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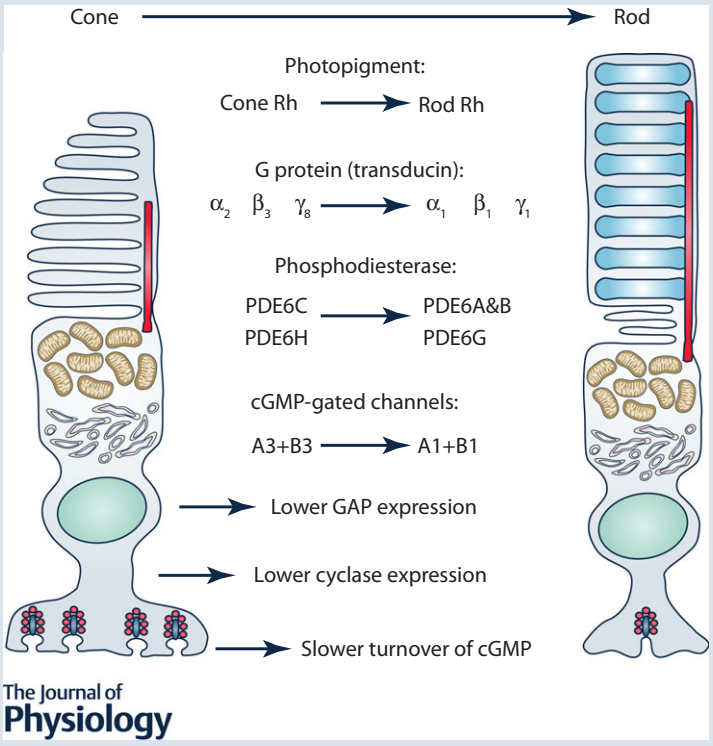
TOPICAL REVIEW

Why are rods more sensitive than cones?

Norianne T. Ingram[1], Alapakkam P. Sampath[2] and Gordon L. Fain[1,2]

[1]*Department of Integrative Biology and Physiology, University of California, Los Angeles, CA 90095–7239, USA*
[2]*Department of Ophthalmology and Jules Stein Eye Institute, University of California, Los Angeles, CA 90095–7000, USA*



Abstract One hundred and fifty years ago Max Schultze first proposed the duplex theory of vision, that vertebrate eyes have two types of photoreceptor cells with differing sensitivity: rods for dim light and cones for bright light and colour detection. We now know that this division is fundamental not only to the photoreceptors themselves but to the whole of retinal and visual processing. But why are rods more sensitive, and how did the duplex retina first evolve? Cells resembling cones are very old, first appearing among cnidarians; the emergence of rods was a key step in the evolution of the vertebrate eye. Many transduction proteins have different isoforms in rods and cones, and others are expressed at different levels. Moreover rods and cones

Gordon L. Fain is Distinguished Professor of Integrative Biology/Physiology and of Ophthalmology at the University of California Los Angeles (UCLA) and a member of the Jules Stein Eye Institute. His laboratory works on the physiology of vertebrate photoreceptors. **Norianne Ingram** is a graduate student in the Molecular, Cellular, and Integrative Physiology Program at UCLA and is presently doing her thesis on cone photoreceptors and retinal signal processing. **Alapakkam P. Sampath** is Professor of Ophthalmology at UCLA and a member of the Jules Stein Eye Institute. His laboratory works on vertebrate photoreceptor physiology and signal integration in vertebrate retina.

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have a different anatomy, with only rods containing membranous discs enclosed by the plasma membrane. These differences must be responsible for the difference in absolute sensitivity, but which are essential? Recent research particularly expressing cone proteins in rods or changing the level of expression seem to show that many of the molecular differences in the activation and decay of the response may have each made a small contribution as evolution proceeded stepwise with incremental increases in sensitivity. Rod outer-segment discs were not essential and developed after single-photon detection. These experiments collectively provide a new understanding of the two kinds of photoreceptors and help to explain how gene duplication and the formation of rod-specific proteins produced the duplex retina, which has remained remarkably constant in physiology from amphibians to man.

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Corresponding author G. L. Fain: 2129 Terasaki Life Sciences, 610 Charles E Young East, University of California, Los Angeles, Los Angeles, CA 90095–7239, USA. Email: gfain@ucla.edu

Abstract figure legend Rods evolved from cones or their progenitors through the emergence of distinct isoforms and altered expression levels of proteins required for transducing light into an electrical signal. These changes must collectively explain why rods are more sensitive.

Abbreviations *A*, activation constant; cGMP, cyclic guanosine monophosphate; CNG, cyclic-nucleotide gated; GAP, GTPase-accelerating protein; GC, guanylyl cyclase; GCAP, guanylyl cyclase-activating protein; GRK, G-protein receptor kinase; hν, light (photon); mG, mouse 508 nm cone rhodopsin; mS, mouse 360 nm cone rhodopsin; PDE6, phosphodiesterase 6; PDE6*, light-activated phosphodiesterase 6; Rh*, light-activated rhodopsin or metaII; Tα, α subunit of photoreceptor G protein; WT, wild-type.

Introduction

In 2016 we celebrate the 150th anniversary of the groundbreaking article of Max Schultze (1866), who first proposed that rod and cone photoreceptors have different functions. Schultze noticed that retinas of nocturnal animals tend to have a larger proportion of cells with rod-shaped outer segments (Fig. 1*A*), and that diurnal animals have greater numbers of cells with outer segments tapering like cones (Fig. 1*B*). He then proposed the *duplex* theory of vision: that rods mediate perception in dim light and cones are specialized for bright light and colour vision. We now know that his division of visual detection into two systems is fundamental not only to the properties of photoreceptors but also to the connections these cells make with other neurons and to the whole of retinal and visual processing (Masland, 2012).

Since the publication of Schultze's paper, we have wondered why rod vision is more sensitive. The first intracellular recordings showed that most of the sensitivity difference is inherent in the photoreceptors themselves: single rods are more sensitive than single cones (Fain & Dowling, 1973). Soon afterward, biochemists and molecular biologists discovered that the two photoreceptors have many of the same kinds of proteins and detect light in a similar way. Cones are much older than rods: from the sequences of a very large number of vertebrate photopigments, we can infer that gene duplication produced all of the different kinds of cone pigments before the evolution of rod pigments (Nickle & Robinson, 2007; Shichida & Matsuyama, 2009). Along with the pigment came the many other molecular and anatomical differences between the two kinds of cells, with the result that rods are able to integrate incoming light over a longer period and operate at the theoretical limit of single-photon detection, whereas cones are less sensitive but exhibit adaptive properties that allow them to detect luminance changes and motion when the photon flux is less limiting. These differences in physiology must ultimately derive from differences in the mechanism of transduction in the two kinds of photoreceptors.

Recent experiments are beginning to clarify these differences. Some of the most interesting observations have been made from the combined efforts of molecular biologists and physiologists inserting cone genes into mouse rods. These experiments along with more traditional observations by biochemists and single-cell physiologists are gradually clarifying the roles of different proteins in rod sensitivity. Our initial expectation had been that one particular alteration might dramatically change the properties of the photoreceptor. Instead we have discovered what we should have suspected all along, that evolution proceeded by making small changes in many transduction proteins, incrementally increasing sensitivity to produce the rods and cones that emerged as long as 500 million years ago. Although the present state of research leaves many questions unanswered, we can now begin to see how rods became more sensitive.

Mechanism of transduction: rod/cone protein isoforms

Both rods and cones detect light according to the same basic scheme (Fig. 2). They use similar photopigments, which were once given distinct names but are now usually called rod or cone opsin or rhodopsin. The absorption of light produces a change in the pigment conformation to an intermediate called metaII or Rh*, which triggers a G-protein cascade (for an overview, see for example Fain, 2014). The heterotrimeric G proteins of rods and cones (called transducins) are different: rods express α1, β1 and γ1, whereas cones express α2, β3 and γ8 (Sakmar & Khorana, 1988; Kubo *et al.* 1991; Ong *et al.* 1995; Deng *et al.* 2009). The G protein binds to Rh*, and exchange of GTP for GDP on the transducin α subunit (Tα) produces the active form Tα•GTP.

Tα•GTP binds to the photoreceptor effector enzyme, which is phosphodiesterase 6 (PDE6). This protein has four subunits, two catalytic and two inhibitory. The catalytic subunits are slightly different from one another in rods and are called PDE6α and PDE6β (or PDE6A and PDE6B), whereas the two in cones are the same and called PDE6α' (or PDE6C). Each PDE tetramer also has two inhibitory subunits, one for each catalytic subunit, which have somewhat different sequences in the two types of photoreceptors and are called rod or cone PDE6γ, or PDEG (in rods) and PDEH (in cones). Activated PDE6 hydrolyses cGMP, which acts as the second messenger of the cascade by binding to cGMP-gated channels. The channels are tetramers again with different protein subunits called CNGA1 and CNGB1 in rods and CNGA3 and CNGB3 in cones (see Kaupp & Seifert, 2002; Zhong *et al.* 2002; Shuart *et al.* 2011).

Based on this general scheme, the activation of a single rhodopsin molecule is amplified across these stages to lead ultimately to the destruction of as many as one million cGMP molecules per Rh* in rods (Yee & Liebman, 1978). This reduction in cGMP concentration across vertebrate species is sufficient to reduce the cGMP-gated current by more than its intrinsic noise in darkness (Baylor *et al.* 1979, 1984; Nakatani *et al.* 1991). The natural question to ask then is, can the lower sensitivity of cones be the result purely of reduced amplification within these steps? Let us suppose that rod and cone responses were to *inactivate* at the same rate. A reduced rate of *activation* would then cause cone responses to reach smaller peak amplitudes and might account entirely for the difference in sensitivity. But do rod and cone responses inactivate at the same rate? Not even close! In every vertebrate species from lamprey (Morshedian & Fain, 2015; Asteriti *et al*, 2015) to mouse (see Fig. 3), the rate is much faster in cones, and this difference must also contribute to the reduced cone sensitivity.

The rate of inactivation is determined by the rates at which Rh*, transducin and PDE return to their basal

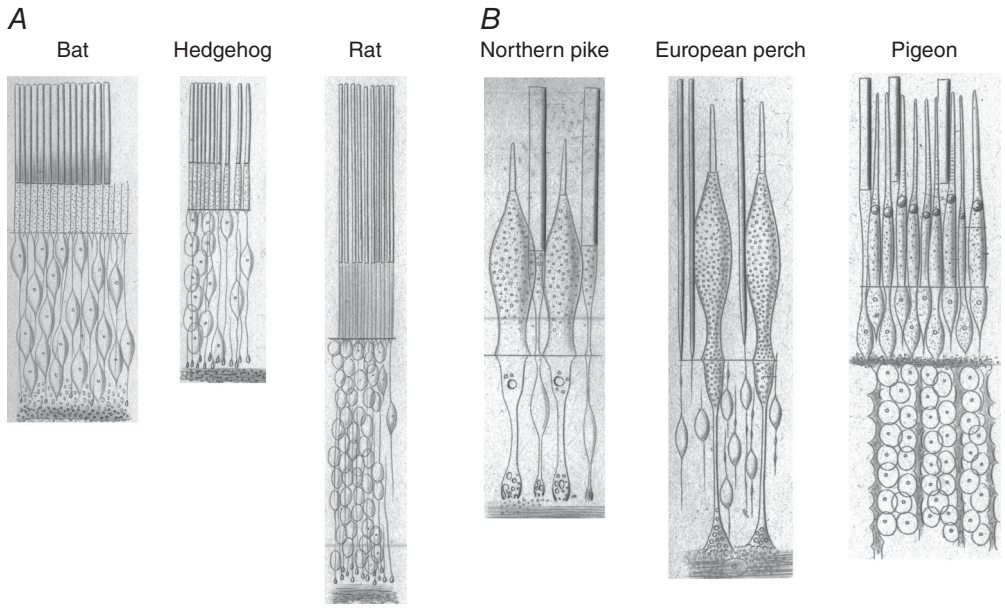

Figure 1. Rods and cones in nocturnal and diurnal animals
Drawings from Schultze's original paper (1866) of photoreceptors from nocturnal animals (*A*) and diurnal animals (*B*), magnification approximately 350–400 times. Schultze claimed that the bat retina lacked even a trace of cones, but in rat he noticed occasional gaps (*Lücken*) which he speculated could possibly correspond to cones, as we now know to be true. Fish and pigeon on the other hand have many easily observable cones in addition to rods. Schultze commented that these observations 'would seem to indicate that rods are more advantageous than cones for quantitative light perception', but that 'cones would seem to be the nerve end-organs for colour perception'.

conformations and the cGMP concentration goes back to its dark level. Rh* is silenced like other G-protein receptors by phosphorylation and binding of arrestin. Rods and cones can have two different G-protein receptor kinases, GRK1 in rods and GRK1 and/or GRK7 in cones, but rodents, including mice, have only GRK1 in both kinds of photoreceptors. Rods in mice have arrestin-1 and cones both arrestin-1 and arrestin-4, though arrestin-1 is by far the predominant species in both kinds of photoreceptors (Nikonov *et al.* 2008).

Activated transducin and phosphodiesterase are extinguished as in other G-protein cascades by hydrolysis of Tα•GTP to Tα•GDP with the assistance of PDEγ and three GTPase-accelerating proteins (GAPs): RGS9-1, Gβ5 and the R9AP-1 binding protein (see Arshavsky & Wensel, 2013). These proteins are required to speed PDE deactivation into the functional range of tens to hundreds of milliseconds, compared to the seconds or tens of seconds required in their absence (Hollinger & Hepler, 2002). Although these GAP complex proteins are the same in rods and cones, expression is significantly higher in cones (Cowan *et al.* 1998; Zhang *et al.* 2003), a point we return to later.

The cGMP concentration is restored by guanylyl cyclase (GC), which in photoreceptors is a member of the membrane guanylyl cyclase family (Potter, 2011). There are two cyclases in mammalian photoreceptors called retGC1 (or GC-E) and retGC2 (or GC-F); in mouse, rods have mostly retGC1 with some retGC2, whereas cones have only retGC1 (Wen *et al.* 2014). This difference is unlikely to be physiologically significant because when the gene for retGC2 is deleted there is little effect on rod sensitivity or response waveform (Baehr *et al.* 2007). The rate of cyclase activity is controlled by small molecular weight Ca^{2+}-binding proteins called guanylyl cyclase-activating proteins or GCAPs. There are again two in mouse, GCAP1 and GCAP2, with somewhat different sensitivities for divalent ion binding (Dizhoor *et al.* 2010); rods express both GCAPs but cones mostly express GCAP1 (Dizhoor *et al.* 1995; Xu *et al.* 2013; Boye *et al.* 2015).

The differences in transduction proteins for rods and cones are summarized in Table 1. Rods and cones also display differences in anatomy: the photopigment in rods is contained almost entirely within the membrane of intracellular discs, whereas cone outer segments are formed from infoldings of the plasma membrane. We have long wondered whether this difference in anatomy might hold the key to the difference in sensitivity, but we now know the answer. Nature did the experiment for us: the rods and cones of lamprey have an identical morphology, which is like that of cones (see for example Dickson & Graves, 1979), but lamprey rods are nearly as sensitive as mouse rods and about 70 times more sensitive than lamprey cones (Morshedian & Fain, 2015; Asteriti *et al.* 2015). The discs of

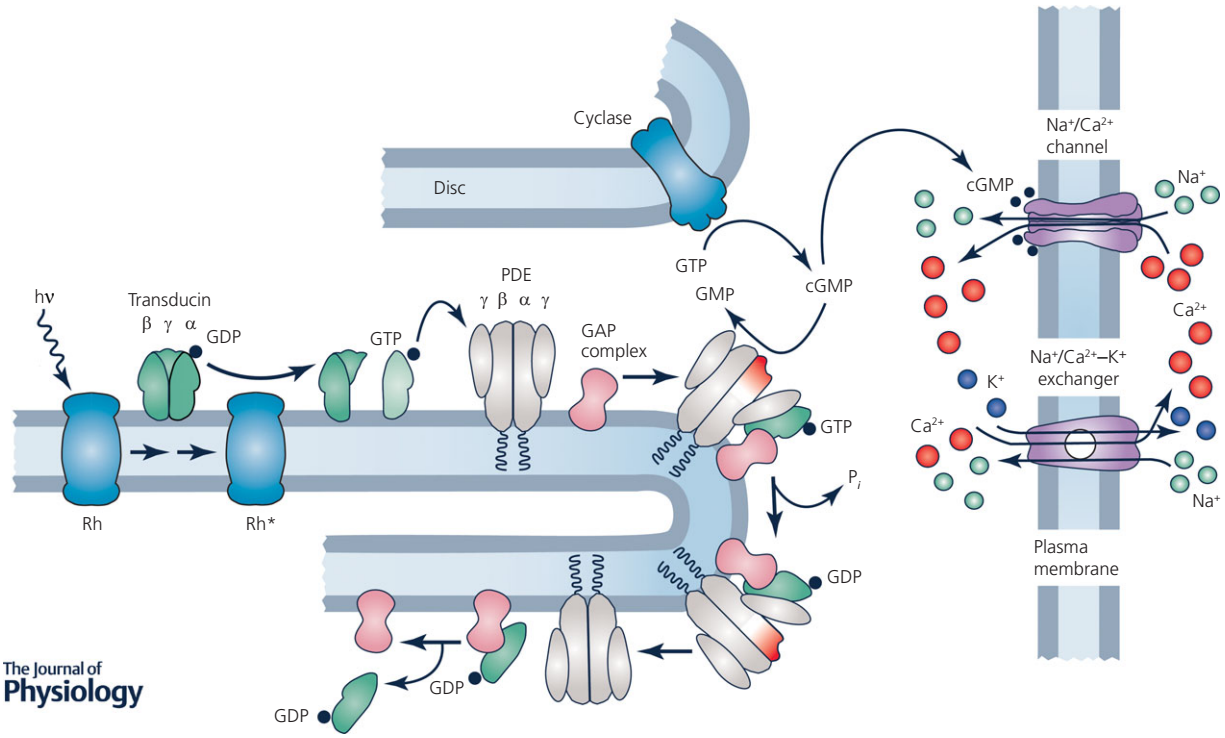

Figure 2. Phototransduction in vertebrate photoreceptors
Redrawn and printed with permission from Fain *et al.* (2010).

rods do not seem to be essential for high sensitivity vision (see also Ma *et al.* 2001) but may instead have evolved to allow more efficient renewal of outer segment membrane (Morshedian & Fain, 2015).

Activation of transduction

Although rods are universally more sensitive than cones, the value of the sensitivity difference varies among vertebrates, ranging from 25-fold in mudpuppy (Fain & Dowling, 1973) to 1000-fold between red-sensitive cones and rods in carp (Tachibanaki *et al.* 2001). In our examination of the cause of this sensitivity difference, we will take as our example the mouse, because many of the most recent experiments have utilized transgenic mice. In Fig. 3*A* and *B*, we show mean responses of mouse rods and cones recorded with suction electrodes. Rod responses decay much more slowly than cone responses (note ten-fold difference in the scale of the abscissa) and are typically about twice as large; after normalizing response amplitudes to their maximum values, rods are a little more than 100 times more sensitive than cones (Fig. 3*C*), as previously reported (see for example Nikonov *et al.* 2006). Part of this difference is the result of the larger volume of the rod outer segment, which increases the probability of absorption of a photon by pigment molecules. We can, however, correct for these differences by calculating the percentage decrease in photocurrent per photon absorbed. Calculations of this kind give about 0.2–0.25% per Rh* for cones (Nikonov *et al.* 2006; Sakurai *et al.* 2011; Cao *et al.* 2014) and 5% for rods (Sampath *et al.* 2005; see Reingruber *et al.* 2015). The resulting factor of between 20 and 30 is the difference in sensitivity produced by the transduction cascade.

One reason rods are more sensitive is that early events in the transduction cascade have greater gain and close channels more rapidly, as alluded to previously. As a consequence, rod responses rise more quickly per photon absorbed; with everything else being equal, rod responses would reach a commensurately larger peak amplitude for the same intensity of stimulus. Following the theoretical treatment of Pugh and Lamb (1993, 2000), we can use the rising phases to calculate values of an amplification constant *A* (see Fig. 4*A* and legend), equal to the product of (1) the rate of formation of light-activated PDE6* by the photopigment, (2) the rate of decline of cGMP

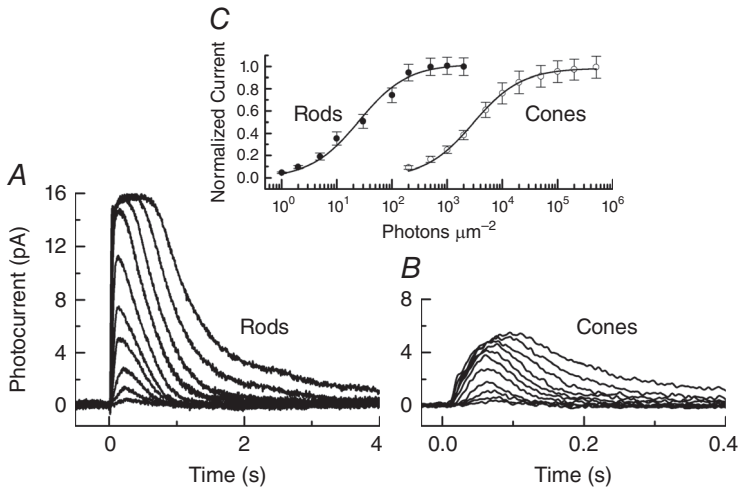

Figure 3. Responses of mouse rods and cones
A, mean responses of 11 WT mouse rods to 20 ms flashes of 500 nm illumination from 0.5 to 2000 photons μm^{-2}. *B*, mean responses of 18 mouse M (508 nm) cones to 20 ms flashes of 500 nm illumination from 200 to 500,000 photons μm^{-2}. Responses in *A* and *B* were filtered with an 8-pole Bessel filter with a low-pass filter setting of 75 Hz. *C*, mean peak amplitudes (with SEM) of responses of mouse rods (•) and mouse cones (○) to 20 ms flashes of 500 nm illumination, normalized to maximum response and plotted as a function of flash intensity. Curves give best-fitting Michaelis–Menten equation with flash intensities at half-maximal amplitude of 25.3 (for rods) and 2960 (for cones) photons μm^{-2}. All recordings were made from C57BL/6 mice from Jackson Laboratory (Bar Harbor, ME, USA), dark adapted for at least 4 h and usually overnight. All experiments were performed on mice of either sex in accordance with the rules and regulations of the NIH guidelines for research animals, as approved by the institutional animal care and use committee (IACUC) of the University of California, Los Angeles. Animals were kept in cyclic 12 h/12 h on/off lighting in approved cages and supplied with ample food and water. Animals in all experiments were killed before tissue extraction by approved procedures, usually CO_2 inhalation or decerebration. Recordings were made at 37°C in Ames solution. Light intensities are given as photons effective at the lambda max of the rod or cone pigment calculated by convolving the spectrum of the stimulating beam with the rod or cone photopigment absorption curves.

Table 1. Photoreceptor transduction protein isoforms in mouse rods and cones

	Rod	Cone
Photopigment	Rod opsin (or rhodopsin)	Cone opsin (or rhodopsin)
G protein (transducin)	α1, β1 and γ1	α2, β3 and γ8
Phosphodiesterase 6	PDE6A and PDE6B	PDE6C
	Rod PDE6γ (PDE6G)	Cone PDE6γ (PDE6H)
cGMP-gated channels	CNGA1 and CNGB1	CNGA3 and CNGB3
Rhodopsin kinase	GRK1	GRK1
Arrestin	Arrestin-1	Arrestin-1 and arrestin-4
GAPs	RGS9-1, Gβ5 and R9AP-1	RGS9-1, Gβ5 and R9AP-1
Guanylyl cyclase	retGC1 and retGC2	retGC1
GCAPs	GCAP1 and GCAP2	GCAP1
Na^+/Ca^{2+}–K^+ exchanger	NCKX1	NCKX2 and NCKX4

concentration per PDE6* molecule, and (3) the Hill coefficient of binding of cGMP to the channels. The value of *A* is somewhat dependent on the frequency response of the recording (Chen *et al.* 2010*b*) but is at least 2–3 times larger in rods than in cones (see Pugh & Lamb, 1993; Nikonov *et al.* 2006; Cao *et al.* 2014). This difference must be produced by the collective properties of the proteins responsible for activation. Since the Hill coefficient of rod and cone cGMP-gated channels is nearly the same (Picones & Korenbrot, 1992; see Kaupp & Seifert, 2002), we can focus our attention on the photopigments, G proteins and PDE6s, which, as we have seen, all have different isoforms in rods and cones.

One way to test the role of these proteins is by exogenous expression of cone proteins in rods or rod proteins in cones. Gene incorporation is easier for rods because there is only one rod photopigment in mouse with a reliable and widely used promoter, and rods are more convenient for physiology; so most experiments have put cone genes into rods. There is one complication: the value of *A* depends upon the rate of change of the cGMP concentration which is inversely proportional to cytoplasmic volume, because the larger the volume, the smaller the change in concentration per activated enzyme. Since mouse rods are about 2.5 times larger in volume than mouse cones, *A* would be 2.5 times smaller in rods even if the properties of all of the proteins were the same. To account for the *greater* value of *A* actually recorded from rods, activation would need to proceed at a rate at least 5–10 times faster (Nikonov *et al.* 2006). That is, if we could express the cone variants of all the activation proteins in a rod, activation should be at least something like 5–10 times slower. No one has yet expressed all of the proteins together, but many attempts have been made to express them one by one.

We begin with the photopigments. Sakurai and colleagues (2007) inserted the mouse 508 nm cone pigment gene (*mG*) in place of mouse rhodopsin. They found that *mG/mG* rods were about a factor of 3–4 less sensitive than wild-type (WT) rods and gave smaller values for the activation constant *A*, but *mG/mG* rods expressed considerably less pigment and transducin, had smaller outer segments, and showed signs of degeneration. Clearer perhaps were experiments expressing the mG pigment on a background of mutant E112Q rod rhodopsin (Sakurai *et al.* 2007), whose peak absorbance is shifted into the blue so that rod and cone pigments in mG/Rh^{EQ} rods can be stimulated selectively. The cone mG pigment produced a response per Rh* only about a third as large as the rod E112Q rhodopsin.

In a similar study, Shi and colleagues (2007) expressed the mouse short wavelength-sensitive (360 nm) pigment (mS) in mouse rods and recorded from homozygous *mS/mS* rods lacking rod rhodopsin as well as from heterozygous photoreceptors expressing both the mS pigment and rod rhodopsin. Although the single-photon response of *mS/mS* rods was smaller than in WT rods, confirming the study of Sakurai *et al.* (2007), recordings from heterozygotes expressing both the mS cone and WT rod pigments and selectively stimulated with short- and long-wavelength light showed no differences in sensitivity or response waveform. The two pigments seemed to produce nearly identical responses when expressed in the same rod.

Fu and colleagues (2008) then expressed the human long-wavelength pigment in mouse rods. Responses to the rod and cone pigments were indistinguishable in sensitivity and waveform. The cone pigment produced greater dark noise as also in the experiments of Sakurai *et al.* (2007; but see Shi *et al.* 2007), perhaps as a result of the lower stability of cone pigments generally (Rieke & Baylor, 2000; Sampath & Baylor, 2002; Kefalov *et al.* 2003; Kefalov *et al.* 2005; but see Angueyra & Rieke, 2013). This increase in noise was, however, not large enough to affect photoreceptor sensitivity. In conclusion, cone pigments expressed in rods either have no effect on sensitivity or reduce it by as much as a factor of 2–3.

The first experiments expressing transducin used a viral vector approach to inject the rod or cone Tα gene into

a mouse line that lacked both rod and cone transducins (Deng *et al.* 2009). Only a small fraction of the rods had any light response, probably reflecting the variability in expression level. From the few cells that could be recorded, there was no marked difference between cells expressing rod Tα and those expressing cone Tα. Since, however, the sensitivity of the rod is heavily dependent on transducin expression level (Sokolov *et al.* 2002), which was not (and could not) be measured for individual cells with this technique, the results were inconclusive.

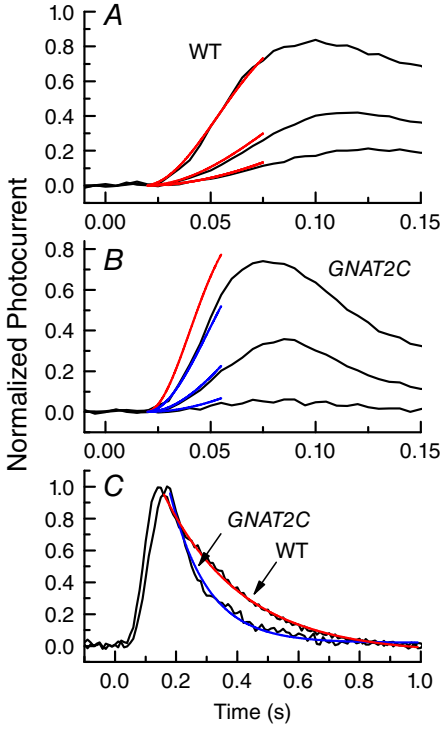

Figure 4. Differences in rate of activation and decay of WT and *GNAT2C* rods
A, black traces are mean initial time courses of responses of 16 WT rods to 10 ms flashes at intensities of 8.6, 21 and 79 photons μm^{-2}, after filtering with an 8-pole Bessel filter with a low-pass filter setting of 70 Hz. Responses have been normalized to the peak amplitude of the response. Red traces are fits to the data of the function $\frac{r}{r_{max}} = 1 - \exp[-\frac{1}{2}IA(t - t_{eff})^2]$ where r/r_{max} is the normalized flash response, I is the flash intensity in photoisomerizations, A is the amplification constant, t is time, and t_{eff} is the effective delay time of transduction (Pugh & Lamb, 1993), with the same mean values of A of 20.5 s^{-2} and t_{eff} of 18 ms at all three intensities. *B*, black traces are mean initial time courses of responses recorded and normalized as in *A* but of 14 *GNAT2C* rods to 10 ms flashes at intensities of 21, 79 and 227 photons μm^{-2}. Blue traces are fits to the data with an A of 10.2 s^{-2} and t_{eff} of 19.3 ms. Single red curve gives prediction for brightest intensity with WT rod value of A (20.5 s^{-2}). The value of A is about two times smaller in *GNAT2C* rods. *C*, mean small-amplitude responses of 21 WT rods and 9 *GNAT2C* rods to flashes of intensities 17 photons μm^{-2} (WT) and 79 photons μm^{-2} (*GNAT2C*). Responses have been normalized rod by rod to the peak amplitude of the response to compare waveforms of response decay. Responses have been fitted with single exponentials of 258 ms (red trace, WT) and 122 ms (blue trace, *GNAT2C*). Responses of *GNAT2C* rods decay significantly more rapidly. (Panels *A*–*C* reprinted with permission from Chen *et al.* 2010*b*).

Chen *et al.* (2010*b*) used a more traditional transgenic approach to express cone transducin in *Gnat1*−/− mice lacking rod transducin. They were fortunate to isolate a *GNAT2C* line in which the level of cone transducin was nearly the same as the WT rod transducin level. Sensitivity in *GNAT2C* rods was reduced by a factor of about 3, and the amplification constant A was about a factor of 2 smaller. This effect on amplification can be seen in Fig. 4*A* and *B*, which shows that the initial phase of the WT response rises more rapidly than that of *GNAT2C* rods.

Mao and colleagues (2013) then did a similar experiment also using a transgenic approach but with a different result. Rods in their mice expressed less cone Tα than *GNAT2C* rods and were less sensitive than WT rods, but the decrease in sensitivity seemed to depend only upon the expression level of the transducin and not upon the properties of cone Tα. They concluded that the species of transducin has no effect on the sensitivity difference between rods and cones. Thus incorporation of cone Tα in rods either has no effect on sensitivity or decreases it by as much as a factor of 3. No attempts have been made to express cone β3 or γ8 in place of rod β1 or γ1.

Two groups have attempted to express cone PDE6C in rods. Deng *et al.* (2013) injected viral vectors containing the *PDE6C* gene into the eyes of *rd10* mice, a line that is deficient in rod PDE6 but does not lack it entirely. Rods with cone PDE6C were surprisingly about twice as sensitive as those with the rod PDE6 proteins and showed a slower time course of decay. This anomalous result may have been produced by an unphysiological level of expression of PDE6, which again could not be measured. A clearer result was obtained by Majumder and colleagues (2015), who used a transgenic approach and were able to compare rod and cone PDE6 at the same expression level. Rods with cone PDE6C had a higher PDE6 basal activity and a single-photon response between 1.5 and 2 times smaller than WT rods, with a more rapid time course of decay (Fig. 5*A*). No attempt has been made to substitute cone PDE6γ for rod PDE6γ. This experiment could be revealing in view of Muradov *et al.* (2007), who showed that lamprey rods and cones have the same catalytic PDE6 subunits but different γ subunits. In conclusion, substitution of cone PDE6 for rod PDE6 either has no effect or decreases sensitivity by about a factor of two.

In summary, activation in mouse cones is at least 2- to 3-fold slower than activation in mouse rods. Taking outer segment volumes into account, we would predict that expression of cone pigment, cone transducin and cone PDE into a rod should together decrease the rate of activation by at least a factor of 5 with a commensurate decrease in sensitivity. Experiments expressing cone isoforms have, however, given conflicting results, with some

showing a 2- to 3-fold difference and some none at all. There are three possibilities: either papers showing significant differences are at least partially correct, or cone isoforms have to be expressed together (for example cone pigment with cone transducin), or other proteins (such as PDEγ or G-protein β and γ) also have a role. One conclusion, however, seems clear: the contribution of any one isoform is individually small, such that no one protein by itself is responsible for the entire difference in activation or sensitivity between the two kinds of photoreceptors.

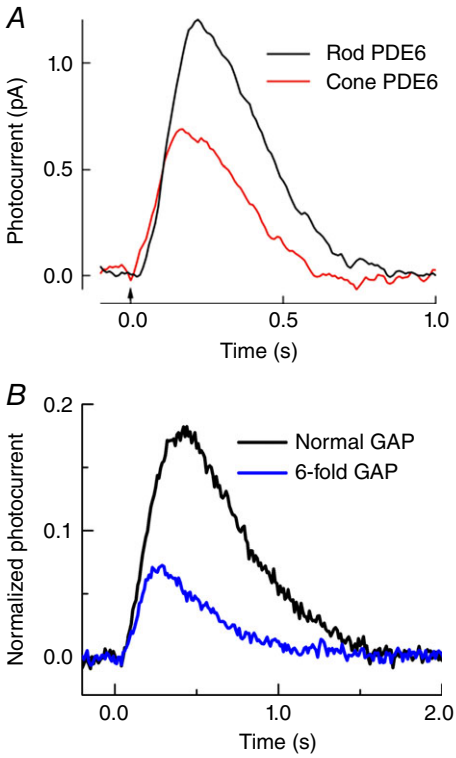

Figure 5. Single-photon responses of mouse rods with altered transduction proteins
A, derived average single-photon responses from control rods (black; rod PDE6) and cone-PDE6C-expressing rods (red; cone PDE6) (redrawn and reprinted with permission from Majumder *et al.* 2015). *B*, superimposed single-photon responses of WT mouse rods and of R9AP95 rods with six times the normal expression of GAP proteins (Chen *et al.* 2010*a*). Responses were plotted as a fraction of the peak current of the rod, effectively giving the fraction of channels closed per photon. Recordings were made from animals on a *GCAPs−/−* background to remove the effects of cyclase modulation on response amplitude and waveform (Gross *et al.* 2012). All experiments were performed on pigmented mice of either sex in accordance with the rules and regulations of the NIH guidelines for research animals, as approved by the institutional animal care and use committees (IACUCs) of the Virginia Commonwealth University and the University of California, Los Angeles. Animals were kept in cyclic 12 h/12 h on/off lighting in approved cages and supplied with ample food and water. Animals in all experiments were killed before tissue extraction by approved procedures, usually CO_2 inhalation or decerebration. Rods were perfused at 37°C with Dulbecco's modified Eagle's medium (Sigma Chemical, St Louis, MO, USA), supplemented with 15 mM $NaHCO_3$, 2 mM sodium succinate, 0.5 mM sodium glutamate, 2 mM sodium gluconate, and 5 mM NaCl, bubbled with 95% O_2–5% CO_2 (pH 7.4). Unless otherwise indicated, data were filtered at 35 Hz (8 pole, Bessel) and sampled at 100 Hz. (M. L. Woodruff, C. K. Chen & G. L. Fain, unpublished data).

Inactivation

If the response per Rh* is 20–30 times smaller in mouse cones than in mouse rods and activation accounts for only part of this difference, the remainder must emerge from mechanisms of inactivation. The records in Fig. 3 show that rods decay much more slowly than cones and integrate incoming photons over a longer time period. This difference in decay could in theory be produced by any of the reactions terminating the response.

We begin with extinction of Rh*. Rods and cones in mouse both phosphorylate photopigment with the same GRK1 kinase with no marked difference in antibody labelling and presumably expression (Lyubarsky *et al.* 2000; Weiss *et al.* 2001). Moreover both rods and cones use arrestin-1 with the small amount of arrestin-4 in cones unlikely to affect the rate of Rh* decay (Nikonov *et al.* 2008). The mean lifetime of Rh* in rods is probably as short as 40–45 ms (see Burns & Pugh, 2011), which is already so short that it is difficult to understand how even two or three serines or threonines could be phosphorylated and arrestin bind in so little time (Gurevich *et al.* 2011). If phosphorylation is faster in cones as Tachibanaki and colleagues have argued (2005), it is probably not much faster at least in mouse, whose rods and cones both express GRK1 at a similar level. More likely suspects for the slower rate of rod inactivation may be differences in the rates of decay of light-activated PDE6* and restoration of cGMP concentration by the cyclase.

Decay of PDE6* is produced by hydrolysis of Tα•GTP to Tα•GDP and rebinding of the PDE6γ inhibitory subunits to the PDE catalytic subunits. The rate of hydrolysis of Tα•GTP may be affected by the particular isoforms of transducin and PDE6: both cone transducin and cone PDE6C expressed in rods have been reported to produce responses that decay more rapidly than WT rod responses (see Figs 4*C* and 5*A*). The rate of hydrolysis may also be affected by the GAP proteins which, as we have said, are the same in rods and cones but are more abundantly expressed in cones at perhaps a 10-fold higher concentration (Cowan *et al.* 1998; Zhang *et al.* 2003). This difference in expression could have a significant effect on sensitivity. In Fig. 5*B*, we compare single-photon responses from rods with the normal GAP level and mutant *R9AP95* rods in which the GAP proteins are 6-fold over-expressed (Chen *et al.* 2010*a*). This experiment was done on a *GCAPs−/−* background to obviate any effect of cyclase feedback on response waveform or amplitude (Gross *et al.*

2012). Rods with over-expressed GAPs are about a factor of 2–3 less sensitive and decay more rapidly.

There are two ways guanylyl cyclase could produce a difference in the rate of cGMP synthesis between rods and cones and alter sensitivity. Both rods and cones in mouse use the same retGC1 cyclase, but the expression level is likely to be higher in cones. Staining with a retGC1 antibody is brighter in cones than in rods (Dizhoor *et al.* 1994), and unpublished measurements on retinas lacking the neural retina leucine zipper (Nrl) transcription factor (Mears *et al.* 2001), where all photoreceptors are cone-like, indicate that mouse cones may have something like 2–3 times more retGC1 than rods (A. Dizhoor, personal communication). Cone PDE6 expression may also be greater than rod but probably by no more than a factor of 1.5 (Zhang *et al.* 2003; Lobanova *et al.* 2010); however, cone PDE6 has a higher basal activity (Majumder *et al.* 2015). Together the cyclase and PDE6 would produce a higher rate of cGMP turnover in darkness, which in salamander has actually been measured and is about 3-fold greater in cones than in rods (Cornwall & Fain, 1994; Cornwall *et al.* 1995). This increase in turnover rate would produce both an increase in the rate of response decay and a decrease in sensitivity (Rieke & Baylor, 1996; Nikonov *et al.* 2000; Fain *et al.* 2001). Measurements in salamander indicate that if turnover in a rod were increased by a factor of 3, sensitivity would be reduced by about a factor of about 2 (Cornwall & Fain, 1994; Nikonov *et al.* 2000).

The rate of cGMP synthesis is also controlled by GCAP proteins, which in turn are regulated by the outer-segment Ca^{2+} concentration. Although the GCAPs themselves are similar in rods and cones, the change in Ca^{2+} concentration is considerably faster in cones, at least in salamander (Sampath *et al.* 1999). Rods and cones express different isoforms of the Na^+/Ca^{2+}–K^+ exchanger (Lytton, 2007; Vinberg *et al.* 2015) and may have different concentrations or isoforms of Ca^{2+} buffers. This accelerated decline in Ca^{2+} would produce a more rapid modulation of the GCAPs and faster activation of the cyclase, which could in theory decrease cone sensitivity. This notion has, however, been tested by deleting the genes for the GCAP proteins, which increases sensitivity by about the same factor of 3 in both rods (M. L. Woodruff & G. L. Fain, unpublished observations; Gross *et al.* 2012) and cones (Sakurai *et al.* 2011). These observations indicate that GCAP-mediated feedback makes little contribution to the sensitivity difference (however, see Wen *et al.* 2014). A similar conclusion emerges from comparison of salamander rod and cone responses under conditions that suppress changes in outer-segment Ca^{2+} (Matthews *et al.* 1988, 1990; Nakatani & Yau, 1988, 1989).

In conclusion, the rate of inactivation of transduction is slower in rods than in cones, with the major effects apparently produced by the species of transducin and PDE6, the expression level of cyclase, PDE6 basal activity, and the expression level of the GAP proteins. Each of these differences seems, however, to make a relatively small contribution, and once again no single change predominates.

We have based our conclusions on results from mouse, but it is possible and even likely that additional adaptations are present in other species that contribute to the difference in rod and cone inactivation. Fish are of particular interest, because the difference in rod and cone sensitivity can be much larger than in mouse (Tachibanaki *et al.* 2001). Kawamura's laboratory has shown that fish cones have a very high rate of pigment phosphorylation by GRK7 (Tachibanaki *et al.* 2005), an enzyme highly expressed in fish but not present in mouse (Liu *et al.* 2005). Moreover carp also show a much higher rate of cGMP synthesis in cones than in rods, and therefore a higher cGMP turnover rate (Takemoto *et al.* 2009). These changes would collectively cause photoresponses from carp cones to be smaller and faster (Tachibanaki *et al.* 2005;Liu *et al.* 2005; Takemoto *et al.* 2009). In addition, Rebrik and Korenbrot have identified a Ca^{2+}-binding protein present in fish cones but not fish rods that reduces the affinity of cyclic nucleotide-gated ion (CNG) channels for cGMP in high $[Ca^{2+}]_i$, a protein they first called CNG-modulin (Rebrik *et al.* 2012), but later identified as echinoderm microtubule-associated protein-like 1 (EML-1, Korenbrot *et al.* 2013). Knockdown of this protein in zebrafish produced a 5-fold increase in cone sensitivity (Korenbrot *et al.* 2013), presumably by slowing the rate at which CNG channels open following illumination. This protein has not as yet been identified in mammalian cones.

Why are rods more sensitive?

The key step in the formation of the duplex retina of vertebrates was the evolution of more sensitive rods to accompany cones, so that the entire range of light intensities could be encoded by the photoreceptors. Molecular and biochemical studies tell us that rods and cones have many of the same transduction proteins but use different isoforms probably arising by gene duplication (see Table 1); in some cases they use the same isoform but at a different level of expression. No one change accounts for the difference in absolute sensitivity between rods and cones. Instead, each of the differences we have described seems to have produced a small increase in the rate of activation or prolongation of response decay, conferring an incremental advantage to the organism.

Accumulated changes in a large number of proteins eventually produced a sensitivity great enough in the rod to allow it to operate in dim light, with cones remaining for enhanced temporal resolution when photon flux is no longer limiting. These changes also have

implications for the dynamic properties of rods and cones, namely their ability to adapt to increasing light intensity. While we have not discussed these mechanisms in this review, the fundamental tradeoff between sensitivity and dynamic range between rods and cones will also depend upon differences in their transduction mechanisms. The properties of the two receptor types form the basis of our duplex visual system, whose fundamental nature was first proposed by Schultze 150 years ago.

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Additional information

Competing interests

None declared.

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