SUPPLEMENTAL INFORMATION

CHANNEL MODULATION AND THE MECHANISM OF LIGHT ADAPTATION IN MOUSE RODS

Jeannie Chen, Michael L. Woodruff, Tian Wang, Francis Concepcion, Daniel Tranchina, and Gordon L. Fain
Figure S1: Relative levels of transduction proteins in retinal homogenates from CNGβ1ΔCaM, CNGβ1ΔCaM/GCAPs−/− and GCAPs−/− mice as compared to WT mice. Fluorescence signals were detected and quantified using the Odyssey® infrared imaging system (LI-COR Biosciences). The values were normalized to those of actin. Normalized value of each protein was subsequently normalized to that of WT (Mean ± SD, N=3-5).
Supplemental Mathematical Methods

Model equations: modulation of light-activated and spontaneously activated PDE6

The model equations below were used to simulate experiments on wild-type and GCAPs−/− mouse rods presented in the main body of the paper. Numerical solution of the systems of differential equations were used to generate the model fits of experimental sensitivity data and experimental flash-response data shown in Figures 3 and 6, respectively.

All dynamic variables in the model equations below are defined in Table S1. The rate constants and dimensionless parameters are defined in Tables S2 and S3. Our model is substantially the same as that of Hamer et al. (2003; 2005). The minor differences were motivated by our desire to keep the number of parameters to a minimum; they involve modeling the faster reaction in Hamer et al. as instantaneous, and combining several fast discrete steps into one step in a number of places. Our model does not include recoverin-mediated modulation of the lifetime of activated rhodopsin (Rh*) through the control of rhodopsin kinase activity, because of the evidence cited in the main body of this paper that recoverin does not play a detectable role in the manifestations of light adaptation studied here.

In an approach similar to that used by Soo et al. (2008), we started with the basic model for rod phototransduction above and embellished it only as dictated by its failures. A new component in our model is a mechanism for the modulation of the rate of decay of activated phosphodiesterase (PDE6*).

Details of Rh* deactivation are controversial (Mendez et al., 2000; Doan et al., 2006; Bisegna et al., 2008). However, our model behavior is not sensitive to details in the “front end” of the phototransduction signaling pathway, because it pertains to average, large-signal, macroscopic responses (see Comments on Best-Fitting Model Parameters, below). In keeping
with Hamer et al. (2003; 2005), we assume that Rh* is deactivated by multiple, sequential phosphorylations; for sake of simplicity, arrestin binds only to the fully phosphorylated form; each phosphorylated form of rhodopsin has catalytic activity; the catalytic activity of rhodopsin steps down upon each phosphorylation, as does the affinity of phosphorylated rhodopsin for G-protein (transducin), in such a way that the number of activated G-proteins produced by each form of activated rhodopsin is the same on average, namely, $\mu^G$; rhodopsin produces activated G-protein instantaneously upon binding G-protein and simultaneously rhodopsin is released. We define $R_n$ as the number of Rh* with $n$ bound phosphates, and $\Phi(t)$ as the number of photoisomerizations per second. For the dynamics of rhodopsin activation and successive phosphorylations (with a maximum of 6) or final arrestin binding, we have

\[
\frac{d}{dt} R_0 = \Phi(t) - k^{RK}_0 R_0 \tag{S1}
\]

\[
\frac{d}{dt} R_n = k^{RK}_{n-1} R_{n-1} - k^{RK}_n R_n, \quad \text{for } n = 1, 2, ..., 5 \tag{S2}
\]

\[
\frac{d}{dt} R_6 = k^5^{RK} R_5 - k^{Arr} R_6, \tag{S3}
\]

where $k^{RK}_n$ is the effective catalytic rate constant for the phosphorylation of Rh*, with $n$ bound phosphates, by rhodopsin kinase (RK); $k^{RK}_n = k^{RK}_0 \exp(-n\omega)$, for $n = 1, 2, ..., 5$; $k^{Arr}$ is the rate constant for arrestin capping of fully phosphorylated Rh*; and $k^{Arr} = k^{RK}_0 \exp(-6\omega)$, with $\omega = 0.6$ (Hamer et al.; 2003; 2005).

We define $G_n$ as the number of $G_\beta\gamma-G_\alpha$-GTP molecules (G-proteins with GTP bound, synonymous with $T_\alpha$-GTP) that were produced specifically by the form of Rh* with $n$ phosphates bound. The subscript on $G_n$ is convenient for bookkeeping purposes. The rate of change of $G_n$ is given by the difference between the catalytic rate of production of $G_n$, and the rate of its dissociation into activated G-protein ($G_\alpha$-GTP, called $G^*$ for short in
where $k_n^G$ is the rate at which a single Rh* molecule with $n$ occupied phosphorylation sites catalyzes the production of $G_{\beta\gamma}$-$G_\alpha$-GTP molecules; $k_n^G = \mu^G \, k_{n^{RK}}$, for $n = 1, 2, \ldots, 5$; $k_6^G = \mu^G \, k_{Arr}$; $\mu^G$ is the average number of activated G-proteins produced by each phosphorylated form of Rh*, in a probabilistic (stochastic) interpretation of Eqs. (S1)–(S4) (Hamer et al.; 2003; 2005).

We define $G^*$ as the total number of activated G-protein ($G_\alpha$-GTP) molecules. We assume that $G_\alpha$-GTP molecules are produced both catalytically and spontaneously, at a rate given by the total rate of dissociation of $G_{\beta\gamma}$-$G_\alpha$-GTP into its subunits. We assume that the spontaneous rate of $G_\alpha$-GTP production is constant and independent of light level. The rate of change of $G_\alpha$-GTP is given by the difference between the total rate of its production, and the rate at which it binds to the inactivation subunit of phosphodiesterase (PDE6):

$$\frac{d}{dt}G_\alpha^* = \nu^{\text{spon}} + k^f \left( \sum_n G_n \right) - k^{\text{G-PDE}} \, G^*,$$

where $\nu^{\text{spon}}$ is the rate at which $G_\alpha$-GTP molecules are produced spontaneously; $k^f$ is the rate constant for the dissociation $G_{\beta\gamma}$-$G_\alpha$-GTP molecules into $G_{\beta\gamma}$ and $G_\alpha$-GTP; $k^{G-PDE}$ is the rate constant for the binding of $G_\alpha$-GTP to the inactivating subunit of PDE6, assuming that PDE6 is in excess, i.e. that the concentration of inactive PDE6 is not changed substantially over the range of stimuli in this study, so that its concentration is rolled into $k^{G-PDE}$.

We define $P^*$ as the number of activated PDE6 subunits. The rate of change of $P^*$ is given by the difference between the activation and deactivation rates. We assume that the rate of deactivation is controlled in a dynamic manner by free intracellular Ca$^{2+}$. The
resulting equation for the rate of change of \( P^* \) is:

\[
\frac{d}{dt} P^* = k_{G-PDE} G^* - \nu_{PDE} P^*, \quad \text{with} \quad \nu_{PDE} = \nu_{PDE, \text{dark}} z,
\]

where \( \nu_{PDE} \) is the inactivation rate per activated subunit of PDE; \( \nu_{PDE} \) is assumed to be proportional to the concentration of a hypothetical molecule in its active form \( (z) \).

The remaining equations describe the dynamics of free intracellular \( \text{Ca}^{2+} \), \( \text{Ca}^{2+} \)-buffer, cGMP, and a hypothetical molecule that controls the rate constant for PDE6* decay, \( \nu_{PDE} \), in Eq. (S6) above. The number of kinetic parameters is kept to a minimum by using normalized units for these dynamic variables; each concentration is divided by the corresponding value in the dark (Sneyd and Tranchina, 1989; Tranchina et al., 1991) Thus, we define the normalized concentrations by: \( x \) for cGMP; \( y \) for free intracellular \( \text{Ca}^{2+} \); \( u \) for \( \text{Ca}^{2+} \)-bound buffer sites; and \( z \) for the active form of hypothetical molecule that controls the rate of deactivation of activated phosphodiesterase.

The rate of change of the cGMP concentration is given by the difference between the rate at which it is synthesized by guanylyl cyclase and the rate at which it is hydrolyzed by PDE6*:

\[
\frac{dx}{dt} = \beta_{\text{dark}} g(y) - \beta_{\text{sub}} P^* x,
\]

where \( \beta_{\text{dark}} \) is the rate at which cGMP is synthesized (turned over, in units of the dark concentration of cGMP) in the dark; \( g(y) \) describes the dependence of guanylyl cyclase activity on free intracellular \( \text{Ca}^{2+} \) concentration; \( \beta_{\text{sub}} \) is the rate of cGMP hydrolysis (also in units of the dark concentration of cGMP) per activated subunit of PDE6. The function \( g(y) \) in Eq. (S8) is the rate of synthesis of cGMP normalized by its rate of synthesis in the
dark, and for the wild-type model it is given by:

\[
g(y) = \frac{1 + (1/y^*)^m}{1 + (y/y^*)^m}, \tag{S9}
\]

where \( m \) is the Hill coefficient; \( y^* \) is the normalized concentration of \( \text{Ca}^{2+} \) that gives half maximal cyclase activity; and the numerator of Eq. (S9) is determined by our convention on units, which requires that \( g(y = 1) = 1 \). In the GCAPs\(^{-/-} \) model, \( g(y) = 1 \).

The rate of change of the concentration of \( \text{Ca}^{2+} \)-bound buffer sites is given by the difference between the rate at which free sites become bound, and the rate at which bound sites become free:

\[
\frac{du}{dt} = k^B \left( y \frac{b - u}{b - 1} - u \right), \tag{S10}
\]

where \( k^B \) is the rate constant for the unbinding of \( \text{Ca}^{2+} \) from bound buffer sites; \( b \) is total \( \text{Ca}^{2+} \)-buffer concentration divided by the concentration of \( \text{Ca}^{2+} \)-bound buffer in the dark; the product term \( y(b - u) \) reflects the fact that the rate of \( \text{Ca}^{2+} \) binding is proportional to the product of the concentration of \( \text{Ca}^{2+} \) and the concentration of unbound \( \text{Ca}^{2+} \)-buffer sites; and the coefficient multiplying \( y(b - u) \) is determined by the steady-state constraint in darkness, \( du/dt = 0 \), where our convention on units gives \( y = 1 \) and \( u = 1 \), by definition.

The rate of change of free intracellular \( \text{Ca}^{2+} \) concentration is comprised of 2 components, each of which is a difference of 2 terms: (i) the difference between the rate at which \( \text{Ca}^{2+} \) enters the cell through cGMP-gated channels, and the rate at which it is pumped out by the \( \text{Na}^+ / \text{Ca}^{2+}, \text{K}^+ \) exchange pump; (ii) the difference between the rate at which \( \text{Ca}^{2+} \) is freed up by release from \( \text{Ca}^{2+} \)-bound buffer sites, and the rate at which \( \text{Ca}^{2+} \) binds to unbound buffer sites:

\[
\frac{dy}{dt} = \gamma^{Ca} \left[ (1 - y^{\text{min}}) x^3 - (y - y^{\text{min}}) \right] - a \frac{du}{dt}. \tag{S11}
\]

In Eq. (S11) \( \gamma^{Ca} \) is the rate constant for the \( \text{Na}^+ / \text{Ca}^{2+}, \text{K}^+ \) exchange pump, which pumps
Ca\(^{2+}\) out at a rate proportional to the difference between the free intracellular Ca\(^{2+}\) concentration and an assumed minimum to which this concentration can be driven; the term involving \(x^3\) reflects the fact that the inward flux of Ca\(^{2+}\) through cGMP-gated channels is proportional to the third power of the cGMP concentration; the coefficient of \(x^3\) is determined by the constraint that \(dy/dt = 0\) in the dark steady state, where \(x = 1\), and \(y = 1\) (by definition); \(a\) is the concentration of Ca\(^{2+}\)-bound buffer sites in the dark divided by the dark free intracellular Ca\(^{2+}\) concentration; the coefficient \(a\) that links the buffer kinetics in Eq. (S10) to the overall free intracellular Ca\(^{2+}\) kinetics stems from our convention on units, in which both the Ca\(^{2+}\) and Ca\(^{2+}\)-bound buffer site concentrations are measured relative to their dark values.

We assume that the rate of decay of both spontaneously activated and light-activated PDE6* is controlled by a molecule that has an “active” and an “inactive” form; the rate at which this molecule is inactivated is controlled by the free intracellular Ca\(^{2+}\) in a cooperative manner; and that the activation rate is independent of Ca\(^{2+}\). This is a phenomenological model, and we discuss an alternative scheme below. The rate of change of the concentration of the active form of this molecule is given by the difference between the rate at which the molecule transitions from the inactive to active form, and the rate at which it transitions from the active to the inactive form:

\[
\frac{dz}{dt} = (k_{f,z} + k_{r,z,dark}) - (k_{f,z} + k_{r,z,dark} h(y)) z, 
\]

(S12)

where \(k_{f,z}\) is the forward (inactive to active) rate constant; \(k_{r,z,dark}\) is the reverse rate constant in the dark (where \(y = 1\)); \(k_{r,z,dark} h(y)\) is the rate constant for general value of the free intracellular Ca\(^{2+}\) concentration \((y)\); \(h(y)\) gives the dependence on free intracellular Ca\(^{2+}\) concentration of the deactivation (reverse) rate constant. We found that the following empirical Hill equation, which gives a sigmoidal dependence of the reverse rate constant on
Ca$^{2+}$, captures the desired adaptation behavior:

$$h(y) = \left( \frac{(y/\eta)^w}{1 + (y/\eta)^w} \right) \left[ \frac{1 + (1/\eta)^w}{(1/\eta)^w} \right],$$  

(S13)

where $w$ is the Hill coefficient; $\eta$ is a constant that controls the range of normalized Ca$^{2+}$ concentrations over which the rate of decay of PDE6* changes (see below); and the coefficient in square brackets in Eq. (S13) is determined by our convention on units, which requires $h(y = 1) = 1$. Replacing $h(y)$ in Eq. (S13) with $h(y) = y^w$, where $w$ is a fitted parameter, gives similar behavior. In either case, the steady-state value of $z$ increases sigmoidally as the steady-state concentration of intracellular Ca$^{2+}$ falls with increasing background light level. Because the rate of deactivation of spontaneously activated and light-activated PDE6*, $\nu^{PDE}$, is given by $\nu^{PDE,\text{dark}} z$, the rate of PDE6* deactivation, normalized by its value in darkness, is equal to $z$.

The steady-state dependence of $z$ on the normalized Ca$^{2+}$ concentration, $y$, follows by setting $dz/dt$ equal to zero in Eq. S12:

$$z = \frac{k^{f,x} + k^{r,x,\text{dark}}}{k^{f,x} + k^{r,x,\text{dark}} h(y)}.$$  

(S14)

In the dark $y = 1$, $h(y) = 1$, $z = 1$, and $\nu^{PDE} = \nu^{PDE,\text{dark}} z = \nu^{PDE,\text{dark}}$. In very bright background light, $y$ is close to $y^{\min} = 0.08$, where, with our wild-type model parameters, $g(y) \approx 0.0001$, $z \approx 2.2$, and $\nu^{PDE,\text{dark}} z \approx 2.2 \nu^{PDE,\text{dark}}$.

In our model, the time scale for the change of $\nu^{PDE}$ is on the order of a minute. This slow time scale is responsible for the slow partial recovery of photocurrent that the model exhibits in response to a prolonged step of light.

The total outer-segment membrane current, which includes cGMP-gated current and
current produced by the electrogenic Na\(^+\)/Ca\(^{2+}\), K\(^+\) exchange pump, is given by

\[
I = I_{\text{dark}} \left[ \frac{x^3 + \left( \frac{f_{\text{Ca}}}{z_{\text{Ca}}} \right) (y - y_{\text{min}}) / (1 - y_{\text{min}})} {1 + \left( \frac{f_{\text{Ca}}}{z_{\text{Ca}}} \right)} \right],
\]

(S15)

where \(I_{\text{dark}}\) is the dark value of the total outer segment membrane current.

**Alternative model equations: modulation of light-activated PDE6\(^*\) only, and modulation of the CNG channel by a hypothetical non-calmodulin mechanism**

We were inspired by an anonymous reviewer to consider an alternative mechanism of light adaptation in which the rate of decay of light-activated PDE6\(^*\) only is modulated by free intracellular Ca\(^{2+}\), and there is an additional, hypothetical, Ca\(^{2+}\)-mediated modulation of the CNG channels that is not mediated by calmodulin. We found that such a scheme gives adaptation behavior that it indistinguishable from that of our original model above. It also exhibits slow partial recovery of the photocurrent in response to a prolonged step of light, and a pronounced overshoot, with slow recovery to baseline, upon light offset. In the GCAPs KO version, the model requires that the sensitivity of the photocurrent to cGMP increase by a factor of 10 at the highest background light level shown in our GCAPs KO data figures.

In this alternative model with the modulation of light-activated PDE6\(^*\) only: the kinetic Eq. (S5) for \(G^*\) is modified by setting \(\nu_{\text{pon}}\) equal to zero; \(P^*\) in Eq. (S6) is interpreted as light-activated PDE6\(^*\); and the term corresponding to the hydrolysis of phosphodiesterase, \(\beta_{\text{sub}} P^* x\), in Eq. (S8) is replaced by \((\beta_{\text{dark}} + \beta_{\text{sub}} P^*) x\). In the particular scheme for modulation of the CNG channel we explored, the photocurrent is scaled multiplicatively (gated) by a slow dynamic variable, \(s\), controlled by free intracellular Ca\(^{2+}\) according to an equation like
that for \( z \) above:

\[
\frac{ds}{dt} = (k_{fs} + k_{rs}) - (k_{fs} + k_{rs} y_{ws}) s.
\]  

(S16)

In this model, the \( x^3 \) terms in photocurrent Eq. (S15) and in the Ca\(^{2+}\) dynamics Eq. (S11) are replaced by \( s x^3 \). The fitted value for the Hill coefficient in Eq. (S16) was 3.2.

**Supplemental Computational Methods**

Model equations were solved numerically with the built-in ordinary differential equation solver *ode15s* in Matlab (Natick, MA). The best-fit model parameters were found for each genotype by minimizing an objective function that we defined as the sum of the squared differences (errors) between the model computations and experimental data points, over all flash responses and over all background light levels. To do this, we used Matlab’s *fminsearch* function which requires only evaluation of the objective function at each iteration, as opposed to other minimization programs that require computation of the objective function gradient, or still others that require the gradient and the Hessian of the objective function. All model equations above correspond to both genotypes, wild-type and GCAPs\(^{-/-}\), with the exception of Eq. (S9). For wild-type simulations, Eq. (S9) was used as stated, and for the GCAPs\(^{-/-}\) simulations we eliminated Ca\(^{2+}\) feedback onto guanylyl cyclase, mediated by GCAPs, by simply setting \( g(y) \) equal to 1.

**Comment on best-fit parameter values**

The “front-end" of our model — including the activation and deactivation of rhodopsin, production of \( G_\alpha\)-GTP, and the activation of PDE6 — is linear. Consequently, an alternative front end, differing in detail, but producing closely similar \( G_\alpha\)-GTP waveforms, would fit the data equally well. The upshot is that our data and model do not allow an unambiguous
parsing of the contribution made by each front-end reaction to the overall kinetics. In other words, $k_{RK}^0$, $k^f$, and $k^{G-PDE\_PDE}$ cannot be assigned unambiguously. Furthermore, the fact that the rising phase of the rod photocurrent response to a flash is not affected by light adaptation (Nikonov et al., 2000; Soo et al., 2008) makes it seem unlikely that specific details in the front-end reaction in our model matter in the context of our study.

It is worth noting that, in general, we do not expect the best-fitting parameter values for the 2 genotypes in Table S4 to be the same. We see substantial diversity in response waveforms from rod to rod, for a given experimental condition and stimulus, even within the same genotype. Such diversity could only be reproduced by a model with a diversity of parameter values. As stated in the main body of this paper, the circulating dark current is larger in GCAPs$^{-/-}$ rods than in WT rods. This implies that both cGMP and Ca$^{2+}$ concentrations are higher in GCAPs$^{-/-}$ rods. Thus it seems reasonable to expect even greater variation between the 2 genotypes in this study, particularly because enzymes are in environments with systematic differences in cGMP and Ca$^{2+}$ levels. In fact, the differences for the values of $\eta$ and $\beta_{dark}$ between the 2 genotypes are in the direction expected from the higher free intracellular Ca$^{2+}$ concentration in GCAPs$^{-/-}$ rods; the difference in $a$ is in the direction expected from the higher intracellular Ca$^{2+}$ concentration coupled with the removal of the GCAPs Ca$^{2+}$-binding protein in the GCAPs$^{-/-}$ rods. Furthermore, our procedure for finding best-fit values of parameters has made it clear to us that our model is robust in the sense that multiple parameter sets give model behaviors that are not detectably different by visual inspection of the response waveforms. One consequence is that the precise values of the best-fit model parameters, found by minimizing the objective function (see Mathematical Methods in this section), depend to some extent on the initial guess/estimate for the parameters. It seems reasonable to suppose that this robustness reflects true physiological robustness of the phototransduction signaling pathway. Nevertheless, it is worth noting that a number of parameters in Table S4 for GCAPs$^{-/-}$ are well within, or nearly within, a factor
of 2 compared with corresponding values for WT, namely, $k_r$; $k_{G-PDE}$; $\nu_{PDE,\text{dark}}$; $\beta_{\text{sub}}$; $k_B$; $k_{r,z,\text{dark}}$; $\gamma_{\text{Ca}}$; $b$; $a$; and $\eta$. The difference of roughly a factor of 3 in $\beta_{\text{dark}}$ between the 2 species suggests the possibility that the rate at which the dark concentration of cGMP is turned over in the dark is higher in GCAPs$^{-/-}$ mouse rods, which lack guanylyl cyclase accelerating proteins, than in WT rods.
### Dynamic Variable | Definition
--- | ---
\( \Phi \) | Number of photoisomerizations per second
\( R_n \) | Activated rhodopsin with \( n \) occupied phosphorylation sites
\( G_n \) | Number of G-protein (G\( \beta \gamma \)-G\( \alpha \)-GTP) molecules produced by \( R_n \)
\( G^* \) | Number of activated G-protein (G\( \alpha \)-GTP) molecules
\( P^* \) | Total number of light- and dark-activated phosphodiesterase sub-units
\( x \) | Normalized free cGMP concentration
\( y \) | Normalized free Ca\(^{2+} \) concentration
\( u \) | Normalized concentration of Ca\(^{2+} \)-bound buffer
\( z \) | Normalized concentration of hypothetical molecule that controls PDE6* decay
\( g(y) \) | Normalized rate of synthesis of cGMP by guanylyl cyclase
\( h(y) \) | Normalized rate constant for inactivation of the hypothetical molecule that controls PDE6* decay
\( I \) | Total outer segment membrane current

Table S1: Dynamic variables in phototransduction model described by equations (S1)-(S13). Each normalized variable is normalized by its dark value. \( \Phi(t) \) for each simulation was computed by multiplying the corresponding photons per square micron per second in each stimulus by a nominal rod collecting area of 0.5 square microns. The time courses of the dynamic variables \( R_n, G_n, G^*, P^*, x, y, u, \) and \( z \) are governed by ordinary differential equations, Eq.s (S1)-(S6), (S8), and (S10)-(S12). The definitions of \( g(y) \) and \( h(y) \) are given in Eqs. (S9) and (S13), respectively. The model equations were used to generate fits of experimental sensitivity data and experimental flash-response data shown in Figures 3 and 6, respectively.
<table>
<thead>
<tr>
<th>Parameter (s$^{-1}$)</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{n}^{RK}$</td>
<td>Rate constant for phosphorylation of rhodopsin with $n$ bound phosphates</td>
</tr>
<tr>
<td>$k^{Arr}$</td>
<td>Rate constant for arrestin capping of fully phosphorylated rhodopsin</td>
</tr>
<tr>
<td>$k_{n}^{G}$</td>
<td>Rate constant for catalysis of the exchange of GDP for GTP on G-protein by activated rhodopsin with $n$ occupied phosphorylation sites</td>
</tr>
<tr>
<td>$k^{f}$</td>
<td>Rate constant for the dissociation of G-protein into $\alpha$- (activated) and $\beta\gamma$-subunits</td>
</tr>
<tr>
<td>$k_{G-PDE}$</td>
<td>Rate constant for the activation of phosphodiesterase by activated G-protein</td>
</tr>
<tr>
<td>$\nu^{spon}$</td>
<td>Rate of spontaneous activation of G-protein</td>
</tr>
<tr>
<td>$\nu^{PDE, dark}$</td>
<td>Rate constant for decay of activated phosphodiesterase in the dark</td>
</tr>
<tr>
<td>$\beta^{dark}$</td>
<td>Rate of synthesis (turnover) of cGMP in the dark, normalized by the dark concentration of cGMP</td>
</tr>
<tr>
<td>$\beta^{sub}$</td>
<td>Rate constant for the hydrolysis of cGMP per activated phosphodiesterase subunit</td>
</tr>
<tr>
<td>$k^{B}$</td>
<td>Rate constant for the dissociation of bound Ca$^{2+}$ from buffer sites</td>
</tr>
<tr>
<td>$\gamma^{Ca}$</td>
<td>Rate constant for the Ca$^{2+}$, K$^+$/Na$^+$ exchange pump</td>
</tr>
<tr>
<td>$k^{r,z, dark}$</td>
<td>Dark value of the rate constant for deactivation of the hypothetical molecule that controls PDE6* decay</td>
</tr>
<tr>
<td>$k^{f,z}$</td>
<td>Rate constant for the activation of the hypothetical molecule that controls PDE6* decay</td>
</tr>
</tbody>
</table>

Table S2: Rate constants in model equations (S1)-(S13). Ten rate constants in this table were chosen by best-fit criteria. The remaining ones were chosen according to the following prescriptions: (1) $k_{n}^{RK}$, for $n = 1, 2, ..., 5$, and $k^{Arr}$ were computed from $k_{0}^{RK}$ as described in the text just below Eq. (S3); (2) $k_{n}^{G}$ were computed as described in the text below Eq. (S4); (3) The dark steady state requires that $\nu^{spon} = \beta^{dark} \nu^{PDE, dark} / \beta^{sub}$. The model equations were used to generate fits of experimental sensitivity data and experimental flash-response data shown in Figures 3 and 6, respectively.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b$</td>
<td>Total $\text{Ca}^{2+}$-buffer concentration divided by the concentration of $\text{Ca}^{2+}$-bound buffer in the dark</td>
</tr>
<tr>
<td>$a$</td>
<td>Concentration of $\text{Ca}^{2+}$-bound buffer in the dark divided by the dark concentration of free intracellular $\text{Ca}^{2+}$</td>
</tr>
<tr>
<td>$y^\text{min}$</td>
<td>Minimum concentration of free intracellular $\text{Ca}^{2+}$, normalized by the dark value of free intracellular $\text{Ca}^{2+}$</td>
</tr>
<tr>
<td>$y^*$</td>
<td>Concentration of free intracellular $\text{Ca}^{2+}$ that gives half maximal guanylyl cyclase activity, normalized by the dark value of free intracellular $\text{Ca}^{2+}$</td>
</tr>
<tr>
<td>$m$</td>
<td>Hill coefficient for $\text{Ca}^{2+}$ control of guanylyl cyclase activity</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Concentration of free intracellular $\text{Ca}^{2+}$ that gives half maximal rate of deactivation of the hypothetical molecule that controls PDE6* decay</td>
</tr>
<tr>
<td>$w$</td>
<td>Hill coefficient for $\text{Ca}^{2+}$ control of the rate of deactivation of the hypothetical molecule that controls PDE6* decay</td>
</tr>
<tr>
<td>$f_{\text{Ca}}$</td>
<td>Fraction of cGMP-gated current carried by $\text{Ca}^{2+}$</td>
</tr>
</tbody>
</table>

Table S3: Dimensionless parameters in model equations (S1)-(S13). Four of the parameters in this table were chosen by best-fit criteria ($b$, $a$, $y^*$ and $\eta$), and their values are given in Table S4. The remaining parameters were set as follows: $y^\text{min} = 0.08$; $m = 2.2$; $w = 4$; $f_{\text{Ca}} = 0.15$ (Fu and Yau, 2007) The model equations were used to generate fits of experimental sensitivity data and experimental flash-response data shown in Figures 3 and 6, respectively.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild-type</th>
<th>GCAPs(^{-/-})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k^0_{RK}) (s(^{-1}))</td>
<td>524</td>
<td>525</td>
</tr>
<tr>
<td>(k^f) (s(^{-1}))</td>
<td>29</td>
<td>36</td>
</tr>
<tr>
<td>(k^{G-PDE}) (s(^{-1}))</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>(\nu_{PDE,dark}) (s(^{-1}))</td>
<td>5.5</td>
<td>5.9</td>
</tr>
<tr>
<td>(\beta_{dark}) (s(^{-1}))</td>
<td>9.0</td>
<td>2.8</td>
</tr>
<tr>
<td>(\beta_{sub}) (s(^{-1}))</td>
<td>0.0086</td>
<td>0.0068</td>
</tr>
<tr>
<td>(k^H) (s(^{-1}))</td>
<td>12</td>
<td>5.9</td>
</tr>
<tr>
<td>(k^{r,z,dark}) (s(^{-1}))</td>
<td>0.023</td>
<td>0.018</td>
</tr>
<tr>
<td>(k^{f,z}) (s(^{-1}))</td>
<td>0.019</td>
<td>0.0053</td>
</tr>
<tr>
<td>(\gamma_{Ca}) (s(^{-1}))</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>(b)</td>
<td>7.1</td>
<td>13</td>
</tr>
<tr>
<td>(a)</td>
<td>0.85</td>
<td>0.64</td>
</tr>
<tr>
<td>(y^*)</td>
<td>0.42</td>
<td>NA</td>
</tr>
<tr>
<td>(\eta)</td>
<td>0.89</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Table S4: Best-fit parameters, in model equations (S1)-(S13), for WT and GCAPs\(^{-/-}\) mouse rod data. Note that the interpretation of \(\beta_{sub}\) is the number of times per second the dark concentration of cGMP is hydrolyzed per activated subunit of PDE6. The data in the present paper do not allow one to determine the absolute value of \(\beta_{sub}\). Rather, its best-fit value is inversely proportional to the number of activated G-proteins assumed to be produced by each phosphorylated form of activated rhodopsin, \(\mu^G\). We set \(\mu^G\) to a nominal values of 20; if one were to use 4 instead, then \(\beta_{sub}\) would be 5 times larger. The model equations were used to generate fits of experimental sensitivity data and experimental flash-response data shown in Figures 3 and 6, respectively.
Supplemental References


Figure S2: Simulated response of a dark-adapted, wild-type rod to onset and offset of a step of light (Eqs. (S1) through Eq. (S15), with best-fit parameters given in Table S4). Light onset is at time $t = 0$ s, and offset is at $t = 60$ s. The slow, partial recovery of the photocurrent during the prolonged step presentation is a consequence of the slow increase in the rate of deactivation of spontaneously activated and light-activated PDE6*. The increased rate of deactivation persists for some time after light offset; this causes the cGMP concentration, photocurrent, and free intracellular Ca$^{2+}$ concentrations to increase beyond their dark values for many seconds afterwards. As the deactivation rate decreases, in response to Ca$^{2+}$, the cGMP concentration, Ca$^{2+}$ concentration, and photocurrent return to their baseline values.