Investigating the Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent mechanisms for mammalian cone light adaptation

Frans Vinberg\(^1,2\) & Vladimir J. Kefalov\(^1\)

Vision is mediated by two types of photoreceptors: rods, enabling vision in dim light; and cones, which function in bright light. Despite many similarities in the components of their respective phototransduction cascades, rods and cones have distinct sensitivity, response kinetics, and adaptation capacity. Cones are less sensitive and have faster responses than rods. In addition, cones can function over a wide range of light conditions whereas rods saturate in moderately bright light. Calcium plays an important role in regulating phototransduction and light adaptation of rods and cones. Notably, the two dominant Ca\(^{2+}\)-feedbacks in rods and cones are driven by the identical calcium-binding proteins: guanylyl cyclase activating proteins 1 and 2 (GCAPs), which upregulate the production of cGMP; and recoverin, which regulates the inactivation of visual pigment. Thus, the mechanisms producing the difference in adaptation capacity between rods and cones have remained poorly understood. Using GCAPs/recoverin-deficient mice, we show that mammalian cones possess another Ca\(^{2+}\)-dependent mechanism promoting light adaptation. Surprisingly, we also find that, unlike in mouse rods, a unique Ca\(^{2+}\)-independent mechanism contributes to cone light adaptation. Our findings point to two novel adaptation mechanisms in mouse cones that likely contribute to the great adaptation capacity of cones over rods.

Our daytime vision is mediated by cone photoreceptors, which can adapt quickly and over a wide range of ambient light levels. In contrast, rod photoreceptors, which mediate our dim light vision, adapt slower and saturate under bright light. However, the molecular underpinnings explaining the faster and more efficient capability of cones to light adapt, i.e. to regulate their sensitivity in response to increments and decrements of background light, are still poorly understood.

Absorption of a photon by visual pigment molecule triggers a G protein signaling cascade involving identical or homologous rod and cone proteins\(^1,2\). Briefly, a single photoactivated rod or cone pigment can activate several cell-specific heterotrimeric G proteins, transducins\(^3-5\). In turn, each of these transducins disinhibits a rod/cone-specific effector enzyme phosphodiesterase, PDE6\(^6,7\). The resulting upregulated hydrolysis of cGMP by PDE6 leads to a lower cGMP concentration in the outer segments of photoreceptors, followed by closure of cGMP-gated (CNG) channels in their plasma membrane\(^8\), reduced influx of Na\(^{+}\) and Ca\(^{2+}\), and ultimately cell hyperpolarization\(^9,10\). Continuous photoreceptor function requires the inactivation of the visual pigments, transducins and PDE6, and the upregulation of cGMP synthesis by guanylate cyclases (GC) to restore and maintain cGMP concentration\(^11,12\). The concerted activation and inactivation of these transduction components upon light stimulation is modulated by their properties and expression levels, and produces a light response with distinct rod/cone-specific amplitude and kinetics\(^13-22\).

When background light levels change, photoreceptors adjust their sensitivity allowing vision even under rapidly changing ambient illumination. Part of this change in sensitivity is driven by the modulation of cGMP turnover\(^23\). However, the dominant mechanism for modulating photoreceptor sensitivity in background light (referred to below as light adaptation) is thought to be driven by Ca\(^{2+}\)-dependent feedback on the phototransduction cascade triggered by the decrease in outer segment Ca\(^{2+}\) concentration upon light stimulation\(^24-27\). This feedback

1Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri, USA. 2Present address: John A. Moran Eye Center, University of Utah, Salt Lake City, Utah, USA. Correspondence and requests for materials should be addressed to F.V. (email: frans.vinberg@hsc.utah.edu)
is mediated by several Ca$^{2+}$-binding proteins, including 1) Guanylate Cyclase Activating proteins (GCAP1 and GCAP2), which activate guanylyl cyclase and thus accelerate cGMP synthesis in low Ca$^{2+}$; 2) recoverin (Rv), which dissociates from rhodopsin kinase (GRK1) in low Ca$^{2+}$ allowing GRK1 to accelerate visual pigment inactivation in both rods and cones$^{30,34}$ and, in the case of amphibian photoreceptors. 3) calmodulin and/or CNG modulin, which modulate the gating of CNG channels$^{35-37}$. Notably, rods and cones share the same GCAPs and recoverin isoforms, leaving the question of the mechanisms that produce the difference in their light adaptation still open.

It has been demonstrated that light adaptation in amphibian rods and cones is mediated by Ca$^{2+}$e. A recent study showed that the same is also likely true for mammalian rods$^{40}$ but whether mammalian cone light adaptation is mediated exclusively by Ca$^{2+}$-dependent mechanisms is not known. Here we combined electrophysiology, pharmacology, and genetic approaches to dissect the contribution of Ca$^{2+}$-dependent and Ca$^{2+}$-independent mechanisms to the light adaptation capacity of mammalian cones in the absence of the known Ca$^{2+}$ feedbacks mediated by GCAPs and recoverin.

Methods

Ethical approval. All experimental protocols were in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the institutional Animal Studies Committee at Washington University.

Animals. Mice were housed in the University's animal facilities under 12/12 hour light/dark cycle and had free access to water and regular rodent chow. The GCAPs$^{−/−}$ and Rv$^{−/−}$ mice were originally obtained from Dr. Jeannie Chen (University of Southern California) but were backcrossed for several generations to the Gnat$^{1−/−}$ background$^{41}$ to allow for cone-specific recordings. We crossed these GCAPs$^{−/−}$/Gnat$^{1−/−}$ and Rv$^{−/−}$/Gnat$^{1−/−}$ mice. We then compared the functional properties of Gnat$^{1−/−}$ control cones and GCAPs$^{−/−}$/Rv$^{−/−}$/Gnat$^{1−/−}$ mice to evaluate the functional contribution of residual Ca$^{2+}$-dependent and any possible Ca$^{2+}$-independent regulation of cone phototransduction. All tested mice were between 2–3 months of age. Mice were genotyped from tail samples by Transnetyx Inc. and were also tested to be free of Rd8 mutation$^{42}$.

Ex vivo Electroretinogram (ERG) Experiments. Transretinal ERG responses to flashes and steps of cyan LED light (505 nm Luxeon Rebel LED SR-01-E0070) from isolated mouse retinas were recorded as described previously$^{43}$. Briefly, the retinas were mounted on a specimen holder where they were perfused 2 mL/min with Locke's solution heated to 37 °C. The perfusion solution contained (in mM): NaCl, 112; KCl, 3.6; MgCl$_2$, 2.4; CaCl$_2$, 1.2; HEPES, 10; NaHCO$_3$, 20; Na$_2$-succinate, 3; Na-glutamate, 0.5; glucose, 10. The solution was equilibrated with 95% O$_2$/5% CO$_2$ at 37 °C. In addition, 2 mM L-Aspartate, 40 mM DL-AP4 (Tocris Biosciences) and 100 μM BaCl$_2$ were added to the medium to isolate the photoreceptor component of the ERG signal. Low Ca$^{2+}$ solution was prepared by adding 0.4 mM EGTA and substituting the 1.2 mM CaCl$_2$ with only 0.1 mM CaCl$_2$ added to the medium, estimated to produce ~30 mM free Ca$^{2+}$ concentration$^{44}$.

Signals were amplified initially by a differential amplifier (DP-311, Warner Instruments) and then amplified further and low-pass filtered at 300 Hz (8-pole Bessel, Krohn-Hite Corporation, model 3382) and sampled at 10 kHz with 0.03 μV resolution by a digitizer (1440 A Digidata, Molecular Devices) and pCLAMP 10 software (Molecular Devices). Light stimulation was provided by a custom-build LED system via optical cable (Newport, 77536) and the optics of an inverted microscope that produced homogenous light over the effective measurement area of 0.5 mm at the central retina. Light intensity and the length of light flashes (1 ms) and background light steps were controlled by an LED driver (Thorlabs, LDC210C) and neutral density filters. The total light power of the LED stimulus ($\lambda_{max}$ at 505 nm; Rebel star, SR-01-E0070) was measured by calibrated optometer (UDT Instruments, Model 211) near the plane of the retina. The intensity was then calculated based on the light spot area at the plane of the retina (0.23.5 mm) and converted to a number of 505 nm photons μm$^{-2}$ s$^{-1}$.

Analysis. Origin 9.0.0 software (64-bit, SR2, OriginLab) was used for data analysis and figure preparation. A Naka-Rushton function was fitted to the response amplitude ($r$) from Gnat$^{1−/−}$ control and GCAPs$^{−/−}$/Rv$^{−/−}$/Gnat$^{1−/−}$ mouse cones:

$$r = \frac{I_F}{I_{F/2} + I_F},$$

(1)

where $r_{max}$ is the maximal amplitude of a saturated cone response, $I_F$ is the intensity of the light flash (in photons μm$^{-2}$), and $I_{F/2}$ is the light intensity producing a half-maximal photoresponse.

A modified Weber-Fechner function was fitted to the light adaptation data:

$$\frac{S_F}{S_{F,D}} = \frac{I_0^n}{I_0^n + I^n},$$

(2)

where $S_F$ is the sensitivity of cones defined as the amplitude of a response to dim flash divided by the flash strength (in 505 nm photons μm$^{-2}$), $S_{F,D}$ is the sensitivity in darkness defined as the amplitude of a response to dim flash divided by the flash strength with no background light present, $n$ is a slope factor, $I$ is the background light intensity (in 505 photons μm$^{-2}$ s$^{-1}$) and $I_0$ is the background light intensity at which the sensitivity $S_F$ drops to 50% of that in darkness. In all cases, response amplitude was measured at its peak.
where $T_i$ is the integration time of a dim flash response defined as the area between the trace and time-axis along the baseline of the response divided by the response peak amplitude and $r_{\text{max}}$ is the maximal response amplitude of a saturated cone response. Alternatively, a function

$$\frac{s_F}{s_{F,D}} = e^{\frac{s_F}{r_{\text{max}}}}$$

that has been derived by removing all the feedbacks from a phototransduction model was used.$^{45}$

### Results

**Cones can light adapt in the absence of GCAPs and recoverin.** The role of GCAP1, GCAP2 and recoverin (Rv) in mouse rod and cone phototransduction and light adaptation has been well characterized.$^{28,31,32,39,46–49}$ Here, we bred $\text{GCAPs}^{-/-}$ (lacking both isoforms 1 and 2) and $\text{Rv}^{-/-}$ mice to produce $\text{GCAPs}^{-/-}\text{Rv}^{-/-}$ double knockout mice. The rods and cones in these mice lack the Ca$^{2+}$ feedbacks to modulate cGMP synthesis (via GCAP1/2) and active visual pigment lifetime (via recoverin) that are known to contribute to the rod and cone phototransduction termination and light adaptation.$^{28,31,32,46}$ To investigate how the simultaneous deletion of GCAPs and recoverin affects the phototransduction in mouse cones, we performed *ex vivo* ERG recordings from dark-adapted isolated retinas (see Methods, Fig. 1). To facilitate assessment of cone physiology, all of the mice were on Gnat1$^{-/-}$ background to remove the light responses originating from their rod photoreceptors.$^{41}$ We compared the responses from control Gnat1$^{-/-}$ cones with the responses from $\text{GCAPs}^{-/-}\text{Rv}^{-/-}$ Gnat1$^{-/-}$ cones. The calcium feedback deficiency in $\text{GCAPs}^{-/-}\text{Rv}^{-/-}$ Gnat1$^{-/-}$ cones resulted in slower shut-off of their photoresponses, leading to larger response integration time (Fig. 1A,B, and Table 1; see also$^{46}$). The larger integration time also appeared to increase the sensitivity of $\text{GCAPs}^{-/-}\text{Rv}^{-/-}$ cones, as demonstrated by an apparent shift of their response amplitude data to dimmer light (Fig. 1C). As a result, the averaged cone light flash intensity required to elicit 50% of the maximal response ($I_{1/2}$) was reduced by the deletion of GCAPs and recoverin (though the difference was not statistically significant, see Table 1). These results are consistent with previous rod studies showing that $\text{GCAPs}^{-/-}\text{Rv}^{-/-}$ rods are more sensitive to light and have slower response kinetics as compared to wild type rods.$^{28,40}$

A recent study showed that GCAPs- and Rv-deficient mouse rods can still adapt to light.$^{39}$ However, the ability of cones lacking GCAPs and recoverin to modulate their phototransduction cascade has not been investigated. To address this question, we first recorded light responses of cones in isolated retinas to steps of light by using *ex vivo*
To assess the light adaptation capacity of both GCAPs and recoverin, mouse cones were able to regulate their CNG channel current and light-adapt during different backgrounds. (S_F) Normalized sensitivity (C_rmax) for control Gnat1−/− and GCAPs−/− Rv−/− Gnat1−/− data in normal Ca2+; *p < 0.05; †p < 0.01 (paired t-test between normal and low Ca2+ data of GCAPs−/− Rv−/− Gnat1−/− mice). Control Gnat1−/− data (mean ± SEM) is from 4 mice, and GCAPs−/− Rv−/− Gnat1−/− data (mean ± SEM) is from 8 mice.

Table 1. Parameters of photoresponses from control Gnat1−/− and from GCAPs−/− Rv−/− Gnat1−/− mouse cones in normal and low Ca2+: r_{max}, saturated photoresponse amplitude; I_{1/2}, light flash intensity producing a half-maximal light response (see Eq. 1); t_p, time-to-peak of a dim flash response; T_r, integration time of a dim flash response defined as the integrated area between the response and baseline divided by the response amplitude; I_b, background light intensity reducing the sensitivity to 50% of the dark-adapted sensitivity; n, slope of the light adaptation curve (see Eq. 2).

| Condition                  | r_{max} (µV) | I_{1/2} (photons µm−2 s−1) | t_p (ms) | T_r (ms) | I_b (photons µm−2 s−1) | n    |
|----------------------------|-------------|-----------------------------|----------|----------|------------------------|------|
| Control Gnat1−/−           | 56 ± 7      | 5,500 ± 700                 | 56 ± 2   | 71 ± 5   | 48,800 ± 11,800        | 1.0 ± 0.1 |
| GCAPs−/− Rv−/− Gnat1−/− (normal Ca2+) | 30 ± 10     | 5,800 ± 400                 | 142 ± 12† | 240 ± 40† | 12,100 ± 2,400        | 1.5 ± 0.1† |
| GCAPs−/− Rv−/− Gnat1−/− (low Ca2+) | 72 ± 9†     | 5,400 ± 1,100†             | 139 ± 10 | 420 ± 30† | 42,900 ± 6,400†        | 2.0 ± 0.2† |

Figure 2. GCAPs/recoverin-deficient cones can adapt to background light. Responses of control (A) and GCAPs/recoverin-deficient (B) cones to steps of 505 nm light (indicated by green bars) from 2,620 up to 407,300 photons µm−2 s−1 (numbers on the right indicate the background light intensity, identical for A and B). A flash of light was delivered at 4.5 s after the step onset (arrow) to probe the sensitivity of cones (S_p) during different backgrounds. (C) Normalized sensitivity (S_p/S_pD, where S_pD is the sensitivity in darkness, (mean ± SEM) plotted as a function of background light intensity in photons µm−2 s−1 for control cones (black squares, n = 4 mice) and GCAPs/recoverin-deficient cones (blue squares, n = 3 mice). The smooth traces plot Eq. (2) with I_0 = 39,600 µm−2 s−1 and n = 1.0 for control cones (black), and with I_0 = 10,200 µm−2 s−1 and n = 1.4 for GCAPs/recoverin-deficient cones (blue). The blue dashed trace plots Eq. (3) calculated from the data measured from dark-adapted GCAPs/recoverin-deficient cones.

ERGs. In control Gnat1−/− cones, the initial hyperpolarization after the step onset was followed by partial recovery demonstrating the modulation of CNG channel current during steady background light (Fig. 2A). Exposure of cones lacking GCAPs and recoverin to identical steps of light produced larger responses as compared to those of control cones (Fig. 2A,B; note the different scale of the two y-axes). For example, the mean amplitude of step responses at steady state just before the test flash was flash was 2.6 ± 1 µV in control and 17 ± 1 µV at ~41,000 photons µm−2 s−1 background. This difference was highly significant (n = 3, p < 0.0005). Interestingly, despite lacking the two major components of their calcium feedback, GCAPs−/− Rv−/− Gnat1−/− cones also showed a prominent recovery after the initial hyperpolarization following the onset of light step. Thus, even in the absence of both GCAPs and recoverin, mouse cones were able to regulate their CNG channel current and light-adapt during steady background light (Fig. 2B). To assess the light adaptation capacity of GCAPs−/− Rv−/− Gnat1−/− cones, we probed their sensitivity in background lights of varying intensity by delivering a test flash 4.5 s after the onset of the background. As expected, the sensitivity of control Gnat1−/− cones declined according to Weber-Fechner...
Figure 3. Low Ca$^{2+}$ exposure causes large transient increase of $r_{\text{max}}$ and deceleration of flash response kinetics in control mouse cones. (A) Normalized maximal cone response amplitudes ($r_{\text{max}}$, mean ± SEM, n = 4 mice) to a saturating bright test flash plotted as a function of time after exposing the retina to low Ca$^{2+}$ medium. Amplitudes have been normalized to $r_{\text{max}}$ just before the low Ca$^{2+}$ exposure. (B) Averaged normalized responses (mean, n = 4 mice) of control cones to a dim test flash producing a response with amplitude < 20% of $r_{\text{max}}$ just before the low Ca$^{2+}$ exposure (black) and about 10 min after the switch to low Ca$^{2+}$ solution (red).

Modulation of CNG channel current and light response kinetics in cones by lowered Ca$^{2+}$. The contribution of Ca$^{2+}$-dependent mechanisms to the light adaptation of amphibian photoreceptors has been studied by several methods. Those methods include clamping Ca$^{2+}$ concentration of a single photoreceptor to its dark-adapted or light-adapted level. This can be achieved for a brief time by removing Na$^+$ and Ca$^{2+}$ from the extracellular solution in darkness or during steady background light, or by manipulating Ca$^{2+}$ levels in truncated photoreceptor outer segments that can be dialyzed with GTP or cGMP to study cGMP synthesis and hydrolysis, respectively. These methods require rapid changes of extracellular environment of single cells or truncated outer segment compartments of photoreceptors, manipulations that are not well tolerated by fragile and small mouse rod or cone outer segments. Here, we adopted a different approach to force the cone phototransduction to its maximally light adapted state. We achieved that, we exposed isolated mouse retinas to low ~30 nM [Ca$^{2+}$]$_i$ in under these conditions will drop from 250 nM in darkness in normal Ca$^{2+}$ concentration to drive intracellular Ca$^{2+}$ level below that attained in bright light, thus fully engaging the Ca$^{2+}$ feedback. To achieve that, we exposed isolated mouse retinas to low ~30 nM [Ca$^{2+}$]$_i$, during ex vivo ERG recordings to study how mouse cone physiology is affected under low Ca$^{2+}$ environment. Assuming that the extrusion of Ca$^{2+}$ via the cone Na$^+/Ca^{2+}$, K$^+$ exchangers is linearly proportional to the intracellular Ca$^{2+}$ concentration, and that the conductance of Ca$^{2+}$ is increased about 50% by low Ca$^{2+}$ exposure in GCaPs$^{-/-}$ Rv$^{-/-}$ cones (see Fig. 4A below), it can be calculated that [Ca$^{2+}$]$_i$ under these conditions will drop from 250 nM in darkness in normal Ca$^{2+}$ to ~1 nM. Thus, at steady state in low Ca$^{2+}$ exposure, the level of [Ca$^{2+}$]$_i$ in cones would be below its level even in very bright light, fully activating any Ca$^{2+}$-mediated phototransduction feedbacks.

As has been shown previously for other photoreceptor types and/or species, the low Ca$^{2+}$ exposure of retinas from control Gnat1$^{-/-}$ mice led to a significant but only transient increase of the maximal light response amplitude ($r_{\text{max}}$) in mouse cones (Fig. 3A; see also). At steady state about 10 minutes after the switch to low Ca$^{2+}$, the amplitude of $r_{\text{max}}$ stabilized to approximately the same level as in normal Ca$^{2+}$. However, comparison of dim flash responses in normal and low Ca$^{2+}$ at steady state revealed a significant deceleration of response kinetics of cones caused by lowering Ca$^{2+}$ (Fig. 3B), an effect similar to that observed previously in rod photoreceptors. Previous studies have suggested that the abnormally high cGMP in the photoreceptors exposed to low Ca$^{2+}$ in darkness would not be well tolerated by the cells causing gradually declining response amplitudes and slowdown of their phototransduction.
In contrast to control cones, the cones of our GCAPs−/− Rv−/− Gnat1−/− mice lack the GCAP-mediated acceleration of cGMP synthesis at low Ca2+. Therefore, low Ca2+ exposure of these cones would not be expected to produce increase in their cGMP concentration. Consistent with this hypothesis, the large transient increase of the maximal cone response amplitudes observed after the switch to low Ca2+ in cones from control Gnat1−/− mice was absent in cones from GCAPs−/− Rv−/− Gnat1−/− mice (Fig. 4A). However, we still observed about 50% increase of rmax by low Ca2+ exposure even in cones lacking GCAPs and recoverin (Fig. 4A), and these larger amplitudes remained stable for up to 30 minutes in most of the experiments. In striking contrast to the case in control cones, the kinetics of cone responses from GCAPs−/− Rv−/− Gnat1−/− mice were not decelerated by the low Ca2+ exposure (Fig. 4B). The stability of response amplitudes and the lack of phototransduction deceleration indicate that in the absence of GCAPs-mediated acceleration of cGMP synthesis low Ca2+ exposure does not induce any toxic effects. This allowed us to use this low Ca2+ method in GCAPs−/− Rv−/− Gnat1−/− mice to study the contribution of Ca2+-dependent and any possible Ca2+-independent mechanisms to the light adaptation of mouse cones in the absence of GCAPs- and recoverin-mediated regulation.

Cones lacking both GCAPs and recoverin can light adapt via both Ca2+-dependent and Ca2+-independent mechanisms. Finally, we evaluated the contribution of Ca2+-dependent and Ca2+-independent mechanisms of light adaptation in cones already devoid of their two known feedback mechanisms, via GCAPs and recoverin. To do that, we compared the light adaptation of GCAPs−/− Rv−/− Gnat1−/− mouse cones in normal Ca2+, where a potential dynamic light-induced Ca2+-dependent mechanism would be functional, and in low Ca2+, where any residual Ca2+ feedback would be driven to a fully light-adapted state. Exposure of cones lacking GCAPs and recoverin to a step of background light in normal Ca2+ produced an initial hyperpolarization, followed by partial recovery indicative of adaptation of the cone phototransduction cascade (Fig. 5A, blue). As shown in Fig. 2C, the decline in their sensitivity in backgrounds of increasing intensity was steeper than in control cones (i.e. n increased upon deletion of GCAPs and Rv, Table 1), but still well above what would be expected in the lack of any adaptation (Fig. 5B blue). These results suggest the existence of a feedback mechanism contributing to mouse cone light adaptation even in the absence of the known GCAPs- and recoverin-mediated pathways.

When GCAPs/recoverin-deficient cones were exposed to a step of light in low Ca2+, the rapid partial recovery in their response was no longer observable (Fig. 5A, red). In addition, the sensitivity of these cones in low Ca2+ started to decline at somewhat brighter background light intensities (I) than in normal Ca2+ (i.e. Ir increased in low Ca2+, see Table 1). However, the subsequent decline was steeper (i.e. the steepness parameter n was larger in low Ca2+, see Table 1) so that the sensitivities under normal and low Ca2+ appeared to converge at brighter background light intensities (Fig. 5B, blue and red squares and solid lines). To quantitatively confirm convergence of the sensitivity in normal and low Ca2+, we performed statistical comparison of the normalized sensitivity data at five different background light intensities ranging from ~6,000 to 400,000 photons μm−2 s−1 using paired Student t-test. The analysis from six GCAPs−/− Rv−/− Gnat1−/− mice demonstrated that the p-value, indicating significant difference, gradually increased from 0.01 to 0.01, 0.03, and 0.04 for the four dimmest backgrounds, and finally reached 0.13, indicating no significant difference, for the brightest background. To evaluate whether GCAPs/ recoverin-deficient cones in low Ca2+ undergo any light adaptation, we calculated the predicted drop of sensitivity as a function of t under low Ca2+ (Fig. 5B, dashed red and green lines). Although partially suppressed by the pharmacological reduction in Ca2+, these cones appeared to have a wider dynamic range and performed better than expected by the theoretical “no adaptation” model. To confirm the presence of light adaptation under low
which we used identical background light intensities. The theoretical traces plot the mean values for the same 3 photoreceptors. 

ments to prevent the light-induced change in their Ca²⁺ levels. Some studies have suggested that Ca²⁺ concentrations summed linearly leading to a rapid exponential saturation of these photoreceptors at very dim background light intensities. We determined if background light shortened the time-to-peak of a dim flash response of a dark adapted DKO cone under normal Ca²⁺ and with I₀ = 10,000 photons μm⁻² s⁻¹ and n = 1.4 in normal Ca²⁺ (blue) and with I₀ = 27,000 photons μm⁻² s⁻¹ and n = 1.7 in low Ca²⁺ (red). The dashed red and green traces plot Eqs (3) and (4), respectively, with parameter values calculated from dark-adapted responses of these cones in low Ca²⁺. Sensitivity data (mean ± SEM) in (B) is from 3 GCAPs⁻/⁻ Rv⁻/⁻ Gnat1⁻/⁻ mice for which we used identical background light intensities. The theoretical traces plot the mean values for the same 3 GCAPs⁻/⁻ Rv⁻/⁻ Gnat1⁻/⁻ mice. For comprehensive statistical analysis, see Table 1 and text.

Figure 5. Ca²⁺-dependent and Ca²⁺-independent light adaptation mechanisms contribute to the light adaptation capacity of cones lacking both GCAPs and recoverin. (A) A response to a step of light (I = 17,100 photons μm⁻² s⁻¹) superimposed with a test flash (arrow) recorded from GCAPs/recoverin-deficient cones in normal (blue) and low (red) Ca²⁺ (same retina). The test flash strength was 1,600 and 570 photons μm⁻² in normal and low Ca²⁺, respectively. (B) Sensitivity of cones (S_F normalized to the sensitivity in darkness (S_F, D)) plotted as function of background light intensity (I) for GCAPs/recoverin-deficient cones in normal (blue squares) and low (red squares) Ca²⁺. Smooth lines plot Eq. (2) with I₀ = 10,000 photons μm⁻² s⁻¹ and n = 1.4 in normal Ca²⁺ (blue) and with I₀ = 27,000 photons μm⁻² s⁻¹ and n = 1.7 in low Ca²⁺ (red). The dashed red and green traces plot Eqs (3) and (4), respectively, with parameter values calculated from dark-adapted responses of these cones in low Ca²⁺. Sensitivity data (mean ± SEM) in (B) is from 3 GCAPs⁻/⁻ Rv⁻/⁻ Gnat1⁻/⁻ mice for which we used identical background light intensities. The theoretical traces plot the mean values for the same 3 GCAPs⁻/⁻ Rv⁻/⁻ Gnat1⁻/⁻ mice. For comprehensive statistical analysis, see Table 1 and text.

Ca²⁺, we compared measured sensitivities and those calculated by Eq. 4 from six GCAPs⁻/⁻ Rv⁻/⁻ Gnat1⁻/⁻ mice at five different background light intensities ranging from ~6,000 to 400,000 photons μm⁻² s⁻¹. The sensitivity calculated by the model was lower at each of the five backgrounds (paired Student t-test, p < 0.05). We also analyzed if background light shortened the time-to-peak of a dim flash response of a dark adapted DKO cone under low Ca²⁺ perfusion. The time-to-peak shortened 38 ± 6% (mean ± SEM) under background of ~150,000 photons μm⁻² s⁻¹ as compared to that in darkness, a difference found to be highly significant (n = 6, p < 0.005, paired Student t-test). Further increase of background light intensity did not cause a further acceleration of response kinetics. Although voltage-gated channels and capacitive currents can also shape the ERG signals, the robust Weber adaptation observed in our experiments indicates that these findings are not affected by voltage-dependent or capacitive currents. We conclude that, in striking contrast to mouse rods, mammalian cones appear to have a unique mechanism for Ca²⁺-independent light adaptation.

Discussion

Light-induced decrease in the photoreceptor outer segment Ca²⁺ concentration is a signal that mediates several feedback mechanisms via Ca²⁺ sensor proteins GCAPs, recoverin, and calmodulin/CNG modulin to modulate cGMP synthesis, active visual pigment lifetime, and CNG channels conductance, respectively. Many studies have indicated that Ca²⁺ is both necessary and sufficient for the light adaptation of amphibian photoreceptors, i.e. without Ca²⁺ feedbacks the responses of dark-adapted photoreceptors to single photons would be summed linearly leading to a rapid exponential saturation of these photoreceptors at very dim background light levels. Some studies have suggested that Ca²⁺-independent adaptation mechanism(s) could also contribute to the light adaptation of amphibian rods. However, Nikonorov et al. have argued that in salamander rods the acceleration of response shut-off caused by background light is due to a trivial Ca²⁺-independent acceleration of cGMP turnover following activation of PDE by light and does not require existence of any specific feedback pathway. Furthermore, even if this increase in the steady state cGMP hydrolysis rate is taken into account, a significant amount of light adaptation persists even in mouse rods lacking GCAPs and recoverin-mediated feedbacks. In amphibians, the contribution of Ca²⁺-dependent mechanisms to the light adaptation capacity of photoreceptors has been studied mainly by manipulating the ionic environment around photoreceptor outer segments to prevent the light-induced change in their Ca²⁺ concentration. On the other hand, the role of different molecular mechanisms to the light adaptation of mouse, Xenopus and zebrafish photoreceptors has been studied by combining genetic and physiology approaches. Mouse photoreceptors are small and fragile making it very challenging to perform physiological recordings from their rods or cones in isolation. Thus, Ca²⁺ clamp experiments have not been performed successfully from mouse rod or cone photoreceptors.

As an alternative approach, light-induced decrease in Ca²⁺ concentration could be mimicked by reducing the extracellular Ca²⁺ that would drive the intracellular Ca²⁺ concentration to a lower level. However, in contrast to the light-induced acceleration of flash response kinetics, an exposure of photoreceptors to a very low Ca²⁺
in darkness causes a significant deceleration of flash response kinetics.\textsuperscript{44,53,54,59} In addition, after the initial large increase, the CNG channel current gradually decreases following exposure to low Ca\textsuperscript{2+}.\textsuperscript{10,39} A light-induced drop in Ca\textsuperscript{2+} also causes acceleration of cGMP hydrolysis so that cGMP concentration or CNG channel current do not exceed the values observed in dark-adapted photoreceptors. In contrast, exposure to low Ca\textsuperscript{2+} in darkness is expected to increase cGMP concentration due to the GCAP-mediated feedback on guanylyl cyclase activity. The hypothesis that high cGMP is causing the anomalous effects of low Ca\textsuperscript{2+} exposure in mouse rods (directly or indirectly) was tested recently by exposing rods lacking the GCAP-mediated feedback on cyclase to a very low (about 30 nM) [Ca\textsuperscript{2+}]\textsuperscript{−}.\textsuperscript{39} In striking contrast to wild type rods, the flash responses of GCAPs\textsuperscript{−/−} rods were accelerated by low Ca\textsuperscript{2+} exposure. Thus, low Ca\textsuperscript{2+} exposure in the absence of GCAPs-mediated increase of cGMP concentration in rods is a viable approach for dissecting the contribution of Ca\textsuperscript{2+}-dependent and Ca\textsuperscript{2+}-independent mechanisms of phototransduction and light adaptation. Here, we applied this approach to study light adaptation in mouse cones that lack Ca\textsuperscript{2+} feedbacks mediated by GCAPs and recoverin by comparing the light adaptation of GCAP\textsuperscript{−/−}Rv\textsuperscript{−/−}Gnat1\textsuperscript{−/−} mouse cones between normal (1.2 mM) and low (~30 nM) extracellular [Ca\textsuperscript{2+}]\textsuperscript{−}. As in wild type rods, cones expressing GCAPs also showed a significant but only transient increase of their \(r_{max}\) after exposure to low Ca\textsuperscript{2+} (Fig. 3A). Furthermore, similarly to mouse rods, the kinetics of their flash responses slowed down (Fig. 3B). However, the value of \(r_{max}\) of GCAPs/recoverin-deficient cones increased only moderately and remained relatively stable during low Ca\textsuperscript{2+} exposure (Fig. 4A). Importantly, the kinetics of flash responses also did not slow down by low Ca\textsuperscript{2+} exposure in these cones (Fig. 4B).

The Ca\textsuperscript{2+} concentration in the outer segments of photoreceptors is maintained at about 10,000-fold lower level than outside the cells by active extrusion of Ca\textsuperscript{2+} via Na\textsuperscript{+}/Ca\textsuperscript{2+}, K\textsuperscript{+} exchangers (NCKX, 25,69–71). Thus, it is expected that during our low Ca\textsuperscript{2+} treatment, outer segment Ca\textsuperscript{2+} would be below the ~200 nM attained in bright light\textsuperscript{20} and well below the operating range of the potential Ca\textsuperscript{2+} feedback mechanisms. For example, the affinity of Ca\textsuperscript{2+} to mouse GCAP1 is ~130 nM and to GCAP2 ~50 nM.\textsuperscript{72} Consequently, at ~10 nM almost all of the GCAPs would be in a Ca\textsuperscript{2+}-free state and any further reduction caused by light would not have any modulatory effect on light adaptation. Indeed, the converging sensitivity of cones between normal and low Ca\textsuperscript{2+} when background light intensity increases suggests that during our low Ca\textsuperscript{2+} exposure, the cones are at physiological fully light-adapted state with regards to their Ca\textsuperscript{2+} feedback mechanisms (Fig. 5B).

Cones are much less sensitive to light and can operate at significantly brighter ambient illumination levels as compared to rods. A recent review summarized nicely what is known about differences of the phototransduction proteins and their expression levels between rod and cone photoreceptors, and how much these differences could contribute to the sensitivity difference of mouse rods and cones.\textsuperscript{4} Studies comparing fish and avian rods and cones have demonstrated several differences in the activity and expression levels of rod- and cone-specific phototransduction proteins that could potentially explain the physiological differences between rods and cones (for review, see\textsuperscript{2}). Thus, the cone-specific kinase GRK7 (though not expressed in mouse photoreceptors) is more highly expressed as compared to rod GRK1\textsuperscript{11}. The active form of chicken cone visual pigment, Meta II, decays 50 times faster than rhodopsin\textsuperscript{15}. Cone pigments are also less stable\textsuperscript{20,24} and noisier than rod pigment\textsuperscript{5,7,6}. In addition, the enzyme RGS9 known to accelerate inactivation of active transducing-phosphodiesterase complex, the cGMP-synthesizing enzyme guanylyl cyclase, as well as arrestins are expressed at higher levels in carp cones than in rods.\textsuperscript{4,6,16,17} Quantification of expression levels of phototransduction proteins in mouse cones is challenging due to their very small percentage in the mouse retina. Many studies have tried to assess the different properties of cone vs. rod isoforms of visual pigments, G proteins and PDE6 by expressing the cone isoforms in the rod photoreceptors\textsuperscript{3,7,27–81}. These studies suggest that differences in the activity of rod and cone phototransduction enzymes may contribute to the difference in the sensitivity of mouse rods and cones but the molecular origin of the sensitivity difference between mammalian rods and cones remains poorly understood.\textsuperscript{1} It is known that the amphibian outer segment Ca\textsuperscript{2+} concentration changes more rapidly and over wider range in cones than in rods.\textsuperscript{82,83} This could translate into more efficient regulation of cone phototransduction sensitivity by background light via Ca\textsuperscript{2+} feedback mechanisms. However, the main Ca\textsuperscript{2+} feedback, mediated by GCAPs, contributes similarly to the regulation of sensitivity and light adaptation in mouse rods and cones.\textsuperscript{46} Although recoverin appears to contribute slightly more to the regulation of cone than rod phototransduction, its role in the mouse phototransduction and light adaptation is rather small both in rods and cones and certainly cannot explain the large differences between these cells.\textsuperscript{42,46} A recent study demonstrated that rods lacking both GCAPs and recoverin still can light adapt via some other Ca\textsuperscript{2+}-dependent mechanism.\textsuperscript{59} Here, we show the cones of these mice also have a Ca\textsuperscript{2+}-dependent light adaptation component. However, overall this residual Ca\textsuperscript{2+} feedback in GCAPs/recoverin-deficient mouse photoreceptors is rather small and its magnitude is not larger in cones than in rods (Fig. 5B and\textsuperscript{84}). On the other hand, our results reveal a substantial Ca\textsuperscript{2+}-independent light adaptation component in mouse cones that is not present in rods. This adaptation is apparent even when the upregulation of cGMP flux by the background light is taken into consideration (Fig. 5B). Thus, surprisingly it appears that the difference between the adaptation capacities of mammalian rod and cone photoreceptors may not be explained by Ca\textsuperscript{2+}-dependent mechanism. Instead, our findings suggest that future efforts in understanding the functional differences between rods and cones should be focused on identifying Ca\textsuperscript{2+}-independent molecular mechanism(s).

Our results demonstrated a robust Ca\textsuperscript{2+}-dependent light adaptation mechanism even in the absence of GCAPs and recoverin in mouse cones (Fig. 5B). We observed about 50% increase of \(r_{max}\) by low Ca\textsuperscript{2+} exposure in GCAPs\textsuperscript{−/−}Rv\textsuperscript{−/−}Gnat1\textsuperscript{−/−} cones, indicating that some Ca\textsuperscript{2+}-dependent mechanism can potentiate the CNG channel current in these cones (Fig. 4A). On the other hand, lowering Ca\textsuperscript{2+} did not affect much the response kinetics (Fig. 4B). A very small effect of low Ca\textsuperscript{2+} exposure to response kinetics in GCAPs\textsuperscript{−/−}Rv\textsuperscript{−/−}Gnat1\textsuperscript{−/−} cones (and rods) suggest that the Ca\textsuperscript{2+} feedback is not modulating cGMP hydrolysis or synthesis rate (Fig. 4B). Another potential mechanism for the observed Ca\textsuperscript{2+} feedback is the modulation of CNG channels. This modulation may be via a recently discovered CNG modulin or its mammalian homolog Eml1.\textsuperscript{35,36} Future studies combining genetics and cone physiology will be able to test these hypotheses.
Our results clearly demonstrate the robust function of a Ca2+-independent mechanism for light adaptation in mouse cones (Fig. 5B) that is not present in rods. One trivial explanation for this residual light adaptation in cones is a simple light-induced acceleration of cGMP hydrolysis. The reduction in free cGMP will result in easier conformational changes of the fractional cGMP concentration even without upregulating cGMP synthesis. However, using a phototransduction model with all active feedbacks removed to estimate the drop of sensitivity reveals that a significant amount of light adaptation persists in GCAPs/recoverin-deficient cones even in low Ca2+ (Fig. 5B, dashed green trace). Comparing the flash responses in darkness and under various backgrounds in their cones under low Ca2+ exposure shows that background light can progressively accelerate light response termination (see Results). Thus, it is possible that the novel Ca2+-independent mechanism identified here accelerates cGMP synthesis rate. In principle, this could be related to a recently suggested bicarbonate-dependent modulation of guanylyl cyclase although it is not clear whether bicarbonate concentration can be modulated in background light-dependent manner. Another possible mechanism for the Ca2+-independent adaptation could be a recently discovered pathway acting via IGF-1 and all-trans-retinol to regulate the rod CNG channels. This mechanism causes potentiation (increase of light sensitivity) of rod response amplitudes in background light. Whether it can contribute to the light adaptation in mouse cones and explain the Ca2+-independent light adaptation observed here will be an interesting subject for future studies.

Data Availability Statement

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

References

1. Ingram, N. T., Sampath, A. P. & Fain, G. L. Why are rods more sensitive than cones? J Physiol, https://doi.org/10.1113/JRP272556 (2016).
2. Kefalov, V. J. Rod and cone visual pigments and phototransduction through pharmacological, genetic, and physiological approaches. J Biol Chem 287, 1635–1641, https://doi.org/10.1074/jbc.R111.309008 (2012).
3. Fung, B. K., Hurley, J. B. & Stryer, L. Flow of information in the light-triggered cyclic nucleotide cascade of vision. Proc Natl Acad Sci USA 78, 152–156 (1981).
4. Kuhn, H. Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes. Nature 283, 387–389 (1980).
5. Lerea, C. L., Somers, D. E., Hurley, J. B., Klock, I. B. & Bunt-Milam, A. H. Identification of specific transducin alpha subunits in retinal rod and cone photoreceptors. Science 234, 77–80 (1986).
6. Baehr, W., Devlin, M. J. & Applebury, M. L. Isolation and characterization of GTPase phosphodiesterase from bovine rod outer segments. J Biol Chem 254, 11669–11677 (1979).
7. Li, T. S., Volpp, K. & Applebury, M. L. Bovine cone photoreceptor cGMP phosphodiesterase structure deduced from a cDNA clone. Proc Natl Acad Sci USA 87, 293–297 (1990).
8. Fesenko, E. E., Kolesnikov, S. S. & Lyubarsky, A. L. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. Nature 313, 310–313 (1985).
9. Hagins, W. A., Penn, R. D. & Yoshikami, S. Dark current and photocurrent in retinal rods. Biophys. J. 10, 380–412, https://doi.org/10.1016/S0006-3495(70)86308-1 (1970).
10. Yau, K. W., McNaughton, P. A. & Hodgkin, A. L. Effect of ions on the light-sensitive current in retinal rods. Nature 292, 502–505 (1981).
11. Pannbacker, R. G. Control of guanylate cyclase activity in the rod outer segment. Science 182, 1138–1140 (1973).
12. Kondo, H. & Miller, W. H. Rod light adaptation may be mediated by acceleration of the phosphodiesterase-guanylate cyclase cycle. Proc Natl Acad Sci USA 85, 1322–1326 (1988).
13. Tachibana, S., Arinobu, D., Shimauchi-Matsukawa, Y., Tsushima, S. & Kawamura, S. Highly effective phosphorylation by G protein-coupled receptor kinase 7 of light-activated visual pigment in cones. Proc Natl Acad Sci USA 102, 9329–9334, https://doi.org/10.1073/pnas.0501875102 (2005).
14. Tomizuka, J., Tachibana, S. & Kawamura, S. Phosphorylation-independent suppression of light-activated visual pigment by arrestin in carp rods and cones. J Biol Chem 290, 3939–4941, https://doi.org/10.1074/jbc.M114.363453 (2015).
15. Shichida, Y., Imai, H., Imamoto, Y., Fukada, Y. & Yoshizawa, T. Is chicken green-sensitive cone visual pigment a rhodopsin-like pigment? A comparative study of the molecular properties between chicken green and rhodopsin. Biochemistry 33, 9040–9044 (1994).
16. Tachibana, S., Yonetsu, S., Fukaya, S., Koshitani, Y. & Kawamura, S. Low activation and fast inactivation of transducin in carp cones. J Biol Chem 287, 41186–41194, https://doi.org/10.1074/jbc.M112.403717 (2012).
17. Takemoto, N., Tachibana, S. & Kawamura, S. High GMP synthetase activity in carp cones. Proc Natl Acad Sci USA 106, 11788–11793, https://doi.org/10.1073/pnas.0812781106 (2009).
18. Cowan, C. W., Fariss, R. N., Sokal, I., Palczewski, K. & Wensel, T. G. High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. Proc Natl Acad Sci USA 95, 5351–5356 (1998).
19. Zhang, X., Wensel, T. G. & Kraft, T. W. GTPase regulators and photoreponses in cones of the eastern chipmunk. J. Neurosci. 23, 1287–1297 (2003).
20. Nikonov, S. S. et al. Mouse cones require an arrestin for normal inactivation of phototransduction. Neuron 59, 462–474, https://doi.org/10.1016/j.neuron.2008.06.011 (2008).
21. Majumder, A. et al. Exchange of Cone for Rod Phosphodiesterase 6 Catalytic Subunits in Rod Photoreceptors Mimics in Part Features of Light Adaptation. J Neurosci. 35, 9225–9235, https://doi.org/10.1523/JNEUROSCI.3563-14.2015 (2015).
22. Kefalov, V. J. et al. Breaking the covalent bond—a pigment property that contributes to desensitization in cones. Neuron 46, 879–890, https://doi.org/10.1016/j.neuron.2005.05.009 (2005).
23. Nikonov, S. S., Kholodenko, R., Lem, J. & Pugh, E. N. Jr. Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. J Gen Physiol 127, 359–374, https://doi.org/10.1085/jgp.200609490 (2006).
24. Fain, G. L., Matthews, H. R., Cornwall, M. C. & Koutalos, Y. Adaptation in vertebrate photoreceptors. Physiol. Rev. 81, 117–151 (2001).
25. Yau, K. W. & Nakatani, K. Electrogenic Na-Ca exchange in retinal rod outer segment. Nature 311, 661–663 (1984).
26. Nakatani, K. & Yau, K. W. Calcium and light adaptation in retinal rods and cones. Nature 334, 69–71, https://doi.org/10.1038/334067a0 (1988).
27. Matthews, H. R., Murphy, R. L., Fain, G. L. & Lamb, T. D. Photoreceptor light adaptation is mediated by cytoplasmic calcium concentration. Nature 334, 67–69, https://doi.org/10.1038/334067a0 (1988).
28. Mendez, A. et al. Role of guanylate cyclase-activating proteins (GCAPs) in setting the flash sensitivity of rod photoreceptors. Proc Natl Acad Sci USA 98, 9948–9953, https://doi.org/10.1073/pnas.171308998 (2001).

29. Koch, K. W. & Stryer, L. Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions. Nature 334, 64–66, https://doi.org/10.1038/334064a0 (1988).

30. Matthews, H. R., Cornwell, M. C. & Crouch, R. K. Prolongation of action of Ca2+ early in phototransduction by 9-demethylretinal. J. Gen. Physiol. 118, 377–390 (2001).

31. Chen, C. K., Woodruff, M. L., Chen, F. S., Chen, D. & Fain, G. L. Background light produces a recoverin-dependent modulation of activated rhodopsin lifetime in mouse rods. J. Neurosci 30, 1213–1220, https://doi.org/10.1523/JNEUROSCI.4533-09.2010 (2010).

32. Sakurai, K., Chen, J., Kowalski, S. C. & Kefalov, V. J. Regulation of mammalian cone phototransduction by recoverin and rhodopsin kinase. J Biol Chem 290, 9239–9250, https://doi.org/10.1074/jbc.M111.369391 (2015).

33. Dizhoor, A. M. et al. Recoverin: a calcium sensitive activator of retinal rod guanylate cyclase. Science 251, 915–918 (1991).

34. Gorodovskyina, E. N., Gottereau, A. A., Senin, I. I. & Philippov, V. I. Guanylate cyclase-mediates the calcium effect upon rhodopsin phosphorylation and cGMP hydrolysis in bovine retina rod cells. FEBS Lett. 349, 187–190 (1994).

35. Rebrir, T. I., Botchkina, I., Arshavsky, V. Y., Craft, C. M. & Korenbrot, J. C. CNG-modulin: a novel Ca-dependent modulator of ligand sensitivity in cone photoreceptor CNG-gated ion channels. J. Neurosci. 32, 3142–3153, https://doi.org/10.1523/JNEUROSCI.5518-11.2012 (2012).

36. Korenbrot, J. I., Mehta, M., Tietenssoodol, N., Postlethwait, J. H. & Rebrir, T. I. EML1 (CNG-modulin) controls light sensitivity in darkness and under continuous illumination in zebrafish retinal cone photoreceptors. J. Neurosci. 33, 17763–17776, https://doi.org/10.1523/JNEUROSCI.2659-13.2013 (2013).

37. Hsu, Y. T. & Molday, R. S. Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. Nature 361, 76–79, https://doi.org/10.1038/361064a0 (1993).

38. Fain, G. L., Lamb, T. D., Matthews, H. R. & Murphy, R. L. Cytoplasmic calcium as the messenger for light adaptation in salamander rods. J. Physiol 416, 215–243 (1989).

39. Vinberg, F., Turunen, T. T., Heikkinen, H., Pitkanen, M. & Koskelainen, A. A novel Ca2+-feedback mechanism extends the operating range of mammalian rods to brighter light. J Gen Physiol 146, 307–321, https://doi.org/10.1085/jgp.201511412 (2015).

40. Makino, C. L. et al. Recoverin regulates light-dependent phosphodiesterase activity in retinal rods. J Gen Physiol 123, 729–741, https://doi.org/10.1085/jgp.200308994 (2004).

41. Calvert, S. & Newby, P. J. Transient modulation and the mechanism of light adaptation in mouse rods. J. Neurosci 30, 16232–16240, https://doi.org/10.1523/JNEUROSCI.2668-10.2010 (2010).

42. Sakurai, K., Chen, J. & Kefalov, V. J. Role of guanylyl cyclase activity in phototransduction. J. Neurosci. 31, 7991–8000, https://doi.org/10.1523/JNEUROSCI.6650-10.2011 (2011).

43. Makino, C. L. et al. Enzymatic relay mechanism stimulates cyclic GMP synthesis in rod phototransduction: biochemical and physiological study in guanylyl cyclase activating protein 1 knockout mice. PLoS One 7, e47637, https://doi.org/10.1371/journal.pone.0047637 (2012).

44. Chen, C. K. et al. Modulation of mouse rod response decay by rhodopsin kinase and recoverin. J. Neurosci. 32, 15998–16006, https://doi.org/10.1523/JNEUROSCI.1639-12.2012 (2012).

45. Chen, C. K., Woodruff, M. L. & Fain, G. L. Rhodopsin kinase and recoverin modulate phosphodiesterase during mouse phototransduction. J Gen Physiol 145, 213–224, https://doi.org/10.1085/jgp.201411273 (2015).

46. Matsusue, M., Szymanski, J. M. & Siman, R. W. Ion transport by the Na-Ca exchange in isolated rod outer segments. J. Gen. Physiol 107, 19–34 (1996).

47. Hodgkin, A. L., McNaughton, P. A., Nunn, B. J. & Yan, W. K. Effect of ions on retinal rods from Bufo marinus. J. Physiol 350, 649–680 (1984).

48. Matthews, H. R. Effects of lowered cytoplasmic calcium concentration and light on the responses of salamander rod photoreceptors. J Physiol 488 (Pt 2), 267–286 (1995).

49. Sather, W. A. & Detwiler, P. B. Intracellular biochemical manipulation of phototransduction in detached rod outer segments. Proc Natl Acad Sci USA 84, 9290–9294 (1987).

50. Lagnado, L., Cervetto, L. & McNaughton, P. A. Ion transport by the Na-Ca exchange in isolated rod outer segments. Proc Natl Acad Sci USA 85, 4548–4552 (1988).

51. Woodruff, M. L. et al. Measurement of cytoplasmic calcium concentration in the rods of wild-type and transducin knock-out mice. J. Physiol 542, 843–854 (2002).

52. Winkler, B. S. The electroretinogram of the isolated rat retina. Vision Res. 12, 1183–1198 (1972).

53. Bastian, B. L. & Fain, G. L. The effects of low calcium and background light on the sensitivity of toad rods. J Physiol 330, 307–329 (1982).

54. Vinberg, F. J., Strandman, S. & Koskelainen, A. Origin of the fast negative ERG component from isolated aspartate-treated mouse retina. J Vis (9), 1–17, https://doi.org/10.1167/7.12.9 (2009).

55. Robson, J. G. & Frishman, L. J. The rod-driven a-wave of the dark-adapted mammalian electroretinogram. Prog Retin Eye Res 39, 1–22, https://doi.org/10.1016/j.preteyres.2013.12.003 (2014).

56. Pahlberg, J. et al. Voltage-sensitive conductances increase the sensitivity of rod photoreceptors following pigment bleaching. J. Physiol 595, 3459–3469, https://doi.org/10.1113/JP233988 (2017).

57. Nikonov, S., Lamb, T. D. & Pugh, E. N. Jr. The role of steady phosphodiesterase activity in the kinetics and sensitivity of the light-adapted salamander rod photoreceptor. J. Gen. Physiol 116, 795–824 (2000).

58. Nicola, G. D. & Bownds, M. D. Calcium regulates some, but not all, aspects of light adaptation in rod photoreceptors. J. Gen. Physiol 94, 233–259 (1989).

59. Kawamura, S. & Murakami, M. Regulation of cGMP levels by guanylate cyclase in truncated frog rod outer segments. J. Gen. Physiol 94, 649–668 (1989).

60. McKeown, A. S., Pitate, P. M. & Kraft, T. W. Signalling beyond photon absorption: extracellular retinoids and growth factors modulate rod photoreceptor sensitivity. J Physiol 594, 1841–1854, https://doi.org/10.1113/JP271650 (2016).
67. Fadool, J. M. & Dowling, J. E. Zebrafish: a model system for the study of eye genetics. Prog Retin Eye Res 27, 89–110, https://doi.org/10.1016/j.preteyeres.2007.08.002 (2008).
68. Fu, Y. & Yau, K. W. Phototransduction in mouse rods and cones. Pflugers Arch 454, 805–819, https://doi.org/10.1007/s00424-006-0194-y (2007).
69. Reilander, H. et al. Primary structure and functional expression of the Na/Ca+ exchanger from bovine rod photoreceptors. EMBO J. 11, 1689–1695 (1992).
70. Primsen, C. F., Szerencsei, R. T. & Schnetkamp, P. P. Molecular cloning and functional expression of the potassium-dependent sodium–calcium exchanger from human and chicken retinal cone photoreceptors. J. Neurosci. 20, 1424–1434 (2000).
71. Vinberg, F. et al. The Na+/Ca2+, K+ exchanger NCKX4 is required for efficient cone-mediated vision. Elife 6, https://doi.org/10.7554/eLife.24550 (2017).
72. Peshenko, I. V. et al. Enzymatic properties and regulation of the native isozymes of retinal membrane guanylyl cyclase (RetGC) from mouse photoreceptors. Biochemistry 50, 5590–5600, https://doi.org/10.1021/bi200491b (2011).
73. Kawamura, S. & Tachibanaki, S. Rod and cone photoreceptors: molecular basis of the difference in their physiology. Comp Biochem Physiol A Mol Integr Physiol 150, 369–377, https://doi.org/10.1016/j.cbpa.2008.04.660 (2008).
74. Wald, G., Brown, P. K. & Smith, P. H. Iodopsin. J. Gen. Physiol. 38, 623–681 (1955).
75. Rieke, F. & Baylor, D. A. Origin and functional impact of dark noise in retinal cones. Neuron 26, 181–186 (2000).
76. Kefalov, V., Fu, Y., Marsh-Armstrong, N. & Yau, K. W. Role of visual pigment properties in phototransduction. Nature 425, 526–531, https://doi.org/10.1038/nature01992 (2003).
77. Sakurai, K. et al. Physiological properties of rod photoreceptor cells in green-sensitive cone pigment knock-in mice. J. Gen. Physiol. 130, 21–40, https://doi.org/10.1085/jgp.200609729 (2007).
78. Shi, G., Yau, K. W., Chen, J. & Kefalov, V. J. Signaling properties of a short-wave cone visual pigment and its role in phototransduction. J Neurosci 27, 10084–10093, https://doi.org/10.1523/JNEUROSCI.2211-07.2007 (2007).
79. Fu, Y., Kefalov, V., Luo, D. G., Xue, T. & Yau, K. W. Quantal noise from human red cone pigment. Nat Neurosci 11, 565–571, https://doi.org/10.1038/nn.2110 (2008).
80. Chen, C. K. et al. Replacing the rod with the cone transducin subunit decreases sensitivity and accelerates response decay. J Physiol 588, 3231–3241, https://doi.org/10.1113/jphysiol.2010.191221 (2010).
81. Mao, W. et al. Functional comparison of rod and cone Galpha(t) on the regulation of light sensitivity. J. Biol. Chem. 288, 5257–5267, https://doi.org/10.1074/jbc.M112.430058 (2013).
82. Sampath, A. P., Matthews, H. R., Cornwell, M. C., Bandarchi, J. & Fain, G. L. Light-dependent changes in outer segment free-Ca2+ concentration in salamander cone photoreceptors. J Gen Physiol 113, 267–277 (1999).
83. Sampath, A. P., Matthews, H. R., Cornwell, M. C. & Fain, G. L. Bleached pigment produces a maintained decrease in outer segment Ca2+ in salamander rods. J Gen Physiol 111, 53–64 (1998).
84. Duda, T., Wen, X. H., Isayama, T., Sharma, R. K. & Makino, C. L. Bicarbonate Modulates Photoreceptor Guanylate Cyclase (ROS-GC) Catalytic Activity. J. Biol. Chem. 290, 11052–11060, https://doi.org/10.1074/jbc.M111.560408 (2015).
85. McKeown, A. S., Kraft, T. W. & Loop, M. S. Increased visual sensitivity following periods of dim illumination. Invest Ophthalmol Vis Sci 56, 1864–1871, https://doi.org/10.1167/iovs.14-15958 (2015).

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Author Contributions
F.V. and V.J.K contributed to study conception, design and data interpretation. F.V. and V.J.K wrote the manuscript. F.V. conducted the experiments and analyzed the data.

Additional Information
Competing Interests: The authors declare no competing interests.

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