

A kinetic analysis of mouse rod and cone photoreceptor responses

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Key points

- Most vertebrate eyes have rods for dim-light vision and cones for brighter light and higher temporal sensitivity.
- Rods evolved from cone-like precursors through expression of different transduction genes or the same genes at different expression levels, but we do not know which molecular differences were most important.
- We approached this problem by analysing rod and cone responses with the same model but with different values for model parameters. We showed that, in addition to outer-segment volume, the most important differences between rods and cones are: (1) decreased transduction gain, reflecting smaller amplification in the G-protein cascade; (2) a faster rate of turnover of the second messenger cGMP in darkness; and (3) an accelerated rate of decay of the effector enzyme phosphodiesterase and perhaps also of activated visual pigment.
- We believe our analysis has identified the principal alterations during evolution responsible for the duplex retina.

Abstract Most vertebrates have rod and cone photoreceptors, which differ in their sensitivity and response kinetics. We know that rods evolved from cone-like precursors through the expression of different transduction genes or the same genes at different levels, but we do not know which molecular differences were most important. We have approached this problem in mouse retina by analysing the kinetic differences between rod flash responses and recent voltage-clamp recordings of cone flash responses, using a model incorporating the principal features of photoreceptor transduction. We apply a novel method of analysis using the log-transform of the current, and we ask which of the model's dynamic parameters need be changed to transform the flash response of a rod into that of a cone. The most important changes are a decrease in the gain of the response, reflecting a reduction in amplification of the transduction cascade; an increase in the rate of turnover of cGMP in darkness; and an increase in the rate of decay of activated

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phosphodiesterase, with perhaps also an increase in the rate of decay of light-activated visual pigment. Although we cannot exclude other differences, and in particular alterations in the Ca^{2+} economy of the photoreceptors, we believe that we have identified the kinetic parameters principally responsible for the differences in the flash responses of the two kinds of photoreceptors, which were likely during evolution to have resulted in the duplex retina.

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Introduction

In most vertebrate retinas there are rod photoreceptors, which are more sensitive and mediate vision in dim light; and cone photoreceptors, which are less sensitive but respond at higher temporal frequencies and are used for most bright-light vision. As Schultze first proposed over 150 years ago (Schultze, 1866), these two photoreceptor types form a duplex retina, which we now know must have appeared very early during the evolution of vertebrates (Asteriti *et al.* 2015; Morshedien & Fain, 2015). Rods appear to have evolved from more primitive cone-like photoreceptors through gene duplication, modification of transduction enzymes, and changes in protein expression levels to accommodate vision in dimmer light (see Fain *et al.* 2010; Morshedien & Fain, 2017; Lamb, 2019).

Although rods and cones both use a similar transduction cascade, there are many molecular differences in the proteins they utilize, which are thought to be responsible for their different response properties. For several of the components of transduction, the two kinds of photoreceptors use different protein isoforms (see Ingram *et al.* 2016). That is true of their photopigments, transducin G proteins, phosphodiesterase (PDE6) effector enzymes, and cyclic nucleotide-gated channels. In other cases, the rods and cones are thought to use the same protein isoform but with different levels of expression. The GTPase-activating proteins (GAPs) and guanylyl cyclase are both more highly expressed in cones than in rods (Cowan *et al.* 1998; Zhang *et al.* 2003; Takemoto *et al.* 2009). These molecular differences must in some way have been responsible for the evolution of the differences in the physiology of the two photoreceptor types. Rods and cones also differ in the morphology of their outer segments, with rods having cytoplasmic discs and cones membrane lamellae. This difference is, however, now known not to contribute to the different sensitivities and kinetics of the rod and cone photocurrent responses (Asteriti *et al.* 2015; Morshedien & Fain, 2015).

Many attempts have been made to determine which of the different isoforms or different expression levels are responsible for the differences in sensitivity and response kinetics of rods and cones. The cone isoforms

of visual pigment (Sakurai *et al.* 2007; Shi *et al.* 2007; Fu *et al.* 2008), transducin (Deng *et al.* 2009; Chen *et al.* 2010b; Mao *et al.* 2013), and the phosphodiesterase-6 effector enzyme (PDE, Deng *et al.* 2013; Majumder *et al.* 2015) have been expressed in rods with variable results, sometimes producing alterations in response properties and sometimes producing no change at all. In other experiments, the expression of the GAP proteins in rods was increased (Krispel *et al.* 2006; Chen *et al.* 2010b), which may mimic the greater expression of these proteins in cones. In none of these experiments did a single molecular change in a rod produce responses similar to those of a cone.

These studies indicate that many different alterations in isoforms and expression levels must have been required during evolution to produce the duplex retina. It is, however, still unclear which alterations were most important. Because it may be difficult to make further progress with purely experimental methods, we approached this problem by combining experiment and theory. We began with a model of the mouse rod photoreceptor response that incorporates the principal features of the transduction cascade (Pugh & Lamb, 1993; Rieke & Baylor, 1996; Andreucci *et al.* 2003; Hamer *et al.* 2003; Hamer *et al.* 2005; Reingruber & Holcman, 2008; Chen *et al.* 2010c; Gross *et al.* 2012a; Reingruber *et al.* 2013). Although similar models have been proposed for cones (see, for example, Klaus *et al.* 2019), these attempts have suffered from a paucity of cone recordings. Suction-electrode recordings from mouse cones have been obtained by several investigators (Nikonov *et al.* 2005, 2006; Sakurai *et al.* 2011, 2015; Cao *et al.* 2014), including our own laboratory (Ingram *et al.* 2016; Kaylor *et al.* 2017), but these recordings are unlikely to provide an accurate reflection of the kinetics of the light-dependent change in outer-segment conductance because of the rapid kinetics of the cone response and the high capacitance of the cone membrane (Perry & McNaughton, 1991). Whole-retina measurements of massed cone responses (as in Sakurai *et al.* 2011, 2015; Morshedien *et al.* 2019) reflect the change in the cone membrane potential rather than the change in outer-segment conductance.

A more accurate assessment of changes in cone conductance could be obtained from voltage clamp

(Perry & McNaughton, 1991). We have recently described voltage-clamp recordings from dark-adapted mouse cones in retinal slices (Ingram *et al.* 2019), not only from wild-type (WT) cones but also from cones with mutations in key transduction proteins. We now use these responses together with responses from mouse rods to define the minimal changes required to transform the dynamics of a rod response into those of a cone. We focus our study on dark-adapted responses to dim and non-saturating flash intensities, from which dynamical parameters can already be inferred.

In agreement with the molecular biology, we show that no one change is sufficient but that several changes need to be made, particularly in the amplification of the G-protein cascade, the dark rate of cGMP turnover, the rate of decay of activated PDE, and perhaps also the rate of decay of activated visual pigment. Our analysis also predicts that a much lower number of PDEs are activated per photon in cones than in rods. These studies help clarify the physiological differences between the two photoreceptor types and may indicate which molecular changes were most important in forming the duplex retina over 500 million years ago.

Materials and methods

Animals and ethical approval

The data we used to determine model parameters came from experiments performed in accordance with the rules and regulations of the NIH guidelines for research animals, as approved by the Institutional Animal Care and Use Committee of the University of California, Los Angeles, USA, and were in conformity with regulations as described by Grundy (2015). Mice were kept under cyclic light (12-on/12-off) with *ad libitum* food and water in approved cages. Retinas lacking the genes for the two guanylyl-cyclase-activating proteins (GCAPs) ($GCAPs^{-/-}$) were obtained from Jeannie Chen of the University of Southern California, Los Angeles, CA (Mendez *et al.* 2001). Mice in which the rod-specific alpha subunit of the G-protein transducin had been deleted ($Gnat1^{-/-}$) were originally made in the laboratory of Janice Lem at Tufts University, Boston, MA (Calvert *et al.* 2000), and were obtained locally from the laboratory of Dr Gabriel Travis at UCLA in Los Angeles, CA. Mice with deletions of both $Gnat1$ and the genes for the GCAPs ($Gnat1^{-/-};GCAPs^{-/-}$) were made by mating animals from the two lines. At least three generations of double-deletion animals were produced before data collection commenced. All animals were on a C57BL/6J background; approximately equal numbers of male and female mice were used to obtain the recordings. Mice were killed by cervical dislocation.

Recordings

Wild-type (WT, C57BL/6J) rod responses and all cone responses were recorded with patch electrodes and voltage clamp from retinal slices (Ingram *et al.* 2019). Rod $GCAPs^{-/-}$ responses were recorded with suction electrodes and have been previously published (Chen *et al.* 2010c). These earlier studies should be consulted for details of the experimental procedure. In both sets of experiments, temperature was measured with a thermocouple inside the chamber and was adjusted to 37°C for the suction recordings and 35°C for the voltage-clamp recordings; the value of the temperature was regulated to within 1°C with feedback controllers (TC-324B; Warner Instruments). Cone recordings were made from mice that lacked the rod transducin alpha subunit ($Gnat1^{-/-}$), in order to prevent rod signals contributing to cone responses (Ingram *et al.* 2019). Cell membrane potential was clamped near the dark resting membrane potential (−40 mV for rods and −50 mV for cones).

Rod WT responses and all cone responses were recorded in Ames' medium bubbled with 95% O₂/5% CO₂ and buffered with 1.9 g per litre sodium bicarbonate, pH 7.3 to 7.4. Rod $GCAPs^{-/-}$ responses were recorded in Dulbecco's Modified Eagle's medium supplemented with 15 mM NaHCO₃, 2 mM Na succinate, 0.5 mM Na glutamate, 2 mM Na gluconate, and 5 mM NaCl, bubbled with 5% CO₂, pH 7.4. Although rods in these two media have been reported to have somewhat different kinetic properties (Azevedo & Rieke, 2011), these differences are small in comparison with the differences in kinetics between rods and cones and are likely to result from a difference in Ca²⁺-dependent cyclase feedback, which would not affect responses from $GCAPs^{-/-}$ rods. We attempted to record from mouse $GCAPs^{-/-}$ rods with patch clamp in retinal slices under the same conditions we used for WT rods and cones. Despite considerable effort (four animals, over 30 successful seals), we were unable to obtain stable voltage-clamp recordings from these cells, which seemed fragile and were unusually noisy in comparison with WT rods. The $GCAPs^{-/-}$ rod responses even to dim flashes decayed with an extremely slow time course, and responses to bright flashes often 'hung up' and failed to return to baseline during the duration of the recording. From that point onward, the rod no longer responded even to the brightest flash. We will show, however, that WT rods with patch clamp in Ames' medium (Fig. 1A) can be fitted with the same parameters as $GCAPs^{-/-}$ rods recorded with suction electrodes, with the addition to the WT model of GCAP-dependent Ca²⁺ feedback (see Fig. 4). Though the fits are not as good for WT as for $GCAPs^{-/-}$ rods, we think this difference is more likely the result of oversimplification in our model for Ca²⁺ and feedback than methods of recording and perfusion solutions. Data were analysed either with pCLAMP and Origin (for suction

recordings) or in MATLAB with custom scripts (for patch clamp recordings).

Derivation of the phototransduction model

We have used a simplified, spatially homogeneous model for photoreceptors (see, for example, Hamer *et al.* 2005; Reingruber & Holzman, 2008; Chen *et al.* 2010; Korenbrot, 2012; Reingruber *et al.* 2013; Wang *et al.* 2018). The photoreceptor current, I , is taken to be a function of the cyclic guanosine nucleotide (cGMP) concentration c_{cg} according to:

$$I = I_d \left(\frac{c_{cg}}{c_{cg,d}} \right)^{n_{ch}} \quad (1)$$

where I_d and $c_{cg,d}$ are the current and cGMP concentration in darkness, and n_{ch} is the cooperativity constant of the channel, which we will take to be 2.5 (see, for example, Haynes & Yau, 1985; Zimmerman & Baylor, 1986; Yau & Baylor, 1989). We will make the simplifying assumption that changes in Ca^{2+} during the flash response are

sufficiently rapid for mouse cones that they can be assumed to occur in proportion to the change in current, that is:

$$\frac{c_{ca}}{c_{ca,d}} = \frac{I}{I_d} \quad (2)$$

where c_{ca} is the calcium concentration, and $c_{ca,d}$ is the calcium concentration in darkness. We make this assumption because light-dependent changes in Ca^{2+} are much faster in cones than in rods in salamanders (Sampath *et al.* 1999), and because this simplifying assumption gave a reasonable fit of the model to our cone responses. We further assume that α , the Ca^{2+} -dependent rate of synthesis of cGMP, quickly adapts to the changing Ca^{2+} concentration. We then compute the rate from the Ca^{2+} concentration according to:

$$\alpha(c_{ca}) = \alpha_{min} \frac{c_{ca}^2 + r_{\alpha} K_{\alpha}^2}{c_{ca}^2 + K_{\alpha}^2} \quad (3)$$

where K_{α} determines the sensitivity of the rate to Ca^{2+} , α_{min} is the minimum value of the cyclase rate at high

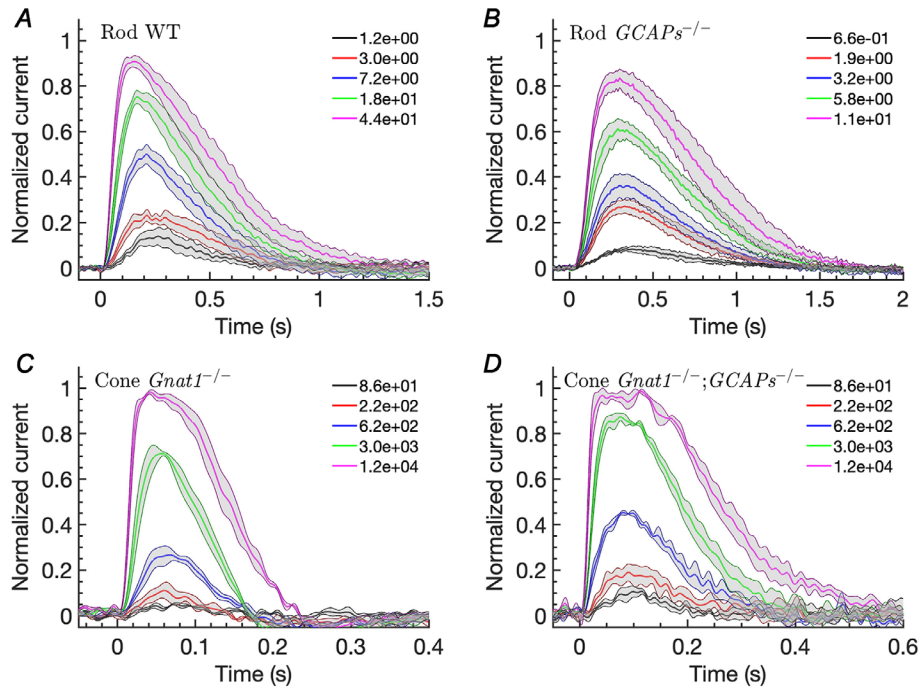


Figure 1. Experimental current recordings from mouse rods and cones

Currents from individual rods and cones have been normalized to their corresponding steady-state current in darkness I_d , with $\hat{I} = \frac{I}{I_d}$ (such that $\hat{I} = 1$ in darkness) and then averaged point by point among cells to compute mean responses. Ordinates give the mean current $\hat{I} = 1 - \hat{I}$ (such that $\hat{I} = 0$ in darkness), which is equivalent to the change in current normalized to the change produced by a bright light closing all of the outer-segment channels. The shaded regions indicate standard error of the mean computed point by point. Saturating responses to the brightest stimuli were not used in our model calculations (see Methods) and are not shown. A, mean responses of seven wild-type (WT) rods. B, rod $GCAPs^{-/-}$. Mean responses of five rods from Chen *et al.* (2010c). C, cone $Gnat1^{-/-}$ (equivalent to WT or control, see text). Mean responses from three cones. D, cone $Gnat1^{-/-}; GCAPs^{-/-}$ (equivalent to cone $GCAPs^{-/-}$, see text). Mean responses from three cones. Flash durations are 10 ms in (A), 20 ms in (B) and 5 ms in (C, D). The figure inserts give the expected number of pigment isomerizations R^* , computed with collecting areas = 0.2 (A) or 0.5 (B) for rod responses, and = 0.013 for cone responses.

Ca^{2+} , and r_α is the ratio of maximum to minimum rates, i.e. $\alpha_{\max}/\alpha_{\min}$.

To simplify the solution of the equations, we introduce dimensionless quantities by normalizing with steady-state values in darkness. That is, we set:

$$\hat{I} = \frac{I}{I_d}, \hat{c}_{cg} = \frac{c_{cg}}{c_{cg,d}}, \hat{c}_{ca} = \frac{c_{ca}}{c_{ca,d}},$$

$$\hat{\alpha} = \frac{\alpha}{\alpha_d}, \text{ and } \hat{K}_\alpha = \frac{K_\alpha}{c_{ca,d}} \quad (4)$$

The rate of synthesis of cGMP in darkness is $\alpha_d = \beta_d c_{cg,d}$ where β_d is the rate constant of cGMP hydrolysis in darkness (turnover rate). With our assumption that the Ca^{2+} concentration is directly proportional to the current, $\hat{c}_{ca} = \hat{I}$, we can express the normalized Ca^{2+} -dependent rate of synthesis of cGMP as a function of the current as:

$$\hat{\alpha}(\hat{I}) = \frac{\hat{I}^2 + r_\alpha \hat{K}_\alpha^2}{\hat{I}^2 + \hat{K}_\alpha^2} \frac{1 + \hat{K}_\alpha^2}{1 + r_\alpha \hat{K}_\alpha^2} \approx \frac{1 + \hat{K}_\alpha^2}{\hat{I}^2 + \hat{K}_\alpha^2}. \quad (5)$$

The approximation to the right in eqn (5) is valid for $r_\alpha \hat{K}_\alpha^2 \gg 1$ and was used in most of our calculations. Using the full expression, we verified r_α largely satisfied this condition.

The light-dependent rate constant of cGMP hydrolysis by PDE depends on the number of activated PDE molecules, P^* , according to $\beta_{sub} P^*$, where β_{sub} is the rate constant of cGMP hydrolysis by a single light-activated PDE. The equation for the change in normalized cGMP concentration is:

$$\frac{d}{dt} \hat{c}_{cg} = \beta_d \hat{\alpha} - (\beta_d + \beta_{sub} P^*) \hat{c}_{cg} \quad (6)$$

Log-transform of current

To facilitate the analysis of the rod and cone flash responses, we introduce the log-transform of the normalized current $y = -\ln \hat{I}$ so that $\hat{I} = e^{-y}$. We reasoned as follows. The initial phase of the light response was shown by Pugh & Lamb (1993) to follow to a first approximation an equation of the form:

$$\hat{I} = e^{-\frac{1}{2} \kappa \phi \Delta t A (t - t_{eff})^2} \quad (7)$$

with κ the collecting area, ϕ the light intensity, Δt the flash duration, t_{eff} an effective delay at the beginning of the response, and A the amplification constant. If we let $\hat{I}(t) = e^{-y(t)}$, then the initial waveform of $y(t)$ becomes linear with the flash intensity ϕ . We shall show that for responses to dim flashes, and over much of the intensity range when the GCAPs have been knocked out and there is no Ca^{2+} -dependent cyclase feedback (Mendez *et al.* 2001), not only the initial phase but the entire waveform of $y(t)$ is, to a good approximation, linear with

the flash intensity ϕ . If, therefore, the waveforms $y(t)$ of responses at different flash intensities are normalized by their corresponding peak amplitudes, the waveform of the normalized log-transform of a flash response is to a good approximation invariant with light intensity (see Fig. 2). In this way, the kinetics of flash responses can be pooled and analysed together.

From eqn (1) after normalization from eqn (4), we get $\hat{I} = \hat{c}_{cg}^{n_{ch}} = e^{n_{ch} \ln \hat{c}_{cg}}$. From this expression we get $y = -n_{ch} \ln \hat{c}_{cg}$, and with eqn (6) we obtain:

$$\frac{d}{dt} y = n_{ch} \beta_{sub} P^* - \beta_d H(y) \quad (8)$$

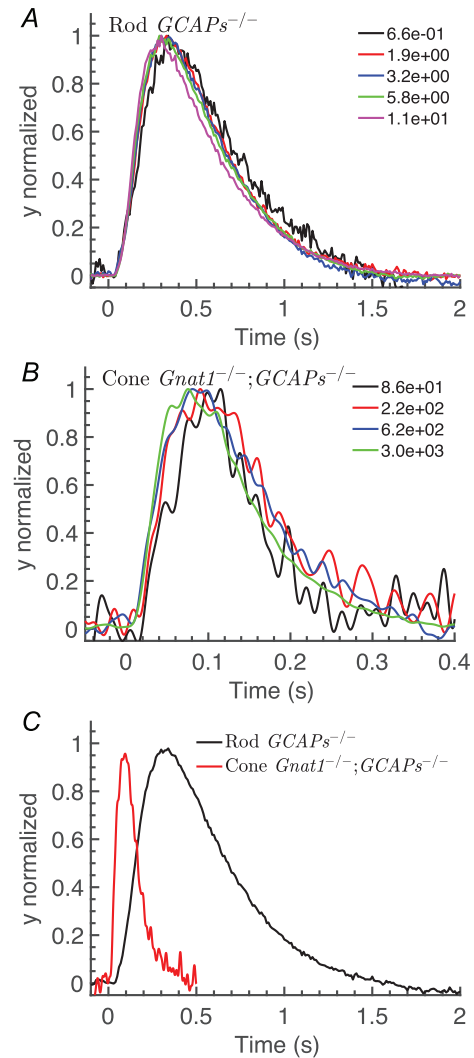


Figure 2. Normalized log-transforms of response waveforms
The $\text{GCAPs}^{-/-}$ responses from Fig. 1B and D were transformed, first by taking the negative log of the current, $y(t) = -\ln \hat{I}(t)$, and then by normalizing the value of $y(t)$ to give $\hat{y}(t) = \frac{y(t)}{y_{\text{peak}}}$. A, plots of $\hat{y}(t)$ for rod $\text{GCAPs}^{-/-}$ responses of Fig. 1B. B, plots of $\hat{y}(t)$ for cone $\text{Gnat1}^{-/-}; \text{GCAPs}^{-/-}$ responses of Fig. 1D. C, comparison of mean values of $\hat{y}(t)$ from (A) and (B).

with

$$H(y) = n_{ch} \left[e^{\frac{y}{n_{ch}}} \hat{\alpha} (e^{-y}) - 1 \right] = n_{ch} \left[e^{\frac{y}{n_{ch}}} \frac{1 + \hat{K}_\alpha^2}{e^{-2y} + \hat{K}_\alpha^2} - 1 \right]. \quad (9)$$

Transduction equations

To obtain a closed system of equations for the rod or cone light response, we additionally need equations for the production and decay of the components of the transduction cascade. We assume that light-activated visual pigment activates the G-protein transducin, which activates PDE6. We let R^* be the number of pigment molecules activated in the rod or cone by the flash, T^* be the number of activated transducins, and P^* be the number of activated PDEs. We have:

$$\begin{aligned} \frac{d}{dt} R^* &= \phi(t) \kappa - \mu_{rh} R^* \\ \frac{d}{dt} T^* &= k_{act} R^* - \mu_{tr} T^* \end{aligned} \quad (10)$$

$$\begin{aligned} \frac{d}{dt} P^* &= \mu_{tr} T^* - \mu_{pde} P^* \\ \frac{d}{dt} y &= n_{ch} \beta_{sub} P^* - \beta_d H(y) \end{aligned}$$

For flashes with duration Δt and flash intensity ϕ we have $\phi(t) = \phi(\theta(t) - \theta(t - \Delta t))$, where θ is the unit step or Heaviside function (i.e. $\theta(t) = 0$ for $t < 0$ and $\theta(t) = 1$ for $t \geq 0$). All of the parameters in eqn (10) are defined in Table 1. For the collecting areas κ we used $0.5 \mu\text{m}^2$ for $GCAP5^{-/-}$ rods recorded with suction electrodes (Field & Rieke, 2002), and $0.2 \mu\text{m}^2$ for WT rods and $0.013 \mu\text{m}^2$ for $Gnat1^{-/-}$ and $Gnat1^{-/-}; GCAP5^{-/-}$ cones recorded in retinal slices with voltage clamp (Ingram *et al.* 2019). The parameter k_{act} is the rate of activation of transducin by a single activated visual pigment, μ_{rh} is the deactivation rate of a light-activated visual pigment, μ_{pde} is the deactivation rate of a light-activated PDE, and μ_{tr} is the rate by which a T^* is converted into P^* but can be more generally viewed as an effective parameter that accounts for intermediate processes that contribute to PDE activation and delay the response without amplifying it.

To simplify the analysis of eqn (10), we transform the variables according to $\tilde{P}^* = n_{ch} \beta_{sub} P^*$, $\tilde{T}^* = n_{ch} \beta_{sub} \mu_{tr} / \mu_{pde} T^*$ and $\tilde{R}^* = n_{ch} \beta_{sub} k_{act} / \mu_{pde} R^*$. With the transformed variables, eqn (10) becomes:

$$\begin{aligned} \frac{d}{dt} \tilde{R}^* &= \mu_{rh} (\phi(t) \kappa \xi - \tilde{R}^*) \\ \frac{d}{dt} \tilde{T}^* &= \mu_{tr} (\tilde{R}^* - \tilde{T}^*) \end{aligned} \quad (11)$$

Table 1. Parameter descriptions for rod and cone model

Parameter	Description
κ (μm^2)	Photoreceptor collecting area
n_{ch}	Cyclic-nucleotide-gated channel cooperativity (Hill coefficient)
β_d (s^{-1})	Rate constant of cGMP hydrolysis in darkness (cGMP turnover rate in darkness)
μ_{rh} (s^{-1})	Rate of deactivation of an activated visual pigment
μ_{pde} (s^{-1})	Rate of deactivation of a light-activated phosphodiesterase (PDE)
μ_{tr} (s^{-1})	Rate for the transformation of light-activated transducin into light-activated PDE
$\hat{K}_\alpha$	Sensitivity of the cyclase activity on the Ca^{2+} concentration scaled by the dark Ca^{2+} concentration
ξ	Gain $\xi = \frac{n_{ch} \beta_{sub} k_{act}}{\mu_{pde} \mu_{rh}}$
k_{act} (s^{-1})	Rate of transducin activation by an activated visual pigment
β_{sub} (s^{-1})	Rate constant of cGMP hydrolysis by a light-activated PDE

$$\frac{d}{dt} \tilde{P}^* = \mu_{pde} (\tilde{T}^* - \tilde{P}^*)$$

$$\frac{d}{dt} y = \tilde{P}^* - \beta_d H(y)$$

where we introduced the transduction gain:

$$\xi = \frac{n_{ch} \beta_{sub} k_{act}}{\mu_{pde} \mu_{rh}}. \quad (12)$$

Equation (11) reveals that $y(t)$ and the current do not depend upon the individual values of κ , n_{ch} , β_{sub} and k_{act} but only on their product. We call ξ the gain because it determines the amplitude of $\tilde{P}^*(t)$ and that of the dim flash responses (see next paragraph).

Result for PDE activation

The solution of eqn (11) for PDE is $\tilde{P}^*(t) = \kappa \xi \int_0^t \phi(s) g_p(t-s) ds$ with Green's function:

$$\begin{aligned} g_p(t) &= \mu_{rh} \mu_{tr} \mu_{pde} \left(\frac{e^{-\mu_{rh} t}}{(\mu_{rh} - \mu_{tr})(\mu_{rh} - \mu_{pde})} \right. \\ &\quad + \frac{e^{-\mu_{tr} t}}{(\mu_{tr} - \mu_{rh})(\mu_{tr} - \mu_{pde})} \\ &\quad \left. + \frac{e^{-\mu_{pde} t}}{(\mu_{pde} - \mu_{rh})(\mu_{pde} - \mu_{tr})} \right) \end{aligned} \quad (13)$$

Note that $g_p(t)$ is symmetrical in μ_{rh} , μ_{tr} and μ_{pde} . For a short flash of duration Δt we have $\tilde{P}^*(t) = R_0^* \xi g_p(t)$, where $R_0^* = \phi \kappa \Delta t$ is the number of isomerizations generated by the flash. The number of activated PDEs is $P^*(t) = \frac{\tilde{P}^*(t)}{n_{ch}\beta_{sub}} = R_0^* \frac{\xi}{n_{ch}\beta_{sub}} g_p(t)$. The number of PDEs activated during a single-photon response is obtained with $R_0^* = 1$.

Asymptotic result for dim flashes

The equation for $y(t)$ is non-linear due to $H(y)$ (see eqn (9)) and cannot be solved analytically. However, for $GCAPs^{-/-}$ photoreceptors where non-linear Ca^{2+} feedback is absent, and for dim flashes where $y(t)$ remains small, we can use the first order approximation $\beta_d H(y) \approx \beta y$ with:

$$\beta = \beta_d (1 - n_{ch}\hat{\alpha}') \quad (14)$$

and $\hat{\alpha}' = \frac{d}{dx} \hat{\alpha}(x)|_{x=1} = -\frac{2}{1+\hat{\alpha}_0^2}$ to derive an asymptotic expression for the current. The solution of eqn (11) with the linear approximation $\beta_d H(y) = \beta y$ is $y(t) = \kappa \xi \int_0^t \phi(s) g_y(t-s) ds$, where:

$$g_y(t) = \mu_{rh}\mu_{tr}\mu_{pde} \left(\frac{e^{-\mu_{rh}t}}{(\mu_{tr} - \mu_{rh})(\mu_{rh} - \mu_{pde})(\mu_{rh} - \beta)} + \frac{e^{-\mu_{tr}t}}{(\mu_{rh} - \mu_{tr})(\mu_{tr} - \mu_{pde})(\mu_{tr} - \beta)} + \frac{e^{-\mu_{pde}t}}{(\mu_{rh} - \mu_{pde})(\mu_{pde} - \mu_{tr})(\mu_{pde} - \beta)} + \frac{e^{-\beta t}}{(\mu_{rh} - \beta)(\beta - \mu_{tr})(\beta - \mu_{pde})} \right). \quad (15)$$

For short flashes we get $y(t) = \phi \kappa \Delta t \xi g_y(t) = R_0^* \xi g_y(t)$. For photoreceptors in which the genes for the GCAPs have been deleted and which therefore lack Ca^{2+} -dependent feedback of cyclase activity, $\hat{\alpha}' = 0$ and $\beta = \beta_d$. Because the dim flash analysis is valid for WT and $GCAPs^{-/-}$ photoreceptors, we conclude that, to first approximation, a WT cell behaves like a $GCAPs^{-/-}$ cell with an increased cGMP turnover rate of $\beta_d(1 - n_{ch}\hat{\alpha}')$.

By dividing $y(t)$ by its peak value y_{peak} we define the normalized log-transform:

$$\hat{y}(t) = \frac{y(t)}{y_{peak}} \approx \frac{g_y(t)}{g_{y,peak}}. \quad (16)$$

For dim flashes the normalized log-transform is independent of the light intensity ϕ and the gain ξ and depends only on the dynamic parameters, and the waveform of $\hat{y}(t)$ characterizes the shape of a flash response.

Fitting procedure

Fitting was done with the Data to Dynamics framework (Raue *et al.* 2013, 2015). The optimization algorithm that we used for fitting and parameter estimation is the non-linear least-squares solver LSQNONLIN from MATLAB. Because the model is non-linear in parameters, we give goodness of fit values as root-mean-squared error (RMSEs; see, for example, Dekking *et al.* 2005). Lower values of RMSE indicate a better fit of the model to the data.

Results

Figure 1 illustrates dark-adapted photoresponses of rods and cones, normalized to the maximum response to light bright enough to close all of the outer-segment channels. Saturating responses to the brightest stimuli were not used in our model calculations (see Methods) and are not shown. For our analysis we consider two different expressions for the normalized currents: first $\hat{I}$, which is unity in darkness and decreases with light; and the complementary current $\hat{i} = 1 - \hat{I}$, which is zero in darkness and increases to unity in bright light. The latter expression is more common in the literature and is depicted in Fig. 1.

The responses of WT mouse rods in Fig. 1A are averaged responses from seven rods recorded with voltage clamp from retinal slices (see Methods). As we explain in the Methods, we were unable to obtain similar voltage-clamp recordings from $GCAPs^{-/-}$ mouse rods which were sufficiently stable to be used for model calculations. The responses of $GCAPs^{-/-}$ mouse rods in Fig. 1B are therefore averaged suction-electrode recordings from five rods taken from previously published experiments (Chen *et al.* 2010c). The $GCAPs^{-/-}$ rods lack the GCAPs, which are closely associated with guanylyl cyclase in both rods and cones and mediate Ca^{2+} -dependent modulation of the rate of the cyclase. The use of this mutant to disable Ca-dependent feedback simplifies the fitting of the model.

Cone responses are recent voltage-clamp recordings from mouse retinal slices recorded under the same conditions as the WT rod responses in Fig. 1A (Ingram *et al.* 2019). Recordings from cones were made from $Gnat1^{-/-}$ retinas lacking Gnat1, the alpha subunit of the rod G-protein transducin. Cones in the mouse retina receive input from rods through connexin-36 gap junctions (Asteriti *et al.* 2017; see Fain & Sampath, 2018), and this input is deleted in $Gnat1^{-/-}$ cones (Ingram *et al.* 2019). In Fig. 1C, we show averaged responses from three $Gnat1^{-/-}$ cones, which are effectively WT cone responses lacking rod input. Responses of a very similar waveform were recorded from $Cx36^{-/-}$ retinas lacking connexin 36 (Ingram *et al.* 2019). In Fig. 1D we show averaged responses from three $Gnat1^{-/-}; GCAPs^{-/-}$ cones lacking

both Gnat1 and the GCAP proteins, which are effectively $GCAPs^{-/-}$ responses lacking any rod input.

From the recordings in Fig. 1, we will proceed in the following way: we first use the normalized log-transform of $GCAPs^{-/-}$ current responses from Fig. 1*B* and *D*, which lack Ca^{2+} feedback, to compare the striking difference in kinetics between mouse rods and mouse cones (Fig. 2). We then analyse both WT and $GCAPs^{-/-}$ rod responses together to define a set of rod parameters (Fig. 4), which we use as reference and starting values for our cone analysis. In the model calculations of Fig. 5 we identify those dynamic parameters that have to be considerably different between rods and cones. We then concurrently fit the cone WT and $GCAPs^{-/-}$ responses to estimate values for the unknown cone parameters, including the gain and Ca^{2+} sensitivity of the cyclase rate; and we probe our cone model by fixing certain parameters and varying others (Fig. 6). Finally, we use our simulations to compare the single-photon response and the number of PDE molecules activated per photon between a rod and a cone (Fig. 7).

Normalized log-transform of the rod and cone light response

We begin by considering the log-transform of $\hat{I}$ of rod and cone $GCAPs^{-/-}$ responses, since we have shown (see eqn (15) in the Methods) that the entire waveform of the log-transform of the photoreceptor response in the absence of cyclase feedback should be proportional to the strength of the stimulus over much of the range of stimulus intensities. To demonstrate this phenomenon, we have taken the logarithms of the $GCAPs^{-/-}$ responses $\hat{I} = 1 - \hat{i}$ of Fig. 1*B* (for rods) and Fig. 1*D* (for cones) and have normalized each curve to its peak value. That is, we have calculated the negative log of the current, $\gamma = -\ln \hat{I}$, and then normalized it to the peak value to give $\hat{\gamma} = \frac{\gamma}{\gamma_{peak}}$ (see also eqn (16) in the Methods). For both rods (Fig. 2*A*) and cones (Fig. 2*B*), $\hat{\gamma}$ follows a very similar time course at each light intensity which is, however, quite different for the two kinds of photoreceptors. In Fig. 2*C*, we compare on the same time base the mean waveforms of $\hat{\gamma}$ for rods and cones from Fig. 2*A* and *B*. This figure illustrates the considerable difference in the kinetics of the rod and cone responses.

Model for the rod and cone response

As we describe in the Methods section, we used a parsimonious, spatially homogeneous model of vertebrate phototransduction. Our model was derived from earlier models (see, for example, Hamer *et al.* 2005; Reingruber & Holcman, 2008; Chen *et al.* 2010c; Korenbrot, 2012; Reingruber *et al.* 2013; Wang *et al.* 2018), which were based on the known transduction reactions (see eqn (10)

in the Methods). In brief (see Fig. 3), we assume that light-activated visual pigment (R^*) activates the G-protein transducin (T^*), which activates PDE6 (P^*). The P^* hydrolyses the second messenger cGMP, which controls the opening of the cyclic-nucleotide-gated (CNG) channels in the outer-segment plasma membrane. In a WT rod or cone, the synthesis of cGMP by guanylyl cyclase is modulated by Ca^{2+} feedback via the GCAPs (not shown in Fig. 3). This feedback is eliminated in $GCAPs^{-/-}$ photoreceptors. We use the same equations to model both the WT and $GCAPs^{-/-}$ responses of both kinds of photoreceptors, because the biological transduction pathway is very similar between rods and cones (see Ingram *et al.* 2016). This molecular similarity suggests that, to a first approximation, the same model structure can be used to describe both rods and cones with differences faithfully captured by adjustment of model parameters, which we have circled in Fig. 3. Equations (15) and (16) in the Methods show that the difference in the kinetics of the rod and cone responses in Fig. 2 are generated by differences in the deactivation rate of a light-activated pigment μ_{rh} , the rate μ_{tr} by which an activated transducin is converted into an activated PDE, the deactivation rate of a light-activated PDE μ_{pde} , and the cGMP turnover rate in darkness β_d . The question then becomes: how many of these parameters are different, and which ones are most important?

Adjusting the rod model

To answer this question, we begin with the model for rods, where we have used kinetic parameters for the most part derived from the literature (see Table 2). We assumed a value for μ_{rh} of 28 s^{-1} (for a time constant of 36 ms, see Krispel *et al.* 2006; Chen *et al.* 2010a), a value for μ_{pde} of 5 s^{-1} (for a time constant of 200 ms, see for example Krispel *et al.* 2006; Tsang *et al.* 2006), and a value for β_d of 4.1 s^{-1} (Gross *et al.* 2012b; Reingruber *et al.* 2013). In contrast to μ_{rh} , μ_{pde} , and β_d , no clear estimate for μ_{tr} can be found in the literature. The value of μ_{tr} is often assumed to be very large, in which case the model in eqn (11) can be further simplified by removing the intermediate state T^* such that R^* directly activates PDE (Pugh & Lamb, 2000; Gross & Burns, 2010; Gross *et al.* 2012b). More generally, μ_{tr} can be viewed as an effective parameter that delays the initial rising of the response but does not contribute to amplification, provided each activated transducin (or each pair of transducins, see Qureshi *et al.* 2018) activates only a single PDE. To obtain a better fit to the initial rising phase of the responses, we decided to keep μ_{tr} as a model parameter.

In addition to the kinetic parameters μ_{rh} , μ_{tr} , μ_{pde} , and β_d , the flash response further depends on the dimensionless gain parameter $\xi = \frac{\eta_{ch}\beta_{sub}k_{act}}{\mu_{pde}\mu_{rh}}$ (see eqn (12) in the Methods) and the cyclase Ca^{2+} -sensitivity

Table 2. Parameter values for rod and cone models. Fixed input values are in black and fitted parameter values are in blue. For each cone scenario the same *Gnat1*^{-/-} and *Gnat1*^{-/-};*GCAP5*^{-/-} data have been used for the fitting. The root-mean-squared error (RMSE) makes possible the comparison of the goodness of fit between the different cone scenario (ul = the fitted parameter value reached the upper limit of the parameter range that we imposed)

Parameter	Rod	Cone Scenario 1	Cone Scenario 2	Cone Scenario 3	Cone Scenario 4
κ (μm^2)	0.2 (wild-type) 0.5 (<i>GCAP5</i> ^{-/-})	0.013	0.013	0.013	0.013
n_{ch}	2.5	2.5	2.5	2.5	2.5
ξ	0.45	0.0018	0.0019	0.0020	0.0024
β_d (s^{-1})	4.1	11.0	12.4	12.4	16.6
μ_{pde} (s^{-1})	5	37.8	85.9	40 (ul)	20 (ul)
μ_{rh} (s^{-1})	28	70.7	28	40 (ul)	101.5
μ_{tr} (s^{-1})	23.8	70.7	85.9	100 (ul)	101.5
$\hat{K}_\alpha$	0.87	0.84	0.82	0.78	0.80
RMSE		0.031	0.034	0.033	0.044

parameter $\hat{K}_\alpha$. In the expression for ξ , n_{ch} is the cooperativity constant (Hill coefficient) of the channel, β_{sub} is the rate constant by which a single light-activated PDE molecule hydrolyses cGMP, and k_{act} is the rate constant of activation of T^* by a single R^* . To estimate the parameters ξ , μ_{tr} and $\hat{K}_\alpha$ we concurrently fit the WT and *GCAP5*^{-/-} rod responses from Fig. 1A and B to the non-linear eqn (11) in the Methods using known kinetic

parameters from Table 2 (indicated in black) as input and ξ , μ_{tr} and $\hat{K}_\alpha$ as fitting parameters. In Fig. 4A and B we show the agreement between the best-fitting simulation and the data for the current $i = 1 - e^{-y(t)}$. We emphasize that the fitting and simulations for WT and *GCAP5*^{-/-} rod were performed with exactly the same parameter values but with the cyclase feedback switched off for the *GCAP5*^{-/-} simulations. The best-fitting parameter

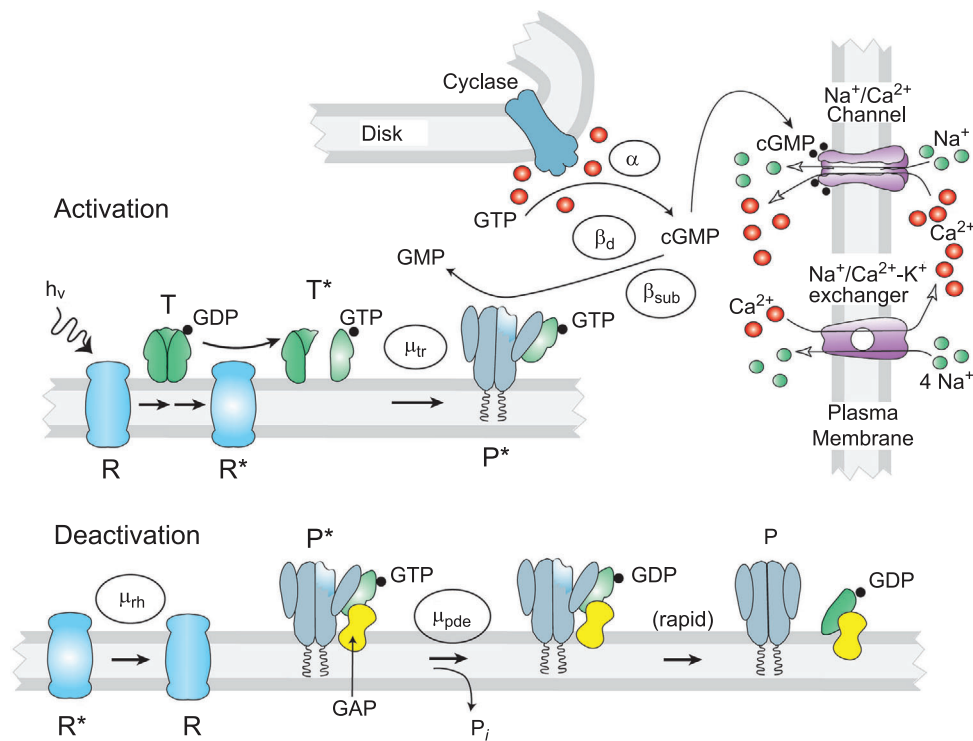


Figure 3. Schema of transduction cascade showing rates of activation and decay used in the model
Model parameters are given in bold and are circled; they are defined in Table 1. Abbreviations: $h\nu$, light; R, visual pigment; R^* , activated visual pigment; T, transducin; T^* , activated transducin; P, phosphodiesterase; P^* , activated phosphodiesterase; GAP, GTPase-activating proteins; GTP, guanosine triphosphate; GDP, guanosine diphosphate; GMP, guanosine monophosphate; cGMP, 3',5'-cyclic guanosine monophosphate.

values were $\xi = 0.45$, $\mu_{tr} = 23.8 \text{ s}^{-1}$ and $\hat{K}_\alpha = 0.87$. The agreement between simulation and data is reasonably good for both WT and $GCAPs^{-/-}$ rods, even though recordings were made with different techniques and in different perfusion solutions (see Methods). Discrepancies are greater for WT rods, probably because our assumptions about Ca^{2+} feedback were over-simplified.

Because rod fittings were performed with the averaged data from Fig. 1, we checked these values by additionally performing a single-cell analysis. We chose the five $GCAPs^{-/-}$ cells whose mean currents are given in Fig. 1B together with five WT cells, randomly chosen from among the seven WT cells used for the averaging in Fig. 1A. We then performed concurrent fittings with 25 different combinations of single-cell data. We fitted the single-cell data in exactly the same way as we performed the fittings with the averaged data. The results for the fitted parameters were (mean $\pm$ SD) $\xi = 0.49 \pm 0.12$, $\mu_{tr} = 27.9 \pm 37 \text{ s}^{-1}$, $\hat{K}_\alpha = 0.85 \pm 0.47$. Although there was considerable variability, the mean values were very close to the values we obtained from the fit to the mean responses. Confidence intervals calculated by Monte Carlo simulations of individual cell fit parameters showed that, for all rod parameters, values obtained by fitting to mean responses were within calculated 95% confidence intervals of values obtained by fitting to individual responses. Thus

we conclude that, within an alpha rate of 5%, fitting to the mean response does not yield a value that is significantly different from the mean of fitting to individual responses.

In Fig. 4C, we plot data and simulations of the log-transforms of the $GCAPs^{-/-}$ responses, and in Fig. 4D we show the agreement between simulation and data for the normalized log-transforms corresponding to Fig. 2B. Table 2 provides a summary of all the rod parameters, with black parameter values giving fixed input values, and blue parameter values giving results from fittings.

Transforming rod dynamics into cone dynamics

We next used the $GCAPs^{-/-}$ responses to examine which of the parameters μ_{rh} , μ_{pde} , μ_{tr} , and β_d have to be modified in order to turn the dynamics of rod responses into those of cones, as shown in Fig. 2C. In Fig. 5A–D we show the effect of changing only a single one of μ_{rh} , μ_{pde} , μ_{tr} , and β_{dark} (indicated in the panels). That is, we kept three of the four parameters the same as in the rod model and determined the best fit to the cone responses for the fourth using eqn (11). For all four possibilities, fits were poor. RMSE values were 0.14 for β_d alone (Fig. 5A), 0.21 for μ_{rh} alone (Fig. 5B), 0.15 for μ_{pde} alone (Fig. 5C) and 0.20 for μ_{tr} alone (Fig. 5D). For each of parameters, the fitting program always returned the value we set for the

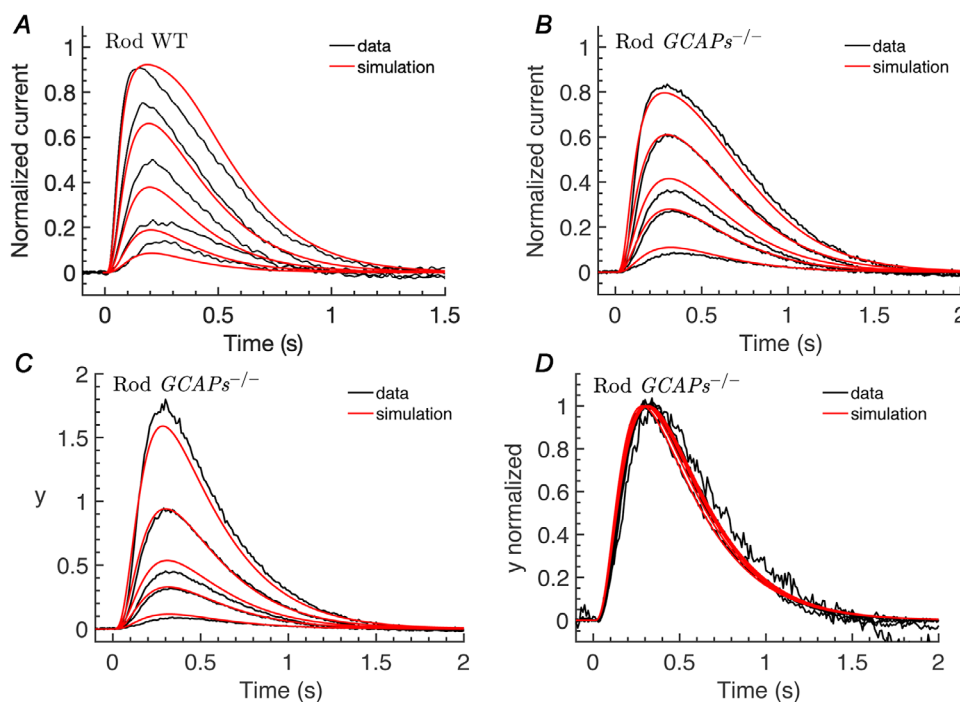


Figure 4. Adjusting the rod model

A–B, to estimate the rod parameters ξ , μ_{tr} and $\hat{K}_\alpha$ we concurrently fit the wild-type (WT) and $GCAPs^{-/-}$ traces from Fig. 1A and B to the non-linear eqn (11) in the Methods using the kinetic parameters from Table 2 (in black) as input and ξ , μ_{tr} and $\hat{K}_\alpha$ as fitting parameters. The best fit was obtained with $\xi = 0.45$, $\mu_{tr} = 23.8 \text{ s}^{-1}$ and $\hat{K}_\alpha = 0.87$. Black traces show the data, red traces show simulations. C, comparison of the log-transforms from (B). D, comparison of the normalized log-transforms from (C).

upper limit of the fitting range of 200 s^{-1} (corresponding to a time constant of 5 ms). Higher values did not seem reasonable to us and, in addition, did not alter the results. Even with this very high value, a change in only a single parameter was insufficient to capture the kinetics of decay of the cone response.

We then tested whether we could turn a $GCAPs^{-/-}$ rod into a $GCAPs^{-/-}$ cone by altering only the dynamic parameters μ_{rh} , μ_{pde} and μ_{tr} . We therefore fitted the $GCAPs^{-/-}$ responses using μ_{rh} , μ_{pde} , μ_{tr} as fitting parameters while keeping β_d equal to the rod value. Again, no reasonable fit could be obtained (Fig. 5E, RMSE of 0.1). Similarly, when we used β_d as a fitting parameter and kept rod values for μ_{rh} , μ_{pde} , μ_{tr} we could not obtain good fits (not shown, RMSE of 0.11). This shows that both β_d and PDE kinetics have to be altered. When we let both μ_{pde} and β_d vary, we could obtain a reasonable fit to the normalized log-transform (RMSE of 0.02, Fig. 5F). In contrast, when we varied only μ_{rh} and β_d , or only μ_{tr} and β_d , we obtained poor fits; the fitting program returned rates for both parameters at the value of 200 s^{-1} we had set for the upper limit of the fitting range, and RMSE was 0.11 and 0.12 (not shown). In summary, we found that, at a minimum, the rate of turnover of cGMP (β_d) and the

PDE deactivation rate (μ_{pde}) need to be increased to fit the kinetics of the cone waveform.

Adjusting the cone model

To examine in more detail the alterations we need to make in the parameters of the model to fit cone responses, we first fitted concurrently both the $Gnat1^{-/-}$ and $Gnat1^{-/-};GCAPs^{-/-}$ cone data from Fig. 1C and D with eqn (11) from the Methods. We did not make any assumptions about the cone parameter values and used all the parameters ξ , μ_{rh} , μ_{tr} , μ_{pde} , β_d and $\hat{K}_\alpha$ as unconstrained fitting parameters. Although the gain $\xi = \frac{n_{ch}\beta_{sub}k_{act}}{\mu_{pde}\mu_{rh}}$ depends on μ_{rh} and μ_{pde} , the product $\beta_{sub}k_{act}$ is unknown. We therefore used ξ as an independent fitting parameter. The best-fitting values for ξ , μ_{rh} , μ_{tr} , μ_{pde} , β_d and $\hat{K}_\alpha$ are given in Table 2 as scenario 1 (RMSE was 0.031). The reason the RMSE is now higher than in Fig. 5F (RMSE was 0.02) is that in Fig. 5F we fitted only $Gnat1^{-/-};GCAPs^{-/-}$ responses, and in addition these responses were normalized, which reduced the variability due to the response amplitude. We verified that the model and the fitting parameters are identifiable (Raue *et al.* 2009). We show the results of these fits in Fig. 6A and B.

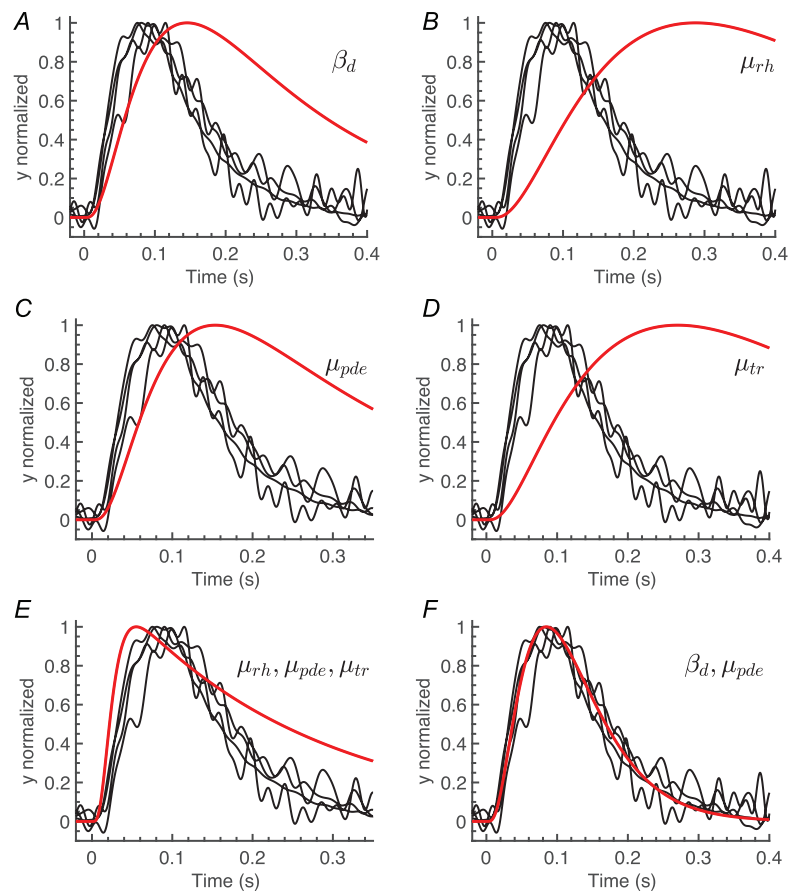


Figure 5. Transition from rod to cone kinetics

The black traces are normalized log-transforms $\hat{y}(t)$ from cone $Gnat1^{-/-};GCAPs^{-/-}$ responses from Fig. 2B. The red traces show a simulation of a normalized log-transform computed with rod parameter values for μ_{rh} , μ_{tr} , μ_{pde} , and β_d as given in Table 2, except for the parameter indicated in each of the panels, which has been estimated by fitting the normalized cone $Gnat1^{-/-};GCAPs^{-/-}$ responses with eqn (11). A–D, the best-fitting parameter values were $\beta_d = 200 \text{ s}^{-1}$, $\mu_{rh} = 200 \text{ s}^{-1}$, $\mu_{tr} = 200 \text{ s}^{-1}$, and $\mu_{pde} = 200 \text{ s}^{-1}$, corresponding to the upper limit of 200 s^{-1} that we imposed for the parameter range. Root-mean-squared errors (RMSEs) were as follows: 0.14 (A), 0.21 (B), 0.15 (C), and 0.20 (D). E, the best-fitting parameter values were $\mu_{rh} = \mu_{tr} = \mu_{pde} = 130 \text{ s}^{-1}$. RMSE was 0.1. F, the best-fitting parameter values were $\beta_d = 31 \text{ s}^{-1}$ and $\mu_{pde} = 183 \text{ s}^{-1}$. RMSE was 0.02.

We compare the current $\hat{i} = 1 - e^{-y(t)}$ for the *Gnat1*^{-/-} and *Gnat1*^{-/-}; *GCAPs*^{-/-} data (black traces) to simulations (red traces) obtained with eqn (11) from the Methods and the fitted parameter values. These simulations show that the model, together with the fitted parameter values, faithfully reproduces the *Gnat1*^{-/-}; *GCAPs*^{-/-} and the *Gnat1*^{-/-} cone responses.

As for the rod fittings, we performed a single-cell analysis with the three *Gnat*^{-/-} and three *Gnat*^{-/-}; *GCAPs*^{-/-} cells that were used for the averaged data in Fig. 1. We concurrently fitted nine different combinations of single-cell data for scenario 1. We found parameter values of (mean $\pm$ SD) $\xi = 0.0019 \pm 0.005$, $\beta_d = 11.8 \pm 2.6 \text{ s}^{-1}$, $\mu_{rh} = 65.1 \pm 20.2 \text{ s}^{-1}$, $\mu_{tr} = 69.9 \pm 19.9 \text{ s}^{-1}$, $\mu_{pde} = 43.4 \pm 8.1 \text{ s}^{-1}$, $\hat{K}_\alpha = 0.81 \pm 0.11$. These values were again very close to the values we obtained from fitting the mean responses (Table 2). Confidence intervals calculated by Monte Carlo simulations showed that, for all cone parameters, parameters fit to mean responses were within the calculated 95% confidence intervals of parameters fit to

individual responses. Thus we again conclude, with an alpha rate of 5%, that fitting to the mean response does not yield a value that is significantly different from fitting to the mean of the individual responses.

We next varied possible values of the parameters μ_{rh} , μ_{tr} and μ_{pde} . As we showed in the Methods section (see eqn (13)), we cannot decide which of the fitted parameter values corresponds to which parameter because of the symmetry among μ_{rh} , μ_{tr} and μ_{pde} ; that is, interchanging the values of μ_{rh} , μ_{tr} and μ_{pde} with fixed ξ results in identical responses. We also note that because $\beta_{sub}k_{act} = \xi \frac{\mu_{rh} \mu_{pde}}{n_{ch}}$, the predicted value of $\beta_{sub}k_{act}$ might depend upon the way the fitted parameter values are attributed to μ_{rh} , μ_{tr} and μ_{pde} . For the parameters in Table 2 for cone scenario 1 we chose $\mu_{pde} \leq \mu_{rh} \leq \mu_{tr}$.

In order to better explore the parameter space, we adopted three additional fitting scenarios. In scenario 2 we performed exactly the same fitting procedure as in scenario 1, except that μ_{rh} was fixed to the rod value. The fitting waveforms were very similar to the ones from scenario 1 (not shown), and the RMSE was only slightly increased from 0.031 to 0.034. This result indicates that a

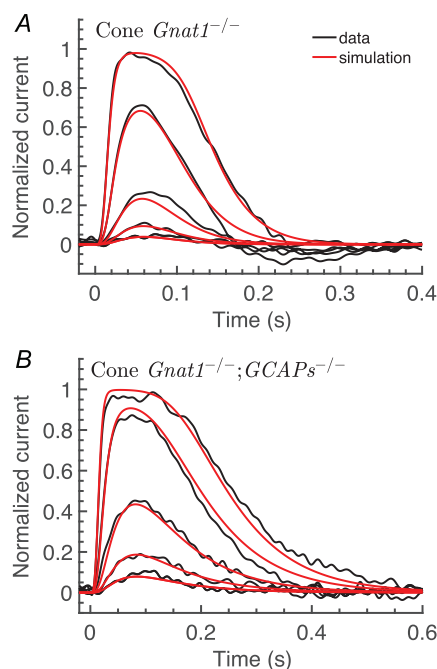


Figure 6. Adjusting the cone model

To estimate cone parameter values, the currents $\hat{i} = 1 - e^{-y(t)}$ of *Gnat1*^{-/-}; *GCAPs*^{-/-} cones and *Gnat1*^{-/-} cones (effectively wild type) from Fig. 1C and D have been fitted concurrently with the non-linear model in eqn (11) from the Methods with fitting parameters ξ , μ_{rh} , μ_{tr} , μ_{pde} , β_d , and $\hat{K}_\alpha$. We explored four different fitting scenarios as explained in the text. The best-fitting parameter values for each scenario are summarized in Table 2 (fixed parameter values are in black, fitted values are in blue). A, and B, current data (black traces) and the corresponding simulation results obtained with the parameters for cone scenario 1 (red traces) for *Gnat1*^{-/-} (A) and *Gnat1*^{-/-}; *GCAPs*^{-/-} cones (B).

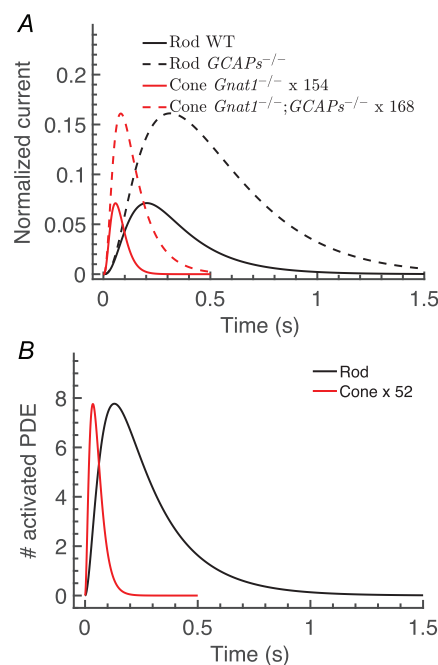


Figure 7. Single-photon response (SPR) for rod and cone model

A, comparison of the SPR current for wild-type (WT) and *GCAPs*^{-/-} rod with *Gnat1*^{-/-} (effectively wild type) and *Gnat1*^{-/-}; *GCAPs*^{-/-} cones. The currents are computed with the analytic formula $\hat{i}_{spr}(t) = 1 - e^{-\xi g_y(t)}$ with $g_y(t)$ from eqn (15) in the Methods and parameter values from Table 2 (scenario 1 for cone). B, comparison of the time-dependent number of activated PDEs corresponding to the response in (A). The number of activated PDEs is computed as $P^*(t) = \frac{\xi}{n_{ch}\beta_{sub}} g_p(t)$, with $g_p(t)$ from eqn (13) in the Methods with parameter values from Table 2 and $\beta_{sub} = 0.07$.

satisfactory fit can be obtained by accelerating μ_{pde} alone without any change in μ_{rh} .

In scenarios 1 and 2, either the rate of decay of activated visual pigment (μ_{rh}) or of activated phosphodiesterase (μ_{pde}) was rapid. In scenario 3 we tested the possibility that both rates are slow and below 40 s^{-1} (for time constants of decay greater than 25 ms). We also constrained the range for μ_{tr} to 100 s^{-1} . The best-fitting parameter values for this scenario were the upper limits $\mu_{rh} = \mu_{pde} = 40 \text{ s}^{-1}$. The fitting results were again very similar to scenarios 1 and 2, and the RMSE was 0.033. This result, together with scenarios 1 and 2, shows that μ_{rh} can be the same as the rod value or faster, but it is unlikely to be slower.

In our final scenario, 4, we tested the possibility that the PDE lifetime in cones is longer than 50 ms, corresponding to the upper limit of 20 s^{-1} for the fitting range of μ_{pde} . The best-fitting values were the upper limit $\mu_{pde} = 20 \text{ s}^{-1}$, and the fitted values for μ_{rh} and μ_{tr} were very large (101 s^{-1}), indicating extremely rapid activation of transducin and pigment decay. Because in mice the extinction of visual pigment is catalysed by the same enzyme (GRK1) in both rods and cones, we think it unlikely that the rate of decay of visual pigment is several-fold larger in cones than in rods. Combined with the around 30% larger RMSE for scenario 4 (0.044) compared with the three other scenarios (0.031–0.034), we conclude that μ_{pde} in a cone is very likely to be larger than 20 s^{-1} (with a time constant of PDE decay less than 50 ms) and several-fold larger than the value in rods. When we retained μ_{pde} at its rod value and attempted to fit the cone responses, the fit was again poor (RMSE 0.18).

The best-fitting parameter values corresponding to each scenario are summarized in Table 2 (fixed input values are in black, fitted parameter values are in blue). From these values we draw the following conclusions. First, the gain parameter ξ varies little among the various scenarios and is at least 200 times smaller for cones than for rods. The value of β_d is consistently 3–4 times larger in cones than in rods, indicating a higher rate of cGMP turnover in darkness. The rate μ_{pde} is consistently higher in cones, indicating a much shorter time constant of decay of activated PDE probably reflecting the higher concentration of GAPs in cones. Because of the similarity of the RMSE for the first three scenarios nothing definite can be said about the relative rates of pigment decay (μ_{rh}), which may or may not be higher in cones than in rods.

Response to a single photon

The difference in sensitivity and dynamics between rods and cones can be well exemplified by comparing responses to a single-photon excitation. To compute the single-photon response current, we used the analytic formula $\hat{i}_{spr}(t) = 1 - e^{-\xi g_y(t)}$ with $g_y(t)$ from

eqn (15) and with parameter values from Table 2. Because $y(t)$ is small during the single-photon response, this analytic result is in excellent agreement with a single-photon response simulation obtained with eqn (11). In Fig. 7A we compare the single-photon response for WT and $GCAPs^{-/-}$ rods with those for $Gnat1^{-/-}$ and $Gnat1^{-/-};GCAPs^{-/-}$ cones, using for cones the parameters of scenario 1. Results with parameters from the other three scenarios were similar both in magnitude and waveform.

The calculated rod single-photon current in Fig. 7A is similar in amplitude and waveform to those of recorded mouse single-photon responses (for example, Mendez *et al.* 2001; Sampath *et al.* 2005; Chen *et al.* 2010; Azevedo & Rieke, 2011), with normalized peak amplitudes (r/r_{max}) and integration times (t_i) of 0.07 and 390 ms (WT), and 0.165 and 640 ms ($GCAPs^{-/-}$). The rod responses are larger by about a factor of 150 (WT) and 165 ($GCAPs^{-/-}$) than in cones (Fig. 6A), similar to previous estimates (Nikonov *et al.* 2006). The amplitude of the single-photon current is about 2.3 times larger in a $GCAPs^{-/-}$ rod than in a WT rod and a 2.1 times larger in a $GCAPs^{-/-}$ cone than in a WT cone, somewhat smaller than found by Sakurai *et al.* (2011).

Next, we investigated how the number of activated PDEs varies between rods and cones during a single-photon response. The number of activated PDEs does not depend on Ca^{2+} feedback and is therefore the same for WT and $GCAPs^{-/-}$ photoreceptors. To compute the number of activated PDEs we used the analytic expression $P^*(t) = \frac{\xi}{n_{ch}\beta_{sub}} g_p(t) = \frac{k_{act}}{\mu_{rh} \mu_{pde}} g_p(t)$, where $g_p(t)$ is given by eqn (13) in the Methods. For a rod we have assumed $k_{act} = 350 \text{ s}^{-1}$ (Reingruber *et al.* 2013), which leads to a maximum of around eight PDEs activated at any one time during the response. With $k_{act} = 350 \text{ s}^{-1}$ and the rod parameters from Table 2, we compute $\beta_{sub} = \frac{\xi \mu_{rh} \mu_{pde}}{n_{ch} k_{act}} \approx 0.07 \text{ s}^{-1}$, which is similar to the value used by Reingruber *et al.* (2013). The parameter β_{sub} is the rate of hydrolysis of cGMP by a single P^* and is proportional to the catalytic activity of an activated PDE (k_{cat}) and inversely proportional to the outer-segment volume (V_{os}); that is, $\beta_{sub} \sim k_{cat}/V_{os}$ (Pugh & Lamb, 2000; Reingruber & Holcman, 2008, 2009). Experiments expressing cone PDE in a rod indicate that k_{cat} is smaller by about a factor of 2 for cone PDE than for rod PDE (Majumder *et al.* 2015). Since the ratio of the cone-to-rod volumes of the outer segments is about 0.4 (Nikonov *et al.* 2006), the value of β_{sub} for a cone may be similar to the rod value. We therefore assumed $\beta_{sub} = 0.07 \text{ s}^{-1}$ for both rods and cones, and we computed the number of activated PDEs from $P^*(t) = \frac{\xi}{n_{ch}\beta_{sub}} g_p(t)$. In Fig. 7B we compare the number of activated PDEs corresponding to the single-photon simulations shown in Fig. 7A. The peak number of PDEs activated at any one time during the single-photon response is reduced by a factor of around 52 in a cone compared with a rod.

Discussion

During the evolution of the vertebrate eye, more ancient cone-like precursors gave rise to photoreceptors with greater sensitivity, in part from a greater gain of the transduction cascade, and in part from a slower decay of the light response and longer time of integration of incoming photons (see Fain *et al.* 2010; Morshedien & Fain, 2017; Lamb, 2019). This process resulted in the duplex retina of the vertebrates with highly specialized rod and cone photoreceptors, which greatly differ in their sensitivity and response kinetics. Results from molecular biology (summarized in Ingram *et al.* 2016) indicate that evolutionary changes were produced gradually by small effects on several transduction parameters rather than a single effect on only one, but it is still unclear which adaptations were most important in determining the different response properties of the two kinds of photoreceptors.

We have approached this problem by comparing rod responses and recent voltage-clamp recordings of mouse cone responses. To quantify the differences between rods and cones, we used a spatially homogeneous model that focuses on the main features of the transduction cascade (Fig. 3). Because of similarities in the molecular transduction pathway (see Ingram *et al.* 2016), we used the same model for rods and cones but with different parameter values. In this way we could classify the differences between the two kinds of photoreceptors. Our analysis shows that the most important changes between rods and cones are likely to be the rate of turnover of cGMP in darkness (β_d), the rate of decay of the activated phosphodiesterase P^* (μ_{pde}) and the gain $\xi = \frac{n_{ch}\beta_{sub}k_{act}}{\mu_{pde}\mu_{rh}}$, where n_{ch} is the cooperativity constant of the CNB channels, β_{sub} is the rate constant of cGMP hydrolysis by an activated PDE and k_{act} is the rate of activation of transducin by activated visual pigment. The difference in ξ reflects the accelerated decay of P^* and perhaps R^* , but we will argue below that the product $\beta_{sub}k_{act}$ must also be significantly altered, indicating that the amplification of transduction at the level of the rates of activation of transducin by visual pigment and/or cGMP hydrolysis by PDE must be lower in a cone. These changes alone, provided they were large enough, would have been sufficient during evolution to alter the response of a cone to produce a rod capable of responding to single photons and mediating dim light vision. There may be other differences, which we made no attempt to include in this work. In particular, there may be differences in the Ca^{2+} economy of the two photoreceptors including buffering, mechanisms of Ca^{2+} extrusion, and differences in the Ca^{2+} permeability of the cyclic-nucleotide-gated channels (Perry & McNaughton, 1991; Sampath *et al.* 1999). These differences may be especially important during adaptation.

From our current model we cannot distinguish whether the changes in parameter values are generated by differences in the properties of transduction enzymes or differences in expression levels, but earlier studies have given some indication of the molecular basis for the changes we postulate. Our analysis indicates that the rate of cGMP turnover in darkness may be around four times faster in cones than in rods (see Table 2). A six-fold difference was inferred from the rate of decline of the salamander cone photocurrent in zero- Na^+ solution (Perry & McNaughton, 1991; see also Cornwall *et al.* 1995), and an even higher rate of turnover was estimated for carp cones (Takemoto *et al.* 2009). One possible explanation for the increased turnover is an increase in the expression level of both the cyclase (Takemoto *et al.* 2009) and PDE (Reingruber *et al.* 2013). Direct biochemical measurement of the expression level of a cone protein is difficult in mouse because there are so few cone photoreceptors.

Our calculations indicate that the rate of PDE decay (μ_{pde}) is also higher in cones such that the decay time constant may be reduced from about 200 ms in rods (Krispel *et al.* 2006; Tsang *et al.* 2006) to as little as 25 ms in cones (see Table 2). Such a short time constant may result from a higher level of expression of GAP proteins in cones (Cowan *et al.* 1998; Zhang *et al.* 2003; Takemoto *et al.* 2009) and is close to the value for *Gnat1*^{-/-} cones of the single-exponential time constant of decay (τ_{rec}), which is about 30 ms for small-amplitude responses (Ingram *et al.* 2019). It is possible that the rate of decay of light-activated visual pigment (μ_{rh}) is also different and more rapid in cones. On the one hand, mouse rods and cones use the same GRK1 kinase and similar arrestins (see Ingram *et al.* 2016), and we might expect that the rates of decay of R^* would also be similar. On the other hand, it is possible that the expression levels of these proteins or the accessibility of pigment phosphorylation sites may be different for rods and cones. We hope to resolve this question by recording from cones with increased expression of cone visual-pigment kinase.

Our fittings in Table 2 indicate that the gain $\xi = \frac{n_{ch}\beta_{sub}k_{act}}{\mu_{pde}\mu_{rh}}$ is smaller in cones by a factor of 200 to 250. Of the five gain parameters, the Hill coefficient of the channels n_{ch} seems not to be significantly different between rods and cones (see, for example, Haynes & Yau, 1990). We can think of the other four parameters as given by the ratios k_{act}/μ_{rh} and β_{sub}/μ_{pde} . Since k_{act} is the rate of transducin activation by visual pigment, and since the inverse of μ_{rh} is the lifetime of R^* , this first ratio gives the response amplification due to activation of transducin. For the second ratio, β_{sub} gives the rate of hydrolysis of cGMP by a single P^* , and the inverse of μ_{pde} is the time constant of P^* decay. This second ratio therefore gives the response amplification due to hydrolysis of the second messenger cGMP by PDE. Our simulations in Table 2 indicate that the product of these

two ratios is of the order of 200 to 250 times smaller in cones. That is to say, amplification in cones is around 200 to 250 times less effective than in rods. This large difference in ξ is also the primary reason for the discrepancies in the single-photon response (SPR) amplitudes between rods and cones in Fig. 7A. The ratios of the SPR amplitudes in Fig. 7A are not exactly equal to the ratios of ξ because the amplitude of the function $g_y(t)$ from eqn (15) is slightly different between rods and cones.

With the values for ξ , μ_{pde} and μ_{rh} from Table 2, we can compute that the product $\beta_{sub}k_{act}$ must be around 13 times smaller in cones. In the Results section (Response to a single photon), we argued that the value of β_{sub} may not greatly differ between rods and cones despite the difference in outer-segment volume and the molecular species of the PDE. These considerations would then imply that k_{act} , which is the rate of transducin activation by visual pigment, is likely to be much smaller in cones. A smaller rate of transducin activation may explain the reduced amplification constant of mouse cones (Nikonov *et al.* 2006; Ingram *et al.* 2019).

In this work we have focused on brief flashes to estimate the minimal changes that have to be made in a dark-adapted retina to transform rod dynamics into those of cones. We made a number of assumptions and constructed a parsimonious model which focused on the principal mechanisms of transduction to facilitate the analysis of the responses. For example, we assumed that the Ca^{2+} concentration changes instantaneously in proportion to the level of the current, we neglected effects of Ca^{2+} buffering, and we assumed that the cGMP synthesis rate by guanylyl cyclase changes instantaneously with the Ca^{2+} concentration. We also modelled cyclase modulation as the only form of transduction modulation occurring in a rod or cone so that, in a $GCAPs^{-/-}$ photoreceptor, there is no modulation of the response of any kind within the time course of the flash response. These assumptions are unlikely all to be true, and these over-simplifications may explain why our model is better able to fit responses of $GCAPs^{-/-}$ photoreceptors in the absence of Ca^{2+} feedback than WT photoreceptors in its presence.

All of these assumptions will have to be modified for longer-duration stimuli where adaptation becomes important. A more extended model will be necessary to incorporate time-dependent changes in Ca^{2+} concentration and cyclase activity, perhaps including additional mechanisms of transduction modulation, to explain why rods saturate in dim light but cones do not. As models become more sophisticated, our estimated parameter values are likely to alter, providing a more accurate reflection of the dynamics of the transduction cascade. We believe, however, that our calculations have already revealed some of the principal differences between the two kinds of photoreceptors, which we will use as input

for the more detailed future models required to explain rod and cone differences in their totality.

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Additional information

Data availability statement

Not applicable

Competing interests

The authors affirm that they have no competing interests.

Author contributions

Reingruber contributed to the conception and design of the work, designed the model, did all of the model calculations, and worked on the drafting and revision of the manuscript; Ingram and Griffis obtained the original data; and Fain contributed to the conception and design of the work and the drafting and revision of the manuscript.

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Keywords

cone, mathematical model, photoreceptor, retina, rod, vision

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document