Optimization of Single-Photon Response Transmission at the Rod-to-Rod Bipolar Synapse

Our ability to see in dim light is limited by the statistics of light absorption in rod photoreceptors and the faithful transmission of the light-evoked signals through the retina. This article reviews the physiological mechanisms at the synapse between rods and rod bipolar cells, the first relay in a pathway that mediates vision near absolute threshold.

Across mammalian species, the retinal circuitry underlying visual processing in dim light is well conserved. Individual rod photoreceptors, themselves capable of reliably signaling the absorption of individual photons (6, 9), are pooled in a specialized circuitry referred to as the rod bipolar pathway (17, 49). Following a series of convergent connections in this pathway, the ganglion cells, which are the output cells of the retina, send signals from thousands of pooled rods (57) to higher visual centers. It has been appreciated for more than a half-century that absolute behavioral threshold for light detection requires only a few photon absorptions in this pool (Refs. 4, 26, 53; reviewed in Ref. 23). Several factors influence the transmission of the rod photoresponse through the retina including the fidelity of the rod photoresponse itself, the great degree of convergence of rod signals, and the number of stages of processing. These factors collectively place fundamental limits on the performance of rod vision and thus must each be optimized for single-photon transmission near absolute threshold.

Of particular interest is the very first synapse of the rod bipolar pathway, which pools the responses of 20–100 rods and has the unenviable task of discriminating the small graded potential change in the few rods absorbing photons from the remainder of the rods generating electrical noise.

The process of rod-to-rods bipolar cell signal transmission has strong implications for setting the absolute threshold for seeing. Indeed, recent experiments on transgenic mice with altered rod photoresponses, due to the lack of the rod photoreceptor protein recoverin, indicate that deficits in absolute visual threshold can be attributable to the properties of rod-to-rods bipolar signal transfer (44). Furthermore, mutations in pre- and postsynaptic proteins that alter the function of this synapse are known to produce visual disorders like congenital stationary night blindness (13, 27, 34, 36, 37). The goal of this review is to highlight the process by which the rod photoresponse traverses the rod-to-rods bipolar synapse to ensure its reliable transmission through the retina. We emphasize the mechanisms that 1) maximize the voltage change sensed by the rod synaptic terminal (called a spherule) following photon absorption, 2) convert this voltage change to a reduction in glutamate release, and 3) remove rod noise postsynaptically. These mechanisms collectively improve the fidelity of the rod single-photon response and allow the retina to transmit these signals to higher visual centers where they contribute to visually guided behavior.

Maximizing the Presynaptic Voltage Change

Near absolute threshold, perhaps the most important goal for the rod photoreceptor is to signal the largest possible voltage change per absorbed photon. Two fundamental processes are involved in signaling this voltage change to the rod spherule, where glutamate release is controlled (FIGURE 1). First, the rod phototransduction cascade must amplify the signal generated by a single rhodopsin molecule to yield the largest possible change in the outer segment current with respect to the underlying noise. Second, the voltage change produced by the closure of the outer segment current needs to be transmitted to the rod spherule with the least amount of loss.

Phototransduction

The phototransduction cascade in vertebrate rods is arguably the best-elucidated G-protein-coupled signal transduction mechanism and the subject of many review articles (14, 19, 33). The activation of a single rhodopsin in the outer segment by light is known to trigger a cascade of events that ultimately results in the closing of cGMP-gated channels that are normally open in darkness. Through a series of amplifying steps, this cascade leads to the degradation of more than $10^7$ cGMP per photon, generating a robust rod photoresponse (59).

A great deal is known about the biophysical mechanisms that underlie the rod photoreceptor, specifically the forms of the intrinsic noise that limit the fidelity of rod signals in the retina. Light-detection near threshold ultimately requires that light-evoked responses can be distinguished from two types of
noise intrinsic to the rod photoreceptor: discrete noise and continuous noise (7). Discrete noise is produced by the thermal activation of the visual pigment and has an identical form to the rod single-photon response (58). The identity of discrete noise to the rod single-photon response prevents the implementation of any mechanism to eliminate this noise. In fact, the rate of occurrence of discrete noise events has historically been thought to be what fundamentally limits absolute threshold, since it generates a “dark light” that the frequency of light-evoked events needs to exceed for detection (Ref. 4; but see also Ref. 23). To some degree, nature has mitigated this form of noise through the evolution of rhodopsin molecules with great thermal stability (9).

Although discrete noise events need to be considered in downstream retinal neurons pooling thousands of rods, their contribution at an individual rod-to-rod bipolar synapse is relatively small. Near absolute threshold, an individual synapse will rarely “see” a discrete noise event. If discrete noise events occur at a rate of 0.006 s⁻¹ • rod⁻¹ (9) and the integration time of the rod response is 0.2 s (57), then an individual rod-to-rod bipolar synapse will see a discrete event on average once every ~833 integration times. The constraints for the detection of a photon instead rely on discriminating a light-evoked event from continuous noise resulting from thermal activation of the rod phosphodiesterase (40). At the rod-to-rod bipolar synapse, a linear convergence of rod inputs would sum this noise and occlude single-photon responses that are sparse in the array of rods at absolute visual threshold. Instead, as described below, nonlinear signal transmission at this synapse can help to differentiate signals from rods absorbing a photon from those generating continuous noise (22, 43).

**Gap-junctional coupling in the outer retina**

The light-evoked closure of outer segment cGMP-gated channels by the phototransduction mechanism is processed by the rod’s input impedance and inner segment conductances to yield a hyperpolarizing voltage change (~1 mV per photon; Ref. 46) that is passively transmitted to the rod spherule (FIGURE 1). Near absolute threshold, the form of this single-photon voltage change appears to mirror the outer segment current (8), but at higher light levels the voltage and current begin to deviate as the voltage is shaped by inner segment hyperpolarization-activated and Ca²⁺-activated conductances (3, 20).

The coupling of rod photoreceptors to both rods and cones in the mammalian retina can provide one...
source of voltage loss to the rod spherule (FIGURE 1A). Thus, at the rod spherule, the light-induced hyperpolarization and reduction in glutamate release per photon will become progressively diminished the more extensively the rod is electrically coupled to its neighbors. It has been suggested that, near absolute threshold, the rods are poorly coupled to their neighbors to allow the spherule to sense the largest voltage change in the rods absorbing photons (49). Recent recordings from primate rods and cones indeed show that, in the dark-adapted retina, many but not all rods are dye-coupled to neighboring rods and cones, and this coupling is suggested to impair absolute visual threshold (29). However, the functional consequences of dye coupling remain to be seen and highlight an important issue. The fact that rods are coupled at all to neighboring photoreceptors is perplexing if the main goal of rod vision is the discrimination of individual photons. The observed coupling would suggest that the retina is simultaneously balancing single-photon detection near absolute threshold (where coupling will degrade the signal), with light detection at higher levels where almost every rod receives a photon (where it is postulated that coupling improves rod signal-to-noise by averaging across neighboring cells; Ref. 49).

Presynaptic Optimization for Signal Transmission

The transmission of the rod photoreceptor to rod bipolar cells is controlled at a specialized synapse at the rod spherule. As shown in FIGURE 2, the bipolar cell dendrite (as well as horizontal cell dendrites) projects into an invaginated structure that is opposite the release site for glutamate. In darkness, a consequence of the open cGMP-gated channels in the rod outer segment is a relatively depolarized resting membrane potential and a steady influx of Ca$^{2+}$ at the synapse. This steady Ca$^{2+}$ influx results in continuous glutamate release from a specialized synaptic ribbon (48). The released glutamate is sensed postsynaptically by a metabotropic glutamate receptor, mGluR6, and leads to the closure of cation channels (FIGURE 2B). The light-evoked hyperpolarization produced by the closing of outer segment cGMP-gated channels is transmitted passively to the rod spherule where it reduces Ca$^{2+}$ influx and transiently reduces glutamate release, thereby reducing postsynaptic G-protein-coupled signaling, opening cation channels, and depolarizing the rod bipolar cell. In the transformation of the rod photovoltage to a bipolar cell voltage, the time course of signaling also becomes speeded (see FIGURE 1), which may allow the rod system to encode accurately the arrival time of photons (11, 41).

Effective signal transmission across this synapse requires that the rod photovoltage is sufficient to alter the open probability of Ca$^{2+}$ channels and change the internal Ca$^{2+}$ concentration to reduce significantly the level of glutamate release. The presynaptic properties of signal transmission also have the important role of setting the dark release rate of glutamate, which may play an instructive role in nonlinear signal transmission at this synapse (see below; Ref. 43).

Voltage sensitivity of Ca$^{2+}$ channels

In the face of continuous depolarization, as experienced by vertebrate rods and cones in darkness (rod voltage of approximately –40 mV; Ref. 46), the photoreceptor synapse must be sensitive to changes in membrane voltage under circumstances where many types of voltage-activated Ca$^{2+}$ channels are in the inactivated state (28). Two fundamental properties of Ca$^{2+}$ channels must hold to account for the strong dependence of glutamate release on Ca$^{2+}$ concentration (2, 10, 51). First, the Ca$^{2+}$ channels must support a stable Ca$^{2+}$ flux that can maintain the dark release rate of glutamate. Second, the voltage sensitivity of the channels must be tuned to the range of voltages experienced that coupling improves rod signal-to-noise by averaging across neighboring cells; Ref. 49).

FIGURE 2. Structure and signal transfer at the rod-to-rod bipolar synapse

A: the rod spherule is a specialized invaginating structure where the dendrites of horizontal (H) and rod bipolar cells (RB) are apposed to a glutamate release site controlled by a ribbon. Adapted from Ref. 39. Ca$^{2+}$ channels (Ca$_{V1.4}$) are located near the active zone (AZ) and allow the continuous release of glutamatic vesicles in darkness. In “on” rod bipolar cells glutamate is sensed by mGluR6 receptors located near the mouth of the invagination. Inset: release of glutamate is dependent on Ca$^{2+}$ influx through Ca$_{V1.4}$ Ca$^{2+}$ channels whose voltage sensitivity can be optimized by the binding of internal factors, like CABP4. CABP4 binding to Ca$_{V1.4}$ pushes the activation curve to more negative voltages into a regime where Ca$^{2+}$ concentration changes in the synapse are more pronounced at physiological voltages. Adapted from Ref. 24. B: the signaling cascade in rod bipolar cell dendrites is poorly understood. mGluR6 activation leads to the activation of G$_{\alpha_i}$ which through unknown mechanisms leads to the closure of a nonselective cation channel whose identity is also unknown. The light-evoked reduction in glutamate release relieves activity in this cascade and opens cation channels leading to depolarization.
rienced by the rod in darkness. It is known that the Ca\(^{2+}\) channels expressed at the rod synapse are Ca\(_v_{1.4}\) L-type channels (35), whose properties appear to be suited ideally for rod-to-robin bipolar cell transmission.

“A requirement for signal transmission near absolute visual threshold is that Ca\(_v_{1.4}\) must be sensitive to small voltage changes.”

A striking feature of Ca\(_v_{1.4}\) channels, even compared with other L-type channels in their family, is the lack of Ca\(^{2+}\)-dependent inactivation and the relatively slow voltage-dependent inactivation. Either form of inactivation would gradually reduce the dark Ca\(^{2+}\) flux and thus glutamate release. Indeed, recent work has identified a specialized domain in the COOH terminus of Ca\(_v_{1.4}\) that prevents Ca\(^{2+}\)-dependent inactivation (56). Furthermore, voltage-dependent inactivation in these channels appears slow, with a time constant on the order of seconds and dependent on the combination of alpha and beta subunits expressed (32). What is intriguing about these findings is that they suggest that the Ca\(^{2+}\) change in the synapse should track the hyperpolarizing rod photovoltage (see also below), thereby raising questions about the mechanism that transforms the slow rod light response into the fast bipolar cell light response (see also FIGURE 1). If the Ca\(^{2+}\) change at the synapse is not accelerated with respect to the rod photovoltage and the bipolar cell tracks faithfully the change in synaptic glutamate (47), then fast glutamate changes in the synaptic cleft must result from fast reuptake (25) or an additional presynaptic mechanism(s). For instance, this may result from Ca\(^{2+}\)-induced changes in the efficacy of release (30). One potential mechanism subserving this speeding may thus be a rapidly shifting Ca\(^{2+}\) dependence of glutamate release to lower Ca\(^{2+}\) concentrations, allowing glutamate release to reach dark levels before the Ca\(^{2+}\) concentration recovers.

A requirement for signal transmission near absolute visual threshold is that Ca\(_v_{1.4}\) must be sensitive to small voltage changes. Indeed, an amplification of voltage, or voltage gain, in rod-to-bipolar cell signal transfer increases the detectability of the light response above synaptic and cellular noise (1, 15). Thus, in an ideal situation, the rod voltage should be positioned at or near the steepest point in the relationship between voltage and channel opening (2, 10, 51). As alluded to previously, mutations that alter the voltage sensitivity are known to impair low-light-level vision (27, 60). More recently, proteins like calcium binding protein-4 (CABP4) have proven essential in altering the position of this voltage dependence toward ideal by shifting it to more negative membrane potentials where small changes in voltage will alter more significantly the Ca\(^{2+}\) current (24). It is possible that CABP4, as well as perhaps other unidentified proteins, give the synapse the ability to alter the voltage sensitivity of Ca\(_v_{1.4}\) as the rod voltage changes with light history. This control of the voltage sensitivity of the Ca\(^{2+}\) channel allows the synapse to signal robustly changes in the rod’s voltage and to set the release rate of glutamate over a wide range of light intensities (see below). Lastly, the low open probability and low unitary conductance of Ca\(_v_{1.4}\) channels are ideal for generating voltage-dependent changes in Ca\(^{2+}\) concentration that are essential for the low noise transmission of small graded signals (18).

Properties of glutamate release

Unlike conventional synapses where action potentials create transient spots of high Ca\(^{2+}\) concentration near Ca\(_v_{1.4}\) channels, rod synapses must sustain a high rate of vesicle release under the low Ca\(^{2+}\) concentrations normally present in the spherule under physiological conditions (rod voltage of approximately –40 mV; 0.5–3 μM [Ca\(^{2+}\)]; Ref. 42). Indeed, rod terminals carry a vesicle pool sensitive to Ca\(^{2+}\) concentration as low as 1 μM, which likely provides sustained vesicle release by sensing averaged Ca\(^{2+}\) concentrations instead of high Ca\(^{2+}\) concentrations near channels (51). The resulting vesicle release is asynchronous and thus will reduce fluctuations in synaptic glutamate (42). The properties of Ca\(_v_{1.4}\) described previously assist in creating the uniform and low noise Ca\(^{2+}\) distribution, and thus are suitable for asynchronous release. Within the narrow range of the physiological Ca\(^{2+}\) concentration, the cooperativity of Ca\(^{2+}\) ions on the vesicle release rate becomes negligible, and the exocytosis rate appears to correlate linearly with the Ca\(^{2+}\) concentration (51). Collectively with other evidence that shows linearity in processes involved in the transmission of rod dim flash responses (22, 42, 46, 51), the properties of rod exocytosis allow the graded release of glutamate that faithfully represents the amplitude of rod single-photon response. This linearity enables rod bipolar cells to interpret glutamate concentrations as scaled rod voltages and may form the basis for an instructive signal that sets their threshold (see below).

The unique invaginating structure of the rod synapse is also desirable for low noise transmission of small graded signals (FIGURE 2). Release sites at rod terminals are spread longitudinally along the bottom of the ribbon, whereas mGluR6 receptors are located on the shaft of the rod bipolar dendrites near the mouth of the invagination (55). The diffusion of glutamate molecules through this distance reduces the glutamate concentration at the mGluR6 receptors, thereby placing it in a range where high-affinity mGluR6 receptors are most sensitive in darkness (25, 38, 39). This mGluR6 location also reduces variability in distance from discrete release sites, thus suppressing potential noise. Based on the structure of the spherule and a diffusion model, the dark release rate that realizes this glutamate
concentration at mGluR6 and that does not generate frequent false-positive events was estimated as ~100 vesicles/s (38, 45, 54). Additionally, it has been proposed that vesicle release might be regular rather than stochastic because Poisson fluctuations in the vesicle release would overwhelm rod continuous noise, making the release rate resulting from single-photon absorptions indistinguishable from the dark release rate (45). Such regularity in the vesicle release may be partly achieved by imposing a refractory period after vesicle release at individual release sites (61).

**Postsynaptic Thresholding and the Elimination of Noise**

Of the three known pathways for rod signals to reach ganglion cells in the mammalian retina (rod bipolar, rod-cone, and rod-off pathways; reviewed in Ref. 12), only the rod bipolar pathway pools enough rods to account for the high sensitivity of rod vision near absolute visual threshold. It has been recognized for more than 20 years that, to account for this high sensitivity, where one can detect few photoisomerizations in thousands of pooled rods (c.f. Ref. 5), rod outputs cannot be pooled linearly. Early measurements of dark noise from primate rods indicate a noise variance that would swamp out a single-photon response in a rod bipolar cell pooling 20–100 rods if the rod output were simply summed (Ref. 9; see also FIGURE 3). A threshold-like mechanism at the synapse between rods and rod bipolar cells has been suggested as a way of eliminating noise from rods and has been studied analytically (16, 22, 54).

As mentioned above, a main source of noise that must be considered at an individual rod-to-rod bipolar synapse is the continuous noise generated in the phototransduction cascade by the spontaneous activation of cGMP phosphodiesterase (40). If a threshold is going to be effective in distinguishing single-photon events from the continuous noise, then it must be precisely positioned. First, the amplitude of the threshold must be high enough to exclude as much of the continuous noise as possible. Second, the amplitude of the threshold must not be too high to exclude single-photon events. Such positioning becomes problematic when the amplitude distribution of the rod continuous noise overlaps significantly with the amplitude distribution of single-photon responses, requiring a tradeoff between these two parameters.

Field and Rieke (22) approached this issue in the mouse retina by measuring the distributions of noise amplitude and single-photon response amplitude in...
rods and determining how these shape rod-to-rod bipolar signal transmission. They found that the responses of rod bipolar cells depended supralinearly on flash strength compared with the linear relation in rods. By combining their data on rod bipolar cell responses with a model where rod signals are passed through a sharp threshold and summed (see also Ref. 54), Field and Rieke (22) determined that, to explain the data, the threshold would need to be positioned at 1.3 times the average amplitude of the rod single-photon response. This is close to the optimal position they determined for the ideal separation of the rod single-photon response from the underlying rod (continuous) noise. In other words, the threshold eliminates rod single-photon responses with amplitudes of <1.3 times average, whereas those larger than 1.3 times average are retained. A striking feature of this conclusion is that the threshold eliminates almost 75% of the rod single-photon responses. Although the elimination of such a large fraction of the rod single-photon responses seems high for a system trying to maximize visual sensitivity, such a nonlinear operation improves the signal-to-noise ratio of rod signals by ~350-fold over their simple linear pooling. Some debate exists about the exact position of this nonlinearity depending on the model (0.85 times amplitude; Ref. 50), but nonetheless a postsynaptic threshold that eliminates a significant fraction of the rod single-photon responses appears to optimize the transfer of rod signals downstream to the retina. One might expect that the position of such a postsynaptic threshold would vary by species depending on the signal-to-noise ratio of their rod single-photon responses (see below). Along these lines, one may also expect that near absolute threshold the extent rods are electrically coupled to their neighbors (see FIGURE 3) will raise the position of the threshold, since coupling will increase noise variance in the spherule of the rod absorbing a photon and thus degrade the signal-to-noise ratio of the single-photon response (29). Such coupling may explain why the position of the threshold measured by Field and Rieke (22) is higher than the optimal position determined for uncoupled rods.

Sampath and Rieke (43) further investigated the physiological mechanism underlying this nonlinear threshold. Previous studies have shown linearity in the mechanisms that convert the rod voltage to changes in glutamate release rate in the rod spherule (22, 42, 46, 51). However, given the high rate of release in darkness of glutamate (52), a reasonable hypothesis is that saturation at the synapse might be responsible for suppressing small single-photon responses that do not reduce the synaptic glutamate enough (54). Sampath and Rieke (43) were able to demonstrate that the extent of nonlinearity of rod bipolar cell responses depends on the magnitude of G-protein-coupled signaling. By utilizing high-affinity agonists and antagonists to activate or inactivate selectively a fraction of the postsynaptic glutamate receptor mGluR6, they found that they could exacerbate or ameliorate the extent of nonlinearity. In fact, for many cells where they applied the mGluR6 antagonist LY341495, they found that the synapse can be restored to linearity. This result indicates that a majority of nonlinearity can be explained by saturation within the G-protein cascade that controls the rod bipolar response and not by saturation at the level of mGluR6 receptors.

Taken collectively, the information presented above allows us to describe the role of nonlinear signaling at the rod-to-rod bipolar synapse and how it is controlled. Near absolute threshold, the ability of the retina to distinguish single-photon absorptions from noise will ultimately be dependent on individual rods. In the mouse retina, the signal-to-noise ratio of the rod single-photon response is ~3, with distributions of noise amplitude and single-photon response amplitude overlapping significantly (22). However, in other mammalian species with a higher signal-to-noise ratio (~5 in primates and ~4 in guinea pigs; Ref. 21) signal transmission should require less nonlinearity. Under these circumstances, the rod single-photon response is more distinguishable from the underlying (continuous) noise, and thus the threshold can be positioned lower with respect to the average single-photon response amplitude to provide optimal separation between signal and noise. We predict a strong negative correlation between the rod signal-to-noise ratio (or any other measure of the fidelity of rod signals; Ref. 16) and extent of nonlinearity across all mammalian species.

The origin of nonlinear signaling within the G-protein cascade, rather than at the level of the mGluR6 receptors (43), provides a site for potential modulation. It would seem that the extent of nonlinearity could be set by the relative expression of the G-protein, $G_{s}$, or other components of the signaling cascade that remain largely unknown. However, if the expression of some component of the G-protein cascade is setting the extent of nonlinear signaling at the rod-to-rod bipolar synapse, especially given the near-optimal position of the threshold in mice (21), how its concentration is set becomes an interesting problem. In particular, the expression level of these signaling elements would either need to be encoded genetically, guided by an instructive signal, or some combination of both. Near absolute threshold, where a single rod in 10,000 may be absorbing a photon, the benefit of nonlinear signal transmission is significant, providing ~350-fold improvement of the signal-to-noise ratio over the linear combination of rod signals (22). However, this benefit diminishes as the light level increases. Perhaps the extent of nonlinearity is genetically encoded for optimal separation of signal and noise near absolute threshold, but even at higher light levels some modulation of nonlinearity may modestly improve the rod signal-to-noise ratio. Such modulation may result from an instructive signal, which in this case could be the dark release rate and fluctua-
tions in synaptic cleft glutamate concentration, since these define the functional properties required for the separation of light-evoked signals from noise.

A problem with the idea of an instructive signal controlling the extent of nonlinearity is that from the perspective of a single rod-to-bipolar cell synapse or a single rod bipolar cell, this signal may not occur at a high enough frequency to guide this mechanism. Under these circumstances, the instructive signal needs to originate at a level of the retina that integrates over many rods (i.e., all amacrine cell). Alternatively, at light levels above 1 photon absorbed in 100 rods per rod integration time (i.e., where a rod bipolar cell “sees” at least 1 absorbed photon in its receptive field per integration time), the rod bipolar cell itself could act as the integrator. These instructive signals could guide the expression of $G_o$ or downstream elements either at the level of nuclear expression or by local protein synthesis in the dendrites for faster changes (31).

Early psychophysical studies of detection thresholds and recordings from ganglion cells have emphasized the notion that the visual system can detect few photon absorptions in large pools of rod photoreceptors. Such sensitivity can only be borne out of early biophysical mechanisms that preserve information about single-photon absorptions in individual rods and allow those signals to be propagated efficiently through the retina. In this review, we emphasize that precise tuning of the rod-to-bipolar cell synapse is mediated by both pre- and postsynaptic mechanisms. Presynaptic mechanisms must amplify small graded signals generated by a single rhodopsin molecule, transmit the voltage change with the least loss to the synapse, and reduce significantly the synaptic Ca\textsuperscript{2+} concentration and glutamate release. Furthermore, the dark release rate and fluctuations in synaptic cleft glutamate concentration appear to serve as the instructive signal for the postsynaptic nonlinear removal of noise. Thus coordinated pre- and postsynaptic mechanisms at the rod-to-bipolar cell synapse are crucial for the transmission of the rod single-photon response and our exquisite visual sensitivity.

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