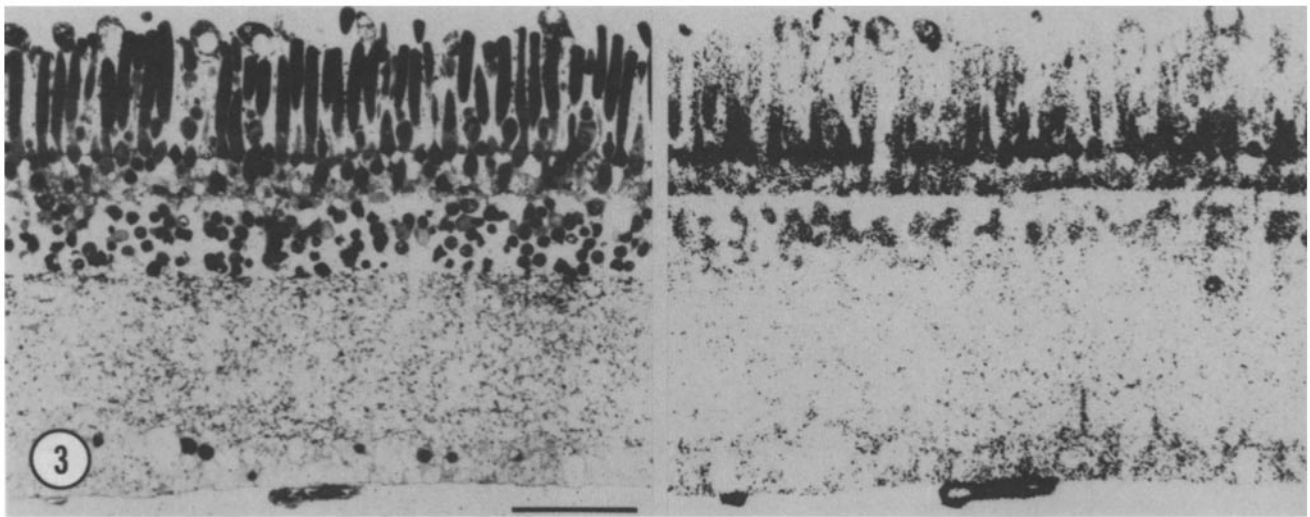
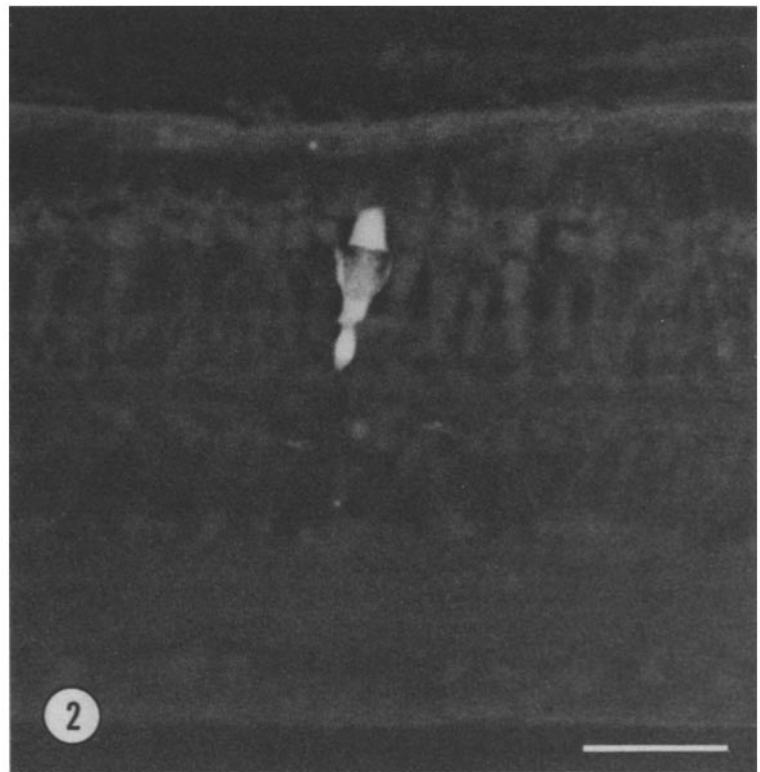
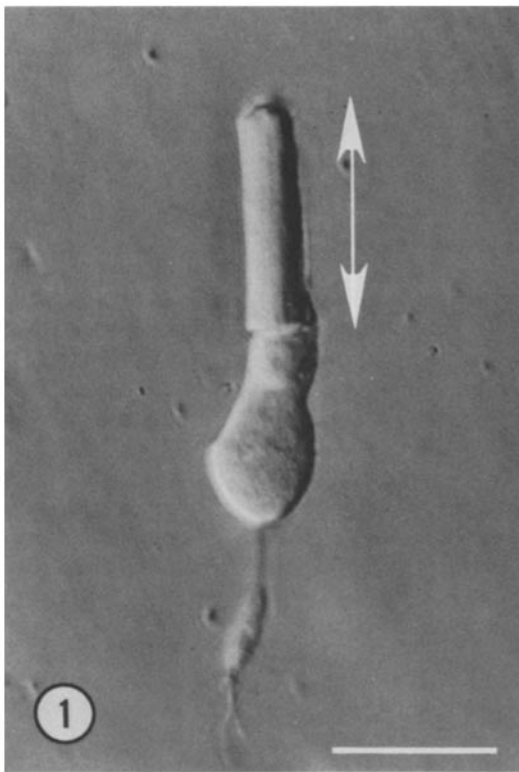


FIGURE 1 A solitary rod isolated from the eye of the salamander, *Ambystoma tigrinum*. Arrows indicate the outer segment; below is the inner segment from which dangles a synaptic process. From Fig. 1 of reference 2. Bar, 20 μ m.

FIGURE 2 A cross section of a retina from the turtle, *Pseudemys scripta elegans*. The section was photographed with dark-field illumination and contains a rod that had been injected with the fluorescent dye Procion Yellow M4R. The section is oriented with the pigment epithelium at the top and the vitreal surface at the bottom. From plate 1 of reference 21. Bar, 50 μ m.

FIGURE 3 A cross section of a retina taken from the toad, *Bufo marinus*, and treated to produce the selective survival of photoreceptor cells. Afterward, the preparation was cultured for 24 h in a mixture of 15 [3 H]amino acids. Subsequently, the preparation was processed for histology and autoradiography. A photograph of autoradiographically developed silver grains is at the right, and a photograph taken after the grains were removed and the section stained is at the left. A small blood vessel is at the vitreal surface.² Bar, 50 μ m.

biochemical system that does not change its basic properties during the entire response. It is not necessary to assume a large autocatalytic or adaptational change in the underlying events. Instead, a prominent adaptation that does change the shape of the response arises from another source (see below).

The signal generated in the outer segment can spread to the

inner segment without significant attenuation. When voltage-dependent currents (see below) are poisoned, and inner and outer segments simultaneously penetrated by micropipettes, the difference in response is only 2% (1). A high membrane resistance insures that little loss of amplitude occurs as a signal spreads from the outer to the inner segment. In some species,

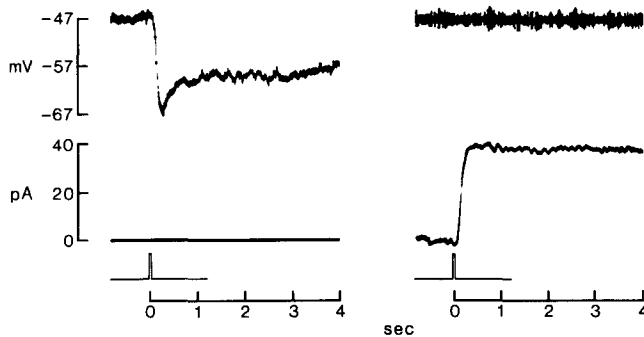


FIGURE 4 Voltage clamp of the response produced by light. The normal voltage response to a bright flash is shown at the upper left. No current was injected during the response (as indicated by the lower left trace). When the same stimulus was applied during voltage clamp, the membrane voltage was maintained constant (upper right) by injecting a time varying current (lower right). The timing trace below each pair of records indicates the duration of the flash. From Fig. 3 of reference 1.

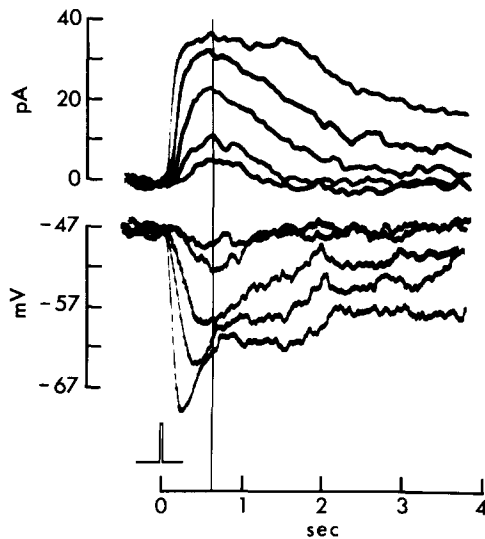


FIGURE 5 Comparison of the normal voltage response and the light-suppressed current measured during voltage clamp. Superimposed in the lower part of the figure are the normal voltage responses to a series of flashes in which the intensity was increased $\times 4$ for each successive trial. Superimposed above are the light-suppressed currents for the same series of stimuli. From Fig. 5 of reference 1.

such as the turtle, synapses are formed at the base of the inner segment; in other species, such as the salamander, synapses are formed at the end of a synaptic pedicle.

The signal generated by a change in current flow through the outer segment is modified as it spreads to the inner segment. Comparison of the two sets of records in Fig. 5 reveals that current and voltage responses have different time-courses. The difference is most apparent in the responses to the brightest flash, where the voltage has an initial transient followed by a decline to a plateau that is absent from the current record. The distinctive shape of the voltage response is due to the effect of additional currents. These currents are controlled by the membrane voltage and produced by channels (or carriers) in the inner-segment membrane.¹ During a voltage-clamp experi-

¹ C. R. Bader, D. Bertrand, and E. A. Schwartz. Manuscript in preparation.

ment, a constant voltage is maintained and these currents do not intervene. During the normal response to light, the voltage changes and these currents have a large effect. Their influence can be clearly demonstrated by injecting steps of constant current (Fig. 7). Hyperpolarizing current elicits a voltage that resembles the response following a flash. Both have an initial transient followed by a decline to a plateau of lesser polarization.

INTACT RETINA

Experiments with solitary rods identify the processes that are intrinsic to a single rod. To understand the complete activity of a rod, it is necessary to record from cells in the intact retina. For these experiments, the eye of a turtle was placed in a chamber so that a micropipette could be advanced into the retina and a rod impaled. To verify that the micropipette had impaled a rod, the pipette was often filled with a dye which was injected into the cell after recording responses to light. Subsequently, the retina was processed for histology and the dye-filled cell identified. An example is shown in Fig. 2. The responses recorded from rods in the intact retina did not always resemble the responses of solitary rods. In particular, the response depended upon the area of retina illuminated (Fig. 8). When a small diameter spot was enlarged, the response grew in size and changed in shape, indicating that there were synaptic inputs from neighboring cells (22). By stimulating with lights of different colors, it was possible to take advantage of the difference in spectral sensitivity between rods and cones to show that a major part of the synaptic input comes from distant rods (23) and a minor part comes from cones (24).

The synaptic mechanism for the component that originates in cones is still not well understood. In contrast, the interaction between the rods themselves has been intensively studied. The distance over which this interaction occurs has been determined by measuring the amplitude of responses to monochromatic, dim spots of increasing diameter (7, 12, 17, 23, 25). In Fig. 9, the dotted line indicates the expected result if responses were determined only by light which impinged upon the impaled rod (the response would change with spot size because intensity at the center of small spots is attenuated by scatter within the retina). Responses from rods (open symbols) increase in amplitude until the spots are $>250 \mu\text{m}$ in radius, a distance much greater than can be explained by light scatter. The large

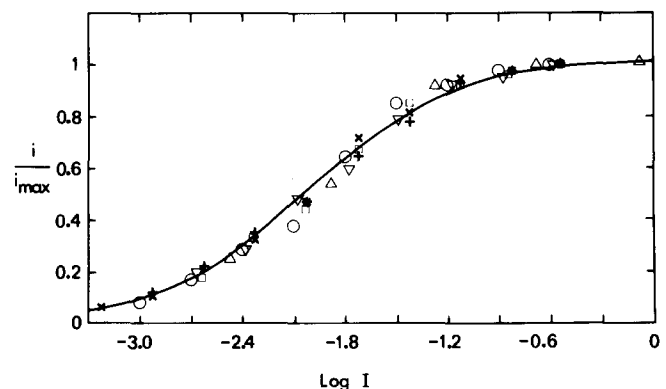


FIGURE 6 The normalized amplitude of the light-suppressed currents are plotted as a function of the logarithm of the flash intensity. Data from six cells. The data from individual cells have been adjusted laterally to bring the data from all six cells into coincidence along a common curve. The continuous line is the best fit of a Michaelis equation. From Fig. 7 of reference 1.

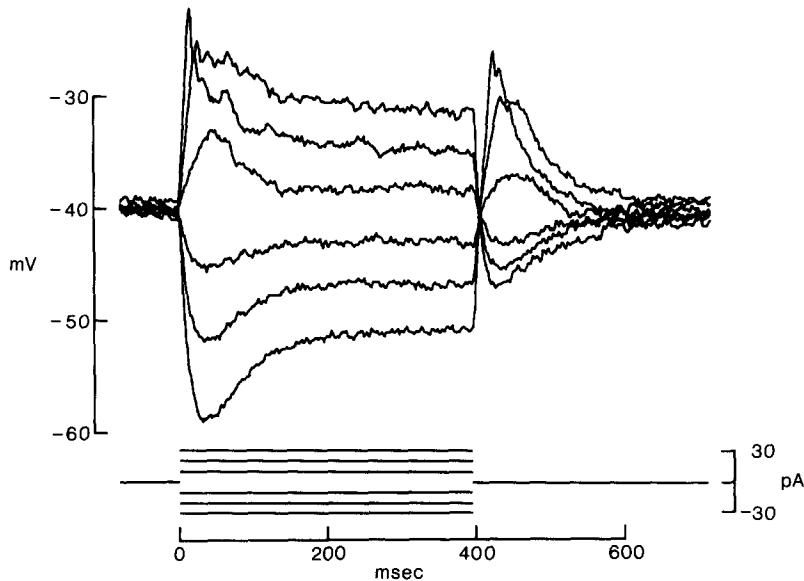


FIGURE 7 Superimposed are six responses to steps of current injected into a solitary rod. The amplitudes of the current are shown below. There is a time-dependent change in the voltage during a constant current step. The time course of the voltage during a hyperpolarizing step resembles the normal response to light.¹

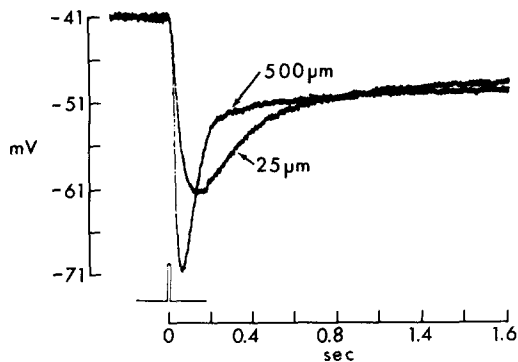


FIGURE 8 Superimposed are responses recorded when a rod in the intact retina was stimulated with bright lights covering either a small or large area. The diameter of each spot is indicated next to each trace. Both spots delivered the same quantity of light to the impaled rod. From Fig. 1 of reference 24.

distance indicates that each rod must interact with many of its neighbors. For comparison, the closed symbols plot the results of similar measurements on three cones. Their responses increase until the spots are $75 \mu\text{m}$ in radius, slightly more than can be attributed to scatter. Consequently, a cone interacts with only a few of its neighbors (see also reference 3).

The interaction between rods can be explained by electrical connections. Evidence for electrical synapses comes in part from experiments in which chemical transmission was blocked (25). This can be accomplished by continually superfusing a retina with a salt solution that lacks calcium but contains cobalt, an ion that blocks calcium influx (see below) and transmitter release. Although chemical transmission between photoreceptor and second-order cells is blocked by this solution (6, 15, 25), the amplitude and shape of responses recorded from rods are unmodified (25). Additional evidence for electrical connections comes from electron microscopic studies demonstrating the presence of gap junctions in some species (8, 11).

Electrical synapses and voltage-dependent currents can explain why the responses produced by small and large diameter

spots differ in amplitude and shape. When a retina is uniformly illuminated all rods produce the same voltage response and therefore no current flows between rods. Consequently, each rod behaves as though it is isolated and the response to a large spot resembles that produced by solitary rods. Both have an initial transient followed by a plateau of lesser polarization. This characteristic shape is produced by the contribution of voltage-dependent currents. In contrast, when only one rod is stimulated, adjacent rods have different voltages and current can flow across the electrical connections between neighboring rods. The spread of current polarizes the neighboring rods. Thus, there is a decremting wave of polarization that spreads from the illuminated rod. Because the current change produced by the absorption of light in one outer segment is shared by a group of adjacent rods, the amplitude of the voltage in each is much smaller than would be produced by uniform illumination (the absorption of one photon in a single turtle rod produces a response of $30 \mu\text{V}$; the simultaneous absorption of one photon in every rod produces an estimated response of $300 \mu\text{V}$ (7, 23)). Consequently, a much smaller change in voltage-dependent currents occurs when only one rod is stimulated, and the effect of these currents is also attenuated by spread to neighbors. Hence, voltage-dependent currents influence the polarization produced by uniform illumination but scarcely modify the voltage response generated when only one rod is stimulated. Thus, it appears that an early step in information processing takes place at the photoreceptor level by taking advantage of voltage-dependent mechanisms and electrical coupling (10, 25).

A consequence of electrical coupling and voltage-dependent currents is a neural adaptation that attenuates the responses to dim, large objects but allows full sensitivity to small objects. The effect is illustrated in Fig. 10. The experiment involves a quantitative comparison of the responses produced by dim lights. In *A* is the response to the flash of a dim, small diameter spot. The intensity of the flash was sufficiently dim so that the response amplitude was proportional to the light intensity. For the response in *B*, the spot was attenuated $\times 25$ and delivered as a step. The intensity was attenuated so that the response would remain only a few millivolts in size. If each photon

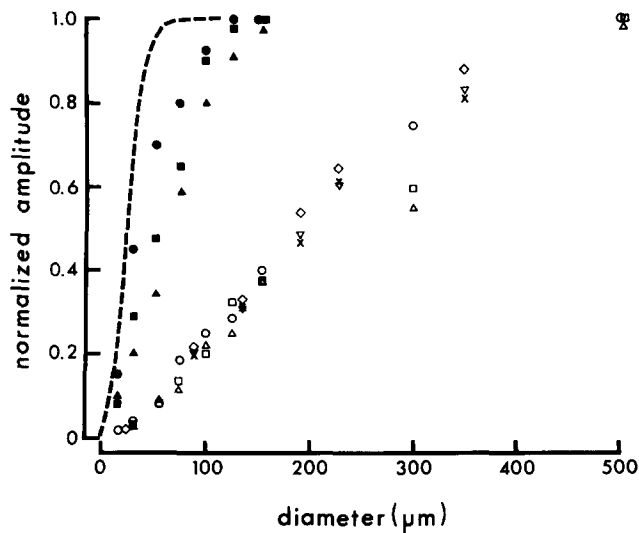


FIGURE 9 The amplitude of rod and cone responses depends upon the area of retina illuminated. Average amplitudes were measured for dim, monochromatic stimuli. Open symbols are for rods; filled symbols are for cones; dotted line indicates expected amplitude in the absence of interaction. Adapted from Fig. 5 of reference 22.

delivered during the step made an identical contribution, then the response to the step should be predicted by the integral of the response to a flash (after, of course, correcting for the difference in light intensity). This is in fact the case. Consequently, no adaptation occurs because each photon continues to contribute the same response. However, the situation observed with a large diameter spot is different. In *C* and *D* are responses recorded from the same cell when the stimulus was a large diameter spot that was half as bright. The response to the flash of a large spot is shown in *C*. For comparison, the response produced by the flash of a small spot has been rescaled (closed circles) to account for the effect of electrical coupling and the change in light intensity (the method is described in the original references). The shapes of the observed and predicted responses differ because of voltage-dependent currents which are initiated by even small degrees of polarization. Their cumulative effect is illustrated by the record in *D* obtained when the large diameter stimulus was attenuated $\times 25$ and delivered as a step. Also shown is the integral of the response to a flash (line of open circles) and the integral of the response predicted to occur in the absence of voltage-dependent currents (line of closed circles). The observed response diverges from both curves. The deviation cannot be attributed to photochemical adaptation in the outer segments of the illuminated rods, for a light twice as bright (as shown above) gave no evidence of photochemical adaptation. Instead, the deviation is due to voltage-dependent currents that grow in size during prolonged polarization and, together with electrical synapses, are able to produce a neural adaptation that depends upon the area of retina illuminated.

A CALCIUM CHANNEL

So far, I have described the direct electrical effect of light and how this signal is modified by voltage-dependent currents and electrical coupling. Next, I would like to consider how the composite signal is transmitted to second-order neurons. Charles Bader, Danielle Bertrand, and I have begun to study the mechanism of transmitter release by measuring the calcium

current that regulates the exocytosis of synaptic vesicle. The membrane current carried by calcium can be recorded in solitary inner segments with the voltage-clamp technique (Fig. 11). Our approach was to block other currents with pharmacologic agents and then to measure the current flowing through the membrane at a series of different membrane voltages. Afterward, the solution bathing the cell was changed to one that lacked calcium but contained cobalt, and the current-

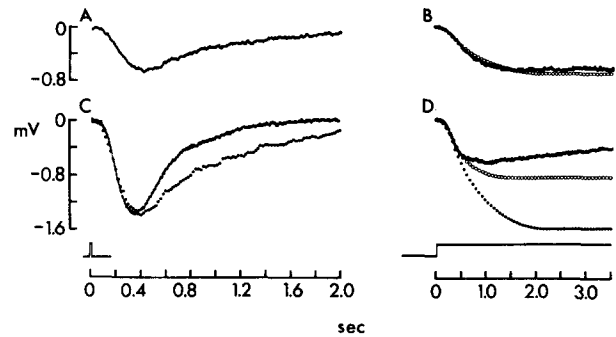


FIGURE 10 A neural adaptation occurs during illumination with a large diameter spot. *A*: response to a dim, small-diameter spot delivered as a flash. *B*: response when the small-diameter spot was further dimmed $\times 25$ and delivered as a step; open circles are the integral of the response to a flash (*A*) after correcting for the change in light intensity. *C*: response to a large-diameter spot of half the intensity used previously; closed circles replot the responses produced by the small spot after correcting for the difference in light intensity and the effect of electrical coupling. This is the response expected to occur in the absence of voltage-dependent currents. *D*: response when the large-diameter spot was further dimmed $\times 25$ and delivered as a step; open circles are the integral of the response to a flash (*C*) after correcting for the change in light intensity; closed circles are the integral of the response that is predicted to occur in the absence of voltage-dependent current. Responses are computer averages obtained from one rod. Adapted from Fig. 7 of reference 22.

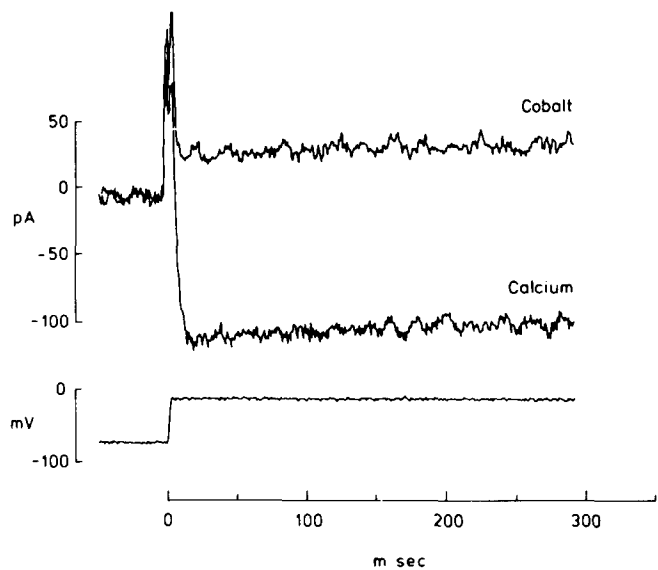


FIGURE 11 Measurement of the calcium current during a voltage clamp. The voltage was stepped from -70 to 0 mV (lower trace). After the change in voltage there was first a surge of current, required in part to charge the membrane capacity, and then a new maintained value. When the experiment was repeated in a medium in which cobalt replaced calcium, a different maintained value was observed.¹

voltage relationship was remeasured. Cobalt blocks the calcium current. The current observed at different membrane potentials in calcium and cobalt-containing solutions is plotted in the upper part of Fig. 12. The difference between the current observed during the two conditions, the calcium current, is plotted in the middle part of Fig. 12. In this case, the current approaches a null value at $\sim +48$ mV, and this value has been taken as the approximate reversal potential, E_{Ca} , for the calcium current. If each current value I observed at a voltage V is divided by $V - E_{Ca}$, then it is possible to define a conductance in analogy with Ohm's Law. The calculated values of the conductance are plotted against the membrane voltage in the lower part of Fig. 12. The sigmoid curve describes how the calcium conductance changes with membrane potential. Rods in darkness have a membrane potential of ~ -40 mV. Light increases their membrane polarization. Consequently, their membrane potential normally rests near the bottom of the sigmoid curve; and light polarizes them so that they reduce the constant, small flow of calcium that enters. Hence, the release of transmitter is continuous in the dark and stopped when the cell is hyperpolarized. A considerable amount of circumstantial evidence obtained from experiments in the intact retina is in accord with continuous release during darkness (6, 15, 25, 26).

PHOTORECEPTOR TRANSMITTERS

The identities of the compounds that are released when calcium enters have been the subject of frequent study. Several possibilities have been suggested, in particular, the amino acids aspartate and glutamate. It has been known for 25 years that these agents have little effect on photoreceptors but eliminate the responses to light of postsynaptic neurons (14). Several studies have shown that these agents produce a continuous stimulation of postsynaptic neurons (5, 20, 21, 27). Although these agents mimic the natural transmitter, nevertheless, several difficulties have prevented the acceptance of either molecule as a true transmitter. First, it has not been possible to demonstrate the release of either compound from photoreceptors. Second, all physiological experiments have required a very high concentration (on the order of 10 mM) of these agents to produce a significant response. Therefore, the possibility of release and a postsynaptic effect at a physiological concentration have been recently investigated. Transmitter release was studied using a preparation of "isolated" photoreceptors obtained by culturing intact toad retina in a mixture of toxins.² To determine which cells survived and were metabolically active, preparations were then incubated for 24 h in a medium that contained 15 ³H amino acids. Afterward, retinæ were processed for histology and autoradiography (Fig. 3). The micrograph at the left, photographed after silver grains were removed and the section was stained, shows the histology of the preparation. The photoreceptors appear normal, but most other cells are lysed. At the right is a micrograph of the same section taken before the removal of silver grains and staining. Comparison of the two micrographs shows that silver grains are densely distributed over the cell bodies and synaptic pedicles of photoreceptors. Far fewer grains are localized over some bipolar cell somata. Hence, following culture in the selective medium, photoreceptors preferentially incorporate radioactivity from a broad mixture of amino acids.

² A. M. Miller and E. A. Schwartz. Evidence for aspartate and glutamate as transmitters released by photoreceptors of the toad retina. Manuscript in preparation.

Isolated photoreceptors were incubated with radioactive aspartate. A report that the neuronal, "high-affinity" uptake of aspartate does not distinguish between D and L isomers (9) suggested incubating isolated photoreceptors with [¹⁴C]L-aspartate and [³H]D-aspartate. It was expected that the D isomer would be less readily metabolized. After incubation, individual preparations were first superfused with normal medium (1.5 mM K, 3 mM Ca), a medium with an elevated potassium concentration (41.5 mM K, 3 mM Ca), for the purpose of depolarizing the photoreceptors, and finally a medium in which cobalt was substituted for calcium (41.5 mM K, 3 mM Co) for the purpose of terminating Ca-mediated release. The superfusion fluid was collected during sequential 1-min intervals. An aliquot from each 1-min sample was subjected to cation-exchange high-pressure liquid chromatography (HPLC). Examples of chromatograms of samples collected from one preparation during superfusion with normal, high potassium, and cobalt media are shown in Fig. 13. Radioactivity derived from both D- and L-aspartate appears in a peak at fraction 24–28. The material of this peak coeluted with authentic aspartate in cation-exchange HPLC, and its dansyl derivative coeluted with authentic dansyl-aspartate in reverse-phase HPLC. Thus, this material is identified as aspartate. The amount of recovered aspartate is small in the chromatogram of a sample collected during superfusion with the normal medium (upper chromatogram), increased in the chromatogram of a sample collected when the superfusion medium contained an elevated potassium concentration (middle chromatogram), and decreased in the chromatogram of a sample collected after calcium was replaced with cobalt (lower chromatogram). More complete information is shown in Fig. 14. The data are from the experiment also illustrated in Fig. 13. For each chromatogram the amount of ³H and ¹⁴C radioactivity in fractions 24–28 was measured. These values are plotted along the ordinate against the time at which the individual sample was collected. The amounts of both labels were increased during superfusion with an elevated

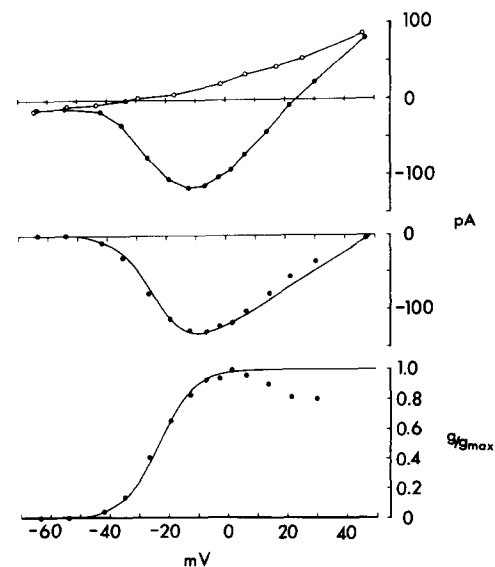


FIGURE 12 Current-voltage relation of the calcium current. Above, the maintained current observed in calcium (closed symbols) and cobalt medium (open symbols) is plotted as ordinate against voltage as abscissa. Middle: the difference between the two curves is plotted to show the calcium current. Below: the calcium conductance, g , was normalized by its maximum value and plotted against membrane voltage.¹

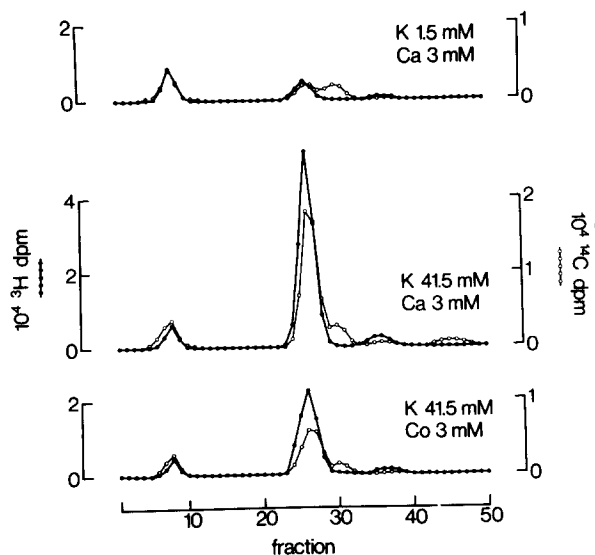


FIGURE 13 Isolated photoreceptors were incubated with [^3H]-D-aspartate and [^{14}C]-L-aspartate and subsequently superfused with three media: normal (1.5 mM K, 3 mM Ca), high potassium (41.5 mM K, 3 mM Ca), and one in which cobalt replaced calcium (41.5 mM K, 3 mM Co). The superfusate was collected during sequential 1-min intervals. Each sample was subjected to cation exchange high pressure liquid chromatography. Examples of chromatograms of individual samples are shown. Authentic aspartate eluted in fractions 24–28.

potassium concentration and reduced when calcium was replaced with cobalt. Similar experiments have demonstrated that glutamate is also released from isolated photoreceptors.

A postsynaptic effect of aspartate and glutamate was demonstrated by using a preparation of “isolated” horizontal cells. These cells normally receive synapses from photoreceptors. The preparation was obtained by a selective culture method.³ A considerable amount of indirect evidence indicates that horizontal cells use gamma-aminobutyric acid (GABA) as a transmitter (for example, references 16 and 18). The ability of aspartate and glutamate to release GABA from horizontal cells was used as an assay for a postsynaptic effect. Accordingly, isolated horizontal cells were first loaded with [^3H]GABA and [^{14}C]glycine and then placed on a small filter and superfused to detect the leakage of labeled molecules. Glycine was included to indicate any nonspecific effect. In Fig. 15 are plotted the amounts of ^3H (closed circles) and ^{14}C (open circles) recovered in the superfusate during sequential 1-min intervals. The addition of $1\ \mu\text{M}$ L-glutamate to the superfusion fluid produced no change in efflux; $0.1\ \text{mM}$ produced a moderate increase in GABA efflux; and $10\ \text{mM}$ produced a large increase. L-aspartate produced effects that were similar to those produced by L-glutamate. Hence, both aspartate and glutamate are released and have a significant postsynaptic effect at $0.1\ \text{mM}$. This concentration is ~ 10 higher than that in extracellular brain fluid but is comparable to the concentration estimated to occur when other transmitter compounds are released into a synaptic cleft.

SUMMARY

The generation of responses in vertebrate rods involves a sequence of events that is now partly known. During darkness,

³ E. A. Schwartz. Calcium-independent release of GABA from isolated horizontal cells of the toad retina. Manuscript in press.

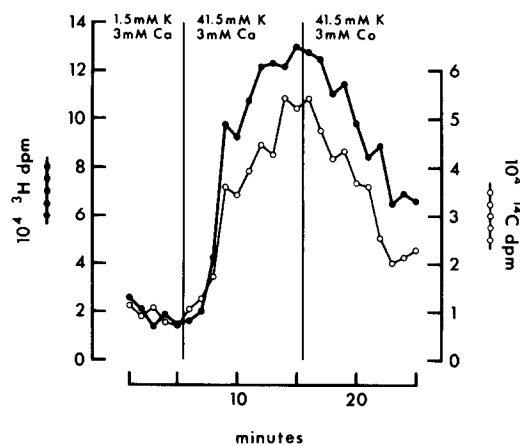


FIGURE 14 Amount of radioactivity in fractions 24–28 for each sample collected during the experiment illustrated in Fig. 13.

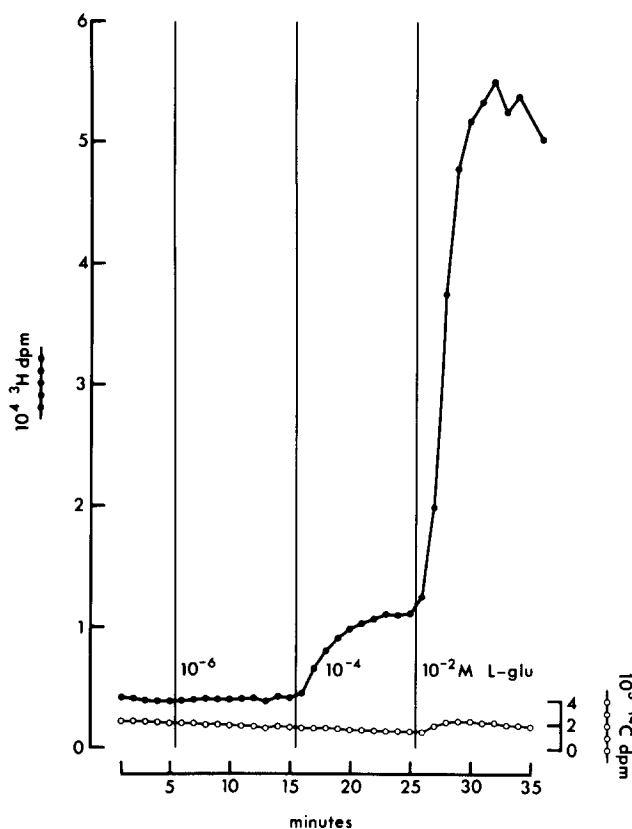


FIGURE 15 Effect of L-glutamate on the release of GABA from isolated horizontal cells. The preparation was incubated with [^3H]GABA and [^{14}C]glycine and then superfused to detect the leakage of labeled molecules. Samples were collected during sequential 1-min intervals. The amounts of ^3H (closed circles) and ^{14}C (open circles) recovered in each sample are plotted against time. The superfusing fluid was initially minimal medium. After the fifth minute, L-glutamate was added in three increasing concentrations as indicated in the figure.

a sodium current enters the outer segment (Fig. 16A, current path 1) and keeps a rod depolarized. Absorbed photons transiently reduce this current. If we assume that every photon initiates the same intracellular effect, then we can predict the current change produced by many photons by using a Michaelis equation. Diminution of the current hyperpolarizes a rod. The final polarization produced depends upon whether one or many rods are illuminated. When only one rod is illuminated

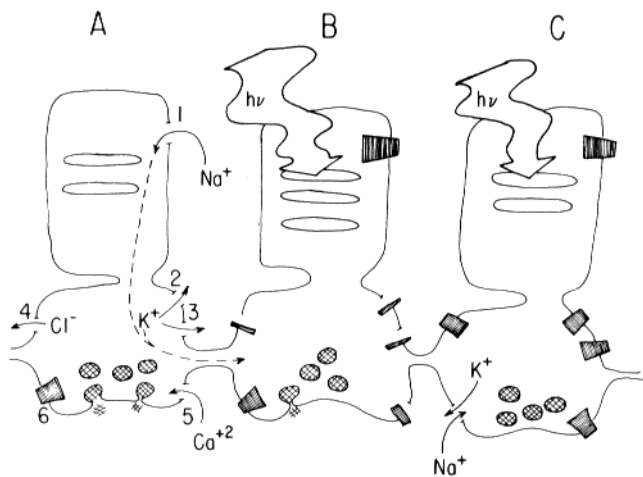


FIGURE 16 A cartoon to illustrate the physiology of rods. **A:** situation in darkness; **B:** when only one rod absorbs light ($h\nu$); **C:** when many neighboring rods are uniformly illuminated. Sodium entry into the outer segment (current path 1) is regulated by the photo-transduction process. Several channels (or carriers, current paths 2-6) of the inner segment are either voltage- or calcium-activated.¹ Gap junctions connect neighboring rods. See text.

(Fig. 16 B), current flows from unilluminated neighbors across gap junctions and enters the illuminated cell; thereby, the polarization initiated by light is reduced. At the same time, neighboring, unilluminated rods are polarized. Thus, the absorption of light in only one rod produces a potential of reduced amplitude that spreads to a cluster of adjacent rods. In contrast, when many rods are illuminated, all have the same voltage and, therefore, no current flows between them. In this situation, the voltage produced in each rod is not reduced by current entering from neighbors and therefore is larger than when only one rod adsorbs light. The large change in potential is sufficient to produce a large effect on voltage-dependent currents of the inner segment (Fig. 16 C: current paths 2-5 are closed and 6 is opened¹).

In the absence of voltage-dependent currents, dim light absorbed by one rod would produce the same change in transmitter release whether or not rods are coupled. Without coupling, the illuminated cell alone would change its rate of transmitter release, whereas, because of coupling, each of a cluster of neighboring rods produces a smaller change. If the change in transmitter release expected from each of the neighboring rods is summed, then the total change in transmitter release in the two cases would be the same (25). However, in the presence of voltage-dependent currents, responses to large spots are smaller than otherwise anticipated. This will reduce the voltage and should have the effect of a neural adaptation selectively produced by large spots of light.

Normally, during darkness, a rod is depolarized and several channels of the inner segment are opened. Among these are a calcium channel (current path 5). The influx of calcium is believed to promote the exocytosis of synaptic vesicles. Light stops the current entering the outer segment, which in turn

causes hyperpolarization of the inner segment, closure of calcium channels, and a decrease in the exocytosis of transmitter vesicles. Evidence for identifying synaptic transmitters comes from experiments in which retinæ are cultured under conditions that allow only selected cell types to survive. Aspartate and glutamate are released from photoreceptors and have a post-synaptic effect on horizontal cells at a physiological concentration. Because a preparation of isolation photoreceptors includes both rods and cones, it has not yet been possible to associate each compound with an individual photoreceptor type.

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REFERENCES

- Bader, C. R., P. R. MacLiesh, and E. A. Schwartz. 1979. A voltage-clamp study of the light response in solitary rods of the tiger salamander. *J. Physiol. (Lond.)*, 296:1-26.
- Bader, C. R., P. R. MacLiesh, and E. A. Schwartz. 1978. Responses to light of solitary rod photoreceptors isolated from the tiger salamander retina. *Proc. Natl. Acad. Sci. U. S. A.* 75:3507-3511.
- Baylor, D. A., M. G. F. Fuortes, and P. M. O'Bryan. 1971. Receptive fields of cones in the retina of the turtle. *J. Physiol. (Lond.)*, 214:265-294.
- Baylor, D. A., T. D. Lamb, and K. W. Yau. 1979. The membrane current of single rod outer segments. *J. Physiol. (Lond.)*, 288:589-611.
- Cervetto, L., and E. F. MacNichol, Jr. 1972. Inactivation of horizontal cells in the turtle retina by glutamate and aspartate. *Science (Wash. D. C.)*, 178:767-768.
- Cervetto, L., and M. Piccolino. 1974. Synaptic transmission between photoreceptors and horizontal cells in the turtle retina. *Science (Wash. D. C.)*, 183:417-419.
- Copenhagen, D. R., and W. G. Owen. 1976. Functional characteristics of lateral interactions between rods in the retina of the snapping turtle. *J. Physiol. (Lond.)*, 259:251-282.
- Custer, N. V. 1973. Structurally specialized contacts between the photoreceptors of the retina of the axolotl. *J. Comp. Neurol.* 151:35-55.
- Davies, L. P., and G. A. R. Johnston. 1976. Uptake and release of D- and L-aspartate by rat brain slices. *J. Neurochem.* 26:1007-1014.
- Detwiler, P. B., A. L. Hodgkin, and P. A. McNaughton. 1978. A surprising property of electrical spread in the network of rods in the turtle's retina. *Nature (Lond.)*, 274:562-565.
- Fain, G. L., G. H. Gold, and J. E. Dowling. 1976. Receptor coupling in the toad retina. *Cold Spring Harbor Symp. Quant. Biol.* 40:547-561.
- Gold, G. H. 1979. Photoreceptor coupling in retina of the toad, *Bufo marinus*. II. Physiology. *J. Neurophysiol. (Bethesda)*, 42:311-328.
- Hagins, W. A., R. O. Penn, and S. Yoshikami. 1970. Dark current and photocurrent in retinal rods. *Biophys. J.* 10:380-412.
- Furukawa, T., and I. Hanawa. 1955. Effects of some common cations on electroretinograms of toad. *Jpn. J. Physiol.* 5:289-300.
- Kaneko, A., and H. Shimazaki. 1975. Effects of external ions on the synaptic transmission from photoreceptors to horizontal cells in the carp retina. *J. Physiol. (Lond.)*, 252:509-522.
- Lam, D. M. K., E. M. Lasater, and K. I. Naka. 1978. γ -Aminobutyric acid: a neurotransmitter candidate for cone horizontal cells of the catfish retina. *Proc. Natl. Acad. Sci. U. S. A.* 75:6310-6313.
- Leeper, H. F., R. A. Norman, and D. R. Copenhagen. 1978. Evidence for passive electrotonic interactions in red rods of toad retina. *Nature (Lond.)*, 275:234.
- Marc, R., W. K. Stell, D. Bok, and D. M. K. Lam. 1978. GABA-ergic pathways in the goldfish retina. *J. Comp. Neurol.* 182:221-245.
- Miller, W. H., editor. 1981. Molecular Mechanisms of Photoreceptor Transduction. Academic Press, Inc., New York.
- Murakami, M., K. Ohtsu, and T. Ohtsuka. 1972. Effects of chemicals on receptors and horizontal cells in the retina. *J. Physiol. (Lond.)*, 227:899-913.
- Murakami, M., T. Ohtsuka, and H. Shimazaki. 1975. Effects of aspartate and glutamate on the bipolar cells in the carp retina. *Vision Res.* 15:456-458.
- Schwartz, E. A. 1973. Responses of single rods in the retina of the turtle. *J. Physiol. (Lond.)*, 233:503-514.
- Schwartz, E. A. 1975. Rod-rod interaction in the retina of the turtle. *J. Physiol. (Lond.)*, 246:617-638.
- Schwartz, E. A. 1975. Cones excite rods in the retina of the turtle. *J. Physiol. (Lond.)*, 246:639-651.
- Schwartz, E. A. 1976. Electrical properties of the rod syncytium in the retina of the turtle. *J. Physiol. (Lond.)*, 257:379-406.
- Trifonov, Yu. A. 1968. Study of synaptic transmission between the photoreceptor and the horizontal cell using electrical stimulation of retina. *Biofizika*, 13:809-817.
- Wu, S. M., and J. E. Dowling. 1978. L-aspartate: evidence for a role in cone photoreceptor synaptic transmission in the carp retina. *Proc. Natl. Acad. Sci. U. S. A.* 75:809-817.