[12] Mechanisms of Single-Photon Detection in Rod Photoreceptors

By FRED RIEKE

Introduction

At low light levels the visual system detects and counts photons with a reliability limited by statistical fluctuations in the number of absorbed photons and photoreceptor noise. This remarkable sensitivity is not merely an obscure laboratory phenomenon; reliable photon detection is crucial for normal rod vision, much of which occurs at light levels where individual rod photoreceptors receive photons rarely. The rod's ability to detect single photons has been appreciated for many years. In the 1940s, Hecht *et al.*[1] found that dark-adapted humans could see flashes producing fewer than 10 absorbed photons spread over an area of the retina containing about 500 rods. This sensitivity is possible only if the rods detect single photons. Subsequent behavioral experiments by Sakitt[2] suggest that the rods produce distinguishable responses to zero, one, and two absorbed photons, permitting the visual system to count photon absorptions. Sakitt's work also provides an estimate of the dark noise that limits absolute visual sensitivity.

Behavioral measurements of the sensitivity of rod vision guide studies of the biophysical and biochemical mechanisms of photon detection as they impose stringent constraints on how single photons are transduced by the rods and how the resulting signals are processed within the retina. Three important constraints on phototransduction are: (1) the single-photon response must be amplified to produce a macroscopic electrical signal from activation of a single rhodopsin molecule; (2) the dark noise in the rod's phototransduction cascade must not consume the single-photon response and render it undetectable; and (3) individual single-photon responses must have similar shapes so that one photon can be reliably distinguished from two. Although the ability of the visual system to detect and count photons has been appreciated for many years, only in the last 10–15 years have we begun to understand the mechanisms that permit the rod to meet these functional requirements. This chapter describes some of the experimental and theoretical methods that have made

[1] S. Hecht, S. Shlaer, and M. Pirenne, *J. Gen. Physiol.* **25,** 819 (1942).
[2] B. Sakitt, *J. Physiol.* **233,** 131 (1972).

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this progress possible and points out some of the important unresolved issues.

Experimental Methods

Three experimental challenges in studying the rod's single-photon response are as follows: (1) keeping the cells fully dark adapted through all steps in the experiment, (2) developing recording techniques with the resolution to detect the single-photon response and the stability to collect enough responses to study the response statistics, and (3) interpreting the properties of the response in terms of the elements of the transduction cascade.

Tissue Preparation

Current techniques for measuring the rod's single-photon response require separating the retina from the pigment epithelium to provide direct access to the rod outer segment. Once the retina is removed from the pigment epithelium, rhodopsin can no longer be regenerated and the rods cannot fully dark adapt after bright light exposure. We maintain the cells in a dark-adapted state by performing all the necessary experimental procedures with infrared light and infrared/visible image converters. Rods are about a factor of 10^9 less sensitive to 880-nm light than to 500-nm light, but even this residual sensitivity to infrared light can cause adaptation. Toad rods begin to adapt at steady light intensities that produce 1–10 photoisomerizations (Rh*) per second[3,4]; when these relatively dim lights are extinguished the rods recover their dark sensitivity quickly and completely. Thus a conservative goal is to use infrared light that produces less than 1 Rh*/sec during all experimental procedures.

For experiments on toad or salamander rods we keep the animal in complete darkness for at least 12 hr prior to the experiment. The animal is quickly decapitated and pithed and the eyes are removed using night vision goggles with a built-in infrared illuminator (5001 Night Invader; ITT Night Vision, Roanoke, VA). All subsequent procedures are performed under a dissecting microscope (SMZ-2B; Nikon, Garden City, NY) equipped with infrared/visible converters (NiteMare 4100 Pocketscope; B. E. Meyers, Redmond, WA) and an illuminator made with an infrared LED (276-143C RadioShack, Ft. Worth, TX) whose output is collimated and

[3] D. A. Baylor, G. Matthews, and K.-W. Yau, *J. Physiol.* **309,** 591 (1980).
[4] K. Donner, D. R. Copenhagen, and T. Reuter, *J. Gen. Physiol.* **95,** 733 (1990).

filtered (RG-850 filter; Schott Glass, Duryea, PA) to eliminate any light reaching the retina with wavelength shorter than 850 nm. The intensity of the infrared light reaching the preparation corresponds to about 0.1 Rh*/sec. Each eye is hemisected under the dissecting microscope using a double-edged razor blade. The back half of each eye is then cut into several pieces and put in Ringer solution, where the retina is gently peeled from the pigment epithelium. Pieces of retina are stored in a light-tight container at 4° for up to 36 hr. Isolated rods are obtained by shredding a small piece of retina (roughly 1–2 mm^2) with fine needles in a 100-μl drop of Ringer solution. The drop is then transferred to a recording chamber mounted on the stage of an inverted microscope equipped with an infrared sensitive camera (e.g., Cohu 4815-2000; San Diego, CA). The cells are visualized using >850-nm light with an intensity in the image plane of the microscope corresponding to less than 0.5 Rh*/sec.

General Recording Issues

The basic operation of the rod is shown in Fig. 1. In darkness a circulating current carried primarily by Na^+ ions flows into the outer segment through cGMP-gated channels in the surface membrane. This circulating current sets the voltage across the cell membrane to a relatively depolarized level, about −40 mV, and causes continual release of neurotransmitter from the synaptic terminal. Incident light activates the photopigment rhodopsin, which triggers the series of biochemical reactions that make up the phototransduction cascade. The end result of activation of the transduction cascade is a reduction in the cGMP concentration, closure of channels in the surface membrane, and a decrease in the circulating current. This current decrease permits the rod to hyperpolarize and slows the rate of transmitter release from the synaptic terminal.

An important consequence of the rod's operation is that the light-induced changes in membrane voltage are a property of both the circulating current and voltage-activated conductances in the inner segment[5,6]; in contrast, the outer segment current is only weakly voltage dependent,[6] and thus provides an electrical signal controlled almost entirely by the transduction process. To measure the light response of rods the circulating current is rerouted through a current-measuring amplifier. These measurements are typically made using patch-clamp recordings from detached outer segments

[5] C. R. Bader, D. Bertrand, and E. A. Schwartz, *J. Physiol.* **331,** 253 (1982).
[6] D. A. Baylor and B. J. Nunn, *J. Physiol.* **371,** 115 (1986).

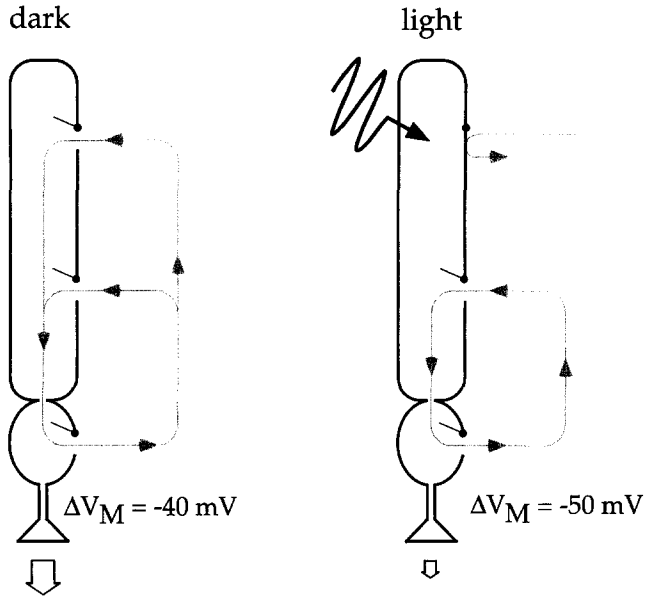

FIG. 1. Basic operation of rod photoreceptor. In darkness a circulating current flows into the outer segment and out the inner segment, depolarizing the rod and causing continual transmitter release from the synaptic terminal. This current is carried primarily by the movement of Na^+ ions, which flow down their electrochemical gradient into the outer segment and are pumped out the inner segment. Light activates the transduction cascade and suppresses the circulating current, permitting the cell to hyperpolarize and slowing transmitter release.

or suction electrode recordings from intact rods or truncated outer segments. Each recording technique faces several challenges:

The single-photon response is small: In a toad rod the single-photon response has a maximum amplitude of about 1 pA, comparable to the current flowing through a typical ion channel while it is open. Measuring this small signal is difficult, a problem that is exacerbated because it is desirable that the measured responses be free of distortions from instrumental noise and thus represent the true signals of the rod. The single-photon response can be measured accurately if the instrumental noise is considerably smaller than the cellular noise in the rod current. The cellular noise is dominated by occasional discrete photon-like events generated by spontaneous isomerization of rhodopsin and continuous current fluctuations caused by spontaneous phosphodiesterase (PDE) activation.[3,7] In a toad rod the continuous noise component has a root-mean-square amplitude

[7] F. Rieke and D. A. Baylor, *Biophys. J.* **71,** 2553 (1996).

of about 0.1 pA; this sets a practical goal for the maximum allowable instrumental noise. The limited bandwidth of the single-photon response is helpful in reaching this low noise level, as the measured currents can be low-pass filtered at 5–10 Hz without significantly affecting the light responses.

Long recordings are required to measure response statistics: To investigate how the rod generates a reproducible response to each absorbed photon it is necessary to characterize the trial-to-trial response variability and how this variability changes when the operation of the transduction cascade is altered. Measuring the variability of the single-photon response requires collecting a minimum of a few hundred responses to a fixed dim flash; the large number of responses is required in part because it is not possible to deliver a light stimulus that deterministically produces a single isomerized rhodopsin (see below). In an amphibian rod, we typically collect 200–400 responses over a period of 1–2 hr. In addition to the problems of instrumental noise already discussed, these long recordings require that the rod response does not change systematically during the course of the measurements, as such changes could be mistaken for response variability. To check stability during the recording the response to a moderate intensity flash is measured every 5–10 min; experiments are aborted if this response changes significantly. During data analysis, we reject experiments that show systematic changes in the single-photon response over time.

Interpretation of results in terms of events in transduction cascade: Electrophysiology provides a limited view of the transduction process: the single-photon current response is measured, and not the activity of the components of the transduction cascade that produce the response. Several methods are helpful in understanding how the transduction cascade shapes the measured currents; these include studies of phototransduction in transgenic mice, experiments on internally dialyzed outer segments, and modeling approaches that combine evidence from electrophysiology and biochemistry. An important characteristic of recording techniques, and one that differs between techniques, is the ability to manipulate elements of the transduction cascade.

Suction Electrode Recording

One approach to recording the rod's light-sensitive current is to draw the outer segment into a tight-fitting glass electrode (Fig. 2A). The electrode collects the current flowing into the outer segment and changes in this current in response to light can be directly monitored. Baylor *et al.*[8,9]

[8] D. A. Baylor, T. D. Lamb, and K.-W. Yau, *J. Physiol.* **288,** 589 (1979).
[9] D. A. Baylor, T. D. Lamb, and K.-W. Yau, *J. Physiol.* **288,** 613 (1979).

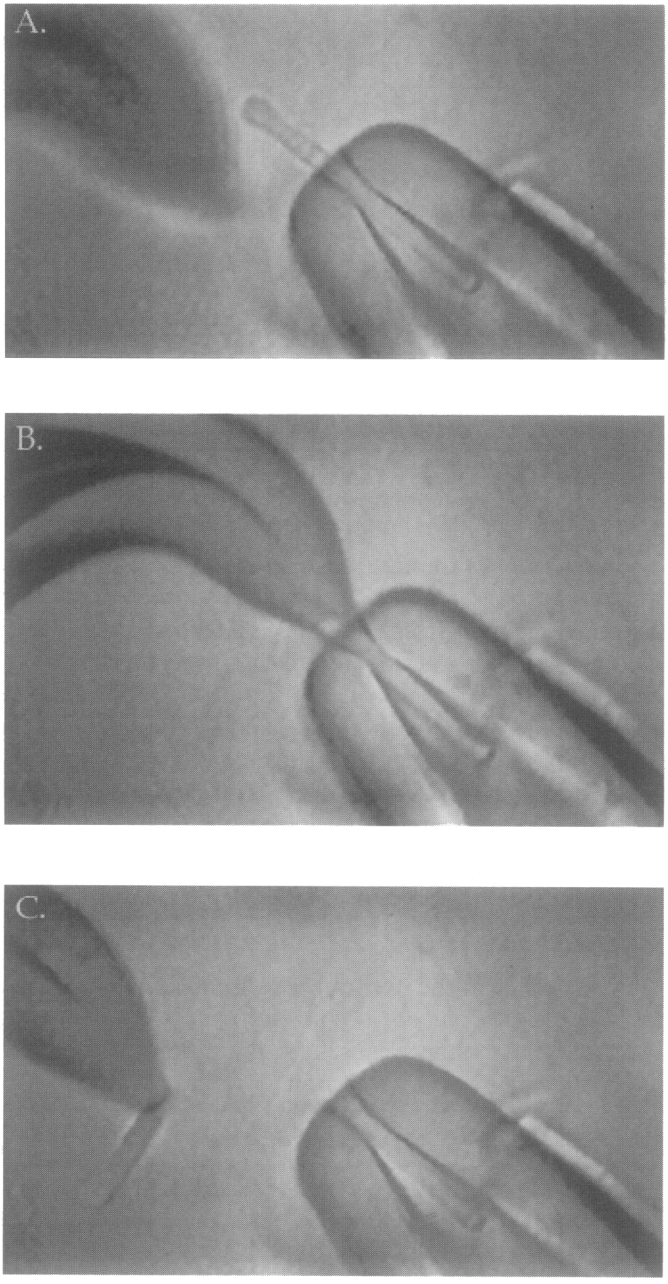

FIG. 2. Truncation of a toad rod. In truncated outer segment experiments the rod is first drawn into the suction electrode (A). The inner segment and a small piece of the outer segment are then cut off with a sharp glass probe (B), leaving the outer segment open and easily dialyzed with various solutions (C).

developed this technique and used it to make the first recordings of single-photon responses from vertebrate rods. Suction electrode recordings can be very stable, permitting a cell to be studied for several hours without a noticeable change in its responses. Suction electrode techniques also permit experiments on small cells, such as mammalian rods, that are difficult or impossible to record from using other methods.

The success of suction electrode recording depends greatly on the electrode itself. Three characteristics of a good suction electrode are a round (not oval) opening, so that the outer segment is not distorted as it is drawn in; walls that taper relatively quickly to the tip opening, so that the electrode resistance is minimized and the current is effectively collected; and a non-stick surface, so the outer segment is not damaged as it is drawn in. We make suction electrodes from borosilicate glass tubing pulled on a horizontal electrode puller. The electrodes are cut with a diamond knife to a diameter four to five times larger than the desired diameter of the final opening (e.g., for toad a 5 to 6-μm-diameter opening is used and the electrodes are cut initially to a diameter of 20–30 μm). The cut electrodes are fire polished until the opening reaches the desired size; electrodes that are more than 10% out of round are discarded. The electrodes are coated with silane to prevent the outer segment from sticking. For recording, the suction electrode is filled with Ringer solution and placed in an electrode holder with one port connected to a pressure/suction source made from glass syringes. Electrical connections to the bath ground and suction electrode are made by Ringer solution-filled agar bridges that contact calomel half-cells. A voltage-clamp amplifier (Axopatch 200B; Axon Instruments, Foster City, CA) is used to measure the circulating current while the bath and electrode are held at the same voltage.

An important property of suction electrode recordings is the electrical resistance of the seal formed between the cell membrane and the wall of the suction electrode. The seal resistance is typically about 5 MΩ, roughly five times the resistance of the electrode itself; this is in contrast to the seal resistances of >1 GΩ achieved in conventional patch-clamp recordings. The relatively low seal resistance causes two limitations of suction recording. First, some of the light-sensitive current flows across the seal resistance and thus goes undetected; if the seal is five times the electrode resistance, about 20% of the current continues to flow in its normal current loop and 80% flows through the current measuring amplifier. Second, a major component of the instrumental noise in suction electrode recordings comes from the thermal movement of ions across the seal resistance. This noise—Johnson noise—is inherent in any resistor at room temperature and its variance scales inversely with the resistance. The instrumental noise can be isolated from cellular noise by exposing the rod to a bright light that

closes all the channels in the outer segment membrane (Fig. 3A). Instrumental noise measured in this way is usually close to the Johnson noise limit and considerably less than the cellular dark noise of the rod (Fig. 3B); however, noise sources such as outer segment channel noise can have an amplitude similar to or smaller than the seal noise and thus are difficult to resolve and study by suction electrode methods. Attempts to increase the seal resistance by making the opening in the suction electrode smaller or inducing the cell to stick to the electrode usually result in damage to the outer segment as it is drawn into the electrode.

The main advantages of suction electrode recording—stability and noninvasiveness—also present some disadvantages. Suction electrode recording does not permit the contents of the outer segment to be changed, making it difficult to alter the operation of particular elements of the transduction cascade. Studies of phototransduction in transgenic mice provide one way around this limitation.[10-12] Suction electrode recording also does not allow control of the intracellular voltage; instead, changes in the outer segment current lead to changes in voltage as they would under normal conditions (Fig. 1).

Truncated Outer Segment Recording

One of the main limitations of suction electrode recordings from intact cells is the difficulty in manipulating the transduction cascade. Yau and Nakatani[13] introduced a variation of suction electrode recording—the truncated outer segment preparation—that permits the solution inside the outer segment to be changed and the operation of individual elements of the transduction cascade to be altered. A rod is drawn into a suction electrode and the inner segment and a small piece of outer segment are cut off with a sharp probe (Fig. 2), providing diffusional access to the inside of the outer segment. After truncation the contents of the outer segment can be changed by flowing different solutions across the cut end and allowing them to exchange with the solution inside the outer segment by diffusion. These solution changes require only 5–10 sec to complete, permitting several conditions to be tested in the same outer segment and even allowing solution changes to be made during the flash response. We fill the suction electrode with a Ringer solution containing low Ca^{2+} (0.25 m*M* Ca^{2+} and 1 m*M*

[10] J. Chen, C. L. Makino, N. S. Peachey, D. A. Baylor, and M. I. Simon, *Science* **267,** 374 (1995).
[11] J. Xu, R. L. Dodd, C. L. Makino, M. I. Simon, D. A. Baylor, and J. Chen, *Nature* (*London*) **389,** 505 (1997).
[12] S. H. Tsang, M. E. Burns, P. D. Calvert, P. Gouras, D. A. Baylor, S. P. Goff, and V. Y. Arshavsky, *Science* **282,** 117 (1998).
[13] K.-W. Yau and K. Nakatani, *Nature* (*London*) **317,** 252 (1985).

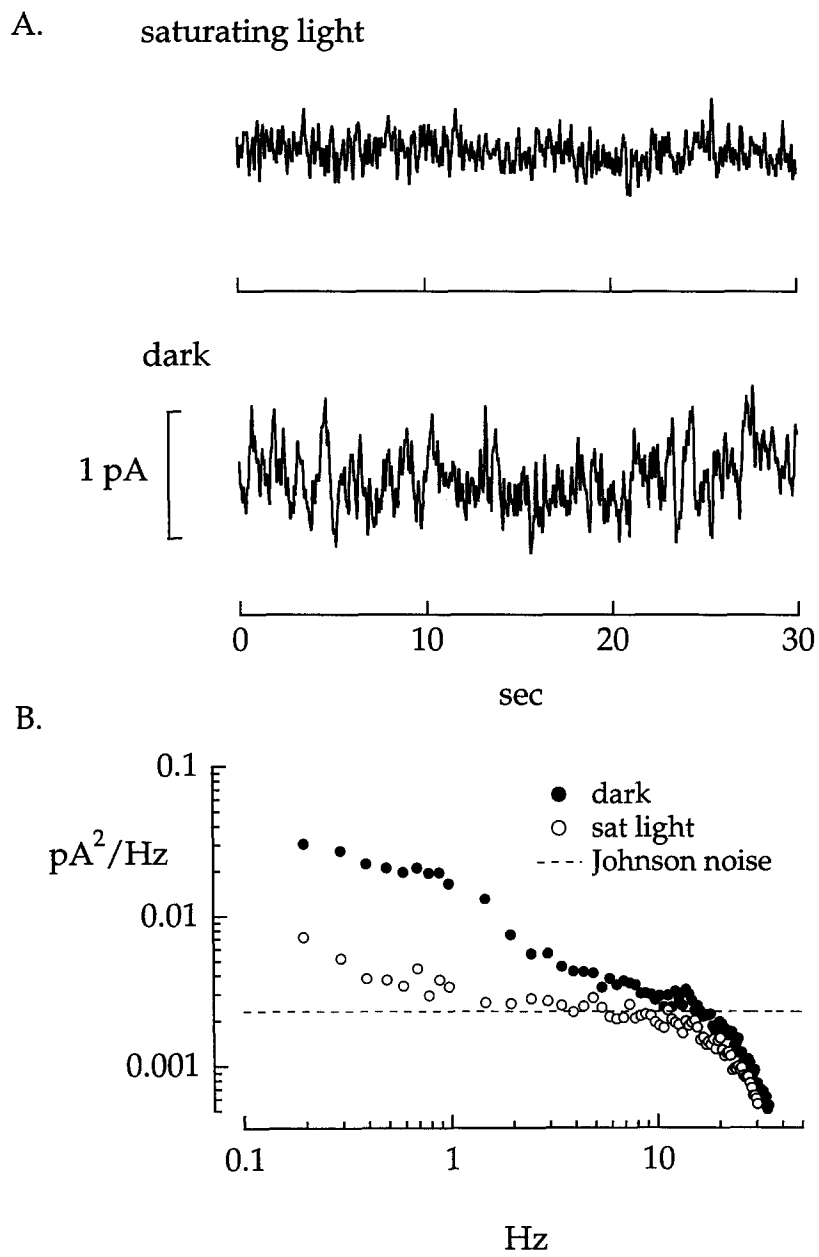

FIG. 3. Comparison of instrumental and cellular dark noise in a suction electrode recording from a salamander rod. (A) Sections of current record measured in saturating light and in darkness. The saturating light closed all the channels in the outer segment surface membrane,

EGTA) and dialyze the outer segment with a solution containing mostly arginine–glutamate. This choice of ionic compositions ensures a large driving force for Na^+ through the cGMP-gated channels in the outer segment.

Usually the first reaction to truncation experiments is surprise that they work at all and concern that important components of the transduction cascade are leaking from the outer segment. Long-term stable recordings are an important limitation of truncation experiments, but light responses can often be measured for 30 min before they begin to change systematically. It is likely that this eventual rundown is due to diffusional loss of components of the transduction cascade. During truncation experiments the bath and the suction electrode are clamped at the same voltage to isolate light-induced changes in current. This preparation does not, however, permit the voltage dependence of the light-activated currents to be studied, as changing the outer segment voltage with respect to the bath creates unmanageable instrumental noise due to the low resistance seal between the outer segment and the electrode. The processes shaping the flash response are also altered in truncation experiments, as diffusion of cGMP and Ca^{2+} from the solution bathing the cut end of the outer segment adds an additional component to the balance of cGMP creation and destruction and Ca^{2+} influx and efflux. Comparison of responses in truncated outer segments and intact cells requires modeling the spatial profiles of Ca^{2+} and cGMP.[7]

Dialyzed Outer Segment Recording

Patch-clamp recordings from isolated outer segments provide another means of characterizing how manipulations of the transduction cascade affect the rod's light response. This technique has been used extensively by Detwiler and colleagues[14–16] and is described in detail by Detwiler and Gray-Keller ([9] in this volume[16a]).

[14] K. Palczewski, G. Rispoli, and P. B. Detwiler, *Neuron* **8,** 117 (1992).
[15] G. Rispoli, W. A. Sather, and P. B. Detwiler, *J. Physiol.* **465,** 513 (1993).
[16] M. P. Gray-Keller and P. B. Detwiler, *Neuron* **13,** 849 (1994).
[16a] P. B. Detwiler and M. P. Gray-Keller, *Methods Enzymol.* **316,** 9, 1999 (this volume).

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eliminating the circulating current (Fig. 1) and isolating instrumental noise. The record in darkness contains this instrumental noise as well as cellular dark noise. The records have been digitally low-pass filtered at 5 Hz. (B) Power spectral densities of currents recorded in saturating light (○) and in darkness (●) as in (A). The dashed line shows the Johnson noise level (0.0023 pA^2/Hz) expected for the seal resistance of 7 MΩ in this experiment. Currents have been filtered at 20 Hz (eight-pole Bessel low-pass).

Patch-clamp experiments, like truncation experiments, permit the solution inside the outer segment to be changed. In patch-clamp experiments the desired internal solution is used to fill the electrode; when the membrane occluding the electrode tip is ruptured the solution diffuses into the outer segment. It is difficult to compare light responses in the same outer segment with different internal solutions, as this requires perfusing the patch electrode to change its contents during recording. The resistance between the electrode and outer segment is usually >1 GΩ in patch-clamp experiments. This high seal resistance permits the transmembrane voltage of the rod to be controlled without a large leak current and produces low instrumental noise, permitting, for example, noise due to stochastic channel gating to be measured.[17,18] As for experiments on truncated outer segments, light responses can usually be measured for about 30 min before changing significantly in shape.

Identification of Single-Photon Responses

One of the primary difficulties in studying single-photon responses is that it is not possible, on command, to cause the isomerization of one and only one rhodopsin molecule. Several stochastic processes contribute to trial-to-trial fluctuations in the number of isomerized rhodopsin molecules produced by a dim flash of nominally fixed intensity; these include the generation of photons by a typical incoherent light source, the attenuation of the light by neutral density filters, and the absorption of photons by rhodopsin. Thus, while a flash that produces *on average* a single photoisomerization can be delivered, some flashes will produce no photoisomerizations, some one, and so on. This raises two issues: determining how many photoisomerizations on average the flash produces, and isolating the single-photon responses of the rod from responses to multiple photons.

Estimating Average Number of Effective Photon Absorptions

Two methods are used to estimate the average number of effective photon absorptions (i.e., number of photoisomerized rhodopsin molecules) produced by a dim flash. The first relies on measuring the photon flux (in photons μm^{-2} sec^{-1}) reaching the preparation and estimating the rod collecting area (in μm^2). We measure and control the photon flux with a light power meter (268R; Graseby Optronics, Orlando, FL) and calibrated neutral density filters. The rate of photoisomerizations for a

[17] R. D. Bodoia and P. B. Detwiler, *J. Physiol.* **367,** 183 (1984).
[18] P. Gray and D. Attwell, *Proc. R. Soc. Lond. B* **223,** 379 (1985).

given photon flux is determined by the rod collecting area. Several factors influence the collecting area: the absorption cross-section of an individual rhodopsin molecule, the rhodopsin concentration in the outer segment, the outer segment dimensions, and the probability that rhodopsin isomerizes on photon absorption and triggers an electrical response.[19] The absorption properties of the rod can also be measured directly by measuring the fraction of light absorbed by the rod before and after bleaching rhodopsin. In toad and salamander rods the estimated collecting areas are 15–20 μm^2.

A second method to determine the average number of photoisomerizations produced by a flash makes use of the Poisson statistics that govern photon absorption and the reproducibility of the rod single-photon response (see below). A defining characteristic of a Poisson process is that the variance in the event count is equal to the mean count. In the case of repeated trials of a dim flash, the variance in the number of photoisomerizations is equal to the mean number. Thus if each photoisomerization produces a response $\hat{r}(t)$ and a flash producing an average of $\bar{n}$ photoisomerizations is delivered, the mean response will be $\bar{r}(t) = \bar{n}\hat{r}(t)$ and the time-dependent variance of the ensemble of responses will be $\sigma_r^2(t) = \bar{n}\hat{r}^2(t)$. By measuring the mean flash response $\bar{r}(t)$ and the time-dependent ensemble variance of the flash response $\sigma_r^2(t)$ the mean number of photoisomerizations per flash can be estimated as $\bar{n} = \bar{r}^2(t)/\sigma_r^2(t)$ and the single-photon response as $\hat{r}(t) = \bar{r}(t)/\bar{n}$. This procedure is illustrated in Fig. 4. Figure 4A shows the mean response and Fig. 4B the ensemble variance of the flash responses and the variance measured in darkness; the difference (light − dark) is the ensemble variance of the flash response itself. Figure 4C compares the variance of the flash response with the square of the mean response; the ratio of the scales of the left and right axes provides an estimate of the mean number of photoisomerizations per flash of $\bar{n} = 0.6$. This is comparable with the estimate of 0.8 obtained from calibration of the light intensity assuming a collecting area of 15 μm^2.

Isolating Single-Photon Responses

Trial-to-trial fluctuations in the single-photon response place important constraints on the operation of the phototransduction cascade.[20,21] To separate the variability in the single-photon response itself from variability due to fluctuations in the number of photons absorbed, we first record several hundred responses to repetitions of a dim flash. Next, we separate single-

[19] G. J. Jones, M. C. Cornwall, and G. L. Fain, *J. Gen. Physiol.* **108,** 333 (1996).
[20] L. Lagnado and D. A. Baylor, *Neuron* **8,** 995 (1992).
[21] F. Rieke and D. A. Baylor, *Biophys. J.* **75,** 1836 (1998).

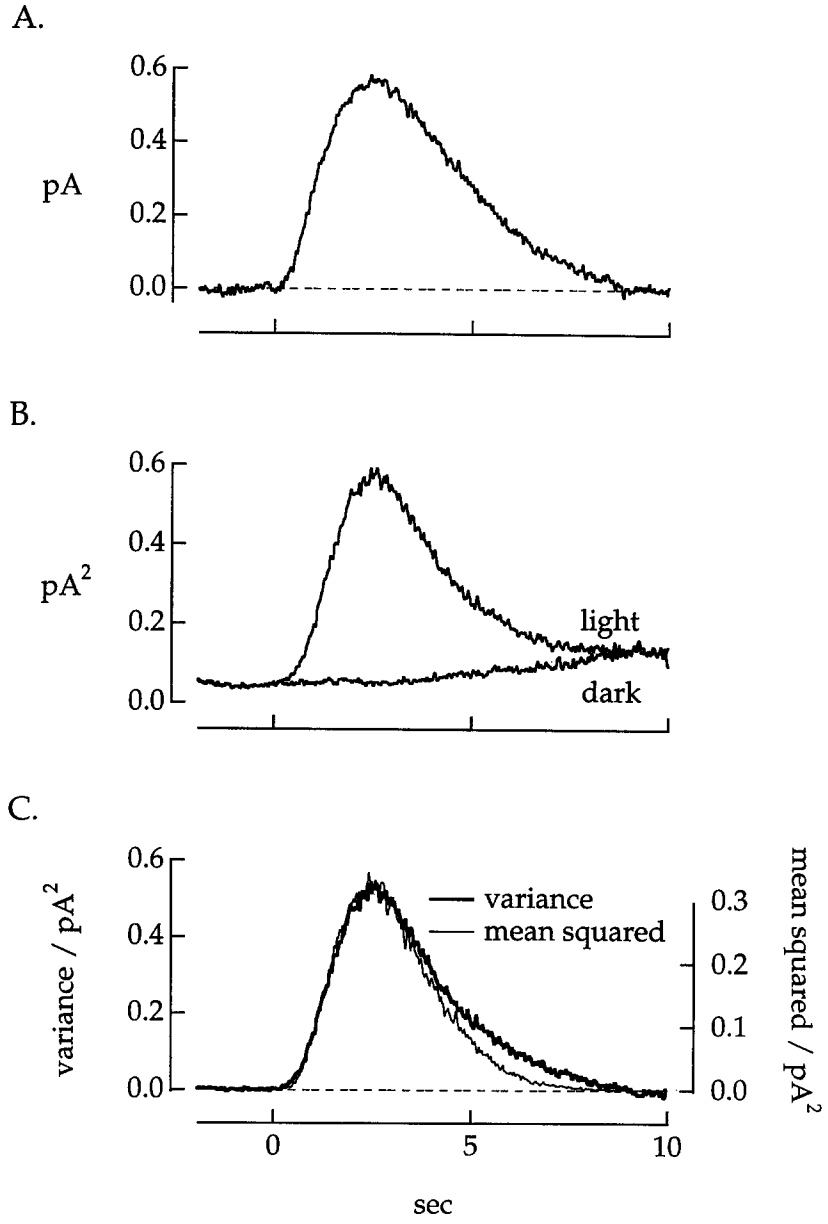

FIG. 4. Estimating the average number of photoisomerizations produced by a dim flash. (A) Average response to a dim flash measured in an intact toad rod, using a suction electrode. The flash was 10 msec in duration, delivered at time 0. The flash strength was 0.053 photon μm^{-2}; for a collecting area of 15 μm^2 this corresponds to 0.8 Rh*. (B) Time-dependent

photon responses from responses to multiple photons and to zero photons by constructing a histogram of the response amplitudes, such as that shown in Fig. 5A. The peaks in the histogram correspond respectively to zero, one, and two photoisomerizations. As these peaks are reasonably distinct, responses to single photons ("singles") can be separated from responses to zero or multiple photons. For example, in Fig. 5A responses with amplitudes between 0.43 and 1.63 pA were taken as singles. This procedure isolates from the original several hundred responses a smaller group of responses, 79 in this case, that are predominantly responses to single photons; 50 of these responses are superimposed in Fig. 5B. Differences between individual single-photon responses represent variability in the response of the transduction cascade to a single isomerized rhodopsin molecule. This variability in the single-photon response is surprisingly small given our intuition from other signals originating from single particles,[9,21] e.g., the time to decay of a radioactive particle, which exhibits large trial-to-trial fluctuations. Understanding the origin of this reproducibility is an important unresolved question in phototransduction.

Interpretation of Results in Terms of Transduction Cascade

Models of the transduction cascade provide a useful tool for interpreting the results of physiological measurements of the cellular current response in terms of the underlying biochemistry. The transduction cascade is usually described in qualitative terms: e.g., photoisomerized rhodopsin activates transducin, which in turn activates phosphodiesterase, etc. But the function of the cascade can be described by a set of coupled differential equations (see Fig. 6) with many of the important rate constants fixed or constrained by quantitative physiological or biochemical data.[7,22,23] These differential equations can be solved either numerically or approximated and solved analytically.[21,22] Because these models have few or no free parameters, this approach provides testable predictions for the transduction process based on the underlying biochemistry.

[22] E. N. Pugh and T. D. Lamb, *Biochem. Biophys. Acta* **1141,** 111 (1993).
[23] Y. Koutalos, K. Nakatani, and K.-W. Yau, *J. Gen. Physiol.* **106,** 891 (1995).

ensemble variance of the dim flash responses ("light") and responses measured in darkness ("dark"). (C) Variance of the flash response [light − dark from (B)] and square of the mean response from (A). The scale factor between the left and right axes (i.e., the mean response squared divided by the variance) provides an estimate of the mean number of photoisomerizations produced by the flash (see text), 0.6 in this experiment.

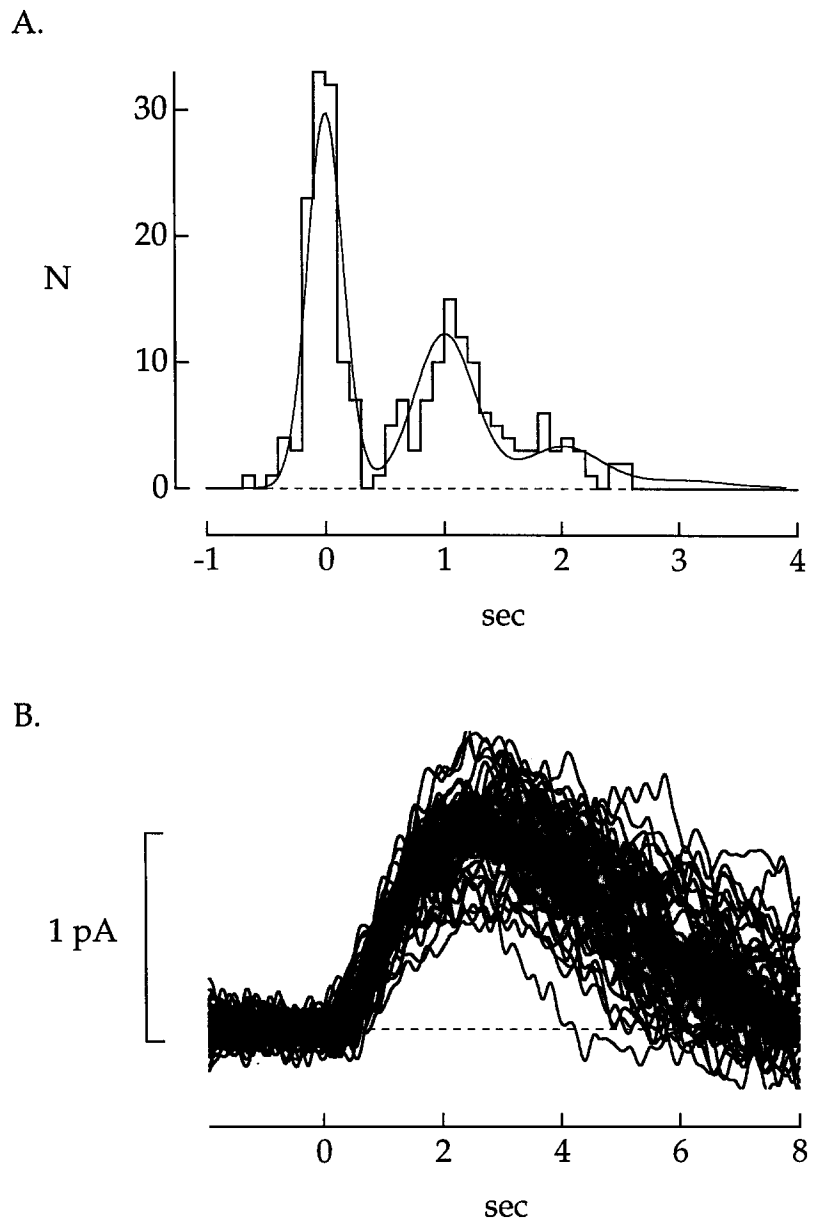

FIG. 5. Separation of single-photon responses from failures and responses to multiple photons. (A) Histogram of peak response amplitudes from 225 responses to a fixed dim flash. The smooth curve fit to the measured histogram was calculated by assuming that the noise in darkness and noise in the elementary response amplitude are independent and additive

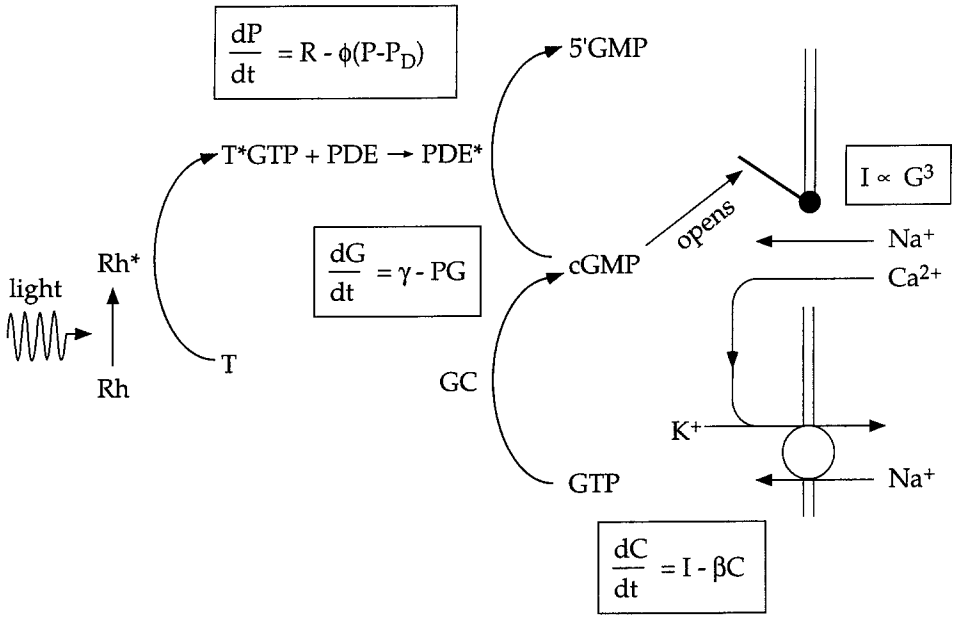

FIG. 6. Differential equations modeling the transduction cascade. Each of the boxed equations describes part of the transduction cascade. Symbols: R, rhodopsin catalytic activity; P, PDE activity; P_D, basal PDE activity; ϕ, decay rate constant of PDE; G, cGMP concentration; γ, rate of cGMP creation by guanylate cyclase; I, membrane current; C, Ca^{2+} concentration; and β, rate constant for Ca^{2+} extrusion. These coupled differential equations can be solved either numerically or approximated and solved analytically; see Pugh and Lamb[22] or Rieke and Baylor[21] for details.

Models of the transduction process such as the one outlined in Fig. 6 serve several roles in studying the single-photon response. First, studies of different classes of models can help pose experimental questions. D. Baylor and I have used this approach to study the reproducibility of the rod's single-photon response.[21] Second, models can provide mechanistic explanations for properties of the light response based on measurements from more reduced preparations—biochemical measurements or experiments on truncated outer segments. Pugh and Lamb used this approach to

and that the number of photoisomerizations per flash obeys Poisson statistics.[9,21] The peaks in the histogram correspond to responses to zero, one, and two photoisomerizations. (B) Fifty superimposed responses with amplitudes between 0.43 and 1.63 pA. From the amplitude histogram in (A) these are primarily single-photon responses. Each response has been shifted vertically to correct for baseline drift and digitally low-pass filtered at 5 Hz.

account for the amplification of the single-photon response,[22] and Koutalos *et al.* combined modeling and experiment to study the relative contributions of different mechanisms mediating light adaptation in rods.[23]

Summary

Rod photoreceptors detect and encode incident photons exceptionally well. They collect sparse photons with high efficiency, maintain a low dark noise, and generate reproducible responses to each absorbed photon. The mechanisms involved in single-photon detection—control of the effective lifetime of a single active receptor molecule, amplification of the activity of this single molecule by a second-messenger cascade, and reliable transmission of small synaptic signals—recur throughout the nervous system. Indeed, several other sensory systems reach or approach limits set by quantization of their input signals. For example, olfactory receptors can detect single odorant molecules.[24]

Although our understanding of visual transduction and signal processing has advanced rapidly during the past 10–15 years, fundamental questions still remain: What mechanisms are responsible for the reproducibility of the rod's elementary response? What are the tradeoffs of speed and sensitivity in the transduction cascade? How are the rod single-photon responses reliably transmitted to the rest of the visual system? Future technical innovations, particularly better methods to monitor the activity of intermediate steps in transduction, will play an important role in providing answers.

[24] A. Menini, C. Picco, and S. Firestein, *Nature (London)* **373,** 435 (1995).

[13] Electroretinographic Determination of Human Rod Flash Response *in Vivo*

By DAVID R. PEPPERBERG, DAVID G. BIRCH, and DONALD C. HOOD

Introduction

The electroretinogram (ERG), a multicomponent electrical signal that can be recorded at the cornea of the vertebrate eye, originates from the responses of retinal neurons to a test flash.[1] The first component of the ERG elicited by a brief flash of moderate or high intensity is a cornea-

[1] R. Granit, *J. Physiol.* **77,** 207 (1933).

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