Light responses of primate and other mammalian cones

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Retinal cones are photoreceptors for daylight vision. For lower vertebrates, cones are known to give monophasic, hyperpolarizing responses to light flashes. For primate cones, however, they have been reported to give strongly biphatic flash responses, with an initial hyperpolarization followed by a depolarization beyond the dark level, now a textbook dogma. We have reexamined this primate-cone observation and, surprisingly, found predominantly monophasic cone responses. Correspondingly, we found that primate cones began to adapt to steady light at much lower intensities than previously reported, explainable by a larger steady response to background light for a monophasic than for a biphatic response. Similarly, we have found a monophasic cone response for several other mammalian species. Thus, a monophasic flash response may in fact be the norm for primate and other mammalian cones as for lower-vertebrate cones. This revised information is important for ultimately understanding human retinal signal processing and correlating with psychophysical data.

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**Results**

**Flash-Response Sensitivity and Kinetics.** We recorded from a total of 112 cones from 12 macaque monkeys (Materials and Methods), and found 97 of them (~90%) to give monophasic flash responses regardless of spectral type (Fig. 1 A–C, Left). Fig. 1D (Left) shows biphatic responses from a minority of the cones, consisting of an initial light-induced reduction in the inward dark current with respect to the outer-segment membrane that, upon recovery from light, is followed by an undershoot, i.e., an enhanced inward dark current. The amplitude of the response undershoot first increased with flash intensity, then decreased with further flash-intensity increase beyond saturation of the inward-current reduction, as found previously (12). Separately, we performed whole-cell voltage-clamp recordings from the inner segment of five macaque cones (Materials and Methods), and likewise found only one cell to show a (rather mild) response undershoot (Fig. S1) (refs. 17–19, but cf. ref. 20).

We have also examined, with suction-pipette recording, cones from pig, ground squirrel, Nile grass rat, and mouse, and found the norm to be an absence of the flash-response undershoot (5 of 5, 27 of 27, 9 of 9, and 30 of 34 cells, respectively; Fig. 1 E–H). Previously, others have found ground-squirrel cones to show monophasic flash responses, but approximately one third of them develop over time a small response undershoot during recordings (21); a substantial fraction of chipmunk cones also gave biphatic responses (22) (Discussion). For mouse cones, no response undershoot has been reported (23).

In lower vertebrates, different spectral cone types of a given animal species show quite dissimilar flash sensitivities, with blue cones being the most sensitive (4–6, 24). In contrast, monkey L-cones (red), M-cones (green), and S-cones (blue) were found to have similar sensitivities (11, 12). We confirmed the latter observation, obtaining half-saturating flash intensities ($\alpha$) of $1.845 \pm 740, 1.665 \pm 920$ and $1.640 \pm 800$ photons$\cdot\mu$m$^{-2}$ (mean $\pm$ SD; $n = 10, n = 8$, and $n = 5$), respectively, for macaque L-, M-, and S-cones at near their respective wavelengths of maximal sensitivity ($\lambda_{max}$) (Fig. 1 A–C, Right, Table 1, and Materials and Methods), matching previous measurements (12). Thus, the monophasic or biphatic nature of the response does not affect flash sensitivity, which is inversely proportional to $\sigma$. Pig was similar to monkey in cone sensitivity (Table 1). The M- and S-cones of ground squirrel likewise were similar to each other in sensitivity (see also ref. 21), but both were $\sim$10-fold less sensitive than monkey cones (Table 1). Nile grass rat was broadly similar to ground squirrel, and mouse was in between monkey and ground squirrel (see also ref. 23) (Table 1). Overall, rodents showed substantially lower cone sensitivity than primate and pig, although the associated functional significance and underlying mechanism remain unclear. This difference does not appear to be related to nocturnal vs. diurnal habitat because macaque monkey (diurnal) and pig (arguably diurnal) cones are much more photosensitive than ground squirrel (diurnal) and Nile grass rat (arguably diurnal; ref. 25) cones, whereas mouse (nocturnal) cones are in between.

The single-photon response amplitude, $a$, is calculated as $S_d/A_d$, where $S_d$ is dim-flash sensitivity in picamperes per photon $\times$ micrometer square ($\text{pAphotons}^{-1}\mu\text{m}^2$) and $A_d$ is the effective

**Significance**

We aimed to solve a longstanding conundrum about the light response of primate cones. Unlike those of lower vertebrates, the primate cones’ response to light has long been reported as being biphatic. This surprise has also raised a yet-unanswered question about the requisite signal processing in the retina. More recently, human paired-flash electroretinographic data have challenged the biphasic waveform of the primate cone response. Our suction-pipette recordings from single primate cones now show directly that the light responses of primate and other mammalian cones are in fact very predominantly monophasic, much like those in invertebrates.


The authors declare no conflict of interest.

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collecting area of the cone outer segment, both at $\lambda_{\text{max}}$ (Table 1 and Materials and Methods). Across the animal species studied here, a covaries qualitatively with $1/\sigma$ (Table 1). The dim-flash response's time to peak ($t_{\text{peak}}$), which reflects to some degree the speed of response termination, also broadly covaries with $\sigma$ across species (Table 1), such as would happen if the Ca$^{2+}$ feedback that regulates the cone light response (26) somehow differed in degree across species. It is currently unclear whether this is the case, and, if so, why. Whether for monophasic or biphasic responses, the $t_{\text{peak}}$ of primates as found by us ($\sim$40 ms, with low-pass filtering at DC to 200 Hz) is generally faster than previously reported (with low-pass filtering at DC to between 20 and 150 Hz; see refs. 9–13), although our value is still slower than that extracted from human ERG recordings ($\sim$20 ms) (15, 16). In the latter case, the cone response is extracted from the ERG recordings by using a rod-saturating background (15), so its $t_{\text{peak}}$ is likely shortened by light adaptation. Table 1 lists the dim-flash response's integration time, $t_{\text{int}}$ (see legend to Table 1), which, except for the biphasic cells, broadly covaries with $t_{\text{peak}}$ (Adaptation to Background Light).

The saturated cone-response amplitude ranged mostly from 20 to 40 pA across species, but was distinctly lower for pig and mouse cones (Table 1). For mouse cones, this difference at least partly reflects the recording method. Mouse cones, being buried among the rods, cannot be individually identified; at the same time, their outer segment is quite fragile (see also ref. 23). Thus, instead of recording from a single targeted outer segment with a suction pipette as conventionally done, we drew several inner segments/somata in the distal-most two rows of cell bodies of the outer nuclear layer (where cone somata are situated) of the Gnat1$^{-/-}$ (i.e., rod-transducin KO) mouse into a recording suction pipette with a tip inner diameter intentionally large enough to fit several cells so as to increase the chance of including a cone cell (Materials and Methods) (23). By trial and error, a cone cell could be recorded from along with several nonphotoresponsive rods. As such, a fraction of the cone's dark current was probably not recorded. Also, considering the low density of mouse cones ($\sim$3% of all photoreceptors; ref. 27), the chance of more than one cone being recorded was very low. As for pig, a single cone

![Fig. 1. Flash-response families of monkey and other mammalian cones. (A–C) Flash-response families of a monkey L-, M-, and S-cone (Left), with corresponding intensity-response relations at transient peak of response (normalized by the saturated response, $R_{\text{max}}$; Right). Curve fits are with a saturating-exponential function (Materials and Methods). The half-saturating flash intensity, $\sigma$, is 2,114, 2,634, and 1,831 photons $\mu$m$^{-2}$, respectively, at near corresponding $\lambda_{\text{max}}$ values (Materials and Methods). (D) An L-cone showing biphasic responses, with $\sigma$ being 1,765 photons $\mu$m$^{-2}$ at near $\lambda_{\text{max}}$. (A) Outer-segment recording; (B–D) inner-segment recordings. (E–H) Flash responses of pig, ground squirrel, Nile grass rat, and mouse cones (Left), with corresponding normalized intensity-response relations (Right) having $\sigma$ values of 1,006, 20,700, 24,900, and 5,020 photons $\mu$m$^{-2}$, respectively, at near corresponding $\lambda_{\text{max}}$ values (Materials and Methods). In all cases, flash is at time 0, and traces are averages of 2 to 15 responses.](https://www.pnas.org/content/111/7/2753)
reflect a change from the normal balance in amplitude and/or result of negative feedback (e.g., refs. 12, 28). Its emergence may of the response undershoot at near-saturating flash intensities. Flash intensities A monkey L-cone showed spontaneous, all-or-none, appearance/disappearance that happened to show biphasic responses, we lowered the ex-

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<th>Type</th>
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<th>$\sigma$, photons$^{-1}$m$^{-2}$</th>
<th>$t_{\text{trans}}$, s</th>
<th>$t_{\text{f}}$, s</th>
<th>$S_F$, pA photon$^{-1}$m$^{-2}$</th>
<th>$a$, pA photon$^{-1}$</th>
<th>$A_{\lambda,\text{B}}$ s$^{-2}$</th>
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<td>1,845 ± 740</td>
<td>43 ± 2</td>
<td>72 ± 3</td>
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<td>40 ± 4</td>
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<td>27 ± 16*</td>
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<td>16,300 ± 6,800</td>
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<td>20,800 ± 4,200</td>
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<td>70 ± 8</td>
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$t_{\text{trans}}$, integration time of dim-flash response, given by $\int ft(\text{df})$, where $f(t)$ is the response waveform and $t_f$ is the waveform’s transient-peak amplitude. All data, given as mean ± SD, are derived from responses low-pass-filtered at DC-200 Hz (eight-pole Bessel). Monkey cones indicated by “US” and marked by an asterisk gave biphasic responses, i.e., with an undershoot (note the correspondingly low $t_{\text{trans}}$). Eight of the monkey cones listed are from outer-segment recordings (four L-, two M-, and one S-cone, together with one L-cone that showed undershoot), but their results were similar to those obtained with inner-segment recordings. For the parameter $A_{\lambda,\text{B}}$, see Data Analysis in Materials and Methods. The table lists only those cells that were stable enough to provide all of the indicated response parameters, which is why their total number (e.g., monkey cones) do not necessarily match the total number of cells recorded as stated in the text.

was recorded in the standard “outer-segment-in” configuration, but they are very fragile and could have sustained some injury.

**Effect of External Ca$^{2+}$**: The response undershoot is most likely a result of negative feedback (e.g., refs. 12, 28). Its emergence may reflect a change from the normal balance in amplitude and/or time course between the cGMP-phosphodiesterase (PDE) and guanyl cyclase (GC) activities underlying phototransduction, controlled in part via their negative-feedback modulations by Ca$^{2+}$ (reviewed in ref. 29). For several of the recorded monkey cones that happened to show biphasic responses, we lowered the extracellular Ca$^{2+}$ concentration and were indeed able to remove the undershoot (four of four cells; Fig. 2A); this change was reversible upon restoring normal Ca$^{2+}$ concentration. Because the same extracellular Ca$^{2+}$ concentration (1.2 mM) was used in the past (e.g., ref. 12) and the present work, this cannot account for the different response behaviors in the two cases. Nonetheless, this observation does reinforce the notion that the Ca$^{2+}$ feedback is somehow involved. In our experiments, we also found that several macaque cones with a response undershoot (four of 15 cells) showed the phenomenon of spontaneously losing this undershoot in an all-or-none and reversible manner, at least at near-saturating flash intensities (Fig. 2B).

**Adaptation to Background Light**. Because the predominantly monophasic flash responses we observed in macaque cones were unlike previous findings (9, 10, 12), we reexamined their adaptation to steady light. In the incremental-flash-on-background experiment on L-cones (Fig. 3A), the reduction in flash sensitivity by steady light followed the Weber–Fechner relation: $S_F/S_F^0 = (1 + IB/I_0)^{-1}$, where $S_F$ is flash sensitivity in the presence of steady light of intensity $I_B$. $S_F^0$ is dark-adapted flash sensitivity (i.e., no background light), and $I_0$ is the intensity of $I_B$ at which $S_F/S_F^0 = 1/2$. The $I_0$ value, which we found to be 9,000 ± 5,300 photons$^{-1}$s$^{-1}$ at $\lambda_{\text{max}}$ (n = 4), is considerably lower than previous measurements [20,000–140,000 photons$^{-1}$s$^{-1}$, with a mean of 71,000 photons$^{-1}$s$^{-1}$ (12)]. In these previous measurements, because the flash responses were biphasic, they had a very small integration time ($t_{\text{int}}$; Table 1 legend). Consequently, the cone’s steady-state response to a given steady-light intensity would also be small—much smaller than would be the case for a monophasic flash response. It is therefore not surprising that a cone with biphasic responses begins to adapt to background light only at much higher intensities, i.e., has a much larger $I_0$ value (see ref. 30 for a discussion on rods that should apply to cones here). For ground squirrel (Fig. 3B), which is the only other mammal examined here for background adaptation by cones, the $I_0$ value in comparison with that of monkey is almost 20-fold higher (142,000 ± 90,000 photons$^{-1}$s$^{-1}$ at $\lambda_{\text{max}}$; n = 5; note that the data in Fig. 3A, Right, and Fig. 3B are plotted on normalized coordinates). Based on the same reasoning, this finding can be explained (although this should not be pushed too quantitatively) by the ~10-fold lower cone flash sensitivity and approximately twofold shorter flash-response $t_{\text{int}}$ of ground-squirrel cones compared with monkey cones. Incidentally, others have reported an $I_0$ value of 30,000 photons$^{-1}$s$^{-1}$ for mouse M-cones and 70,000 photons$^{-1}$s$^{-1}$ for mouse S-cones at their respective $\lambda_{\text{max}}$ values (23), which are several-fold higher than the $I_0$ value we found for monkey L-cones. Again, this is qualitatively in line with

![Fig. 2. Appearance/disappearance of undershoot of monkey cone flash response.](image-url)
the several-fold lower flash sensitivity, but a comparable flash-response $I_{bas}$ of mouse cones compared with monkey cones.

**Discussion**

At least in our hands, monophasic rather than biphasic flash responses appear to be the norm for primate and other mammalian cones, hence similar to what is long known for lower-vertebrate cones. This finding is also in agreement with the conclusion drawn indirectly from human ERG experiments (15, 16).

By using low (0.1 mM) extracellular Ca$^{2+}$ concentration, we were able to convert the occasionally observed biphasic response into a monophasic response in monkey cones, reinforcing the notion that the Ca$^{2+}$ feedback on phototransduction is involved. The biphasic response is perhaps triggered by an abnormal increase in intracellular Ca$^{2+}$ concentration. Our (also occasional) observation of a spontaneous, all-or-none emergence of the response undershoot and its reversibility, as well as the observation by others that some ground-squirrel cones developed an undershoot during recordings (21), suggests that the balance between the PDE and GC activities underlying the light response may be delicate and labile, so that a response undershoot may appear when this balance is off and favors a domination by GC activity. At least for goldfish cones, we have sometimes noticed an emergence of biphasicity in their response in parallel with a progressive morphological deterioration of the recorded outer segment viewed under the microscope, consequently leading possibly to an accumulation of internal Ca$^{2+}$ caused by an excessive inward Ca$^{2+}$ leak and/or a defective Ca$^{2+}$ extrusion. Incidentally, although rods of primates and other mammals almost invariably give monophasic responses (28, 30, 31), we did notice that primate rod responses sometimes showed an initial biphasicity during recording, but became monophasic over time (28). This opposite time sequence in response behavior between primate rods and cones, when it happens, may arise from quantitative differences between the rod/cone phototransduction processes with respect to the underlying reaction kinetics and the strength of the Ca$^{2+}$ feedback. It further underscores a subtle inefficiency of photoisomerization, which happened also not to give biphasic cone flash responses.

As described in **Results**, we attribute the 10-fold higher $I_r$ value reported by Schnapf et al. (12) to the biphasic flash response they observed.

**Materials and Methods**

**Animals.** Monkey (Macaca fascicularis) eyes were obtained from the laboratories of Stephen Hsiao, Rudiger von der Heydt, and Matthias Ringkamp (The Johns Hopkins University, Baltimore). Eyes from normal-sized pigs (i.e., not miniswine; Archer Farms) were obtained from Sue Eller (The Johns Hopkins University School of Medicine, Baltimore). Thirteen-lined ground squirrels were purchased from TLS Research. Nile grass rats were provided by Laura Smale (Michigan State University, East Lansing, MI). Gnat$^{-/-}$ mice were provided by Ching-Kang Chen (Virginia Commonwealth University, Richmond, VA).

**Monkey Retina Preparation and Recording.** Under light-adapted conditions (at the end of the donor laboratory’s experiments unrelated to ours), an animal was anesthetized and euthanized, and the eyes removed (for one animal, the eyes were removed from the anesthetized animal before euthanasia). During transfer to our laboratory, the eyes were dark-adapted in a light-proof container (with a slit cut on the posterior eye capsule to promote O$_2$ penetration, but not cut open further to keep the pigment epithelium attached to the retina and thus promote visual-pigment regeneration) at room temperature (23 °C) in 95% O$_2$/5% CO$_2$-bubbled HCO$_3$⁻-Locke solution (in mM): 120 NaCl, 3.6 KCl, 1.2 CaCl$_2$, 2.4 MgCl$_2$, 10 glucose, 10 Heps, 20 NaHCO$_3$, 3 Na$_2$-succinate, pH 7.4 with NaOH. Upon arrival at the laboratory (within an hour after enucleation), the anterior part of both eyes was removed, leaving the eyecups, and stored as such in the dark at room temperature under 95% O$_2$/5% CO$_2$-bubbled HCO$_3$⁻-Locke solution for as long as 24 h until use. When needed, a retina in an eyecup was peeled from...
the pigment epithelium under Locke solution, and a small piece was removed by cutting (with the rest returned to storage as before), put on nitrilene, face down, cut into slices 200 μm thickness, transferred into the recording chamber, and anchored with silicone grease.

For two retinas, we also compared the responses of cones under the condition of storing the retina in Locke solution at room temperature (n = 6) vs. in L-15 medium at 4 °C (n = 18), with the latter having been reported to make the stored retina less healthy and to reduce the rod single-photon-response amplitude/accelerate the rod response kinetics (33). Although we found no obvious difference between the two conditions with respect to the cone-response parameters (including the absence/presence of the undershoot), we adhered to Locke solution at room temperature just to be on the safe side.

Light was from a 75-W xenon arc lamp, with most IR removed with a water filter and the wavelength and intensity controlled by 10-nm interference filters and neutral-density filters, respectively. The beam went through an electronic shutter and was delivered to the microscope via a liquid light-guide. Flashes were 12.1 μs (by measurement) in duration and 2.5 to 10.0 s in interflash interval depending on the intensity, sufficient for full recovery after each stimulus. The tpeak of the flash response was measured as the duration between the middle of the flash and the transient peak of the response. A second light beam was used for background light in adaptation experiments. The light intensity was periodically calibrated with a radiometer.

The stimulation wavelengths were 560 nm, 530 nm, and 440 nm for monkey L-, M-, and S-cones, respectively.

Suction-pipette recording was used for almost all experiments, either from a single monkey cone outer segment (for approximately one third of the cones recorded) or from its inner segment (for the rest). The purpose of recording the inner segment was to avoid tosinoelectrical changes in extracellular CaCl2 (instead of our standard 2.4 mM MgCl2, 1.2 mM CaCl2) was also tried; we found no obvious difference between the two conditions with respect to the cone-response parameters (including the absence/presence of the undershoot), we adhered to Locke solution at room temperature just to be on the safe side.

For all rodents, the animal was dark-adapted overnight, euthanized with pentobarbital sodium, and the eye was immediately removed. After washing, the rod outer segments, the inner segments, the outer segments with suction-pipette recording following ref. 23, but using retinal slices and slightly smaller suction pipettes with tip openings of 3.5–4.5 μm. Several cell bodies and adjoining inner segments near the outer boundary of the outer nuclear layer were drawn blindly into the suction pipette to increase the chance of including a cone cell. The use of Gnat-labeled mouse, with nonphotoreceptive rods, facilitated the recording.

Pig has two cone types, L- and S-cones, with λmax at 556 nm and 439 nm, respectively (34); the cones we recorded from turned out to be all L-cones, but we did not determine their exact λmax. The stimulating wavelength was 560 nm. Ground squirrel has M- and S-cones, with λmax reported to be 520 nm and 435 nm, respectively (21); from just a few measurements, we found broadly similar values of 524 nm and 440 nm, respectively. We used stimulation wavelengths of 520 nm and 440 nm, respectively. Nile grass rat also has M- and S-cones (35), but we encountered only M-cones, with λmax measured at 522 nm. We used a stimulating wavelength of 520 nm. Finally, mouse has M- and S-cones, with λmax at 508 nm and 360 nm, respectively (23). Because the great majority of mouse cones coexpress M- and S-opsins, we designated those cones with the spectral-sensitivity ratio 3μg/250 < 1 as M-cones, and those with 3μg/250 > 1 as S-cones. We used stimulating wavelengths of 500 nm and 380 nm, respectively.

The saturated flash responses of some cones showed an early transient that subsequently relaxed to a slightly lower “plateau.” This transient is presumed to reflect a hyperpolarization-activated membrane current (Ih) (23). Thus, for these cells, the nonsaturated cone responses were normalized to the plateau level.

Data Analysis. Intensity-response relations were fit with the saturating-exponential function R = Rmax × 1 − exp(−I/Ic) where R is the flash-response amplitude, Rmax is the maximum response amplitude, Ic is the flash intensity, and ρ is a constant. The half-saturating flash intensity, I1/2, is given by ρ = ρI1/2.

The effective collecting area, Aext, of the cone outer segment for incident light transverse to the outer segment’s long axis is given by

\[ A_{ext} = \frac{2\pi \text{Rmax} \sqrt{V_s}}{f} \]

V_s is the volume of the outer segment, calculated to be 35 ± 7 μm^3 for monkey cones (based on the measured length as well as tip and base diameters of 26 randomly chosen cone outer segments). This gave an Aext of 0.43 ± 0.1 μm² for monkey cones, similar to the 0.37 μm² used by Schnapf et al. (12). Likewise, Aext for pig, ground squirrel, and Nile grass rat was calculated as 0.3 ± 0.06 μm² (n = 8), 0.63 ± 0.09 μm² (n = 16), and 0.2 ± 0.05 μm² (n = 9), respectively. Our Aext value for ground squirrel is similar to that used by Kraft (21). For mouse, we simply adopted the value of 0.2 μm² found by Nikonov et al. (23).

The activation phase of the normalized flash response, R/Aext, was fitted with the Lamb–Pugh model (37) of phototransduction, given by

\[ R/A_{ext} = 1 - \exp\left(-\frac{\Phi}{\Phi_{sat}}\right) \]

where \( \Phi \) is the number of photon-iso-merizations (given by \( I_t \times A_{ext} \)), \( A_{ext} \) is the “amplification constant,” and \( \Phi_{sat} \) is an effective time delay contributed by all short phototransduction steps. By fitting this function to the response’s activation phase with \( A_{ext} \) and \( \Phi_{sat} \) as free parameters, we obtained an \( A_{ext} \) value of 3.4 to 4.2 μm² for \( \Phi_{sat} \) of 9.4 ± 5.5 ms (n = 23) for monkey. In this paper, we did not make use of the \( A_{ext} \) and \( \Phi_{sat} \) values, but the \( A_{ext}< \) values are included in Table 1 for reference.

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