Origin and effect of phototransduction noise in primate cone photoreceptors

Juan M Angueyra & Fred Rieke

Noise in the responses of cone photoreceptors sets a fundamental limit on visual sensitivity, yet the origin of noise in mammalian cones and its relation to behavioral sensitivity are poorly understood. Our work here on primate cones improves understanding of these issues in three ways. First, we found that cone noise was not dominated by spontaneous photopigment activation or by quantal fluctuations in photon absorption, but was instead dominated by other sources, namely channel noise and fluctuations in cyclic GMP. Second, adaptation in cones, unlike that in rods, affected signal and noise differently. This difference helps to explain why thresholds for rod- and cone-mediated signals have different dependencies on background light level. Third, past estimates of noise in mammalian cones are too high to explain behavioral sensitivity. Our measurements indicate a lower level of cone noise and therefore help to reconcile physiological and behavioral estimates of cone noise and sensitivity.

Daylight vision relies on high sensitivity to subtle changes in the spatial pattern, contrast and chromaticity of light inputs. Noise in the responses of cone photoreceptors places a fundamental limit on such sensitivity. Here we sought to improve our understanding of the magnitude, origin and properties of noise in the responses of primate cones, particularly in regards to the implications of cone noise for visual function.

Rods and rod vision provide a useful point for comparison. In darkness, noise in rods consists of occasional photon-like events that originate from the spontaneous activation of the photopigment rhodopsin and continuous fluctuations that originate from spontaneous activity of other components of the phototransduction cascade. The low level of rod noise permits detection of single absorbed photons. Dark-adapted behavioral sensitivity approaches the limits set by rod noise and statistical fluctuations associated with the division of light into discrete quanta. The similarity of rod and behavioral noise requires that the retinal readout of rod responses operate efficiently, a constraint that has guided investigation of the underlying circuitry. In the presence of dim backgrounds, quantal fluctuations dominate rod noise. As a consequence, the detection sensitivity of rod responses scales with the square root of the background light level, this scaling is consistent with the classic Rose-DeVries region of behavioral threshold-versus-intensity curves.

The situation is much less clear for cones and cone-mediated vision. Although the rate of spontaneous activation of cone photopigments is much higher than that of rhodopsin, the kinetics of noise in primate cones suggests that most noise originates downstream of the photopigment. However, measured noise in primate cones is too high to account for behavioral sensitivity, suggesting that one or both estimates are in error. Thus, the functional importance of cone noise remains unclear. Furthermore, over a wide range of backgrounds, behavioral thresholds for cone-mediated vision increase linearly with background (the classic Weber region), a property that is important for coding contrast independently of background light level. It is unclear, however, how the Weber region relates to the background dependence of cone signal and noise.

Here, we characterized signal and noise in primate cone photoreceptors and their dependence on background light level. Our measures of cone noise and detection thresholds were considerably lower than past estimates, helping to reconcile cone physiology with behavioral measures of the sensitivity of cone vision. Furthermore, we found that adaptation affected cone signal and noise very differently, providing a natural explanation for the Weber region of behavioral threshold-versus-intensity curves.

RESULTS

Our results are divided into four parts. First, we measured the empirical properties of noise in primate cone photoreceptors. Second, we manipulated the cone phototransduction cascade to identify where noise originates. Third, we determined how light-adaptation affected the signal and noise of primate rod and cone responses. Fourth, we explored how detection thresholds for rod and cone responses depended on background light level.

Cone noise exhibits several distinct temporal components

We started by characterizing the amplitude and kinetics of noise in the responses of primate cones. Past work indicates that cones are noisy, with most noise originating downstream of the photopigment in the transduction cascade. We felt that it was important to begin with similar experiments, given that past measures of cone noise exceed the noise inferred from behavior and that the properties of cone noise are a foundation for the rest of our study.

We recorded the current responses of voltage-clamped long or middle wavelength–sensitive cones (L cones and M cones, respectively)
to brief 100% contrast flashes (producing ~50 opsin isomerizations, or $R^*$) in the presence of a moderate background (Fig. 1a). Individual responses to such flashes were difficult to distinguish from baseline noise, but the response could be uncovered by averaging multiple trials (Fig. 1a). The recorded current fluctuations included noise arising in the outer segment, noise arising from conductances in the inner segment and noise produced by the recording itself (instrumental noise). Exposing cones to a near-saturating light step closed the inner segment noise up to high temporal frequencies (600 Hz); noise spectra from foveal cones ($\tau_{osc} = 397.1$ ms and $\varphi = -79.5$ degrees). Current recorded in bright light had three clear components and was fit empirically as the sum of a low-frequency component with the shape of the dim flash response and two lorentzian functions with distinct corner frequencies ($10$ Hz and $150$ Hz). In the presence of moderate steady light ($\tau_{osc} = 20.2$ ms, $\tau_{decay} = 75.6$ ms, $\tau_{sc} = 397.1$ ms and $\varphi = -79.5$ degrees). Current recorded in bright light is at the top (gray trace). (b) Power spectra of noise in constant and bright light from the cone shown in a. (c) Power spectrum of the outer segment cone noise (black circles, noise in background minus that in saturating light from b) and fit (red trace) obtained by summing a scaled version of the power spectrum of the fitted average dim flash response and two lorentzian functions (equation (5)) with distinct corner frequencies (18 Hz and 170 Hz, black lines). (d) Average outer segment cone noise measured at 5,000 $R^*$ s$^{-1}$ ($n = 6$, black circles represent mean ± s.e.m.) and fit as in e (red trace). Corner frequencies of lorentzian functions were 24 Hz and 200 Hz. (e) Average outer segment cone noise measured in darkness from foveal cones ($n = 7$, black circles represent mean ± s.e.m.) and fit as in c (red trace). Corner frequencies of lorentzian functions were 10 Hz and 150 Hz.

We characterized noise by calculating average power spectra from stretches of data without flashes (Online Methods). Assuming that outer segment and inner segment/instrumental noise are independent, we isolated outer segment noise by subtracting the spectrum in bright light from that in moderate light (Fig. 1c). This process underestimates the total outer segment noise, as bright light did not fully suppress the current; such errors were small, however, as the subtracted noise was 20–100 fold smaller than that measured in darkness or in the presence of moderate steady light (Fig. 1b). We isolated outer segment cone noise similarly in all subsequent experiments.

Both noise generated in the cone phototransduction cascade (intrinsic noise) and noise generated by quantal fluctuations in photon absorption (extrinsic noise) contribute to outer segment noise; extrinsic noise followed Poisson statistics and was absent in complete darkness (see below). Assuming linearity of the cone response, both extrinsic noise and noise from spontaneous pigment activation should have a power spectrum similar to that of the cell’s dim flash response (Fig. 1c). As has been described previously$^{11}$, cone noise instead extended to much higher frequencies, indicating a substantial contribution from events with faster kinetics than the single photon response. By recording noise in voltage clamp, we characterized outer segment noise up to high temporal frequencies (600 Hz); noise spectra had three clear components and were fit empirically as the sum of a low-frequency component with the shape of the dim flash response and two lorentzian functions with distinct corner frequencies (equation (5)). Adequate fits were obtained on a single cell basis (Fig. 1c) or by averaging over a population of L and M cones recorded with the same background illumination (5,000 $R^*$ s$^{-1}$, Fig. 1d). Based on these fits, the lowest frequency component accounted for ~30% of the total variance, which corresponds to the maximal fraction of noise that could be attributed to both spontaneous and background opsin activation.

To separate phototransduction noise from extrinsic noise, we repeated these experiments in complete darkness, targeting foveal cones to eliminate possible contributions from rod photoreceptors coupled to cones via gap junctions$^{12}$. The average noise showed less power at low frequencies (Fig. 1e), such that at most ~10% of the total current variance could be attributed to spontaneous opsin activation. L and M cones exhibited similar noise and, in particular, did not show the ~50-fold difference that would be expected from the scaling of spontaneous opsin activation rates with wavelength$^{14}$.

Although most low-frequency noise is not associated with spontaneous pigment activation, we can still define a rate of pigment activation that would produce an equivalent level of noise. Experiments described below indicate that this equivalent dark noise is ~600 $R^*$ s$^{-1}$, substantially lower than previous estimates$^{11}$. First, however, we describe experiments identifying the sources of noise in the transduction cascade.

Two dominant sources of noise: pharmacology

Rod and cone photoreceptors share the same basic phototransduction scheme, yet their light responses differ considerably in sensitivity and kinetics. Does noise in rods and cones also differ in origin? Rod noise is dominated by early events in phototransduction, namely spontaneous activation of rhodopsin and phosphodiesterase (PDE)$^{1–3}$. We found that open and close transitions in the cGMP-gated channels and fluctuations in cGMP concentration dominated cone noise.

Because the low- and mid-frequency noise components that we identified overlapped spectrally (Fig. 1), we focused on a frequency range (up to 20 Hz) that includes contributions mainly from both...
low- and mid-frequency components and a range (100–600 Hz) that is dominated by the high-frequency component. To maximize sensitivity to pharmacological manipulations that produced modest changes in the phototransduction cascade, we used two approaches to compare noise before and after drug application in single cells. First, we delivered membrane-impermeable drugs (specifically 8-bromo-cyclic-GMP, 8′Br-cGMP) using a two-electrode recording technique in which both a recording electrode and a drug-delivery electrode were sealed onto a single cone cell body. After attaining whole-cell mode with the recording electrode and getting baseline measurements of noise and light response (in under 30 s), we achieved access with the drug-delivery electrode and monitored noise and signal as the drug was introduced into the cytoplasm. Second, we delivered membrane-permeable drugs (specifically 3′-isobutyl-1′-methylxanthine, IBMX) using a puffer pipette located near the cone outer segment after recording baseline noise and light response.

Control experiments for two-electrode recordings

Because the two-electrode technique has not been used in cones, we started by checking for artifactual changes in noise. We first used a normal internal solution in both electrodes, keeping the phototransduction cascade as intact as possible (Fig. 2a). Rupturing the membrane occluding the tip of the second electrode did not substantially change the holding current (Fig. 2b) or the kinetics or amplitude of the light response (Fig. 2c), although some alterations in noise were apparent (Fig. 2c,d). We collected results across cells by computing the ratio, at each temporal frequency, of the noise spectra before and after rupturing the membrane at the tip of the second electrode (Fig. 2e); only pharmacological manipulations that produced changes in this ratio larger than the control case were deemed real.

Next, we tried to eliminate all outer segment noise pharmacologically. Given that all phototransduction noise sources ultimately cause fluctuations in the current flowing through the cGMP-gated channels, one way to eliminate noise is to promote depletion of the internal cGMP, which will, in turn, cause the cGMP-gated channels to close (Fig. 2f). cGMP is produced from GTP by the guanylate cyclase; thus, we omitted GTP from the electrode solution. We also omitted ATP, as transfer of its high-energy phosphate can produce GTP15. Hydrolysis of cGMP by PDE via transducin activation requires considerably lower concentrations of GTP than synthesis3,16, and will therefore contribute to reducing the cGMP concentration. As expected, currents steadily decreased (that is, cGMP-gated channels closed) in two-electrode recordings using internal solutions lacking both ATP and GTP (Fig. 2g); this was accompanied by a loss in the light response and a marked attenuation of the current fluctuations (Fig. 2h). The decrease in noise spanned all relevant frequencies (Fig. 2i,j) and had the same spectral characteristics as the decrease in noise seen during exposure to near-saturating light steps, which also closed the cGMP-gated channels (Fig. 1a,b). We obtained similar results in recordings with single electrodes filled with a solution lacking ATP and GTP ($n = 3$, data not shown).
This pharmacological manipulation served several purposes. First, it confirmed that most of the noise in our recordings originates in the cone outer segment. Second, it revealed that manipulation of the electrode solutions could affect the phototransduction cascade. Third, the current changes provide a basis for evaluating experiments in which a synthetic agonist is used to open the cGMP-gated channels.

**Channel noise**

We used two-electrode experiments to isolate noise produced by cGMP-gated channels. Changes in cGMP were again suppressed by omitting ATP and GTP from the internal solution, and cGMP channels were activated with 8′Br-cGMP, a potent agonist of the cGMP-gated channels (Fig. 3a). Because 8′Br-cGMP is also poorly hydrolyzed by PDE, it suppresses activity in the PDE arm of the phototransduction cascade. Introduction of 8′Br-cGMP through the second electrode would ideally occur only after the internal cGMP has been depleted, but this process can take up to 5 min (Fig. 2g), and long recordings with two electrodes are technically difficult. Instead, we relied on shorter experiments in which we compared noise in the presence of different concentrations of 8′Br-cGMP with that in its absence (Fig. 2j).

We empirically determined appropriate concentrations of 8′Br-cGMP and found that modest concentrations (27 µM) rescued (and even overshoot) the loss in holding current (Fig. 3b) and noise (Fig. 3c) produced by the omission of GTP and ATP alone (Fig. 2g–i). Under these conditions, channels opened by the added 8′Br-cGMP dominated measured currents and noise. Higher concentrations produced a further increase in holding current (Fig. 3d) and noise (Fig. 3e) compared with baseline.

The concentration of 8′Br-cGMP in the outer segment slowly approaches a steady level for two reasons. First, 8′Br-cGMP delivered in the inner segment has to diffuse to the outer segment to open cGMP channels. Second, PDE can still hydrolyze 8′Br-cGMP at a slow rate. Together, these issues cause a slow drift in holding current and artifactual increases in noise below 10 Hz, making changes in noise in that frequency range difficult to interpret. Thus, we focused on changes in noise between 10 and 600 Hz (Fig. 3c,e).

With activity of the transduction cascade suppressed, activation of the cGMP-gated channels produced fluctuations in current extending from low frequencies to at least 600 Hz. The increase in noise scaled with the concentration of 8′Br-cGMP (Fig. 3f). High-frequency noise was similar under control conditions (without 8′Br-cGMP) and with a concentration of 8′Br-cGMP that matched the initial dark current (Fig. 3b,c). These results indicate that the component of cone noise that extends from low to high temporal frequencies originates from gating transitions in the cGMP-gated channels.

**Noise resulting from fluctuations in cGMP**

Our results indicate that channel fluctuations produce noise extending from low to high temporal frequencies. To test for other sources of low- to mid-frequency noise, we puffed the membrane-permeable PDE inhibitor IBMX onto the outer segments of voltage-clamped cones. IBMX has two effects: it decreases baseline hydrolysis of cGMP, leading to an increase in [cGMP] and opening of cGMP-gated channels, and it decreases the fluctuations in [cGMP] produced by dark activation of any of the transduction components upstream of (and including) PDE (Fig. 4a). We performed recordings in darkness to avoid extrinsic noise and again focused on frequencies above 10 Hz to avoid artifacts induced by current drift.

Inhibition of PDE, as expected, reversibly increased the holding current (Fig. 4b) and slowed the light response (data not shown). This was accompanied by an increase in high-frequency noise, consistent with an increase in channel noise; in addition, noise in the low- to mid-frequency range (10–50 Hz) decreased (Fig. 4c,d). These changes in noise were significantly different from those elicited by puffing a vehicle solution lacking IBMX (Fig. 4e) in the two relevant frequency ranges (t test; 10–50 Hz, P = 0.0004; 100–600 Hz, P = 0.0068).

This experiment served several purposes. First, it corroborated the 8′Br-cGMP experiments, showing that the opening of extra cGMP-gated channels led to an increase in high frequency channel noise.

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**Figure 3** High-frequency noise arises from open and close transitions in the cGMP-gated channels. (a) Channel noise was separated from sources causing fluctuations in [cGMP] by suppressing cGMP synthesis by omitting ATP and GTP from the internal solutions in a two-electrode recording. Different concentrations of the cGMP-channel agonist 8′Br-cGMP were added to the second electrode. (b) Changes in holding current before (black) and after (green) the introduction of a second electrode containing 27 µM 8′Br-cGMP. The filled black and green circles represent 500-ms stretches of noise used to calculate the spectra in c. (c) Average noise power spectra for the cone in b (before) (black) and after (green) the introduction of a second electrode containing 27 µM 8′Br-cGMP. Insets show example noise traces in each condition, corresponding to 1 and 2 in b. (d,e) Data are presented as in b and e for 100 µM 8′Br-cGMP. (f) Average (± s.e.m.) ratio of power spectra before and after introduction of 8′Br-cGMP. The color scale corresponds to the concentration of 8′Br-cGMP (18 µM, n = 10; 27 µM, n = 4; 100 µM, n = 3; 200 µM, n = 2). Changes in noise below 10 Hz are unreliable as a result of slow drift in the measured current and are displayed as open circles. The increase at high frequencies (100–600 Hz) was significant across all concentrations (P < 0.05).
Second, it identified a noise source that was suppressed when PDE was inhibited and that therefore normally causes fluctuations in the cGMP concentration. Notably, this noise source has substantial power in the 10–50-Hz range, where opsin noise makes little or no contribution (Fig. 1c–e). This noise component resembles continuous noise in rods and fish cones.

Effect of adaptation on photoreceptor signal and noise

The effect of adaptation on detection of light stimuli depends on how it alters both signal and noise. For example, over a substantial range of backgrounds, rod signal and noise are equally affected by adaptation, such that the signal-to-noise ratio (SNR) is independent of adaptation7. As a consequence, the detection threshold for such backgrounds is limited by the Poisson statistics of photon absorption. This provides a simple explanation for the Rose-DeVries regime of behavioral threshold-versus-intensity curves, as the s.d. of a Poisson process scales as the square root of the mean8.

Given that much of cone noise originates late in the transduction cascade, it may be less affected by adaptation than cone signals. Thus, the relationship between photoreceptor adaptation and behavioral threshold-versus-intensity curves may be fundamentally different for rods and cones.

Rods

We began by characterizing the dependence of rod signal and noise on background. Although our overall conclusions are consistent with previous studies2, we felt that inclusion of the rod data was important to provide a direct comparison with our cone results.

We measured rod flash responses and noise across a range of backgrounds using suction electrodes18. Background light abbreviated and decreased the amplitude of the estimated single photon response (Fig. 5a); adaptation was pronounced for backgrounds exceeding 8 R* s−1. To facilitate direct comparison, we quantified both signal and noise adaptation using spectral analysis. At each background, we integrated the power spectra of fits to the single photon response (Fig. 5a) from 0.5 to 4 Hz (Fig. 5b) and quantified changes in gain as the square root of this integral, normalized by the value in darkness (Fig. 5c).

Gain changes were well described by a Weber-Fechner function

$$\frac{\gamma_B}{I_D} = \frac{1}{\left(1 + \frac{I_B}{I_D}\right)}$$

(1)

where $\gamma_B$ corresponds to gain at a given background (in pA per R*), $\gamma_B$ to gain in darkness (in pA per R*), $I_B$ to intensity of the background illumination (in R* s−1) and $I_D$ to the background illumination that halves gain. The best fit for $I_B$ was 7.1 R* s−1 (95% confidence interval, 6.0–8.2 R* s−1).

We estimated rod noise at the same backgrounds. Noise in darkness was low and composed of continuous noise and rare discrete events (not present in the example trace; Fig. 5d). Noise increased in dim backgrounds as a result of current fluctuations produced by random photon absorptions (Fig. 5d,e), peaking at ~8 R* s−1 with an almost fivefold increase relative to darkness; higher backgrounds produced a subsequent decrease in noise (Fig. 5f).

These changes in noise can be predicted based on two assumptions: intrinsic phototransduction noise and extrinsic noise are independent and additive, and both extrinsic and intrinsic noise are subject to the same light adaptation mechanism that affects the rod signals. These assumptions are summarized in the following equation, in which the first term represents the decrease in noise produced by adaptation and the second term corresponds to the increase in noise produced by quantal fluctuations in photon arrival

$$\frac{\sigma_B}{\sigma_D} = \frac{1}{\left(1 + \frac{I_B}{I_D}\right)} \times \sqrt{\frac{1 + \frac{I_B}{I_D}}{1 + \frac{I_B}{I_D}}}$$

(2)

Here, $\sigma_B$ corresponds to the s.d. of the noise at a given background (in pA), $\sigma_D$ to the s.d. of the noise in darkness (also in pA), $I_B$ is the intensity of the background (in R* s−1), $I_D$ represents the intrinsic noise expressed as an equivalent ‘dark light’ (in R* s−1; see ref. 5), and the half-desensitizing background, $I_B = 7.1$ R* s−1, was fixed from fits to the background-dependence of signal gain (Fig. 5c and equation (1)).
Cones

Adaptation affected cones quite differently, as a substantial component of cone noise evaded adaptation. To quantify signal adaptation in cones, we estimated and fit single-photon responses (Fig. 6a) and calculated their power spectra (Fig. 6b); gain was estimated as the square root of the integrated power between 1 and 10 Hz across a range of backgrounds (Fig. 6c). Changes in gain were well described by a Weber-Fechner function (equation (1)), the best fit was found for a half desensitizing background of \( I_D = 620 \, \text{R}^* \, \text{s}^{-1} \) (95% confidence interval, 480–835 R* s\(^{-1}\)). Thus, low-frequency cone noise can also be described as the sum of intrinsic and extrinsic noise, both subject to the same adaptational mechanisms. This in turn is simply explained if rods are dominated by extrinsic noise across a wide range of backgrounds.

We estimated cone noise at the same backgrounds by integrating power spectra across a low-frequency (1–10 Hz) and a high-frequency (100–600 Hz) range (Fig. 6d,e) and calculating the square root of these integrals across backgrounds (Fig. 6f). The background dependence of low- and high-frequency noise differed. As with rod noise, low-frequency cone noise initially increased and then began to fall with further increases in background (Fig. 6f). This initial increase was modest (compare with the rod noise in Fig. 5f) and was apparent only for backgrounds exceeding 1,000 R* s\(^{-1}\). The dependence of low-frequency noise on background could be fit with equation (2), using the half-desensitizing background for the signal gain \( (I_0) \) derived above (4,500 R* s\(^{-1}\)) and a best-fit value for an equivalent dark light of \( I_D = 620 \, \text{R}^* \, \text{s}^{-1} \) (95% confidence interval, 480–835 R* s\(^{-1}\)). Thus, low-frequency cone noise can also be described as the sum of intrinsic and extrinsic noise, both subject to the same adaptational mechanism as the cone signals. High-frequency cone noise, however, was little affected by dim backgrounds and began to decline only at much higher backgrounds (Fig. 6f). Furthermore, this decline in high-frequency noise was shallow and was not proportional to the inverse of the background. Changes in noise at intermediate frequencies (20–100 Hz) showed a mixed behavior.

The background dependence of rod and cone signals and noise differed in two important ways. First, rod dark noise was ~100 times smaller than the backgrounds required for substantial adaptation, whereas this difference in cones was less than a factor of 10. Second, the majority of noise in rods was affected by adaptation, whereas a large component of noise in cones evaded adaptation. Taken together, these factors suggest that extrinsic noise contributes substantially to the total cone noise only over a narrow range of backgrounds.

**Figure 5** Adaptation similarly affects rod signal and noise. (a) Linear estimates of the rod single photon responses for a range of backgrounds. Increasing backgrounds resulted in faster and smaller responses. Fits were obtained using equation (6). The correspondence between light levels and color scale is maintained throughout the figure. (b) Power spectra of the fitted single photon responses in a. Dashed lines show integration limits. (c) Square root of the integrated signal power (0.5–4 Hz) normalized by that in darkness for the example rod in a and b (colored circles) and for a population of rods (gray circles). The population data (black circles represent mean ± s.e.m., \( n = 5 \)) was fit with equation (1) with \( I_0 = 7.1 \, \text{R}^* \, \text{s}^{-1} \). (d) Examples of noise traces for the same rod and backgrounds as in a. (e) Power spectra of the noise traces in d. Dashed lines show integration limits. (f) Square root of the power spectrum integral (0.5–4 Hz) of the noise normalized by darkness for the example rod shown in a and d (colored circles) and for a population of rods (gray circles). The population (black circles represent mean ± s.e.m., \( n = 5 \)) was fit with equation (2) with \( I_0 = 0.062 \, \text{R}^* \, \text{s}^{-1} \).

The best fit was found for a dark light \( I_D \) of 0.062 R* s\(^{-1}\) (95% confidence interval, 0.055–0.070 R* s\(^{-1}\)), which is within 30% of previously published values\(^7\). The success of this simple model indicates that, consistent with previous work\(^7\), rod signals and noise are subject to the same adaptational mechanisms. This in turn is simply explained if rods are dominated by extrinsic noise across a wide range of backgrounds.
At lower backgrounds, cone noise is dominated by intrinsic noise from both cGMP fluctuations and cGMP channels, whereas the relative contribution of noise from cGMP channels increases at higher backgrounds.

### Threshold versus background behavior of rods and cones

The differences in the effects of adaptation on signal and noise in rods and cones predict differences in the background-dependence of detection threshold. This relates in turn to how adaptation in the photoreceptors could contribute to behavioral threshold-versus-intensity curves.

We calculated detection thresholds, defined as the flash strength (in R*) required to match the noise in a 200-ms integration time at a given background, from the power spectra exemplified in Figures 5 and 6. First, we calculated the SNR as a function of frequency by dividing the signal power spectrum by the noise power spectrum; this SNR spectrum had very little power at high frequencies, and such frequencies contributed negligibly to detection. We integrated the SNR spectrum between 0.4 and 8 Hz for rods and cones, and such frequencies contributed negligibly to detection. We calculated detection thresholds, defined as the signal strength (in R*) required to match the noise in a 200-ms integration time at a given background, from the power spectra exemplified in (in R*) required to match the noise in a 200-ms integration time.

**Figure 6** Adaptation affects cone signal and noise differently. (a) Estimated cone single photon responses for a range of backgrounds, fit with equation (6). Color scale is maintained throughout the figure. (b) Power spectra of the fitted single photon responses shown in a. Dashed lines indicate integration limits. (c) Square root of the integrated signal power (2–10 Hz) normalized by that in darkness for the example cone shown in a and b (colored triangles) and for a population of cones (gray triangles). Population data (black triangles represent mean ± s.e.m., n = 6) was fit with equation (1) with $I_D = 4,500$ R* s$^{-1}$. (d) Example noise traces for the same cone and backgrounds shown in a. (e) Example noise power spectra for the same cone shown in a. (f) Top, square root of the integrated noise power at low frequencies (2–10 Hz) normalized by that in darkness for the example cone shown in a (colored triangles) and for a population of cones (gray triangles). Dashed line represents the signal gain curve from c. Noise below 10 Hz at the highest backgrounds (black open triangles) was unreliable and was excluded from fitting. Population (black triangles represent mean ± s.e.m., n = 6) was fit with equation (3) with $I_D = 620$ R* s$^{-1}$. Bottom, square root of the integrated noise power at high frequencies (50–600 Hz) as in top panel. The population data was fit with equation (7) with $I_D = 17,500$ R* s$^{-1}$ and $\eta = 0.29$. Open gray triangles represent noise from cones in which signal adaptation was not assessed (n = 6); fit to low-frequency noise used $I_D = 1,200$ R* s$^{-1}$ and $I_0 = 9,950$ R* s$^{-1}$ and fit to high-frequency noise used $I_D = 13,360$ R* s$^{-1}$ and $\eta = 0.34$. 3 and 600 Hz for cones; the inverse of the square root of this integral corresponds to the detection threshold.

Rod and cone detection thresholds have a different dependence on the intensity of the background illumination (Fig. 7), as the shape of these curves is determined by how both signal and noise adapt. Given that rod noise and signal adapt identically, the rod thresholds follow (derived from equations (1) and (2))

$$\text{Threshold}_{R} = \left(1 + \frac{I_{0}}{I_{D}}\right) \times \text{Threshold}_{D}$$

(3) with $I_D = 0.062$ R* s$^{-1}$ (derived from the noise adaptation in Fig. 5f). This curve closely follows the Poisson statistics of the background illumination for backgrounds exceeding the dark noise.

Cone threshold increased more steeply with background. The lack of dependence of threshold on backgrounds <1,000 R* s$^{-1}$ reflects the relatively high cone dark noise, which obscures extrinsic noise. Once signal gain is reduced by adaptation ($I_D > 4,500$ R* s$^{-1}$), cone channel noise, which is relatively unaffected by adaptation, becomes dominant and hence the increase in threshold directly reflects the decreased signal gain. Thus, the curve is well fit with a Weber-like function

$$\text{Threshold}_{C} = \left(1 + \frac{I_{0}}{I_{D}}\right) \times \text{Threshold}_{D}$$

(4)
with a best fit for the threshold-doubling background of \( I_0 = 11,700 \) R* s\(^{-1}\) (95% confidence interval, 7,700–24,400 R* s\(^{-1}\)). The dark threshold was \( \text{Threshold}_D = 10.9 \) R* per cone (95% confidence interval, 6.8–14.9 R* per cone).

Rod and cone threshold versus intensity curves differed in at least three ways. First, as expected, the absolute threshold was higher in cones as a result of noise sources that preclude detecting absorption of single photons. Second, higher backgrounds were required to produce changes in cone threshold compared with rod threshold. Third, cone thresholds scaled linearly with background (that is, Weber behavior), whereas rod thresholds scaled with the square root of the background (that is, Rose-DeVries behavior).

**DISCUSSION**

We sought to improve understanding of the origin and functional importance of noise in the responses of primate cone photoreceptors. Our results support three main conclusions. First, most cone noise originates downstream of the photopigment, with a sizable component coming from gating transitions in cGMP-gated channels. Second, cone noise and detection threshold are consistent with those inferred from behavior. Third, cone noise and fluctuations in the concentration of cGMP produce noise that is restricted to low-to-mid temporal frequencies (below 50 Hz).

Several observations suggest that most of the noise from cGMP fluctuations is not generated by dark activation of the cone opsin. First, the dim flash response, which reflects the activity directly caused by opsin activation, lacked substantial power at frequencies above 10 Hz (Fig. 1c–e), whereas noise resulting from cGMP fluctuations extended well beyond 10 Hz (Fig. 4c). Second, the spontaneous isomerization rate of the L-cone opsin, estimated by its expression in mouse rods, was \( \sim 10 \) R* s\(^{-1}\), \( \sim 60\)-fold less than the rate needed to match the cone dark noise that we observed. Third, preliminary recordings in short wavelength-sensitive cones (where opsin noise should be almost negligible, as short wavelength-sensitive opsins are very stable in comparison with middle and long wavelength-sensitive opsins\(^{9,14}\)) revealed very similar dark noise to that observed for L and M cones. Thus, fluctuations in the cGMP concentration originate from another source, for example, activity of the non-isomerized photopigment resulting from chromophore dissociation\(^2\), spontaneous transducin activation or spontaneous PDE activation. Spontaneous PDE activation accounts for substantial noise in the responses of rods\(^3\), salamander short wavelength-sensitive cones\(^{21}\) and fish cones\(^{17}\) and is therefore a likely culprit here.

**Effect of cone noise on retinal signals**

Comparison of the sensitivity of cone-mediated signals at several locations in guinea pig retina indicates that most noise originates early in the retinal circuitry\(^{22}\). Specifically, a substantial loss of sensitivity was observed between photon capture in the cones and the responses of horizontal cells, consistent with noise intrinsic to the cones or their output synapse. Smaller, but still substantial, losses in sensitivity were observed between horizontal and ganglion cells, consistent with a source of additional noise in the retinal circuitry.

Recordings from primate midget and parasol ganglion cells indicate that most noise in their synaptic inputs at cone light levels originates from the cones and has more rapid kinetics than the cone light response\(^{23}\). These studies highlight the importance of noise intrinsic to cones or their output synapses. Our results, particularly the high level and rapid kinetics of the cone noise, suggest that much of the noise limiting the sensitivity of the cone-mediated output signals from primate retina originates in the cone transduction cascade itself.

**Bridging physiology and behavior**

How close does cone vision come to limits imposed by cone noise? The answer to this question is an important constraint on the retinal and cortical circuits that read out the cone signals. However, noise estimates derived from behavioral sensitivity are substantially lower than past measures of noise in primate cones\(^5\); this discrepancy has hindered our understanding of the relationship between retinal mechanisms and the sensitivity of cone-mediated behavior.

Recent behavioral work provides the lowest estimates of threshold and dark noise for cone vision\(^{24}\), indicating that humans can detect \( \sim 200 \) photons delivered at the cornea or, equivalently, \( \sim 2–5 \) R* per cone in a region containing \( \sim 10 \) cones. Assuming that cone signals are pooled linearly, sensitivity should improve as the square root of the number of cones conveying the signal; thus, the inferred single cone dark threshold estimate would be 6–15 R*, which is consistent with our single cone dark thresholds (7–15 R*; Fig. 7).

Dark noise estimates require additional assumptions about spatial pooling and integration time of the visual system. Assuming spatial pooling across the entire area of the flash and an integration time equal to the flash duration (34 ms), behavioral sensitivity indicates
a dark noise of $180-550 \text{ R}^* \text{s}^{-1}$ (ref. 24). Uncertainties in foveal cone density could further extend this range. This is again consistent with our estimate of $\sim 600 \text{ R}^* \text{s}^{-1}$. Thus, our measures of cone sensitivity and dark noise are consistent with behavior, providing a potential resolution to a long-standing discrepancy. Assuming the most sensitive cones that we recorded from (Figs. 6 and 7) are similar to those in vivo, our results leave little room for noise or inefficiencies introduced by the circuits reading out the cone signals.

Effect of adaptation on threshold versus intensity curves

The effect of adaptation on the sensitivity of sensory signals depends on how it affects both signal and noise. This is a key issue for how adaptational mechanisms relate to the rich history of measurement how it affects both signal and noise. This is a key issue for how adaptation on threshold versus intensity curves produced by the circuits reading out the cone signals.

Our estimate of $\sim 600 \text{ R}^* \text{s}^{-1}$. Thus, our measures of cone sensitivity across a wide range of backgrounds. Given that quantal fluctuations follow Poisson statistics, the noise that they contribute increases as the square root of the background intensity, consistent with the psychophysical Rose-deVries region of rod vision. It has been difficult to explain why cone vision does not similarly exhibit a clear Rose-deVries region; our results suggest that the lack of a prominent Rose-deVries region occurs because extrinsic noise makes a relatively small contribution to cone noise except for a narrow range of backgrounds.

Classic work has shown that cone-mediated behavioral thresholds across a wide range of backgrounds increase linearly with increases in background intensity (Weber-law behavior). The mechanismic basis of this behavior, however, is unclear. The gain of cone signals decreases proportionally with backgrounds. This decrease in gain, together with a post-adaptation, background-independent source of noise, could explain Weber-law behavior. We found that channel noise in cones accounted for a substantial fraction of cone dark noise; furthermore, channel noise was little affected by adaptation and therefore became the dominant source of cone noise across a broad range of backgrounds. The weak dependence of channel noise on background and the decrease in gain of cone signals proportional to the background gives rise to an extended region over which the cone detection threshold follows Weber behavior. If noise downstream of the cones is small relative to cone noise, adaptational mechanisms in the retinal circuitry would equally affect signal and noise. In this case, human threshold-versus-intensity curves could be dominated by the signal and noise adaptation properties of individual photoreceptors. A complete explanation of human threshold versus intensity curves will require understanding where gain controls operate relative to key sources of noise in the circuit.

METHODS

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.M.A. and E.R. conducted experiments, performed analyses and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Tissue, cells and solutions. We made electrophysiological recordings from primate retinas (Macaca fascicularis, Macaca nemestrina and Macaca mulatta of either sex, ages 3–19 years) in accordance with the Institutional Animal Care and Use Committee at the University of Washington. We obtained retina through the Tissue Distribution Program of the Regional Primate Research Center. We performed most enucleations under pentobarbital anesthesia. After enucleation, we rapidly separated the retina-pigment epithelium-sclera complex (<5 min) from the anterior segment, drained the vitreous humour and dark-adapted the retina for 1 h in warm (32 °C) Ames medium bubbled with a mixture of 95% CO₂/5% O₂. We performed all subsequent procedures under infrared (>900 nm) light. For recording, we separated a small piece of retina (~4 mm²) from the pigment epithelium and mounted it photoreceptor side up on a poly-lysine–coated coverslip (BD Biosciences) forming the floor of a recording chamber. We continually superfused retinas with warm (~31–33 °C) oxygenated Ames medium. Treatment with DNase I (Sigma–Aldrich) (30 units in ~250 μl of Ames for 4 min) facilitated access to the photoreceptor outer segments. For rod suction recordings, we shredded small pieces of retina with bent needles and transferred them to a recording chamber.

Recordings. We measured cone signals using whole-cell voltage-clamp recordings (holding potential = −70 mV) with an internal solution containing 133 mM potassium aspartate, 10 mM KCl, 10 mM HEPES, 1 mM MgCl₂, 4 mM ATP, 0.5 mM GTP; pH was adjusted to 7.2 with NMG-OH and osmolarity was ~280 mOsm. The internal solution did not contain any calcium buffer (or calcium), as even low concentrations of calcium buffer caused the light response to become increasingly biphasic during the course of a recording (data not shown). We recorded rod photocurrents using suction electrodes as described previously. Holding potentials have been corrected for a −10-mV liquid junction potential.

In experiments with 8Br-cGMP, we used modified internal solutions lacking ATP and GTP and supplemented with various concentrations of 8Br-cGMP (from 0 to 200 μM). We added IBMX (final concentration of 1 mM) dissolved in DMSO (final DMSO concentration was less than 0.1%) to HEPES-buffered Ames and included it in a puffer pipette. For control experiments, we used the same solution without IBMX.

We acquired data using Axoclamp 200B or Multiclamp 700B amplifiers. We low pass–filtered recorded currents at 3 kHz and digitized the data at 20 kHz. We analyzed recorded data through custom routines in Matlab (Mathworks) and calculated power spectra using built-in fast Fourier transformations and represented them as two-sided power spectral densities (in pA² Hz⁻¹). We excluded from analysis cones that showed unusually rapid run-down of light responses, low sensitivity or short-lived recordings. Sensitive cones had holding currents of at least 150 pA (up to 400 pA), and peak responses to bright flashes of similar magnitude.

Light stimulation. We delivered light stimuli from blue, green and red LEDs (peak wavelengths of 470, 510 and 640 nm, respectively), which permitted quick identification of cone types. The stimuli illuminated a ~150 μm diameter area centered and focused on the recorded cone. We converted photon densities (photons per μm²) to R* per photoreceptor using a collecting area of 0.6 mm², previously measured cone spectral sensitivities and the LED spectra.

Two-electrode technique. We performed two electrode recordings by scaling simultaneously on a single cone cell body with both a recording electrode and a drug delivery electrode. After breaking into the cell with the first electrode, we obtained a baseline recording to assess noise and light response in less than 30 s (in voltage-clamp mode). We then obtained access with the drug delivery electrode (in current clamp with no holding current) while maintaining the original recording.

Fitting and statistical analysis. The fits to the noise power spectra presented in Figure 1 correspond to empirical fits (and are not unique) constructed as the sum of the power spectrum of the estimated single-photon response and two separate lorentzian functions. In Fourier space, a single lorentzian function had the form

\[ L(\omega) = \frac{\alpha}{1 + \left(\frac{\omega}{\omega_c}\right)^2} \]

where \( \alpha_c \) corresponds to the corner frequency (frequency at which the power has dropped by half) and \( \omega_c \) is a scaling constant. We obtained fits on a logarithmic scale through built-in Matlab routines (nlinfit and lsqfit) and we assessed the fits through the coefficient of determination (R²). The fit to the average noise from L and M cones at a background illumination of 5,000 R* s⁻¹ (n = 6) did not depart from the measured data by more than 1 s.e.m., and the R² value for the fit was 0.90, with five fitting parameters (Fig. 1d). The same observation holds for the average noise from L and M foveal cones recorded in darkness (n = 7) with an R² value for the fit of 0.99 (Fig. 1e).

Pharmacology. We determined significance in pharmacological experiments using two-tailed Student’s t tests with α ≤ 0.05, by integrating the average power ratios across the specified frequency ranges. We did not perform a sample size calculation before experiments. We chose sample sizes to either establish statistical significance of the effects measured (Figs. 3 and 4) or to provide relative tight confidence intervals on key parameters. In addition, we did not have a sufficient number of samples to test whether the data used in t tests were indeed normally distributed.

For the experiments involving 8Br-cGMP (Fig. 3), we compared each concentration to the experiments lacking 8Br-cGMP (Fig. 2f–j) across the 10–600 Hz frequency range and found significant differences for all concentrations (18 μM, n = 10, P = 0.0009, df = 14; 27 μM, n = 4, P = 0.022, df = 8; 100 μM, n = 3, P = 0.00002, df = 7; 200 μM, n = 2, P = 0.001, df = 6; retinas were derived from 2 different animals). For the experiments involving IBMX (n = 10, Fig. 4) we compared the changes in noise to those produced by a vehicle solution lacking IBMX (n = 4) and found significant differences for both the 10–50 Hz (P = 0.0004, df = 12) and the 100–600 Hz frequency range (P = 0.0068, df = 12; retinas were derived from 2 different animals).

Signal and noise adaptation. We restricted analysis of signal and noise adaptation to cones that passed several criteria: stability of the holding current, good and stable access resistance, minimal run-down of the light response and high sensitivity to flashes in darkness. We assume that the most sensitive cones that we recorded from are most representative of cone responses in vivo. We estimated single photon responses by delivering non-saturating flashes at a given background, then by averaging the resulting responses and scaling by the nominal flash intensity; this procedure provides a linear estimation of the gain of the light response. We then fitted these estimated single-photon responses with the following equation (modified from ref. 2)

\[ f(t) = \alpha \times \frac{t^{4/3}}{1 + \left(\frac{t}{t_{rise}}\right)^{4/3}} \times \left[ e^{-\left(\frac{t}{t_{decay}}\right)} \right] \times \left[ \cos\left(\frac{2\pi t}{t_{osc}} + \phi\right)\right] \]

We obtained the best-fit values through automatic fitting routines in Matlab (nlinfit and lsqfit). The changes in signal gain were virtually the same whether fits or directly estimated single-photon responses were used, but fits eliminated uncertainty resulting from limited data, especially on mid- to high- frequencies (50–600 Hz). The changes in signal gain were also near identical when using the response integral or peak amplitude rather than relying on power spectra.

The fits for the changes in signal gain and noise had only a few parameters, which made them suitable for maximum likelihood estimation; the fit values reported are then the most likely values, bounded by values for which the likelihood of the fit dropped to 2.5% of the maximum, that is, the 95% confidence intervals. The fitting of the changes in cone noise (Fig. 5cd) did not include the highest background, where isolation of the remaining noise from noise in saturating light was difficult and unreliable.

The changes in high frequency cone noise were described by a modified Weber-Fechner function, with an additional free exponent that would accommodate a different slope.
The best fits for the data in Figure 5g were $I_0 = 17,500$ R·s$^{-1}$ (95% confidence interval, 15,600–19,400 R·s$^{-1}$) and $\eta = 0.29$ (95% confidence interval, 0.28–0.31).

**Threshold versus intensity curves.** We obtained the threshold versus intensity curves shown in Figure 7 by first calculating the spectrum of the SNR for each background, using the corresponding power spectra of signal and noise and assuming a 200-ms integration time. We then integrated the square root of this SNR spectrum between 0.4 and 8 Hz for rods and between 3 and 600 Hz for cones. The inverse of this integral corresponds to the flash response that matches the noise at a given background, expressed in R*, or in other words, the just-detectable flash or detection threshold.