1. Saturation of initial rates and extended time analyses of COS elongation kinetics

To further examine the data for rate- and amplitude saturation, we took running derivatives of the OPL traces obtained in response to the most intense stimuli, and extracted their maximal values (Fig. S1). For the traces of Fig. 1 obtained from Subject 1 in response to the four most intense stimuli (Fig. S1A, black traces), the maximum rates were 4.41, 4.27, 4.04 and 4.26 nm ms\(^{-1}\), negligibly different. Additional results from Subject 1 (blue traces) further confirmed the rate saturation: the average maximal rate, achieved ~ 20 ms after stimulus onset, was 4.4 ± 0.1 nm ms\(^{-1}\) (mean ± SEM, n=7; Fig. S1). Similar results were obtained from Subject 2, for whom the maximal rate was 4.3 ± 0.6 nm ms\(^{-1}\) (mean ± SEM, n=4). To confirm amplitude saturation we performed experiments with ~ 3-fold longer recording epochs with stimuli that produced saturated initial rates (Fig. S1C): for the OPL data after 1.5 s the derivatives of the traces had mean values 0.18 nm ms\(^{-1}\) and average RMS deviation from zero of 0.19 nm ms\(^{-1}\), indicating that the traces were changing negligibly, and thus saturated at such times. These statistics are provided in the “raw” OPL units for consistency with Fig. 1; to convert them to the physical length changes of the COS, they need to be divided by the refractive index, 1.41.

2. Quantifying the relationship between the photosensitivity of cone pigment bleaching by light quantified at the COS waveguide entrance and the photosensitivity of Component 2

Adaptive optics serial scanning reflectometry (1, 2) was employed to measure cone pigment bleaching. Specifically, incremental changes of backscattered light from individual cones were measured with an adaptive optics laser scanning ophthalmoscope (AO-SLO) whose preset power was predicted to bleach a small fraction of pigment in each scan cycle (1, 2). Increases in backscattered light occurred initially in each 30 ms scan cycle, consistent with the brevity (~ 1 ms) of the cone pigment Meta I → Meta II (P*) state transition that underlies bleaching, and the incremental backscatter amplitude saturated along a trajectory expected if each successive AO-SLO scan bleached 3 to 4% of the pigment present at the scan onset (Fig. 3A-C, red curves in middle panels). The photosensitivity of the incremental backscattering, obtained by analyzing the data plotted as a function of the cumulative retinal energy density of the scans (Fig. S2A-C, right hand panels), was found to be similar for each subject at the three retinal eccentricities, but ~ 3-fold higher than the average value from prior reflectometry and psychophysical studies (3-8) (Fig. S2D), and ~3-fold higher than the photosensitivity of
Component 2. These reliable discrepancies raise the questions of whether the AO-SLO single-cone reflectometry paradigm in fact measures cone pigment bleaching, and if so, how the photosensitivity of bleaching can be independent of retinal location, given that light capture by cones is affected by several factors that vary substantially with eccentricity, including the angle of incidence of light on the retina (9), the inner segment diameter (10, 11) and the outer segment length.

Light stimuli are necessarily measured “at the cornea” and the retinal irradiance (e.g., in watts mm⁻²) or illuminance (e.g., lumens mm⁻²) computed. The Troland (Td) unit, which incorporates the area of the pupil, is the standard measure of retinal illuminance and is proportional to $V_\lambda$-equivalent photon flux density at the retinal surface. It has often been assumed that there is a single scale factor for conversion of Td s to isomerizations/cone. The Troland unit, however, neglects the biophysical reality that human cones are dielectric waveguides (11, 12), i.e., light propagating through the eye’s optics to the retinal surface is captured by the cone inner segment (CIS) and guided to the COS. Both the dependence on the angle of incidence of CIS light-capture (13) and the power density increase caused by light propagation between the wider CIS and narrower COS are waveguide phenomena (11, 12, 14), and the dependence of cone pigment bleaching on the light measured at the cornea is necessarily affected by these properties, which vary systematically with retinal eccentricity. We hypothesized that these dependencies could be quantified in terms of several factors which taken together could convert photon energy density at the retinal surface ($Q_{\text{retina}}$) and that ($Q_{\text{COS,inc}}$) incident at the COS waveguide entrance (i.e., at the COS basal discs), where the photosensitivity of bleaching could be predicted with biophysical precision with Eq 1 of the paper:

$$Q_{\text{COS,inc}} = Q_{\text{retina}} f_{\text{WFA}} f_{\text{SCI}} f_{\text{CIS2COS}}$$

The goal of this SI section is to show the factors in Eq 1 can be quantified, to explain how the apparent discrepancies in Fig. S2 associated with the photosensitivity of cone pigment bleaching expressed in retinal illuminance units (Td⁻¹ s⁻¹) by application of Eq 1, and to explain how this leads to an absolute prediction of the measured photosensitivity of bleaching.

Cone pigment bleaching is empirically describable as having an exponential dependence on retinal energy density (Fig S2A-C): thus $f_B$, the fraction of pigment bleached by a stimulus of $Q_{\text{retina}}$ (photons µm⁻² at the retina) at a specific retinal location, can be described by
\[ f_B(Q_{\text{retina}}) = 1 - \exp \left( -\frac{Q_{\text{retina}}}{Q_{e,\text{retina}}} \right) \]  
(S2.1)

where \( Q_{e,\text{retina}} \) is a constant. Equation S1 must also apply with photon energy density specified as that incident on the COS waveguide entrance at that retinal location:

\[ f_B(Q_{\text{inc,COS}}) = 1 - \exp \left( -\frac{Q_{\text{inc,COS}}}{Q_{e,\text{COS,inc}}} \right) \]  
(S2.2)

By hypothesis (Eq 1) energy densities at the retina and at the COS basal discs are related by

\[ Q_{e,\text{COS,inc}} = Q_{e,\text{retina}} f_{\text{WFA}} f_{\text{SCI}} f_{\text{CIS2COS}} \]  
(S2.3)

where \( f_{\text{WFA}}, f_{\text{SCI}} \) and \( f_{\text{CIS2COS}} \) are scaling factors that depend on the distribution of light in the pupil (including its wavefront aberration), on the retinal eccentricity of the individual cone, or on both. Explanations of the scaling factors in Eq S2.3 are provided in the following subsections, and the specific values used to transform the measurements in Fig. 3D to those in Fig. 3E given in Table 1. Some background on pigment photosensitivity is required first.

The intrinsic photosensitivity \( \alpha_{\text{max}} \gamma \) of cone pigment in situ. In CGS units the maximal photosensitivity \( \varepsilon_{\text{max}} \gamma \) of the isomerization/bleaching reaction of a visual pigment is the apparent cross-section of the reaction for light of the wavelength of maximum absorbance (\( \lambda_{\text{max}} \)), and is the product of the extinction coefficient \( \varepsilon_{\text{max}} \) (units: cm\(^2\) mmol\(^{-1}\)) and the quantum efficiency \( \gamma \), the probability that a captured photon leads to a stable structural change in the pigment molecule (15). While \( \varepsilon_{\text{max}} \) has been measured for many opsins in detergent solution, it appears that \( \gamma \) has only been measured for rhodopsin (\( \varepsilon_{\text{max}} = 42,000; \, \gamma=0.67 \) (16-18) and iodopsin (\( \varepsilon_{\text{max}} = 47,000; \, \gamma=0.62 \) (19). The orientation of the 11-cis retinal dipole parallel to the disc membranes increases the photosensitivity for axially propagating light by the dichroic factor \( \sim 1.5 \), so that the intrinsic photosensitivity for the cone pigment iodopsin in situ is \( \varepsilon_{\text{max}} \gamma = 47000 \times 1.5 \times 0.62 = 44,000 \, \text{cm}^2 \, \text{mmol}^{-1} \). The molar extinction coefficient is converted to a cross sectional area per molecule by the relation \( \alpha_{\text{max}} = \varepsilon_{\text{max}} / (N_A \gamma / 1000) \, \text{cm}^2 \, \text{molecule}^{-1} \), where \( N_A \) is Avogadro’s number. Thus, taking iodopsin as the standard for cones, the intrinsic photosensitivity of bleaching of a cone pigment molecule to light propagating axially in the COS is \( \alpha_{\text{max}} \gamma = 7.26 \times 10^{-9} \, \text{µm}^2 \, \text{molecule}^{-1} \). Given the narrow acceptance angle of the cone waveguide (cf. below, “\( f_{\text{SCI}} \)”), the local wavefront of light incident at the base of the COS should deviate little from perpendicularity to the CIS long axis, so the value \( \alpha_{\text{max}} \gamma \) can be taken as a biophysical benchmark against which cone pigment bleaching measurements and calculations can be gauged. The fraction \( p \) of pigment
present in the basal discs of the COS during exposure to a stimulus of wavelength \( \lambda \) that delivers

\[ I_{\text{COS,inc}}(\lambda) \text{ (photons } \mu \text{m}^{-2} \text{ s}^{-1}) \] through the CIS will follow the rate equation

\[
\frac{dp}{dt} = -I_{\text{COS,inc}}(\lambda) \alpha_{\lambda} \gamma P \tag{S2.4}
\]

According to Eq S2.4, for \( \lambda = \lambda_{\text{max}} \) the reciprocal \( 1/\alpha_{\text{max}} \gamma \) defines the photon energy density that isomerizes the pigment at the base of the COS to 1/e of its dark adapted value in 1 s. Thus, for the brief exposures \( \Delta T \) of the serial scanning experiments, the fraction of pigment present in the COS basal discs after exposure to a stimulus that produces a photon density at the COS base of

\[ Q_{\text{COS,inc}}(\lambda) = I_{\text{COS,inc}}(\lambda) \Delta T \]

satisfies

\[
p_{\text{COS,base}}[Q_{\text{COS,inc}}(\lambda)] = \exp[-\alpha_{\lambda} \gamma Q_{\text{COS,inc}}(\lambda)] \tag{S2.5}
\]

with the fraction bleached given by \( f_{\text{B,COS,base}} = 1 - p_{\text{COS,base}} \). The concentration of opsin and the length of the COS are such that “self-screening” occurs, however, diminishing the fraction bleached at the COS tip relative to that at the base, especially at lower energy densities.

Consequently, determination of bleaching over the whole COS require a more complex treatment than Eq S2.4.

**fDens: the effect of the pigment axial optical density on bleaching.** The average rate of cone pigment bleaching over the whole COS can be described by the following rate equation (20-22):

\[
\frac{dp}{dt} = -\frac{I_{\text{COS,inc}}(\lambda) \alpha_{\lambda} \gamma}{2.303 D_{\lambda}} \left[ 1 - \exp(-2.303 D_{\lambda} P) \right] \tag{S2.4’}
\]

Here \( p \) is the average fraction of pigment present in the COS at time \( t \), \( \lambda \) is taken to be \( \lambda_{\text{max}} \), \( \alpha_{\lambda} \) is the molecular extinction coefficient of a single pigment molecule, \( D_{\lambda} = (\alpha_{\lambda} C_{\text{dark}} L_{\text{COS}}) / \ln(10) \) is the total axial density, \( C_{\text{dark}} \) the concentration of pigment referred to the COS envelope volume, and \( L_{\text{COS}} \) the length of the COS. The specific values adopted here are \( C_{\text{dark}} = 3.5 \text{ mM} \), and \( L_{\text{COS}} = 30 \mu \text{m} \), giving a total COS optical density at \( \lambda_{\text{max}} \) of \( D_{\lambda_{\text{max}}} = 0.5 \). (The “low density” approximation of Eq S2.4’ is Eq S2.4, which applies when the argument of the exponential term in Eq S2.4’ has magnitude less than \( \sim 0.2 \). When this latter condition is met, the exponential term can be expanded as the first two terms of its Taylor series, so that
1 − exp(−2.303Dₐp) = 2.303Dₐp. A portion of the COS basal discs up to 5 μm in length satisfies this low density criterion, so the description of bleaching at the COS base given by Eq S2.4 and S2.5 is consistent with the more general relation, Eq S2.4′.)

Solutions \( p(Q_{\text{COS, inc}}) \) to Eq S2.4′ for brief stimuli of various \( \lambda_{\text{max}} \)-equivalent photon densities \( Q_{\text{COS, inc}}(\lambda) = I_{\text{COS, inc}}(\lambda) \Delta T \) are plotted in Fig. S2A for three different values of pigment axial density. There are two key points to be made. First, though the solutions are not exponential functions, they are reasonably approximated as such (Fig S2B). Second, when the exponential approximation is used, the apparent photosensitivity is reduced by a factor \( f_{\text{Dens}} \) that depends on the axial density (21). Thus, the average fraction pigment \( \bar{f}_B \) bleached over the whole COS by a stimulus that produces a \( \lambda_{\text{max}} \)-equivalent photon density \( Q_{\text{COS, inc}} \) at the COS base is approximated by

\[
\bar{f}_B(Q_{\text{COS, inc}}) = 1 − \exp(−\alpha_{\text{max}} \gamma f_{\text{Dens}} Q_{\text{COS, inc}})
\]  

(S2.6)

(cf. Fig. S2B). It follows that the apparent photosensitivity of bleaching over the whole COS is \( 1/Q_{\text{e,COS, inc}} = \alpha_{\text{max}} \gamma f_{\text{Dens}} \). Equating this latter value to that in Eq S2.3 yields the prediction tested in Fig. S2E (Table S1):

\[
Q_{e,\text{retina}} f_{\text{WFA}} f_{\text{SCI}} f_{\text{CIS2COS}} f_{\text{Dens}} = 1/(\alpha_{\text{max}} \gamma)
\]  

(S2.7)

**Stiles-Crawford effect.** One characteristic property of a cylindrical waveguide is its farfield angular aperture function (11, 12). Accordingly, as cylindrical waveguides cones are sensitive to the direction of light impinging on their inner segment, where guiding begins. Cone directional sensitivity was first revealed in the eponymous Stiles-Crawford effect of the 1st kind (SCI) (23), whereby cone visual threshold depends on the position in the pupil through which a narrow beam of light passes to a fixed retinal target area:

\[
\Delta I(x)/\Delta I_0 = 10^{-\rho(x-x_0)^2} = e^{-2.303\rho(x-x_0)^2}
\]  

(S2.8)

Here \( x \) (mm) is the position of a microbeam in the pupil, \( x_0 \) a central location, \( \Delta I(x) \) the threshold for light entering at position \( x \) and \( \Delta I_0 \) the threshold at the central location \( x_0 \), and \( \rho \) (mm\(^{-2}\)) a parameter defining the width of the SCI Gaussian (2nd line of Eq S2.8). For a stimulus beam
with power density $PD_{\text{pupil}}$ (W mm$^{-2}$) uniformly filling the pupil having diameter $d$ (mm), the power density at the retina available to cone inner segments (CIS) from the entire pupil can be calculated by substituting $r = (x - x_0)$ in the right-hand side of Eq S2.8, integrating from $r = 0$ to $d/2$, and dividing by the retinal area of the target, $A_{\text{target, retina}}$. Thus, one obtains an expression for the power density of light captured and propagating in the CIS:

$$PD_{\text{CIS}} = \frac{PD_{\text{pupil}}}{A_{\text{target, retina}}} \int_0^{d/2} e^{-2.303 \rho r^2} 2\pi r dr$$

$$= \frac{\pi PD_{\text{pupil}}}{A_{\text{target, retina}}} \left[ 1 - e^{-2.303 \rho (d/2)^2} \right]$$

(S2.9)

If the SCI effect is neglected, as is implicit in the use of the Troland unit, the Gaussian term in the 1st line of Eq S2.9 is replaced by unity, and in this case Eq S2.9 gives a value proportional to Trolands:

$$PD_{\text{retina}} = \frac{PD_{\text{pupil}} \pi (d/2)^2}{A_{\text{target, retina}}}$$

(S2.9′)

For foveal vision a typical value of the SCI parameter $\rho$ is 0.05 (9), but $\rho$ depends on eccentricity (9), and at the 7 deg eccentricity of our OCT experiments the average value is $\rho = 0.093$ (Fig. S3A). The factor $f_{\text{SCI}}$ in Eq S2.3 is defined by $f_{\text{SCI}} = PD_{\text{CIS}} / PD_{\text{retina}}$. Equation S2.9 implicitly assumes the distribution of power over the pupil plane to be uniform, a condition met for the LED stimulus in the OCT experiments, but not for the AO-SLO stimulus in the serial bleaching experiments. We measured the power as a function of aperture diameter in the pupil plane of both the OCT and AO-SLO serial bleaching apparatuses. From these measurements we derived a density function $p(r)dr$ that was included in the first line of Eq S2.9 to calculate the energy density available to the CIS. The factor $f_{\text{SCI}}$ was calculated for a range of retinal eccentricities encompassing those of the OCT and AO-SLO experiments (Fig. S3B). The vertical shift between the curve (blue) for the stimulus in the OCT experiments and that for the AO-SLO stimulus (red) is mainly due to the different energy density distributions in the pupil, with that for the AO-SLO being more concentrated in the pupil center.

$f_{\text{CIS2COS}}$: the effect of power density concentration by CIS $\to$ COS propagation. A distinctive morphological feature of most cone photoreceptors is that they taper, i.e., the cone...
inner segment (CIS) diameter is greater than that of the COS. Experiments in which a flat-mounted retina is homogeneously illuminated have invariably found that light radiating out of the COS tips has a much higher power density than the light incident on the retina (e.g., refs (11, 24), implying that cones “funnel” light from the CIS to the COS in a manner that increases the power density of light propagating in the COS relative to that impinging on the retina at the ELM, the base of the CIS. This “waveguide condensation” behavior is expected of tapered dielectric waveguides (11, 12), and has long been thought to underlie the relatively higher photosensitivity of cone pigment as compared to rhodopsin bleaching measured in humans with reflection densitometry. We derived CIS cross-sectional areas from the classic histology of Curcio (10) (Fig. S4A), assumed that COS’s at the three eccentricities investigated have a diameter of 1.75 µm, used the CIS/COS cross-sectional areas as an approximate estimate of \( f_{\text{CIS2COS}} \), and sought values consistent with the absolute photosensitivity \( \alpha_{\text{max}} \) of bleaching at the COS waveguide entrance (Fig. S4B red symbols; Fig. 3E). (We note recent progress in calculating the CIS→COS condensation factor with electromagnetic theory, including its expected increase as a function of eccentricity due to the increased CIS vs COS cross-sections (25), and also note that cone density (and thus CIS diameter)(26) varies materially between subjects in the central 1-2 degrees.)

\[ f_{\text{WFA}}: \text{light not captured by cones due to wavefront aberration, and correction for Adaptive Optics (AO) light delivery}. \]

The bleaching stimuli in the serial scanning reflectometry experiments (Fig. 3A-C) were delivered via an AO imaging system, whereas those in the OCT experiments were not. Given two distributions of power over the dilated pupil plane having the same total power, but one with, and the other without AO wavefront correction, the efficiency of light capture by a cone can be expected to be higher for the case with AO delivery, as recently described (25) for stimuli focused on a single CIS. We defined \( f_{\text{WFA}} = 1 \) for AO light delivery in the serial scanning bleaching experiments, and expected \( f_{\text{WFA}} < 1 \) for the non-AO-correct delivery in the OCT apparatus (Table 1). While the specific value \( f_{\text{WFA}} = 0.5 \) applied to the stimuli in the OCT experiments is not rationalized, it highlights the notion that power loss due to wavefront aberration may be material to measurements of cone bleaching photosensitivity.

\[ Factors \text{impacting light transmission from cornea and photon density on retina}. \]

Several factors not included in Eqs 4 and S2.3 were involved in our deriving the photon flux density at the retina from the light power measured at the cornea. These include the transmissivity of the cornea, lens and ocular media, and of the macular pigment for targets imaged on the fovea and
parafovea. Another important factor is that which converts stimulus solid angle (say, in deg²) to area on the retina: this “power density” conversion factor can be taken to be $((\pi \text{ PND})/180)^2$, where PND is the posterior nodal distance. The axial length of the healthy adult eye is $(23.7 \pm 1.7 \text{ mm, mean} \pm \text{ SD, N=250 subjects, 500 eyes})$ (27), and the population standard deviation (N=200) of uncorrected spherical error is 3.0D (28), consistent with 5-7% SD in PND. Though wavefront aberrations are correctable with adaptive optics, PND is not affected, and a 5-7% population SD in PND implies power density variation $(\pm 1 \text{ SD})$ of 20% or more in a sample of healthy eyes.

**Wavelength dependencies.** Several of the factors relating photosensitivity measured “at the retina” to the cone pigment photosensitivity (Eq S2.3) are known to be (or are potentially) dependent on wavelength. As the bleaching stimuli used in our experiments were not spectrally narrowband, known wavelength-dependent factors were included in the application of Eq S2.3, as follows. First, the spectral energy densities of the AO-SLO source and the LED source used in the OCT experiments were directly measured “at the cornea” with a spectrometer: these spectra were scaled so that their integrals matched the radiometric measurement for each bleaching condition. The spectral energy density “at the retina” $Q_{\text{retina}}(\lambda)$ (units: photons nm⁻¹ μm⁻²) was obtained by multiplying the energy spectrum “at the cornea” wavelength-by-wavelength by the ocular media transmissivity spectrum and dividing by the stimulus area (μm²) on the retina; the transmissivity spectrum always included that of the lens, and also included the macular pigment transmission, scaled for the eccentricity (29). Third, the spectral energy density “in the CIS” was calculated by applying the appropriate SCI attenuation factor (Eq S2.4).

Fourth, the spectral energy density $Q_{\text{COS,inc}}(\lambda)$ “incident on the COS” was obtained by applying the scaling factor $f_{\text{CIS2COS}}$, subject to the approximate restriction $f_{\text{CIS2COS}} = (d_{\text{CIS}}/d_{\text{COS}})^2$. Fifth, $Q_{\text{COS,inc}}(\lambda)$ was converted to $\lambda_{\text{max}}$-equivalent photons by integration against pigment absorption spectra created with Lamb-Govardovski extinction spectra templates (30, 31), with $\lambda_{\text{max}} = 533$ nm (M-opsin) or 561 nm (L-opsin) adjusted for an axial pigment density of 0.5.

**Summary of factors linking observed and intrinsic photosensitivity of cone opsin in situ.** The factors used to transform photon energy density at the retinal surface to energy density at the cone outer segment waveguide entrance are summarized in Table S1. The guiding principle is that cone pigment in the basal discs has an intrinsic photosensitivity $\alpha_{\text{max}}$ established by
biochemical spectroscopy (16-19) and microspectrophotometric measurements of pigment dichroism in situ. Consequently, for in vivo measurements of cone pigments, there should be an eccentricity-dependent transformation of the photon energy density at the retina to that at the COS basal discs (Eq S2.7). A critical prediction is that with a reasonable set of factors the observed photosensitivities should be consistent with the absolute photosensitivity \( \alpha_{\text{max}} \) of cone pigment in the basal discs (Fig. 3E). It bears emphasis that systematic variation amongst adult subjects in the factors of Eq 1 and Eq S2.3 certainly occur, and that research is needed to define them with sufficient accuracy and precision as possible in individual subjects in the context of cone pigment bleaching experiments.

3. The saturated level of Component 1 elongation provides a gauge for conversion of the responses to change in osmolarity

The hypothesis that the vertebrate photoreceptor outer segment is an osmo-elastic structure (32, 33) provides a basis for converting Component 1 of COS elongation into an increase in cytoplasmic osmolarity, and estimating the water permeability of the COS plasma membrane. The underlying principle is that of Starling’s classic analysis of water permeation through vascular endothelial cells (34): thus, a phototransduction-triggered increase of soluble metabolites will drive water entry into the COS toward an level that tends toward thermodynamic equilibrium between internal and external osmotic pressures. Interpreting the saturated COS elongation as achievement of osmotic equilibrium, one can apply Van’t Hoff’s law,

\[
\frac{\Delta V_{\text{cyto, Comp1, sat}}}{V_{\text{cyto, rest}}} = \frac{\Delta \Pi_{\text{Comp1, sat}}}{\Pi_{\text{rest}}}
\]

(S3.1)

where \( V_{\text{cyto, rest}} \) is the volume of the dark adapted COS cytoplasm, \( \Delta V_{\text{cyto, Comp1, sat}} \) is the saturated increase in volume due to Component 1, \( \Pi_{\text{rest}} / RT = 300 \) mOsM is the osmolarity of human plasma, and \( \Delta \Pi_{\text{Comp1, sat}} / RT \) the maximal increase in osmolarity. Given an invariant COS diameter of 1.75 µm and a dark adapted COS length at 7 deg eccentricity of 22 µm (of which disc membranes comprise 50% in the dark adapted state), the resting COS cytoplasmic volume is 26.4 µm³ (fL). The average saturated Component 1 elongation of 240 nm (Fig. 2B) corresponds to a cytoplasmic volume increase of 0.57 µm³, and so to an osmolarity increase of \( 300 \times (0.6/26) = 6.5 \) mOsM, equivalent to an incremental osmotic pressure \( \Delta \Pi_{\text{sat}} = 1.7 \times 10^5 \) dyn cm⁻² (17 kPa).
As long as its internal osmotic pressure remains above the equilibrium level, the COS will tend to swell at a rate limited by its water permeability and any restoring force between the disc membranes that resists swelling, as predicted by the Starling relation

\[ J_{H_2O}(t) = L_p S_{COS} [\Delta\Pi_i(t) - P(t)] \]  

(S3.2)

where \( J_{H_2O} \) (units: cm\(^3\) s\(^{-1}\)) is the instantaneous water influx, \( L_p \) (units: cm\(^3\) s\(^{-1}\) dyn\(^{-1}\)) the hydraulic conductivity of the membrane, \( S_{COS} \) (units: cm\(^2\)) the COS membrane surface area, \( \Delta\Pi_i(t) \) (units: dyn cm\(^{-2}\)) the internal incremental osmotic pressure at time \( t \), and \( P(t) \) an elastic restoring force, expressed as a reactive pressure (dyn cm\(^{-2}\)) between the disc membranes. From the saturated rate of swelling attributable to Component 1, 2.9 \( \mu \)m s\(^{-1}\) (Fig. 1B; Fig. S1) and the COS cross section of 2.4 \( \mu \)m\(^2\), the maximal rate of water influx into the COS due to Component 1 is found to be \( J_{H_2O} = 6.8 \mu \)m\(^3\) s\(^{-1}\). Assuming all COS disc membranes to be fully exposed to the extracellular space, \( S_{COS} = 4000 \mu \)m\(^2\). A lower bound on hydraulic conductivity can be obtained by assuming the maximal incremental osmotic pressure \( \Delta\Pi_{sat} \) is achieved soon after stimulation: from Eq 3.2 this yields \( L_p = 10^{-12} \) cm\(^3\) s\(^{-1}\) dyn\(^{-1}\), equivalent to a water permeability coefficient of 0.0014 cm s\(^{-1}\). Consequently, the water permeability of the human COS plasma membrane is more than 50-fold greater than that of mouse rods (33). The exponentially rising kinetics of the saturated Component 1 COS elongation response (Fig. 1C) – unlike the rate-limited kinetics of the rod elongation response (33, 35) – suggests that the relatively high water permeability of the COS membrane enables the elongation response to rapidly equilibrate with the changing concentration of osmolytes driving the response, and so allows dynamic length changes to be converted to changes in osmolarity as follows.

Van’t Hoff’s Law together with invariance of the COS width provides a gauge for converting measured COS length changes into changes in osmolarity. Cancelling the common terms defining the COS cross-section contribution to \( V_{cyto, rest} \) and an arbitrary incremental change in volume \( \Delta V_{cyto} \), from the parallel to Eq 3.1 one finds

\[ \frac{\Delta\Pi / RT}{\Delta L} = \frac{\Pi_{rest} / RT}{(L_{COS,rest} / 2)} = 0.027 \text{ mOsm mm}^{-1} \]  

(S3.3)
This gauge was applied to Component 1 elongation responses (Fig. 2) to convert them to a scale to which a model of osmolytes produced by phototransduction could be applied. For COS at 7 deg eccentricity with length 22 µm and 50% cytoplasmic volume, the average saturated Component 1 elongation of 240 nm corresponds to an osmolarity increase of 6.5 mOsM.

4. Biochemical model of phototransduction reactions

This section of the SI presents the equations of the biochemical model used to test the hypotheses that COS elongation Component 1 can be identified with phosphate (P\textsubscript{i}) produced by RGS9-catalyzed hydrolysis of GTP (Fig. 5), and to provide a kinetic account of Component 2 (Fig. 4). The model is conventional, in the sense that it embodies only known proteins and reactions of the COS. Though the specific reactions generating the two hypothesized osmotic factors are distinct, it was necessary to create a comprehensive model because the reactions are interdependent through the light activation of M-/L-cone opsins, and the quantities of opsin intermediates that arise during deactivation.

Structure of the model cone outer segment (COS). COS were assumed to be cylindrical with length between 22 and 30 µm (Fig. S2) and diameter 1.75 µm. The cytoplasm was assumed to comprise 50% of the COS envelope volume. The center-to-center spacing of the disc membranes was taken to be 26 nm (36), so the model for a 22 µm COS comprises 1660 disc membrane faces, each having a surface area of 2.4 µm\textsuperscript{2}. Cone opsin was assumed present at 30,000 µm\textsuperscript{2}, giving a total of 7.2×10\textsuperscript{4} per disc face. The quantity of the membrane-associated proteins in the model were set relative to the cone opsin values (Table S2). All COS disc membranes were assumed to be exposed to the extracellular space, so that given the specific capacitance of 1 µF cm\textsuperscript{-2}, the capacitance of the 22 µm COS was \(C_m = 9.1\) pF.

General description. The model comprises 37 time-dependent variables (Table S2) governed by 38 ordinary differential equations (ODEs), and several ancillary instantaneous relations (Eqs S4.1-S4.44). (Several rate equations – e.g., those for the quantities of inactive pigment, G-protein, PDE and RGS9, \(P_0\), \(G_0\), \(E_0\), \(Rg_0\), respectively – are redundant due to conservation relations, but including them facilitated testing the internal consistency of the model as described below.) The biochemical reactions can be classified as purely disc membrane-associated or mixed membrane-cytosolic, and the variables and rate equations in both cases are treated as homogeneous over the respective reaction spaces. For the purely membrane-associated reactions (e.g., \(G^*\)=G\textsubscript{i}\(\alpha\)-GTP activating PDE6), the reactants are confined to an
individual disc face, and are quantified as “number per active disc face”, where “active” signifies a disc face with at least one photoisomerized cone opsin, P*. For mixed disc-cytosolic reactions (e.g., PDE6 hydrolysis of cGMP), the sum total of any protein species over all the outer segment was referenced to the cytosol volume \( V_{cyto} \) to determine molar quantities. As no responses are characterized for which the number of P* is less than the number of discs in the COS, treating soluble reactions (e.g., cGMP hydrolysis) as homogenous is reasonable. Table S2 gives the resting (dark adapted) values of the variables, and Table S3 the rate parameters and values adopted. In keeping with mathematical convention variables are italicized (but when reference is made to the proteins \textit{per se}, Roman font is used). For clarity, weakly bound complexes are indicated by a “●” between two variables, while a “×” is used to indicate the product of the concentrations of two variables in a bimolecular reaction– e.g., “\( P^*\text{●GRK} \)” symbolizes the complex of photoactivated pigment with a G-protein receptor kinase (Fig. 4B), while “\( P^*\text{×GRK} \)” indicates the product of the concentrations of the two variables. The proteins involved are typically integral membrane proteins (e.g., P, P*•GRK, P*, POps; Fig. 4B) or anchored to the membrane by lipids (e.g., GRK, PDE; Fig. 5B), and the concentrations of reactants are specified as “number per active disc face”, where “active” indicates that one or more P* is initially present. This compartmentalization automatically treats the exhaustion or binding saturation of reactants, with the absolute ceiling of the reactants set by initial conditions (Table S2). Arrestin (ARR), though soluble when free, was treated as membrane-associated to obviate dealing with axial spatial variation, which would only be material for very weak flash strengths. All-\textit{trans} retinol (at-ROL) and free phosphate (\( P_i \)) were also expressed in rate equations “per active disc face”, but along with cyclic cGMP (cG), calcium (Ca) concentrations were referred to the COS cytoplasm \( V_{cyto} \), as required for calculating their effects, and evaluating them as candidates for Components 2 and 1, respectively. Activating light flashes were expressed initially in photoisomerizations/COS (\( \Phi \)), and recoded into photoisomerizations per active disc face (\( \Phi_{pdf} \)): for \( \Phi \) exceeding the number of disc faces, \( \Phi_{pdf} \) was taken as the average per disc face (Eq S4.1); for \( \Phi \) less than the number of disc faces, \( \Phi_{pdf} = 1 \), with n=\( \Phi \) active disc faces. Following isomerization, cone MetaII (Eq S4.1) was assumed to follow an exponential rise with a MI/MII equilibrium constant of \( \sim 0.5 \text{ ms} \). All the biochemical reactions are invariant with respect to variation in \( L_{COS} \), the length of the outer segment. However, the
absolute number of photoisomerizations per COS and the membrane currents (used in modeling the massed \(a\)-wave electrical response – Fig. S7) scale with \(L_{\text{COS}}\).

Rate equations for \(P^*\) deactivation and at-ROL production. In keeping with the flow of signal in the phototransduction cascade, the model begins with the reactions governing activation and deactivation of M- and L-cone opsins, leading ultimately to at-RAL hydrolysis and its reduction to at-ROL (Eq S4.1-4.10). The driving force (Eq S4.1, 4.2) is the number of photoisomerizations per disc face (\(\Phi_{\text{pdr}}\)). Cone opsin can be in 1 of 6 principal states (\(\text{“P-species”}\)): \(P_0\) (inactive pigment), \(P^*\) (fully active MII state), \(P^*\cdot GRK\) (weakly bound to GRK1 or GRK7) (37), \(P_{\text{nPi}}\) (multiply phosphorylated, with all-trans retinal covalently linked), \(P_{\text{nPi}}\cdot ARR\) (phosphorylated and bound with ARR1 or ARR4), and \(POps\) (unliganded, “naked” opsin), and \(P^*\) can also be in 3 different complexes with G-protein in different nucleotide binding states. Likewise, \(P^*\cdot GRK\), \(P_{\text{nPi}}\) and \(POps\) can potentially be in similar complexes with G-protein (Eqs. S4.12 – 4.23). Multi-phosphorylation of \(P^*\) is treated as a single “Michaelis” reaction, without distinguishing levels of phosphorylation (Eqs S4.3, S4.4, S4.6) with 1\(<n_{\text{Pi}}\leq 3\) phosphates transferred. \(ARR\) (ARR1 or ARR4) (38) binds strongly to \(P_{\text{nPi}}\) (Eq S4.7), but the quantity in the dark adapted COS (\(ARR_{\text{tot}}\)) is a small fraction of the total pigment (39, 40), so that once the bleach level exceeds this quantity, the ARR-bound state is driven rapidly to the total, i.e., \(P_{\text{nPi}}\cdot ARR = ARR_{\text{tot}}\). Hydrolysis of the chromophore to the unbound form \(atRAL\) from its Schiff-base linkage to K112 (Fig. 4A, B) can take place from either \(P^*\) or \(P_{\text{nPi}}\), but not from \(P^*\cdot GRK\) or \(P_{\text{nPi}}\cdot ARR\) (Eq S4.8). Free \(atRAL\) undergoes reduction to the alcohol \(atROL\) via RDH-catalysis (Eqs S4.9 – S4.10), and \(atROL\) is removed from the COS by a 1\(^{st}\)-order process. Rate parameters are indexed by subscripted single letter codes for the proteins involved, with numbers (\(\text{“01”, “10”}\)) or \(f\) and \(b\) indicating “forward” and “backward” directions, respectively (cf. Table S3).

Rate equations governing G-protein cycling, PDE activation and deactivation and \(P_i\) production. The states of cone G-protein can be subdivided into two principal subgroups: states in which the heterotrimeric G-protein or its \(\alpha\)-subunit (\(G_{\text{c}\alpha}\)) are in weak complexes with light-activated cone opsin (\(P^*\)), or with other opsin species produced during deactivation of \(P^*\). This first subgroup, epitomized by the reactions in the pale-green highlighted region in Fig. S5, represents the classic \(P^*\)-catalyzed GDP/GTP exchange cycle, whose output in the signaling
cascade is $G^* = G_t \alpha$-GTP ("G4") (Eqs S4.12, 4.16, 4.20, 4.27). While $P^*$ is expected to have a greater ability to catalyze GDP/GTP exchange than other $P$-species, the relatively short lifetime of $P^*$ in cones and extreme light intensities involved necessitate consideration of G-protein activation by $P^*$ in complex with GRK ($P^* \cdot GRK$), phosphorylated opsin ($P_{\text{pH}}$), or ligand-free opsin ($POps$) as potential activators of G-protein. To evaluate these possibilities, scalar positive coefficients $0 \leq a_{\text{pGRK}}, a_{\text{pPH}}, a_{\text{pPOps}} < 1$ were assigned to each of these $P$-species, with the coefficient for the $P^*$ binding reaction taken to be unity. Otherwise, the activation cycle parameters in each case were the same (Eqs S4.12, 4.13, 4.17, 4.21; Eqs S4.12, 4.14, 4.18, 4.22; Eqs S4.12, 4.15, 4.19, 4.23), with the common output $G^4$ ($G^*$; Eq S4.24). The possibility that some $G^*$ dissociated from the membrane were evaluated via Eqs S4.24-25.

Deactivation of $G^*/ = G_t \alpha$-GTP is expected to be dominated by RGS9-catalysis effected by the multiprotein complexes $Rg_1 \cdot Rg_3$ (Table S2; Eqs S4.24, 4.26), but could also involve spontaneous GTP hydrolysis or temporary solubilization; thus, terms for these latter pathways were included for evaluation (Eq S4.25-4.26). Free G$\beta\gamma$ ("G$\beta$") is a byproduct of each of the $G^*$ activation cycles, and is eliminated by recombination with $G_t \alpha$-GDP ("G5") (Eq S4.27).

The second subgroup of G-protein interactions involves $G^*$ complexes with PDE alone ($E_0$- $E_2$) (Eqs. S4.28-4.30) and/or with $G^*$-PDE complexes with 1 or 2 RGS9 molecules bound ($Rg_1 \cdot Rg_3$) (gray-highlighted regions in Fig. 5A; Fig. S5) (Eqs S4.32-4.34). The rate equation governing $P_i$ includes production by the RGS9-catalyzed reactions, spontaneous hydrolysis of $G^*$, enzymatic removal by GAPDH, and steady low level production and removal by unspecified reactions that combine to determine the resting level of free phosphate $P_{i,\text{rest}}$ (Eq S4.35). (The use of "$P_i$" to represent free phosphate, PO$_4$, is in keeping with a large biochemical literature, but may be confusing given the use of "$P_0$" to represent inactive cone pigment, $P^*$ its excited (Meta II) state and POps its ligand-free state. Other options for symbols for cone pigment and its derivative states were considered, but found mnemonically less helpful.)
\[
\frac{dP_0}{dt} = -\Phi_{pdr} \frac{dM_{11}}{dt} + k_{\text{regen}} P\text{Ops}
\]
(S4.1)

\[
\frac{dP^*}{dt} = \Phi_{pdr} \frac{dM_{11}}{dt} - k_{\text{GRK},f} P^* \times \text{GRK} + k_{\text{GRK},b} (P^* \times \text{GRK}) - k_{\text{hydr}} P^*
\]
(S4.2)

\[
\frac{d(P^* \times \text{GRK})}{dt} = k_{\text{GRK},f} P^* \times \text{GRK} - k_{\text{GRK},b} (P^* \times \text{GRK}) - k_{\text{cat,GRK}} (P^* \times \text{GRK})
\]
(S4.3)

\[
\frac{dP_{\text{nP}i}}{dt} = k_{\text{cat,GRK}} (P^* \times \text{GRK}) - k_{\text{ARR},f} P_{\text{nPi}} \times \text{ARR} + k_{\text{ARR},f} (P_{\text{nPi}} \times \text{ARR})
\]
(S4.4)

\[
\frac{dP\text{Ops}}{dt} = k_{\text{hydr}} P^* + k_{\text{hydr}} P_{\text{nPi}} - k_{\text{regen}} P\text{Ops}
\]
(S4.5)

\[
\frac{d\text{GRK}}{dt} = -k_{\text{GRK},f} P^* \times \text{GRK} + k_{\text{GRK},b} (P^* \times \text{GRK}) + k_{\text{cat,GRK}} (P^* \times \text{GRK})
\]
(S4.6)

\[
\frac{d\text{ARR}}{dt} = -k_{\text{ARR},f} P_{\text{nPi}} \times \text{ARR} + k_{\text{ARR},b} (P_{\text{nPi}} \times \text{ARR})
\]
(S4.7)

\[
\frac{datRAL}{dt} = k_{\text{hydr}} P^* + k_{\text{hydr}} P_{\text{nPi}} - k_{\text{RDH},f} \text{RDH} \times \text{atRAL} + k_{\text{RDH},b} (\text{RDH} \times \text{atRAL})
\]
(S4.8)

\[
\frac{d\text{RDH}}{dt} = -k_{\text{RDH},f} \text{RDH} \times \text{atRAL} + k_{\text{RDH},a} (\text{RDH} \times \text{atRAL}) + k_{\text{cat,RDH}} (\text{RDH} \times \text{atRAL})
\]
(S4.9)

\[
\frac{datROL}{dt} = k_{\text{cat,RDH}} (\text{RDH} \times \text{atRAL}) - k_{\text{atROL}} \text{atROL}
\]
(S4.10)
\[
\frac{dG_0}{dt} = - k_{G_01} P^* G_0 + k_{G10} G_{1a} + k_{G10} G_{1b} + k_{G10} G_{1c} + k_{G10} G_{1d} + k_{G5} G_5 G_b \\
- a_{pGRK} k_{G01} (P^* GRK) \times G_0 - a_{pG1} k_{G01} P_{p} \times G_0 - a_{PGR} k_{G01} POps \times G_0 \tag{S.11}
\]

\[
\frac{dG_{1a}}{dt} = k_{G01} P^* G_0 - (k_{G10} + k_{G12}) G_{1a} + k_{G21} G_{2a} \tag{S.12}
\]

\[
\frac{dG_{1b}}{dt} = a_{pGRK} k_{G01} (P^* GRK) \times G_0 - (k_{G10} + k_{G12}) G_{1b} + k_{G21} G_{2b} \tag{S.13}
\]

\[
\frac{dG_{1c}}{dt} = a_{pGRK} k_{G01} (P^* GRK) \times G_0 - (k_{G10} + k_{G12}) G_{1c} + k_{G21} G_{2c} \tag{S.14}
\]

\[
\frac{dG_{1d}}{dt} = a_{PGRK} k_{G01} POps \times G_0 - (k_{G10} + k_{G12}) G_{1d} + k_{G21} G_{2d} \tag{S.15}
\]

\[
\frac{dG_{2a}}{dt} = k_{G12} G_{2a} - (k_{G21} + k_{G23}) G_{3a} \tag{S.16}
\]

\[
\frac{dG_{2b}}{dt} = k_{G12} G_{2b} - (k_{G21} + k_{G23}) G_{3b} \tag{S.17}
\]

\[
\frac{dG_{2c}}{dt} = k_{G12} G_{2c} - (k_{G21} + k_{G23}) G_{3c} \tag{S.18}
\]

\[
\frac{dG_{2d}}{dt} = k_{G12} G_{2d} - (k_{G21} + k_{G23}) G_{3d} \tag{S.19}
\]

\[
\frac{dG_{3a}}{dt} = k_{G23} G_{2a} - (k_{G32} + k_{G34}) G_{3a} \tag{S.20}
\]

\[
\frac{dG_{3b}}{dt} = k_{G23} G_{2b} - (k_{G32} + k_{G34}) G_{3b} \tag{S.21}
\]

\[
\frac{dG_{3c}}{dt} = k_{G23} G_{2c} - (k_{G32} + k_{G34}) G_{3c} \tag{S.22}
\]

\[
\frac{dG_{3d}}{dt} = k_{G23} G_{2d} - (k_{G32} + k_{G34}) G_{3d} \tag{S.23}
\]
\[
\frac{dG_4}{dt} = k_{G34} (G_{3a} + G_{3b} + G_{3c} + G_{3d}) - k_{G45} G_4 - k_{G4S} G_4 + k_{G4Sb} G_{4S} \\
- k_{GE01} G_4 \times E_0 - k_{GE12} G_4 \times E_1 + k_{GE10} E_1 + k_{GE12} E_1 \\
- k_{GR12} G_4 \times Rg_1 + k_{GR10} Rg_1 + (k_{GR21a} + k_{GR21b}) Rg_2 + k_{GR21c} Rg_3 \\
\text{(S4.24)}
\]

\[
\frac{dG_{4S}}{dt} = k_{G4S} G_4 - k_{G4Sb} G_{4S} \\
\text{(S4.25)}
\]

\[
\frac{dG_5}{dt} = k_{G45} G_4 - k_{G5b} G_5 \times G_b + k_{cat,Rg1} Rg_1 + k_{cat,Rg2} Rg_2 + k_{cat,Rg3} Rg_3 \\
\text{(S4.26)}
\]

\[
\frac{dG_b}{dt} = k_{G34} (G_{3a} + G_{3b} + G_{3c} + G_{3d}) - k_{G5b} G_5 \times G_b \\
\text{(S4.27)}
\]

\[
\frac{dE_0}{dt} = - k_{GE01} G_4 \times E_0 + k_{GE10} E_1 + k_{cat,Rg1} Rg_1 + k_{GR10} Rg_1 \\
\text{(S4.28)}
\]

\[
\frac{dE_1}{dt} = k_{GE01} G_4 \times E_0 - k_{GE10} E_1 - k_{GE12} G_4 \times E_1 + k_{GE21} E_2 \\
- k_{RE01} Rg_0 \times E_1 + k_{RE10} Rg_1 + k_{cat,Rg2} Rg_2 \\
\text{(S4.29)}
\]

\[
\frac{dE_2}{dt} = k_{GE12} G_4 \times E_1 - k_{GE21} E_2 - k_{RE12} Rg_0 \times E_2 + k_{RE21} Rg_2 \\
\text{(S4.30)}
\]
Human cone elongation responses to explain the COS elongation response, the model comprises biochemical reactions integral to phototransduction, and is naturally extended to predict the human cone electrical response (Eqs S4.36-4.44). The equations describing cGMP metabolism (Eq S3.36, 3.39), and governing free Ca$^{2+}$ (Eqs S3.37, 3.42, 3.42) have been thoroughly tested in models of rod phototransduction (e.g., (41)). However, unlike that of rods, the I/V relation of cone CNG channels has a non-negligible-slope I/V relation in their normal voltage operating range, and so a simplified voltage-dependence was included (Eq S3.40), along with a simplified outward K+ current (Eq S3.43), and a standard rate equation for membrane potential changes (Eq S3.38). Application of the model to human cone electrical responses (Fig. S6) served to constrain parameters of deactivation of P* by GRK and ARR, and of RGS9-catalyzed GTP hydrolysis, which deactivates G* and activated PDE, as described below.
\[
\frac{dcG}{dt} = \alpha(t) - \left[ E^*(t) \beta_{\text{sub}} + \beta_{\text{dark}} \right] cG \\
\frac{dCa}{dt} = - \left[ \frac{(f_{\text{Ca}} / 2) J_{cG}(t) - J_{cG}(t)}{F V_{\text{cyto}} B_{\text{Ca}}(t)} \right] \\
\frac{dV_m}{dt} = - J_{\text{ion, tot}}(t) / C_m \\
\alpha(t) = \frac{\alpha_{\text{max}}}{1 + \left[ \frac{Ca(t)}{K_{\text{cyt}}} \right]^{N_{\text{cyt}}}} \\
J_{cG}(t) = \left( \frac{cG(t)}{cG_{\text{dark}}} \right)^{N_{\text{cG}}} \text{g}_{\text{cG}} [V_m - E_{\text{rev,cG}}] \\
J_{\text{Ex}}(t) = J_{\text{ex,sat}} \frac{Ca}{Ca + K_{\text{ex}}} \\
B_{\text{Ca}}(t) = B P_{\text{LA}} + \frac{B P_{\text{cap}} \times K_{\text{buff}}}{[Ca(t) + K_{\text{buff}}]^2} \\
J_K(t) = \text{g}_K [V_m - E_K] \\
J_{\text{ion, tot}}(t) = J_{cG}(t) + J_{\text{Ex}}(t) + J_K(t)
\]
**Implementation.** The ordinary differential equations (“ODEs”: Eq S4.1-4.38) and instantaneous ancillary relations (Eq. 4.39-4.44) of the model were implemented in Matlab™ code and integrated with the “ode23s” predictor-corrector integrator with time steps of 10 to 100 μs. The initial conditions of the variables were determined by the assumption of dark adapted steady-state analysis (cf. Table S2) (cf (41) for a similar model applied to rods).

**Constraints on and parameters of the biochemical/physiological model**

**Dynamic conservation.** The model generates photoresponses over the complete range of possible flash strengths, from a single photoisomerization to a full bleaching exposure (Φ = 1.5×10^8, the total complement of pigment in a 30 μm per COS). In the upper range of intensities (Φ > 10^6) material depletion or saturation of the initial (dark adapted) quantities of various reactants occurs – e.g., of GRK, ARR, RDH, and of PDE and of G-protein at the highest range (Φ > 10^7). One important test of the model is that reactants whose total quantities are fixed must be conserved “dynamically”, i.e., throughout the responses, such that the instantaneous sum over all relevant species must be equal to the initial, dark adapted level. All predictably conserved model variables were subjected to this test, including total opsin (17 species, including various weakly bound states), total bleached pigment, total G_α (21, species, including various weakly bound states to PDE), holo-PDE (5 species), RGS9 (4 species), as well as GRK, ARR, RDH. Dynamic conservation in the model for all intensity light responses was maintained to a very high degree of precision for all relevant molecular species. Examination of the time-courses of members of sets of conserved species in plots with linear and logarithmic ordinates was helpful not only in checking code, but also in visualizing and determining appropriate state transition rates.

**Michaelis modules and low and high substrate enzyme modes.** The model comprises reactions governed by 6 enzymes, P*, GRK1/7 (Fig. 4), RDH8, PDE6 (Fig. 5), RGS9 (Fig. 5) and GAPDH. In the Michaelis-Menten framework of enzyme kinetics, an enzyme (E) and its substrate (S) reversibly bind to form an enzyme-substrate (ES) complex, which may either dissociate or react to produce a product (P):

\[
E + S \xleftrightarrow[k_f]{k_b} ES \xrightarrow[k_{cat}]{\text{kin}} E + P
\]  

(S4.45)
When the enzyme-substrate concentration varies only slowly (Briggs-Haldane kinetics), the scheme Eq 4.45 gives rise to the familiar formula for the saturable dependence of steady-state reaction velocity ($V$) on substrate concentration ($S$) (42):

$$V = \frac{[E]k_{\text{cat}}S}{S + K_m}, \quad \text{with} \quad K_m = \frac{k_b + k_{\text{cat}}}{k_f} \quad (S4.46)$$

The model uses the rate parameters of Eq S4.45 for all enzymatic reactions. (Because the underlying differential equations are explicitly solved no steady-state assumption has been made in the model calculations.) The kinetic behavior of enzyme reactions can be notably different depending on the initial concentration $S_0$ of substrate relative to the $K_m$. When $S_0 << K_m$, substrate is eliminated exponentially with the time constant $\tau = K_m / V_{\text{max}}$ while when $S_0 >> K_m$ substrate is removed at the fixed maximal rate $V_{\text{max}} = [E]k_{\text{cat}}$. Specifically, with the parameter values adopted (Table S3) for low to moderate stimulation ($\Phi < 5 \times 10^5$) GRK “removes” all its substrate from the maximally active $P^*$ state to the weakly active phosphorylated state $P_{nPi}$ with an effective time constant of ~ 4 ms (cf. Fig. S7), but at high bleach levels, GRK deactivates $P^*$ to $P_{nPi}$ at the constant maximal rate $V_{\text{max},\text{GRK}} \sim 14$ mM s$^{-1}$ (Fig. S6, upper row of panels).

Similarly, for low to moderate stimulation levels the singly-bound $G^*E^*-$RGS9 complex (Fig. 5) deactivates $G^*$'s produced by a brief $P^*$ pulse by GTP hydrolysis with an effective time constant of ~ 15 ms (Fig. S7), while at the high bleach levels RGS9-catalyzed GTP hydrolysis (and the rate of $P_i$ production) tends toward the saturated level set by the complete and persistent doubly-activated PDE (Fig. S6, lowermost panel). These light-dependent enzyme “mode switches” are important in determining the system behavior in response to low- to moderate intensity stimuli, and to the intense stimuli that trigger the COS elongation reponse.

Each PDE catalytic has a binding site for $G_{\alpha\epsilon}$-GTP (Fig. 5B), so the maximal quantity of substrate for the RGS9-catalyzed GTP hydrolysis reaction is twice the total holo-PDE6. Given that PDE6 in rods is expressed at 1:300 re rhodopsin (~ 200 µM re the rod cytosol), it appears unlikely that even under saturated substrate conditions (i.e., both PDE catalytic subunits having $G_{\alpha\epsilon}$-GTP bound as in the “Higher Intensity” regime of Fig. 5A) that the RGS9 reaction could produce $P_i$ at a rate of 80 mM s$^{-1}$. Consideration of the Michaelis framework (Eq S4.46) can help understand the problem. Assuming the same level of PDE6 expression in cones as in rods, three constraints that must be met to resolve the conundrum are that (i) the maximum substrate
concentration, 2× PDE6c, exceeds \( K_m \) by 5-fold or more; (ii) \( k_{\text{cat}} \) is about 150 s\(^{-1}\); (iii) RGS9 is 1:11 re P Opsin (7 mM re cytosol). If \( k_{\text{cat}} \) and PDE6c expression are higher than these values, the relative expression of RGS9 required would be lower. The expression for \( K_m \) provides a further constraint, a lower limit on the rate constant \( k_f \) for association of substrate and enzyme. This lower limit is around 6\( \times 10^6 \) M\(^{-1}\) s\(^{-1}\), well within the range known for protein-protein reactions (42, 43). In fact, the association rate constant for the binding reaction between photoactivated rhodopsin (R*) and holo-G\(_{t}\)-GDP in rods at body temperature can be shown to exceed 10\(^6\) M\(^{-1}\) s\(^{-1}\), likely by a factor of 3 or more.

**P* deactivation parameters.** Structural modeling ((44), Fig. 4B) indicates that GRK binding to P* per se will inhibit G* activation: the binding parameters \((k_{\text{GRK},f}, k_{\text{GRK},b})\) in the GRK “Michaelis module” (1\(^{st}\) line of Eq S4.3) and of ARR binding to \( P_{n\text{Pi}} \) were chosen so as to produce an effective P* lifetime of 2 to several ms at flash strengths producing up to \( \Phi = 5\times10^6 \) isomerizations/COS (bleach \( B = 3.3\% \)), corresponding to the total quantity of ARR in the COS. Attributing G*-activation to the \( P^*\bullet GRK \) complex was found incompatible with predictions for both low and high intensities (Fig. S6; Figs. 4D, 5D), and so \( a_{P^*\bullet GRK} \) was set to zero (but included formally to indicate that it was evaluated). At flash strengths that exhaust ARR, GRK-phosphorylation of \( P_{n\text{Pi}} \) per se substantially deactivates \( P^* \) \((a_{P^*P_{n\text{Pi}}} = 0.2; \text{Table S3})\), and hydrolysis of \( at-RAL \) causes further deactivation \((a_{P_{n\text{Pi}}P_{\text{Ops}}} = 0.15; \text{Table S3})\). Attributing a reduced capacity for G* activation to \( P_{n\text{Pi}} \) and \( P\text{Ops} \) was necessary to account for the approximately steady production of free phosphate \((P_1)\) hypothesized to underlie Component 1 (Fig. S6).

**G* activation and deactivation parameters.** The hypothesis that the osmolyte underlying Component 1 corresponds to free phosphate \((P)\) generated by RGS9-catalyzed GTP hydrolysis requires that the total complement of G-protein be completely recycled several times during the \( \sim 1\) s saturated Component 1 response. The need to introduce a complete set of binding states of the G-protein activation cycle arose from the problem of conservation: the occupancy time of all G-protein states is material to the cycle time. The parameters \( k_{G01} \) through \( k_{G14} \) (parameters 14-20 in Table S3; light green box in Fig. S5) were selected based on 2 principles: first, the protein
binding reactions are reversible; second, the overall activation cycle from binding of holo-G (variable “G0” in Table S2) to release of G* (G4 in Table S2) should take no more than 3 ms for a single P* per disc face. The maximum rate of G* production per P* with the parameters in Table S3 was 60 s⁻¹; this latter rate is substantially diminished by the brief effective P* lifetime, and would be ~ 300 s⁻¹ for non-deactivating P*. As established for rods undergoing low to moderate stimulation (45, 46), the model assumes that deactivation of Gₐα-GTP in human cones occurs primarily via RGS9-catalysis of GTP hydrolysis in the G*-E* complexes (Fig. 5A, B).

To accommodate the RGS9 concentration-dependence of the GTPase reaction (47), the latter was treated as a “Michaelis module” with reversible RGS9 binding (46) (Eqs. S4.31-4.34; parameters #27 – #35): for low to moderate flash strengths (Φ <5×10⁵) this yielded an effective “G*-E*” lifetime of ~ 15 ms, consistent with human cone electrophysiology (Fig. S7).

Production and removal of free phosphate (Pᵢ). To account for COS elongation Component 1, phosphate (Pᵢ) needed to be produced in a steady but light-dependent manner (Figs. 2, 5). Analysis of the exponential rise of the saturated Component 1 (Eqs 5, 6) suggested this might be achieved by graded production of G* feeding into the RGS9 catalytic system, with removal of Pᵢ by a reaction such as that governed by GAPDH. Graded production of G* was achieved by assuming that relatively slowly deactivating phosphorylated cone pigment (Pᵦₚₕ) and “naked” cone opsin (POps) weakly activate G-protein (Eqs. S4.11, 4.14, 4.15; Fig. S6).

The rate equation for phosphate (Pᵢ, Eq S4.35) assumes production primarily by RGS9-catalyzed GTP hydrolysis with removal by GAPDH obeying “Michaelis” (saturable) kinetics. GAPDH is highly expressed in outer segments (48, 49), and the high dissociation constant of GAPDH for Pᵢ has been noted (42). The hypothesized value of the maximum velocity (Vₘₐₓ,GAPDH, Table S3) may seem high, but if GAPDH is present at 1:25 re cone opsin, its kₐₜ would be about 1000 s⁻¹, more than 4-fold lower than that measured for triosephosphate isomerase, whose substrate is glyceraldehyde-3-phosphate (Table 4.4, ref (42)). Another potential generator of Pᵢ is the phosphatase (PPase PP2A) that catalyzes removal of phosphate groups from phosphorylated cone pigment, Pᵦₚₕ (50). This hypothesis was examined by including Michaelis-Menten rate equations for PPase, and is discussed below (6. Rejected hypotheses about the molecular identity of the osmolyte underlying Component 1).
5. Prediction of the human cone massed electrical response

The reaction schemes and their implementation in rate equations were aimed primarily at explaining the COS elongation responses. However, the model also provides a consistent account of the electrical responses of human cones as measured with the \(a\)-wave of the electroretinogram (51) (Fig. 7S). Key comparative metrics between data and theory are the times to peak of the subsaturating responses, the recovery phase kinetics (e.g., time to recover 50% of the dark current), and the light sensitivities (Fig. S7C). It bears emphasis that the human ERG Ganzfeld data in Fig. S7 represent the summed activity of many hundreds of thousands of cones that can be expected from their morphological variation over the retina to have somewhat different photocurrent responses – for example, in the higher frequency components of the responses (such as the “ripples” in the recovery traces generated by the calcium buffering and feedback to guanylate cyclase) would likely be “washed out” by spatial averaging. According to the model, for the strongest flash strengths used for the ERGs, both catalytic subunits of PDE6c are never activated – the model functions entirely in the “Lower Intensity” regime of Fig. 5A.
### Table S1. Factors for converting photon density at the retinal surface to that at COS basal discs

| Subj, Exp’t | Eccen. (deg) | Fraction bleached ($B_0$) | $Q_{e, retina}$ (hv µm$^{-2}$) | $f_{WFA}$ | $f_{SCI}$ | $f_{CIS2COS}$ | $f_{Dens}$ | $Q_{e,COS,inc}$ (hv µm$^{-2}$) |
|------------|-------------|---------------------------|-------------------------------|----------|----------|--------------|----------|-------------------------------|
| 1, SSR     | 1.5         | 0.043 ± 0.002             | 2.7×10$^7$                    | 1.0      | 0.60     | 10           | 0.62     | 1.0×10$^8$                    |
| 1, SSR     | 4.5         | 0.038 ± 0.003             | 2.4×10$^7$                    | 1.0      | 0.54     | 15           | 0.71     | 1.4×10$^8$                    |
| 1, SSR     | 7.0         | 0.045 ± 0.004             | 2.2×10$^7$                    | 1.0      | 0.54     | 17           | 0.79     | 1.5×10$^8$                    |
| 1, OCT     | 7.0         | --                        | 8.9×10$^7$                    | 0.5      | 0.35     | 17           | 0.79     | 1.9×10$^8$                    |
| 3, SSR     | 1.5         | 0.060 ± 0.002             | 2.0×10$^7$                    | 1.0      | 0.60     | 10           | 0.62     | 7.1×10$^7$                    |
| 3, SSR     | 4.5         | 0.051 ± 0.004             | 1.9×10$^7$                    | 1.0      | 0.54     | 15           | 0.71     | 1.0×10$^8$                    |
| 2, SSR     | 7.0         | 0.064 ± 0.003             | 1.5×10$^7$                    | 1.0      | 0.54     | 17           | 0.79     | 1.1×10$^8$                    |
| 2, OCT     | 7.0         | --                        | 5.2×10$^7$                    | 0.5      | 0.35     | 17           | 0.79     | 1.2×10$^8$                    |

**Notes.** Column (1) identifies the subject and experiment: SSR = serial scanning reflectometry (Fig. 3A-C); OCT, measurement of COS elongation (Figs 1-2). Column (2) gives the retinal eccentricity of the experiment, and column (3) the fraction cone pigment estimated to be bleached per scan in the SSR experiments. Column (4) gives the energy density in $\lambda_{max}$-equivalent photons “at the retina” that yields a normalized amplitude of $1 - e^{-1}$ for the exponentially rising function fitted to the data. (The spectra of the respective light sources at the cornea were multiplied wavelength-by-wavelength by ocular media transmission spectra appropriate for the eccentricity, and integrated against normalized cone pigment absorption spectra; values for the M- and L-cone pigments differed slightly, and the two were averaged.) Columns (4)-(7) give the factors of Eq 1 (Eq S2.7) used to convert the energy density at the retina to that at the cone outer segment waveguide entrance (Eq S3). The eccentricity dependence of the pigment density scaling factor ($f_{Dens}$) is based on measurements of the average COS length at each location (Methods). Column (8) gives the predicted values, and Figure 3E plots the predictions in logarithmic units with error measurements, and provides statistical analysis.
Table S2: Variables of the phototransduction model and initial values

| Symbol   | # re $P_{tot}$ | Description                                                                 | Initial value; upper limit | Equations |
|----------|----------------|-----------------------------------------------------------------------------|----------------------------|-----------|
| $P_0$    | 1:1            | Regenerated pigment (M- or L-opsin)                                         | $P_{tot} = 72160$          | 4.1       |
| $P^*$    |                | Photoisomerized pigment (M- or L-opsin, MII)                                | $0; \leq \Phi_{pdf}$       | 4.2, 4.3, 4.5, 4.6, 4.8, 4.11, 4.11 |
| $P^*GRK$ |                | $P^*$ in complex with GRK1 or GRK7                                          | $0; \leq GRK_{tot}$        | 4.3, 4.4, 4.6, 4.11, 4.11 |
| $P_{nPi}$|                | Multiply phosphorylated $P^*$                                                | $0; \leq P_{tot}$          | 4.4, 4.5, 4.7, 4.8, 4.11, 4.11 |
| $POps$   |                | Ligand-free pigment                                                         | $0; \leq P_{tot}$          | 4.1, 4.5   |
| $GRK$    | 1:50           | Free GRK1 & GRK7                                                            | $GRK_{tot} = 1443$         | 4.3, 4.6   |
| $ARR$    | 1:30           | Free ARR1 & ARR4                                                            | $ARR_{tot} = 2405$         | 4.7       |
| $RDH$    | 1:30           | Free RDH                                                                    | $RDH_{tot} = 2405$         | 4.8, 4.9   |
| $atRAL$  |                | Non-covalently bound all-trans retinal                                      | $0; \leq 6.6 \text{ mM}$   | 4.8, 4.9, 4.10 |
| $atROL$  |                | Soluble all-trans retinol                                                   | $0; \leq 6.6 \text{ mM}$   | 4.10       |
### Table S2 (cont): Variables of the phototransduction model and initial values

| Symbol | # re $P_{tot}$ | Description | Initial value; upper limit | Equations |
|--------|----------------|-------------|---------------------------|-----------|
| **Variables representing G-protein, PDE and RGS9 states** |
| $G_0$  | 1:8            | $G_{ic} \alpha \beta \gamma \cdot GDP$: inactive heterotrimeric G-protein ($G_{ic} \alpha \beta \gamma \gamma_{i_2}$) | $G_{tot} = 9020$ | 4.11-4.15 |
| $G_{1a}$ | –             | $P \ast \cdot G_{ic} \alpha \beta \gamma \cdot GDP$: inactive holo-G in complex with $P \ast$ | 0; $\leq \Phi_{pdf}$ | 4.11, 4.12 |
| $G_{2a}$ | –             | $P \ast \cdot G_{ic} \alpha \beta \gamma$: unliganded holo-G in complex with $P \ast$ | 0; $\leq \Phi_{pdf}$ | 4.12, 4.16 |
| $G_{3a}$ | –             | $P \ast \cdot G_{ic} \alpha \beta \gamma \cdot GTP$: activated holo-G in complex with $P \ast$ | 0; $\leq \Phi_{pdf}$ | 4.12, 4.16 |
| $G_{4}$  | –             | $G_{ic} \alpha \cdot GTP$: activated, membrane-assoc’d $\alpha$-subunit ($G^*$) | 0; $\leq G_{tot}$ | 4.16, 4.20 |
| $G_{4S}$ | –             | Solubilized $G_{ic} \alpha \cdot GTP$ | 0; $\leq G_{tot}$ | 4.24, 4.25 |
| $G_{5}$  | –             | $G_{ic} \alpha \cdot GDP$: inactive membrane-associated $\alpha$-subunit | 0; $\leq G_{tot}$ | 4.11, 4.26, 4.27 |
| $G_{b}$  | –             | $G \beta \gamma$: separated $\beta \gamma$ subunit | 0; $\leq G_{tot}$ | 4.11, 4.27 |
| $E_0$  | 1:25           | Inactive PDE dimer | $PDE_{tot} = 2886$ | 4.28, 4.29 |
| $E_1$  | –             | $PDE \cdot (G_{ic} \alpha \cdot GTP)$: PDE with 1 active catalytic subunit | 0; $\leq PDE_{tot}$ | 4.28, 4.29, 4.30 |
| $E_2$  | –             | $PDE \cdot 2(G_{ic} \alpha \cdot GTP)$: PDE with 2 active catalytic subunits | 0; $\leq 2 PDE_{tot}$ | 4.29, 4.30 |
| $Rg_0$ | 1:10           | Free RGS9 | $RGS9_{tot} = 7216$ | 4.31, 4.32, 4.33, 4.34 |
| $Rg_1$ | –             | $PDE \cdot (G_{ic} \alpha \cdot GTP) \cdot RGS9$ complex (Fig 4ba) | 0; $\leq PDE_{tot}$ | 4.31, 4.32, 4.33 |
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\[
Rg_2 \quad - \quad PDE \bullet 2(G_{ic} \alpha \bullet GTP) \bullet RGS9 \text{ complex (Fig. 4Bb)} \quad 0; \leq PDE_{\text{tot}} \quad 4.31, 4.32, 4.33, 4.34
\]

\[
Rg_3 \quad - \quad PDE \bullet 2(G_{ic} \alpha \bullet GTP) \bullet 2RGS9 \text{ complex (Fig. 4Bc)} \quad 0; \leq 2 PDE_{\text{tot}} \quad 4.31, 4.32, 4.33, 4.34
\]

**Symbol** | **Description** | **Initial value** | **Equations**
---|---|---|---
\( P_i \) | Free phosphate | 0.25 mM | 4.35
\( cG \) | Cyclic GMP | 1.0 µM | 4.36, 4.40
\( Ca \) | Free calcium | 300 nM | 4.36, 4.39, 4.41, 4.42
\( V_m \) | Membrane potential | -40 mV - | 4.38

**Table notes.** In the transduction model (Eqs S4.1-4.44) protein reactants (variables in column 1) were expressed in numbers per disc face, as required by local membrane confinement of the protein-protein reactions; the initial (resting) quantities in column 4 are expressed in #'s per disc face, and were derived from the membrane density of cone opsin (30,000 µm\(^2\)) and the ratios in column 2. The values \( P_{\text{tot}}, G_{\text{tot}}, \) etc. represent the total number of the respective proteins per disc face; the total per COS is obtained by multiplying by the # of disc faces. G-protein can in principle be activated by several “P-species”: \( P^*\bullet GRK \), \( P_{np} \) or \( POps \). To include these possibilities, 9 additional variables representing the respective G-protein complexes (\( G_{1b}, G_{2b}, G_{3b} \) for \( P^*\bullet GRK \) complexes; \( G_{1c}, G_{2c}, G_{3c} \) for \( P_{np} \) complexes; \( G_{1d}, G_{2d}, G_{3d} \) for \( POps \) complexes) and rate equations parallel to those governing the interaction with \( P^* \) were included for the respective complexes, with identical transition rate parameters between complexes (cf. Eqs S4.11 – S4.24).
**Table S3: Rate constants and other parameters of the phototransduction model**

| Parameters ((#) | Unit | Value | Equations | Comment |
|----------------|------|-------|-----------|---------|
| $k_{\text{regen}}$  | s$^{-1}$ | 0.00833 | 4.1, 4.2 | Rate constant of pigment regeneration; negligible on 1 s time scale |
| $k_{\text{hydr}}$  | s$^{-1}$ | 1.05 | 4.2, 4.8 | Value tightly determined by Component 2 kinetics (Fig. 4) |
| $k_{\text{GRK},f}$  | s$^{-1}$ | 2 | 4.2, 4.3, 4.6 | Association rate constant of GRK Michaelis module |
| $k_{\text{GRK},b}$  | s$^{-1}$ | 0.05 | 4.2, 4.3, 4.6 | Dissociation rate constant of GRK Michaelis module |
| $k_{\text{cat,GRK}}$  | s$^{-1}$ | 100 | 4.3, 4.6, 4.9, 4.10 | Turnover # of GRK Michaelis module |
| $a_{\text{pGRK}}$  | # | 0 | 4.3, 4.11, 4.13, | Nonzero value incompatible for all predictions |
| $a_{\text{pOps}}$  | # | 0.2 | 4.4, 4.11, 4.14 | Dimensionless activity parameter of (re $a_{\text{p}} = 1$; cf Fig. S6) |
| $a_{\text{pOps}}$  | # | 0.15 | 4.5, 4.11, 4.15 | Dimensionless activity parameter of (re $a_{\text{p}} = 1$; cf Fig. S6) |
| $k_{\text{ARR},f}$  | s$^{-1}$ | 10 | 4.7, 4.8 | Bimolecular rate constant for association of $P_{\text{nP}}$ with $ARR$ |
| $k_{\text{ARR},b}$  | s$^{-1}$ | 0.1 | 4.7, 4.8 | Rate constant for dissociation of $P_{\text{nP}} \cdot ARR$ complex |
| $k_{\text{RDH},f}$  | s$^{-1}$ | 100 | 4.8, 4.9 | Association rate constant of RDH Michaelis module |
| $k_{\text{RDH},b}$  | s$^{-1}$ | 50 | 4.8, 4.9 | Dissociation rate constant of RDH Michaelis module |
| $k_{\text{cat,RDH}}$  | s$^{-1}$ | 200 | 4.9, 4.10 | Turnover # of RDH Michaelis module |
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Parameters governing activation, inactivation and recycling of G-protein

| Parameter | Value | S^(-1) | Rate constant |
|-----------|-------|--------|---------------|
| k_{G01}  | 10    | 4.11-4.15 | Rate constant for P*binding to G_0 to form G_{1a} complex |
| k_{G10}  | 150   | 4.11-4.15 | Dissociation rate constant for G_{1a} complex |
| k_{G12}  | 2000  | 4.11-4.15 | Rate constant for GDP dissociation from G_{1a} to form G_{2a} |
| k_{G21}  | 100   | 4.12-4.15 | Rate constant for GDP re-association with G_{2a} complex |
| k_{G23}  | 2000  | 4.16-4.23 | Pseudo 1st order rate constant for GTP binding to G_{2a} complex |
| k_{G32}  | 100   | 4.16-4.23 | Rate constant for GTP dissociation from G_{3a} (P*•G_{lc}αβγ•GTP) |
| k_{G34}  | 2000  | 4.20-4.23, 4.27 | Rate constant for dissociation of G_{3a} to G_{lc}α•GTP and Gβγ |
| k_{G45}  | 0.1   | 4.24-4.26, 4.35 | Rate constant for spontaneous GTP hydrolysis |
| k_{G4sf} | 10    | 4.24, 4.25 | Rate constant for solubilization of G_{4} (G_{lc}α•GTP, G*) |
| k_{G4sb} | 10^3  | 4.24, 4.25 | Rate constant for re-association of G_{4b} with disc membrane |
| k_{Gb5}  | 10    | 4.26, 4.27 | Rate constant for re-association of G_{b} and G_{5} |

Parameters governing the activation and deactivation of PDE

| Parameter | Value | S^(-1) | Rate constant |
|-----------|-------|--------|---------------|
| k_{GE01} | 10    | 4.28, 4.29 | Rate constant for association of G_{4} = G*with E_0 |
| k_{GE10} | 150   | 4.28, 4.29 | Rate const for dissociation of E_1 (PDE•(G_{lc}α•GTP)) complex |
| k_{GE12} | 10    | 4.29, 4.30 | Rate constant for association of G_{4} = G*with E_1 |
| k_{GE21} | 300   | 4.29, 4.30 | Rate const for dissociation of a G_{lc}α•GTP from an
| Reaction | $k$ | Unit | $k_{cat,Rg1}$ | $E_2(\text{PDE} \cdot 2(G_c\alpha \cdot \text{GTP}))$ complex |
|----------|-----|------|---------------|---------------------------------------------------------------------------------|
| Rate const for association of $RGS9$ with $E_1$ complex to form $Rg_1$ enzyme-substrate complex | $k_{RE01}$ | (29) | $s^{-1}$ | 0.097 | $4.29, 4.31, 4.32$ |
| Dissociation rate constant for $RGS9$ from $Rg_1$ complex | $k_{RE10}$ | (30) | $s^{-1}$ | 1000 | $4.29, 4.31, 4.32$ |
| Rate const for association of $RGS9$ with $E_2$ complex to form $Rg_2$ enzyme-substrate complex | $k_{RE12}$ | (31) | $s^{-1}$ | 0.48 | $4.30, 4.31, 4.33, 4.32$ |
| Dissociation rate constant for $RGS9$ from $Rg_2$ complex | $k_{RE21}$ | (32) | $s^{-1}$ | 1000 | $4.30, 4.31, 4.33, 4.32$ |
| Rate const for association of $RGS9$ with $E_2$ complex to form $Rg_2$ enzyme-substrate complex | $k_{RR12}$ | (33) | $s^{-1}$ | 485 | $4.31, 4.33, 4.34$ |
| Rate const for association of $RGS9$ with $Rg_2$ complex to form $Rg_3$ enzyme-substrate complex | $k_{RR21}$ | (34) | $s^{-1}$ | 2000 | $4.31, 4.33, 4.34$ |
| Rate constant for $G_c\alpha \cdot \text{GDP}$ dissociation from $Rg_1$ complex | $k_{GR10}$ | (35) | $s^{-1}$ | 200 | $4.31, 4.32$ |
| Rate constant for association of $G_4 = G^* \rightarrow Rg_1$ to form $Rg_2$ complex | $k_{GR12}$ | (36) | $s^{-1}$ | 20 | $4.32, 4.33$ |
| $G_c\alpha \cdot \text{GTP}$ dissociation from complex without GTP hydrolysis | $k_{GR21a}$ | (37) | $s^{-1}$ | 300 | $4.31, 4.32, 4.33$ |
| $G_c\alpha \cdot \text{GTP}$ dissociation from complex without GTP hydrolysis | $k_{GR21b}$ | (38) | $s^{-1}$ | 300 | $4.31, 4.33$ |
| $G_c\alpha \cdot \text{GTP}$ dissociation from complex without GTP hydrolysis | $k_{GR21c}$ | (39) | $s^{-1}$ | 150 | $4.31, 4.32, 4.34$ |
| Turnover # of $Rg_1$ Michaelis-complex | $k_{cat,Rg1}$ | (40) | $s^{-1}$ | 170 | $4.26, 4.31, 4.35$ |
In the model COS, protein quantities are expressed in #’s per active disc face: these variables can be converted to surface density rates by dividing by the disc face membrane area, 2.4 \( \mu \text{m}^2 \), or to molar units by \( \frac{N_{\text{active disc faces}} \times X(t)}{[N_{\text{Av}} V_{\text{cyto}}]} \), where \( N_{\text{active disc faces}} \) is the # of disc faces with one or more P*, \( X(t) \) is the # of reactants per active disc face at time \( t \), \( N_{\text{Av}} \) is Avogadro’s number, and \( V_{\text{cyto}} \) the volume of the COS cytoplasm. Rate constants for bimolecular disc-associated reactions (e.g., \( k_{G01} \), the rate constant for formation of the P*-G\( \alpha \beta \gamma \)-GTP complex) can be converted to molar units by multiplying the values in the table by \( \frac{1}{2} N_{\text{Av}} V_{\text{cyto}} = 9.5 \times 10^6 \) (unit: M\(^{-1}\)), where \( V_{\text{IDS}} \) is the volume of the interdiscal space (the factor \( \frac{1}{2} \) is required because the proteins of 2 disc faces are associated with each interdiscal space). Rate constants for unimolecular reactions \( \text{s}^{-1} \) are unaltered when the reactants are quantified in molar units. Additional parameters/values for Eqs S4.35 – 4.44 describing the electrical response are as follows: \( P_{i,\text{rest}} = 0.25 \) mM; \( \alpha_{\text{max}} = 165 \) \( \mu \text{M s}^{-1} \); \( K_{\text{cyc}} = 80 \) nM; \( \beta_{\text{sub}} = 0.04 \) s\(^{-1} \); \( \beta_{\text{dark}} = 20 \) s\(^{-1} \); \( J_{\text{G}} = -65 \) pA, \( J_{\text{ex, sat}} = -20 \) pA \( (L_{\text{COS}} = 30 \) \( \mu \text{m}) \); \( K_{\text{ex}} = 1000 \) nM; \( BP_{\text{LA}} = 10 \); \( BP_{\text{cap}} = 10^4 \); \( K_{\text{huff}} = 200 \); \( g_{\text{cG}} = 1.25 \) nS; \( f_{\text{Ca}} = 0.14 \); \( g_{\text{K}} = 1.4 \) nS; \( E_{\text{K}} = -90 \) mV.
6. Rejected hypotheses about the molecular identity of the osmolyte underlying Component 1

Component 1 has striking features that can be used to test hypotheses about its molecular identity, which reveals it to be an amplified byproduct of phototransduction (Figs. 1, 2). A number of soluble byproducts of phototransduction were evaluated as candidates for the osmolyte underlying Component 1, including (i) dissociated and solubilized subunits of the G-protein, (ii) byproducts of cGMP hydrolysis, (iii) byproducts of the synthesis of cGMP guanylate cyclase, (iv) free phosphate (Pi) produced by RGS9-catalyzed GTP hydrolysis, and (v) pyrophosphate (PPi) and Pi generated in P* phosphorylation and dephosphorylation.

Candidate (i): solubilized subunits Ga and Gβ1γ1 of cone G-protein. In rods photoisomerized rhodopsin can cause the dissociated subunits Ga and Gβ1γ1 to solubilize and migrate from the outer to inner segment. Assuming a G-protein expression level relative to opsin comparable to that in mammalian rods (~1:10) (52), full activation of cone G-protein and complete dissociation of the separated subunits from the disc membrane would generate ~1.5 mOsM osmolyte, 5-fold too small to account for the saturated level of Component 2, 6.6 mOsM. Moreover, experiments have demonstrated that the short lifetime of photoactivated cone pigment (P*) (and possibly the prenylation of the cone G-protein subunits) seriously limits their dissociation from the cone disc membrane, so that only artificially extended P* activity produces material subunit dissociation and redistribution between COS and CIS (53). Thus, cone G-protein subunits can be rejected as candidates for Component 1, both in terms of their potential magnitude and their limited dissociation from the COS membrane.

Candidate (ii): byproducts of the cGMP hydrolysis reaction. The resting cGMP concentration of rods and cones has been widely estimated to be in the low micromolar range, certainly less than 10 µM (54, 55). The intense flashes used in these experiments cause massive activation of phosphodiesterase, so that the cGMP is very rapidly hydrolyzed to its products, 5’-GMP and H+, as revealed by saturating cone ERG a-wave responses (Fig. S6). While having a potential net gain of 1 osmolyte per molecule of cGMP hydrolyzed, the quantity produced in a saturating light response is far too small to account for the ~6.5 mOsM level of Component 1: after the initial depletion of the resting cGMP, the rate of hydrolysis (and thus osmolyte production) drops by several orders of magnitude. Moreover, pH buffer power is 50-fold or higher in the outer segment, so the H+ osmolyte is further diminished. Finally, the PDE-catalyzed reduction in cGMP is many orders of magnitude more sensitive to light than that of Component 1 (compare Fig. 2D with Fig. S6).
Candidate (iii): byproducts of guanylate cyclase synthesis of cGMP. During the saturated light responses when the resting cGMP is hydrolyzed, \( \text{Ca}^{2+} \) declines rapidly, maximally stimulating guanylate cyclase (56, 57), which converts GTP into cGMP and pyrophosphate (PP\(_i\)), with a net gain of 1 osmolyte per unit conversion. The maximum rate of synthesis in rods is about 150 \( \mu \text{M s}^{-1} \) (56, 58) and unlikely to exceed 200 \( \mu \text{M s}^{-1} \) in cones. Thus, the cyclase reaction cannot account for the nearly 1000-fold higher production rate underlying Component 1, \( \sim 80 \text{ mOsM s}^{-1} \) (Figs. 1, 2).

Candidate (iv): ionic concentration changes accompanying CNG channel closure and hyperpolarization. Another hypothesis that can be rejected is that the osmotic change underlying Component 1 arises from increased intracellular (or lowered extracellular) concentration changes of the permeant ions, \( \text{Na}^+ \), \( \text{K}^+ \), or \( \text{Ca}^{2+} \). The latter is rejected, as \( \text{Ca}^{2+} \) decreases during the light response, while \( \text{Ca}^{2+} \) undergoes a slight increase (relative to the resting level, \( \sim 2 \text{ mM} \)). In a dark adapted COS with a 36 fL cytoplasmic volume a 70 pA CNG channel current corresponds to an influx of \( \text{Na}^+ \) of \( \sim 20 \text{ mM s}^{-1} \), an influx balanced by extrusion by the \( \text{Na}^+/\text{K}^+ \) pump of the inner segment. The intense light stimuli employed in the OCT experiments rapidly closes the CNG channels (cf. Fig. S6), blocking this influx, so that both decreased influx and continuing extrusion by the \( \text{Na}^+/\text{K}^+ \) pump (NKX) of the inner segment will tend to cause \( \text{Na}^+ \) to decrease. (A smaller influx of \( \text{Na}^+ \) through the NCKX exchanger, which continues and increases somewhat when the COS is hyperpolarized, is smaller than the NKX efflux -- cf (41) for an analysis.) In the dark adapted state, an efflux of \( \text{K}^+ \) predominantly through \( \text{K}_{\text{v}}2.1 \) channels (41, 59) in the CIS is balanced by influx of \( \text{K}^+ \) through the NKX. The saturating electrical response leads to a net influx corresponding to the charging of the cone capacitance to the hyperpolarized potential and continued NKX pump activity. Given a resting \( \text{K}^+ \) of 90 mM or higher, any \( \text{K}^+ \) increase can be shown to be osmotically immaterial relative to the resting osmolarity of \( \sim 300 \text{ mOsM} \) with the analysis in (41), especially on the time scale of the \( \sim 1 \text{ s} \) elongation responses. The corresponding decrease in \( \text{K}^+ \) in the subretinal space in which COS are embedded can also be shown to be negligible relative to the resting plasma osmolarity, \( \sim 300 \text{ mOsM} \).

Candidate (v): phosphates removed from phosphorylated cone opsin by phosphatase. C-terminal residues of phosphorylated cone opsin (P\(_{n\phi}\)) are dephosphorylated by protein phosphatase PP2A (50): the removed phosphates removed could act as osmolytes, potentially
contributing to COS elongation. Moreover, 1 phosphate (P_i) removed from each P_{npi}, after a full bleach corresponds to the saturated amplitude (6.5 mOsM) of Component 1. We think the phosphatase hypothesis is rejected for the following reasons. First, to account for the saturated Component 1 response, P_i must be generated at an initial rate of \( \sim 80 \text{ mM s}^{-1} \) immediately after a full bleach (Fig. 2), implying that \( V_{\text{max, PP2A}} \geq 80 \text{ mM s}^{-1} \). However, before PP2A can desphosphorylate P_{npi}, P^* must first be phosphorylated, so that GRK would have to have a substantially higher velocity – say, minimally 2-fold greater. In the present model, \( V_{\text{max, GRK}} = 14 \text{ mM s}^{-1} \), and even if 3 phosphates on average are transferred to each P^*, GRK phosphorylation would still be 4-fold too low to allow PP2A to achieve the requisite velocity. Moreover, were \( V_{\text{max, GRK}} \) further increased, the apparent lifetime of P^* would be further shortened below 2-3 ms value of the current model, reducing the sensitivity of the cone electrical response to a unacceptably low value. Second, P_i generated by phosphatase must be removed by GAPDH or an equivalent reaction (Eqs. 4-5), and in order to produce the plateau phase of Component 1 P^* would need to be repeatedly phosphorylated and dephosphorylated. Such rapid phosphatase action would revert P_{npi} to its fully active state P^*, causing “flash blindness” rather than recovery of sensitivity. Third, the phosphatase hypothesis was examined by including a rate equation for the \( PP2A \cdot P_{npi} \) enzyme-substrate complex, and appropriate terms to the rate equation governing \( P_{npi} \) (Eq S3.4). For this simulation the RGS9 reaction was “knocked out”, the expression level of PP2A was set equal to that of GRK (1:50 re opsin), and the concentrations of all other reactants and parameter values were otherwise unchanged. Even with the extreme assumptions that 3 phosphates transferred by GRK to P^* and removed by PP2A, and that \( k_{\text{cat, PPase}} = 2k_{\text{cat, GRK}} \), the maximum level of P_i was more than 2-fold below the plateau level for Component 1, i.e., rejected the hypothesis. Fourth, the genetic deletion of PP2A slows mouse cone dark adaptation, whose main phase in WT mice takes place over \( \sim 15 \text{ min} \) after a large bleaching exposure (50). The latter results imply that PP2A dephosphorylation normally occurs on time scale of cone dark adaptation, which has an approximately 2 min time constant in humans, more than 1000-fold slower than the time constant of Component 1.

7. Comparison of mechanisms of COS vs. ROS light-stimulated elongation

Mechanisms of ROS and COS elongation. Four mechanisms are hypothesized to underlie ROS and COS light-stimulated elongation:
(1) All OS elongation is accompanied by osmotically driven water influx.

(2) All photoactivated opsins act as “sponges”.

(3) All photoactivated opsins form transmembrane water conduits.

(4) The water that causes OS elongation originates primarily in the choroidal vasculature.

Mechanism (1). Mouse ROS elongation (33, 60) and Component 1 of COS elongation (this paper) have both been hypothesized to be caused by phototransduction-induced increases in the concentration of cytoplasmic solutes (osmolyte) that osmotically drive water influx into the OS cytosol. The hypothesized osmolytes in the two photoreceptor types are qualitatively different, however.

For mouse ROS, the osmolyte candidates rejected as explaining COS Component 1 (cf. SI 6. Rejected hypotheses about the molecular identity of the osmolyte underlying Component 1) can also be rejected for ROS with similar arguments. In addition, because RGS9 is expressed at a much lower level in ROS, GTPase production of Pi can also be rejected. The only hypothesis of which we are aware that can account for the magnitude of the ROS swelling is as follows. Accordingly, during daily morphogenesis, basal rod disc membranes are sealed and internalized in the ROS, trapping extracellular solutes that become concentrated by mechanical forces that “zipper” together the opposing inner bilayer leaflets of the discs and dehydrate the disc interior. Photoactivation of rhodopsin provides trans-disc membrane conduits for water movement into the intradiscal space, allow it to equilibrate osmotically with the cytosol. The “intradiscal osmolyte” hypothesis is not mutually exclusive with opsin swelling, so both mechanisms may play a role in ROS swelling: however, the contribution of photoactivated rhodopsin swelling is likely to be smaller than that arising from the intradiscal solute hydration.

Opsin swelling also drives water entry through the plasma membrane: thus, to restore osmotic equilibrium, water that moves into the interstices of photoactivated opsins creates an activity gradient that can drive water movement across the plasma membrane of ROS and COS.

Mechanism (2). Photoactivated opsin swelling by water of a magnitude sufficient to explain COS Component 2 has only been measured for rhodopsin (61), but similar and perhaps even larger swelling of cone opsins likely occurs. First, human cone opsins have high sequence homology to rhodopsin, and function by activating a homologous heterotrimeric G-protein (cones: Gα2β3γ2; rods Gα1β1γ1γ1) (62), and no doubt undergo similar conformational changes upon photoactivation as rhodopsin. Second, the chromophore binding pocket of photoactivated
cone opsin is much more accessible to water and small molecules such as hydroxylamine (NH₂OH) than of photoactivated rhodopsin, and photoactivation of cone opsin produces a greater change in entropy than photoactivation of rhodopsin of the same species (63). The water that swells photoactivated pigments must ultimately come from outside the OS, because its movement from the cytosol into opsin and membrane necessarily creates an activity gradient that will drive trans-plasma membrane diffusion.

Mechanism (3). Evidence that photoactivated opsins form transmembrane water conduits has so far been obtained from molecular dynamics studies (64, 65). Homology between cone and rod opsin secondary structures suggest that in all photoactivated opsins access of water to the all-trans chromophore binding site is accompanied by one or more intra- and perimolecular pathways through which water can permeate the membrane. The much more rapid hydrolysis of the chromophore in cones than in rods (66-69) implies that the conformational changes enabling trans-membrane water permeation occur more quickly in cone opsins than rhodopsin. The apparent water permeabilities of the individual photoactivated cone and rod cone opsins appears to be comparable, however. Thus, the water permeability of the COS plasma membrane was herein estimated to be at least $P_{W,COS} = 0.0014 \text{ cm s}^{-1}$ and given a full bleach opsin density of 30,000 µm⁻², the estimated water permeability of a single photoactivated cone opsin is $(1.4 \times 10^{-3} \text{ cm s}^{-1})/(3 \times 10^{12} \text{ opsin cm}^{-2}) = 4.7 \times 10^{-16} \text{ cm}^3 \text{ s}^{-1}$. Zhang et al (33) estimated the mouse ROS plasma membrane to have an average permeability of $2.5 \times 10^{-5} \text{ cm s}^{-1}$; the expression density of rhodopsin in the ROS plasma membrane is 30% to 50% that of the disc membranes (70, 71), say ~ 12,000 µm⁻², and the saturated rate of ROS swelling occurs for a 10% bleach (33). Thus, given for a “rhodopsin-transducin water conduit density” of 1200 µm⁻², the “single channel” water permeability is $(2.5 \times 10^{-5} \text{ cm s}^{-1})/(1.2 \times 10^{11} \text{ opsin cm}^{-2}) = 2 \times 10^{-16} \text{ cm}^3 \text{ s}^{-1}$. For comparison, the water permeability coefficient of single aquaporin molecules is over 100-fold higher, $\approx 5 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$ (AQP1)(72, 73) and $3-9 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1}$ (AQP4) (74). The nearly equal estimates of single photoactivated rod and cone opsin water permeability must be taken with caution for several reasons, including (i) that of the cone plasma membrane is a lower limit, supported by the exponential kinetics of Component 1; (ii) the water permeable state of photoactivated rhodopsin appears to require transducin (33). The requirement for transducin is consistent with saturated permeability occurring with a 10% bleach, but there is as present no evidence for such a requirement in cone opsin. Were the transducin-bound state of
cone opsin (Fig. 3A) the most permeable, its relatively short lifetime (< 5 ms) would require this state to be substantially more permeable than the estimated lower limit, and suggests that transducin might not play a key role in water permeation through cone opsin.

Swelling of, and transmembrane water transport by photoactivated opins are conceptually distinct properties of the structure/function state diagram of an opsin, and while it is reasonable to expect them to be strongly related, they could differ in important ways in individual opsins and across opsins. Thus, for example, many waters could enter a cone opsin from the cytoplasm face prior to achievement of the maximally water permeable state. Clearly, much more detailed empirical and theoretical understanding of the structural details of cone and rod opsin water permeation will be needed to address these issues quantitatively.

Mechanism (4). Observations by Zhang et al. (ref 33, Fig. 1E, H) suggest that ROS swelling involves water movement down an activity gradient from the choroidal vasculature through Bruch’s membrane (BrM) and the RPE layer to the ROS whose cytoplasm had increased osmolarity. First, ROS elongation was invariably accompanied by scattering changes in BrM and choroid. Second, the magnitude of the scattering changes scaled with the derivative of the rate of elongation, consistent with the idea that the greater the water flux into the ROS, the greater the flux across BrM. Third, subsequent investigations have revealed that the RPE layer itself invariably swells during the period of ROS elongation (76). RPE cells strongly express aquaporins (77, 78) and the choriocapillaris is highly fenestrated (79), an unusual feature that is established to greatly increase capillary water permeability (80). Parallel observations of scattering changes in BrM and RPE swelling triggered by COS swelling have not yet been made, but the conserved structure of the posterior ocular layers makes it highly plausible that similar COS-driven water movements from the choroid will be observed in the human eye.
Table S4: Summary Comparison of Hypothesized Mechanisms of Rod and Cone Light-Stimulated Outer Segment Elongation

| Mechanism | Applicability | Comment | References |
|-----------|---------------|---------|------------|
| (1) Osmotically driven water influx into the OS | ROS and COS | All elongation involves osmotically driven water influx into the OS, but some waters may remain in the cytosol (1a, 1b) while others enter the opsin protein and/or associated disc membrane (2) | (33); this MS |
| a. Intradiscal solutes | ROS only | Dehydrated intradiscal space becomes osmotically joined to cytosol | this MS |
| b. P₂ from GTPase | COS Component 1 | GTPase activity much lower in ROS due to lower RGS9 expression | this MS |
| (2) Photoactivated opsins act as sponges; membrane swelling | COS Component 2; ROS | Water uptake into opsins and membrane from cytosol and/or ECS; likely occurs in ROS, but predictably much smaller than (1a) | (81) |
| (3) Transmembrane water transport through photoactivated opsins | ROS and COS | cf. Discussion section “Rod versus cone elongation responses: opsins as sponges and water conduits”; transducin necessary in rods | (33, 64, 65) |
| (4) Water entering OS originates in the choroidal vasculature | ROS and COS | Fenestrated choriocapillaris vasculature is extremely water-permeable; RPE cells express aquaporins | (77-80) |
Supplementary Figures

**Figure S1.** Confirmation of rate and amplitude saturation of the COS elongation response to brief stimuli. A. The traces in Fig. 1B produced in response to the four most intense stimuli are replotted (black) along with three traces obtained in a different session from the same observer to the three most intense stimuli. B. The derivatives of the traces in panel A (obtained with the Matlab “gradient” function) are plotted: the maximum slope was $4.4 \pm 0.1$ nm ms$^{-1}$ (mean ± SEM, n=7). C. The blue traces in A, which were obtained over an extended (3 s) time window, are replotted. D. Derivatives of the traces in C: after ~1.5 s the average RMS deviation of the derivatives from zero is 0.2 nm ms$^{-1}$, negligibly different from zero (red line). (Derivatives were computed numerically with the Matlab “gradient” function.)
Figure S2. The photosensitivity of cone pigment bleaching measured with AO-SLO serial scanning is very similar at different retinal eccentricities, and indistinguishable from that of Component 2 when quantified at the COS waveguide entrance. A. Left panel. AO-SLO image of a portion a 1 deg square region of retina centered on the horizontal meridian 1.5 deg temporal to the fovea. The region was scanned 125 times at a frequency of 33 Hz before and after presentation of a stimulus of 35 nm
bandwidth (FWHM) centered on 520 nm that produced an retinal energy density (illumiance × time) of 85,300 Td s per cycle. Middle panel. Results of a single serial scanning experiment: the average backscattered light (black dots) from 429 cones is plotted as function of the cycle number (Scan #). The red curve describes the data as a saturating geometric progression of backscattered intensity, viz.,

\[ I(n) = I_0 + \Delta I_{\text{max}} [1 - (1 - B_0)^{n-1}] \]

where \( n \) is the scan #, \( I_0 \) is the initial recorded intensity, \( \Delta I_{\text{max}} = I_{\text{max}} - I_0 \) is maximum increase, and \( B_0 = 0.042 \) the nominal fraction of cone pigment bleached per scan. Right panel. The data points of the middle panel are replotted on the identical ordinate scale as a function of the cumulative energy density (Td s). The red curve has the formula

\[ I(Q) = I_0 + \Delta I_{\text{max}} \left[ 1 - \exp\left( -Q / Q_e \right) \right] \]

where \( Q = (n-1)Q_i \), with \( Q_i = 85,300 \) Td s the energy density of a single scan, \( Q_e = Q_i / B_0 \) and \( 1/Q_e \) is the photosensitivity of the incremental reflectance increase. Thus, the red curve in the rightmost panel is completely determined by the parameters of the corresponding curve in the middle panel (cf ref (2)).

B, C. AO-SLO images and backscatter data of single experiments in which the bleaching field was centered at temporal retinal eccentricities of 4.5 deg (B) and 7 deg (C); in the experiments at these two eccentricities the scan field was 1.2 deg ×1.2 deg, and \( Q_i = 59,300 \) Td s. The backscatter data in B were extracted from 356 cones and those in C from 276 cones, respectively, and averaged to generate the plotted points. (Aberrant points near the end of the 3.8 s time series typically result from eye movements, which are suppressed by fixation at earlier times.)

D. Summary of reciprocal photosensitivities (\( Q_e \)) from the experiments of this study as shown in panels A-C (colored bars), and from prior studies by other investigators (gray bars). The error bars are standard errors of the mean (SEM), with between 5 and 10 repetitions comprising the data of each colored bar. The \( Q_e \) values from 6 prior investigations (3-6, 8, 82) with bleach field centered on the fovea (light gray bar), and from one prior AO-SLO study (dark gray bar). The dashed line is plotted at the grand average (\( Q_e = 10^{6.46} \) Td s) of the prior studies, i.e., at the height of the light-gray bar.

E. Test of the prediction that photosensitivities of the reflection increases in the serial scanning experiments and that of Component 2 (panel D) are equal when transformed to photon energy density at the COS waveguide entrance (Eq 1), and equal the absolute photosensitivity of cone pigment bleaching in the basal disc membranes. The lighter-colored bars replot correspondingly colored data from panel D converted to photon energy density units at the retinal surface, while each adjacent darker bar of the same hue plots the value to its immediate left transformed via Eq 1 to energy density at the entrance to the COS waveguide entrance; the error bars are 95% confidence intervals (Methods). The transformed values are statistically indistinguishable (\( p > 0.2 \)) from the reciprocal of the photosensitivity of cone pigment in the basal COS disc membranes, \( Q_e = 1/(\alpha_{\text{max}}/\gamma) = 1.38 \times 10^8 \) photons \( \mu m^2 \), indicated by the thickened dark line, with the exception of that for Subject 2 for bleaching at 1.5 deg (\( p = 0.03 \); * on plot). (The retinal eccentricity-dependent scaling factors used to convert the data in D to those in E are given in Table 1, and explained in detail in the SI with references to results in the prior literature.)
Figure S3. The axial density of photopigment in the COS contributes to underestimation of the apparent photosensitivity of bleaching of the whole COS. A. Solutions to Eq S2.4’ for 3 different pigment axial densities $D_{\text{max}}$: low density ($D_{\text{max}} \rightarrow 0$), 0.25; the stimulus intensity axis is scaled in each case by the constant, $Q_{\text{e,intrinsic}} = 1/(\alpha_{\text{max}} \gamma) = 1.38 \times 10^8$ photons $\mu$m$^{-2}$. As axial density increases the average fraction of pigment bleached by any particular energy density is decreased by self-screening, despite the pigment’s having the same intrinsic photosensitivity. B. Approximating whole-COS bleaching vs. intensity data by an exponentially rising function leads to overestimation of $Q_e$. The red curve gives the solution to Eq S2.4’ which predicts the average bleaching of the entire COS with $D_{\text{max}}=0.5$, and $Q_{e,COS,\text{inc}} = 1/(\alpha_{\text{max}} \gamma)$. Approximating the solution to Eq S2.4’ (as in Fig. 3A-C) with a rising exponential (black curve) shifts the apparent value of $Q_e$ 1.45-fold higher value than the intrinsic value: in other words, bleaching over the whole COS can be approximated by $\tilde{f}_B = 1 - \exp(-\alpha_{\text{max}} \gamma f_{\text{Dens}} Q_{\text{COS,inc}})$ with “adjustment for density” $f_{\text{Dens}} = 1/1.45 = 0.69$. (The exponential approximation is preferable for its simplicity, and ease of use – Fig. 3A-C.). C. Length of COS ($L_{\text{COS}}$) for the subjects of the study measured at the 3 eccentricities of the experiments. Symbols plot mean $\pm$ std for the population. $L_{\text{COS}}$ are well described by a regression line whose slope is $-3.0 \mu$m deg$^{-1}$. Given the same pigment expression, the total axial density is expected to range up to 0.65 at 1.5 deg. D. Shift scale factor $(1/f_{\text{Dens}})$ plotted a function of pigment axial density, along with an empirically derived parabolic relation.
Figure S4. The effect of the angular aperture function of the cone waveguide depends on retinal eccentricity and on the distribution of light in the pupil. A. The steepness parameter $\rho$ characterizing the Stiles-Crawford (SCI) parabola (Eq S2.8) depends on retinal eccentricity. Data points (black symbols) are taken from Enoch & Hope (9) (error bars are SEM, $n=3$). These results are for light traversing the eye’s optics as a small (~1 mm diameter) beam. The smooth curve is a ratio of two polynomials fitted to the data with the least-squares curve fitting tool “cftool” of Matlab™. B. The SCI effect computed for the power distribution of light in the pupil plane for the OCT experiments (blue) and AO-SLO experiments (red). The symbols plotted at the three eccentricities (1.5, 4.5, 7.0 deg temporal) of the AO-SLO experiments and at the eccentricity of the OCT experiments (7.0 deg); represent the values of $f_{SCI}$ given in Table 1 and used deriving the results in Fig. 3E (Eq S2.7). The curves are derived from the smooth curve in panel A as described in SI (cf. Eq S2.9) and the measured distributions of power in the plane of the pupil. The vertical shifts between the curves in panel B arise from the different distributions of power in the pupil.
Figure S5. The eccentricity-dependence of the cone inner segment (CIS) cross section contributes critically to the spatial invariance of cone pigment bleaching. A. Diameter of human CIS measured from micrographs presented in Curcio’s classic study (10). Error bars are SEM’s of at least 10 CIS. The smooth curve has the form $d_{\text{CIS}}(\eta) = a[1 - \exp(-b\eta)] + c$ where $\eta$ is eccentricity (deg), $a = 4.85\ \mu m$, $b = 1.23\ \text{deg}^{-1}$, and $c = 2.93\ \mu m$. B. The factor $f_{\text{CIS}\to\text{COS}}$ of Eq 1 estimated as the ratio $(d_{\text{CIS}}/d_{\text{COS}})^2$, where $d_{\text{CIS}}$ is the diameter of the CIS and $d_{\text{COS}}$ that of the COS, taken to be $1.75\ \mu m$. The error bars are computed with an error propagation formula from those in panel A (note the different abscissa scales in A, B: that is B is obtained from that in A by $0.270\ \text{mm} \ \text{deg}^{-1}$). The red symbols plot the values used in the application of Eq 1 (Eq S2.7) in Fig. 3E (cf. Table 1). Note that the abscissas have different units and scales in the two panels, with the panel B representing only the data in A with eccentricity less than 5 mm.
Figure S6. Biochemical schematic of the G-protein, PDE and RGS9 reactions (cf Fig. 5B) identifying the symbols of the model variables, with rate constants superimposed to facilitate comparison with the information in Tables S2 and S3. The P*-catalyzed G-protein activation cycle (pale green) has intermediate binding complexes identified symbolically as G1a, G2a, G3a, with output G4 = G*. The same cycle can also potentially be driven weakly by other P-deactivation intermediates, phosphorylated Meta II (PnPi) and unliganded opsin (POps), not illustrated in the schematic. In the schematic “*” is used to symbolize an activated species, but this notation has been dropped from the symbols used in the equations for simplicity.
Figure S7. Intermediate variables in the prediction of phosphate (P_i) production by RGS9-catalyzed GTP hydrolysis. Upper nine panels. Each column of 3 graphs gives the predicted time course of 9 variables produced in response to stimuli specified in photoisomerizations per COS (Φ) at the top of the column (the corresponding fractions of pigment bleached are given by Φ/(1.5×10^8), and are 7.3%, 20% and 93%, respectively). The first row of each column plots the activities of 3 species of opsin, P* (fully active MetaII-P), P_{nPi} (phosphorylated MetaII), and POps (unliganded cone opsin); thus, the quantities of the latter two species are scaled by the assigned relative activity coefficients, a_{PnPi} = 0.2 and a_{POps} = 0.15 (Table S3): the sum (black traces) gives the total G-protein activation as a function of time, which is seen to be very slowly declining on this 1.1 s time scale (small quantities of P*•GRK and P_{nPi}•ARR are omitted for clarity); the decline occurs as P* is phosphorylated by GRK operating at maximum velocity, V_{max,GRK} = k_{cat,GRK} [GRK]^\text{tot} (note: the ordinate scale in the 3 upper panels depicting opsin activity of the “P-species” is changed with Φ; the slope of declining P* activity is the same in the 3 panels). The graphs in the middle row plot the predicted quantities of activated PDE without RGS9 bound, i.e., E_1* = PDE6•Gα•GTP and E_2* = PDE6•2Gα•GTP, and the graphs in the lowermost row plot the quantities of activated PDE with RGS9 bound, E_1*•RGS9, E_2*•RGS9 and E_2*•2RGS9. The wide graph at the bottom plots the five species of holo-PDE at 1.1 s after flashes whose strength (Φ) varies in 0.2 log_{10} steps over the range that gives rise to Component 1, and serves to illustrate how the model generates the light dependence of P_i (phosphate) production over this range as the distribution of PDE over the 5 states changes. Also illustrated is the level of total PDE, thick black line at upper right, and the sum of the PDE states. (At the left hand side of the plot PDE activity declines to zero not because no PDE is activated, but because by 1.1 s after the flash it has been largely deactivated.). The ordinate units of all plots are “proteins per disc face”, with the exception of the plots of P_{nPi} and POps, which as noted above are scaled by activity coefficients.
Figure S8. The phototransduction model predicts human cone photocurrent responses. A. Family of human cone responses measured with the $a$-wave of the electroretinogram (symbols) from (51), along with predictions (smooth traces) generated with a model presented in (83). B. Theoretical photocurrent traces generated by the cone phototransduction model presented in the present paper. A dashed “fiducial” line at the 50% response amplitude is presented in A, B to assist in comparison of the recoveries in panels A, B, and filled black symbols are plotted on the traces at their maxima. C. Response amplitude vs. flash intensities for model traces in panels A, B. The filled black symbols replot the filled symbols of panel B, fitted with an exponential saturation function (black smooth curve), while the red filled symbols the $a$-wave peak amplitudes extracted from the data in A, fitted with a hyperbolic saturation function (red smooth curve). The conversion from the retinal illuminant energy density scale (Td s) used in (51) to photoisomerizations ($\Phi$) was obtained by sliding the hyperbolic saturation function fitted to the $a$-wave amplitudes along the intensity axis for approximate correspondence with the exponential saturation curve.
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