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Electrical Coupling between Mammalian Cones

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Summary

Background: Cone photoreceptors are noisy because of random fluctuations of photon absorption, signaling molecules, and ion channels. However, each cone's noise is independent of the others, whereas their signals are partially shared. Therefore, electrically coupling the synaptic terminals prior to forward transmission and subsequent nonlinear processing can appreciably reduce noise relative to the signal. This signal-processing strategy has been demonstrated in lower vertebrates with rather coarse vision, but its occurrence in mammals with fine acuity has been doubted (even though gap junctions are present) because coupling would blur the neural image.

Results: In ground squirrel retina, whose triangular cone lattice resembles the human fovea, paired electrical recordings from adjacent cones demonstrated electrical coupling with an average conductance of approximately 320 pS. Blur caused by this degree of coupling had a space constant of approximately 0.5 cone diameters. Psychophysical measurements employing laser interferometry to bypass the eye's optics suggest that human foveal cones experience a similar degree of neural blur and that it is invariant with light intensity. This neural blur is narrower than the eye's optical blur, and we calculate that it should improve the signal-to-noise ratio at the cone terminal by about 77%.

Conclusions: We conclude that the gap junctions observed between mammalian cones, including those in the human fovea, represent genuine electrical coupling. Because the space constant of the resulting neural blur is less than that of the optical blur, the signal-to-noise ratio can be markedly improved before the nonlinear stages with little compromise to visual acuity.

Introduction

In the turtle retina, neighboring cone terminals contact each other by gap junctions [1, 2] and couple electrically to form a syncytium whose linear length constant for current spread is on average 1.5 cone diameters [3]. Each cone is noisy because of its random photon absorptions and random fluctuations of its signaling molecules and ion channels, but each cone's noise is independent of the others. Consequently, coupling reduces noise but does not greatly affect the visual signals [3, 4] shared by adjacent cones because of correlations in the visual scene and those introduced by blur from the eye's optics.

In mammalian retina, including the primate fovea [5, 6], cone terminals also form small gap junctions, but electrical coupling has not been measured. In the fovea, modest coupling would improve discrimination of signal from noise, but extensive coupling would degrade visual acuity. Therefore, our goals were to (1) determine whether mammalian cone-cone gap junctions are indeed functional and measure their conductance; (2) use the junctional conductance to calculate the neural blur and measure it psychophysically; and (3) compute the benefit and cost to vision of coupling foveal cones.

Results

Physiology

We directly measured the conductance between adjacent cones in the ground squirrel by voltage clamping cell pairs in a retinal slice (Figure 1A). A voltage step in one cone caused a steady current to flow into a neighboring cone whose membrane voltage was held constant (Figure 1B). Plots of transjunctional current as a function of transjunctional voltage show that cone-cone gap junction channels are nearly ohmic over a wide voltage range (Figure 1C). Junctional conductance was as large as 1 nS for some pairs (Figure 1D), the average being about 320 pS (317 $\pm$ 246 pS, n = 31 pairs). Cone-cone transmission was indeed electrical; coupling persisted under conditions in which transmitter release was blocked by Cd^{2+} (0.5–1.0 mM, n = 4), Co^{2+} (2 mM, n = 1), or the removal of external Ca^{2+} (0 mM Ca^{2+}, 7 mM Mg^{2+}, n = 1). In addition, coupling was unchanged during exposure to the retinal neuromodulators dopamine (100 μM), forskolin (25 μM), and nitric oxide (300 μM sodium nitroprusside). If one assumes a single-channel conductance of 20–150 pS [7], coupling would involve on the order of 10–100 connexons. These experiments establish that the cone-cone gap junctions observed by electron microscopy actually function as electrical synapses and have a conductance on the order of 100–1000 pS. How would this affect human foveal vision?

Psychophysics

If coupling between human foveal cones resembles that between ground squirrel cones, we can estimate the lateral current spread in the foveal cone syncytium. The inner segments of foveal cones form a triangular array (Figure 2A), and their synaptic terminals are only slightly larger and less orderly. Therefore, each terminal has 4–6 immediate neighbors with which it forms gap junctions

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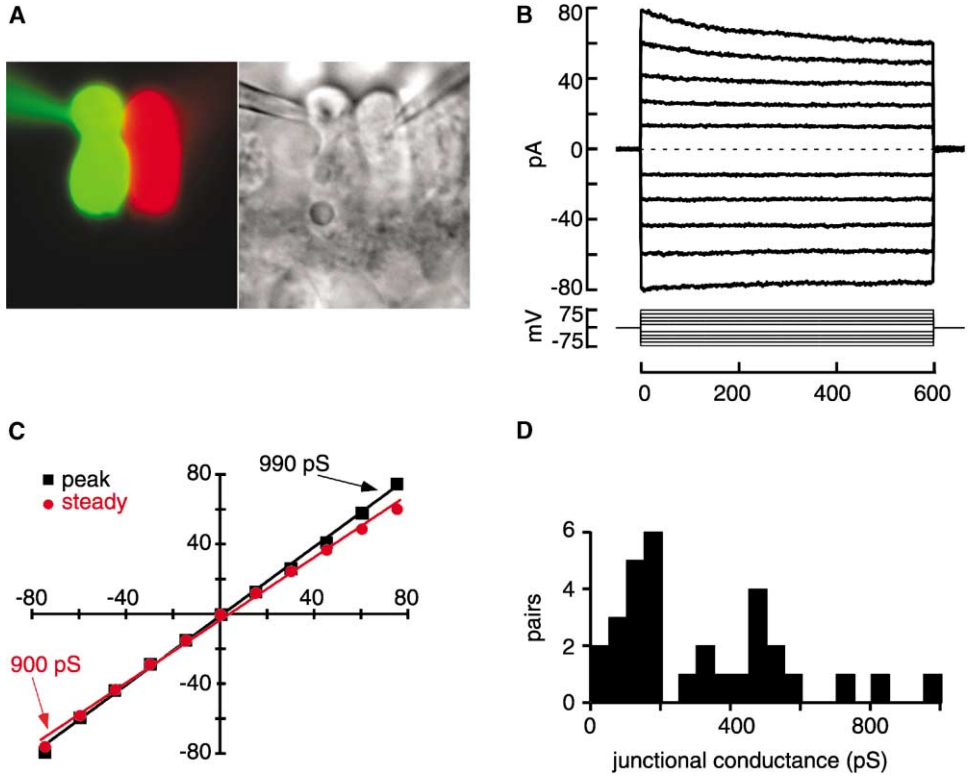

Figure 1. Adjacent Cones in Mammalian Retina Are Electrically Coupled

(A) A retinal slice from the ground squirrel was viewed with epifluorescence (left) and DIC optics (right). Patch pipette solutions contained BODIPY 492/515 (green) or sulforhodamine 101 (red).

(B) Transjunctional currents (above) measured during successive transjunctional voltage steps (below).

(C) Peak and steady transjunctional currents were linearly related to transjunctional voltage with conductances of 990 and 900 pS, respectively. (A) through (C) are from the same pair.

(D) Distribution of junctional conductances for 31 cone-cone pairs.

[5]. One can calculate the length constant (λ) for voltage spread by assuming a rectangular cone array [3] (a triangular array gives nearly identical results), an input resistance (r_m) of an isolated macaque cone equal to 0.5 GΩ (this value was calculated from Schneeweis and Schnapf [8]; a similar value, 0.55 $\pm$ 0.03 GΩ, was obtained for poorly coupled ground squirrel cones, n = 4), and an average cone-cone coupling resistance (r_s) equal to 3.1 GΩ (i.e., 1/320 pS):

$$\lambda = \frac{1}{\operatorname{acosh}\left(\frac{r_s}{2r_m} + 1\right)} \quad (1)$$

Equation 1 predicts a length constant of about 0.5 cone spacings and suggests that only the first ring of cones that surrounds a given cone contributes to its receptive field. To test this, we measured spatial summation at the first linear filter, i.e., the stage preceding the visual system's first nonlinearity. Earlier work [9, 10] had shown this linear filter to be extremely fine and to correspond to the optical aperture of a single foveal cone, but here we looked in more detail to see if we could also detect small signals from neighboring cones.

The experiment was nearly identical to these previous studies: two fine gratings (>30 cycles/degree) were created on the retina by optical interference during brief flashes (2 ms) and superimposed at a slight angle so that the bars of one grating crossed those of the other at (1/6)° intervals. The gratings themselves were not visible, but their distortion product, created at the visual system's first nonlinear stage, produced a grating that was just visible at 6 cycles/degree. The amplitude of this distortion grating is proportional to the product of the contrasts of the interference gratings at the site of the nonlinearity—after attenuation by the cone's linear filter. We measured this attenuation (modulation transfer function) by varying the spatial frequency of both interference gratings while adjusting the contrast of one to hold the distortion grating at visual threshold.

In this manner, the linear filter was measured for three observers across spatial frequencies. The experiment suggested two branches separated by an inflection at about 50 cycles/degree (Figure 2B, solid curves). The points did not fit a single Gaussian (shown for comparison in Figure 2B, dashed curve). However, they were well fit in the spatial domain by a single Gaussian plus a ring convolved with a Gaussian, whose Fourier transform is:

$$N_1(f) = A\, e^{\left(-\frac{f^2}{2\sigma^2}\right)} + K\, e^{\left(-\frac{f^2}{2\sigma^2}\right)} \times \pi R J_0(2\pi R f) \quad (2)$$

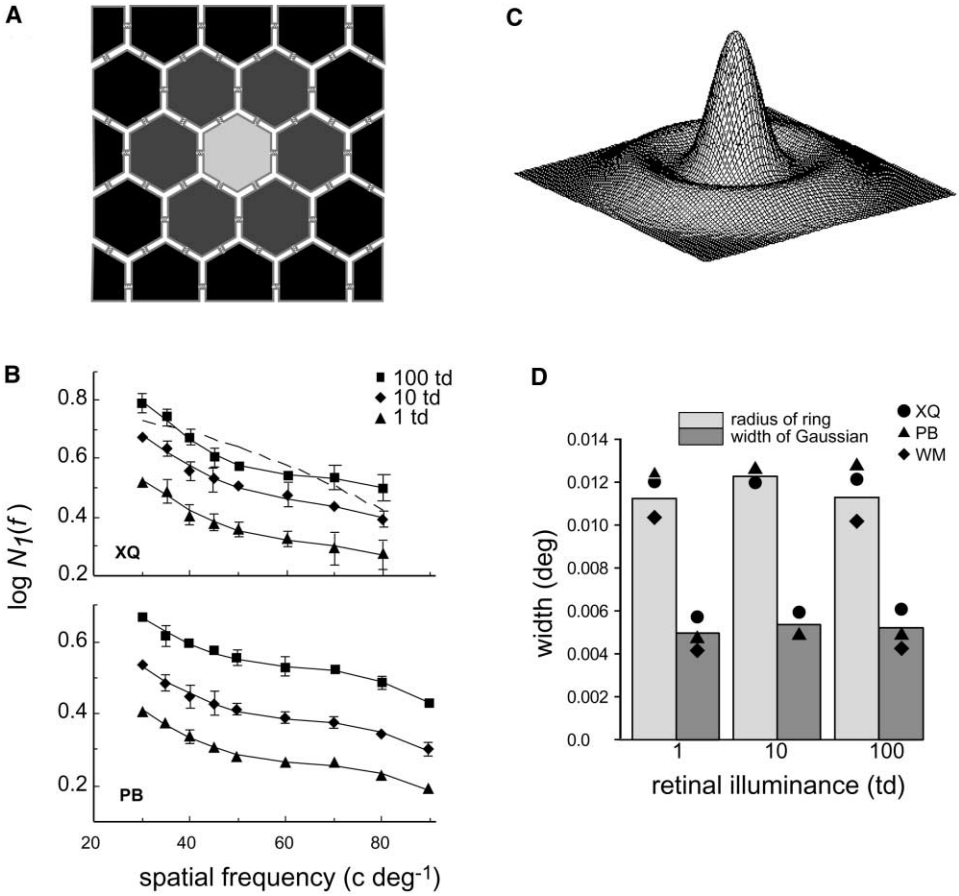

Figure 2. Psychophysical Measurements Suggest that Cones in Human Fovea Are Coupled and that Coupling Is Invariant with Adaptation Level

(A) Cone inner segments in human fovea pack triangularly [44]. Gap junctions couple all nearest neighbors [5].

(B) Modulation transfer function for the first linear filter measured by interferometry across spatial frequency and retinal illuminance. The curves were poorly fit by a single Gaussian (dashed line in upper panel) but were well fit by a Gaussian plus a ring convolved with a Gaussian (Equation 2). PB and XQ are different subjects.

(C) Sensitivity surface (Gaussian + ring) that best fits the psychophysical experiment (points in [B]).

(D) The radius of the ring is roughly twice the width of the Gaussian peak, as expected if the ring represented contributions from neighboring cones. Relative amplitudes of the ring and the peak fit the space constant for electrical coupling calculated from Equation 1, and this relationship was invariant over 3 log units of retinal illuminance (in photopic trolands, td). Histograms show the means of the points from three observers; XQ, PB, and WM.

where f = frequency, σ = Gaussian radius, R = radius of annulus, J_0 = Bessel function of 0th order, A = amplitude of central peak, and K = amplitude of annulus. The first term is the transform of the Gaussian center and provides the sensitivity to the higher spatial frequencies; the second term is the transform of the ring, which enhances sensitivity to low spatial frequencies enough to produce the inflection in the curve.

We make no claim that this particular equation uniquely fits the data. However, these data exclude any model (equation) that does not produce an inflection in the modulation transfer function at about 40 cycles/degree. Among those thereby excluded is any unimodal, radially symmetrical sensitivity profile (receptive field), such as a cylinder, hemisphere, parabola, or single Gaussian. Moreover, changing these profiles has no significant effect on the basic spatial properties (such as the width of the center at half height or the radius of the ring) described below.

These data also exclude any model that is based on parallel or concentrically organized spatial filters (receptive fields) in which the larger receptive field is outside the range, 2.0–2.5 times the size of a foveal cone, because they could not transfer the high spatial frequencies known to pass through this system. Among those thereby excluded are models that might produce an inflection by contributions from more than one class of bipolar cell or horizontal cell. Finally, these data exclude models based on aliasing [11] because aliasing in the fovea produces its first transmission minimum at spatial frequencies greater than 80 cycles/degree [12], far from 40 cycles/degree for the inflection observed here.

The model embodied by Equation 2 quantitatively fits the retinal anatomy (Figure 2C). For the model receptive-field center, peak width at half height averaged 0.005° for all observers and all retinal illuminances (Figure 2B). This represents the cone's optical aperture, about half the inner-segment diameter, in agreement with previous

measurements [9, 10, 13]. The distance from the center of the peak to the center of the ridge (i.e., radius of the ring) averaged 0.0119° ± .0002° (Figure 2D). This radius, about twice the width of the central peak (Figure 2D), corresponds (within observer variability) to the center-to-center spacing of triangularly packed foveal cones (Figure 2A). The profiles of both the central peak and the ring have the same width. Thus, in all respects these dimensions fit the hypothesis that the ring component of the cone receptive field is caused by coupling of the center cone to its immediate neighbors. Finally, comparing the amplitudes of the center and the ring at 0 frequency (dc response) puts the contribution of each cone in the ring at about 5% of the center cone. This acceptably matches the amplitude calculated from the coupling conductance (8.6%).

Although previous studies did not detect this low-amplitude ring [10], our estimate of the cone's optical aperture (0.005° ± 0.0006°) is statistically indistinguishable from that obtained in those studies. Furthermore, one subject (WM) served in both studies with nearly identical results for the cone aperture. Thus, the difference in results cannot be attributed to differences in subject or technique—except that in previous studies the stimuli were prolonged (500 ms), whereas here they were brief (2 ms). The interaction of eye movements with stimulus duration will not explain the difference for reasons discussed [10]. We suspect that the ring due to cone coupling is more obvious with a brief stimulus because it is attenuated by negative feedback during a longer stimulus.

Feedback through a low-pass filter reduces low spatial frequencies, but it is slow [14] and thus not apparent after a brief stimulus. The H1 horizontal cell, which in the fovea connects to 6–7 cones [15], has just the right spatial scale and time course [14] to serve this function. Nevertheless, cone-cone coupling should be present continuously and should improve the signal-to-noise (S/N) ratio independently of stimulus duration.

The overall shape of the linear filter that precedes the first nonlinear stage of the visual system and the width of both components (peak and ring) were invariant with retinal luminance over three log units (Figures 2B and 2D). Therefore, if the model is correct (within the precision of the experiment), coupling strength is invariant with adaptation level. This is consistent with previous reports [16–18] that varying the mean luminance affects the shape of the contrast sensitivity curve only below the lowest spatial frequency tested here (8 cycles/degree); thus, psychophysics suggests that, at least over two log units of intensity, cone coupling is constant.

Computations

If the neural blur due to electrical coupling were too great, visual acuity would be impaired. Therefore, we calculated the neural blur for primate cones by using junctional conductances from the ground squirrel (Equation 1) and compared it to the blur due to optical factors in the human eye. A junctional conductance of 320 pS causes a blur narrower than the line spread due to optical factors, even for the narrowest pupil, which serves in bright light and gives the least optical blur (Figure 3A). Only when the junctional conductance was greater than about 10,000 pS did the electrical blur approach the smallest optical blur. Thus, to a first approximation, coupling in the fovea is no disaster. Yet what precisely are the benefit and the cost?

The benefit was shown qualitatively by simulation of the response of a cone array to a "photon distribution" representing eight cycles of an 8 cycles/degree grating that varies from high contrast at the center to low contrast toward the edges (Figure 3B). When the cones are isolated, the grating's high-contrast region is obvious in the response (four cycles at the middle), but its low-contrast regions are lost in noise attributable to photon fluctuation, transduction noise, and channel noise. When the cones are coupled at 1 nS (the highest value measured for the ground squirrel, chosen here for a qualitative illustration), noise is reduced, and the grating's low-contrast regions are clearly visible. The reason was elucidated long ago in turtle retina [3]: coupling reduces signal amplitude, but only modestly because signals in neighboring cones are correlated (from correlations across the visual scene and optical blur). However, coupling reduces noise amplitude markedly because noise events in neighboring cones are *un*correlated. Thus, coupling improves the S/N ratio to a degree that the simulation suggests might be useful.

To quantify the improvement, we used a compartmental model to compute contrast sensitivity (S/N ratio) for a single cone at the center of a coupled and uncoupled array (Figure 3C; details in Experimental Procedures). At high luminance, with a coupling conductance of 320 pS and a grating of low spatial frequency, sensitivity improves by approximately 77% (Figure 3D; Experimental Procedures). This calculation depends inversely on the value used for cone input resistance, but the value we used seems reasonable because the same value was both directly measured for the ground squirrel and calculated for primates (from [8]). As grating frequency rises, the benefit of coupling declines, and the curve for the coupled array crosses the curve for the uncoupled array around 40 cycles/degree. Thereafter, coupling *decreases* sensitivity because finer gratings cause progressively smaller correlations in neighboring cones, so coupling attenuates the signal nearly as much as the noise [3]. At low luminance, coupling improves sensitivity to the same degree as at high luminance and shows a similar "crossover" point (Figure 3D); however, because high frequencies are already obscured by photon noise and worse optics from a larger pupil, the benefit of coupling is nearly free.

Sensitivity improves with coupling conductance approximately as a square root function [3] (Figure 3E). Stronger coupling, by increasing neural blur, shifts the benefit/cost "crossover" toward lower frequencies. Therefore, 320 pS coupling seems to represent a sensible compromise in maximizing sensitivity while minimizing the cost to spatial acuity. There is an additional cost to coupling foveal cones; trichromatic color vision requires comparisons between cones of different spectral sensitivity, yet coupling must blur their spectral differences. The receptor mosaic minimizes this cost [19] by distributing each spectral type randomly. This results

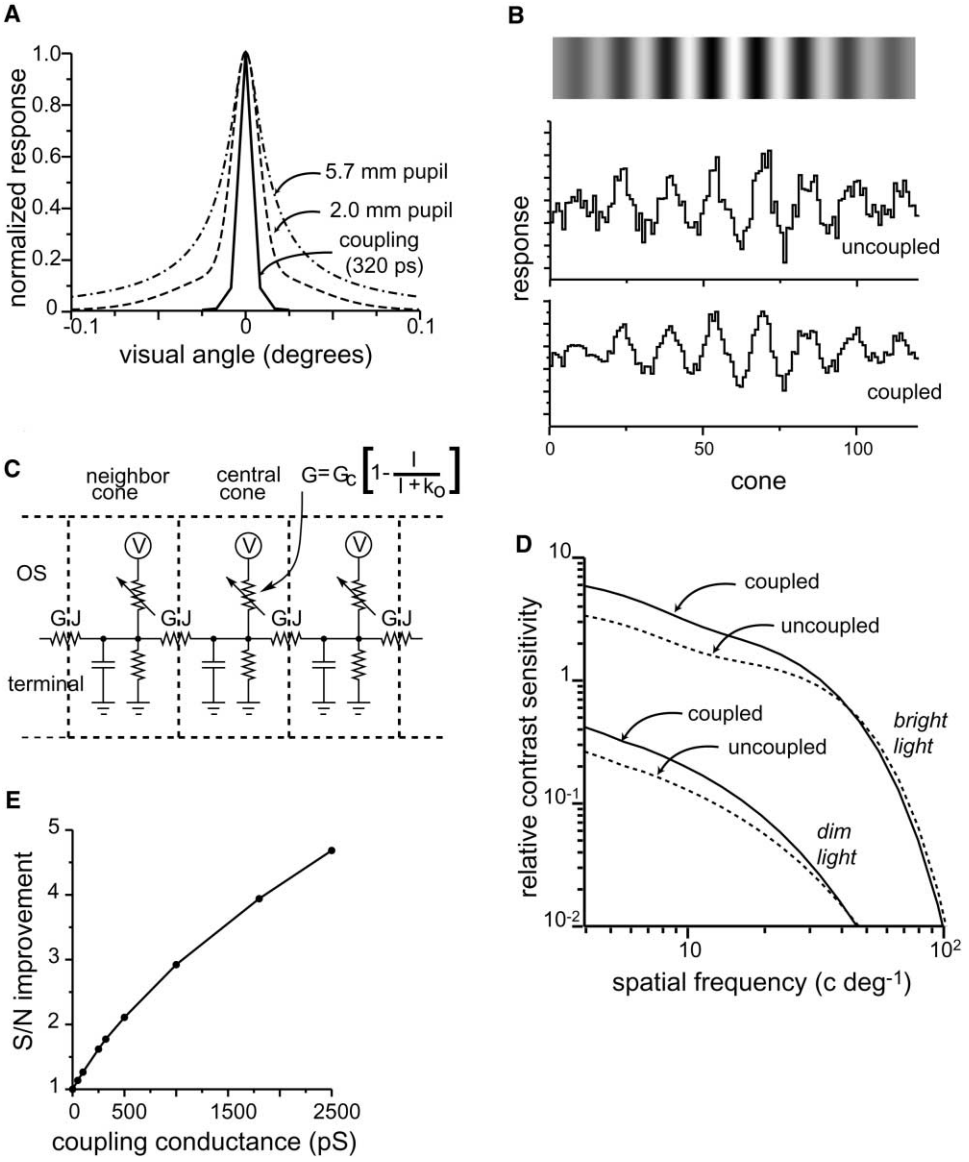

Figure 3. Cone Coupling Improves Contrast Sensitivity with Little Cost to Acuity

(A) The signal spread calculated for cone coupling is narrower than the spread due to optical factors for high photopic (2 mm pupil) and low photopic/mesopic (5.7 mm pupil) backgrounds [40].

(B) Upper panel: the sinusoidal grating envelope decreases from a center contrast of 20%. Middle panel: simulated response of uncoupled cone array to a brief flash of the grating (8 cycles at 8 cycles/degree). Poisson fluctuations obscure the low-contrast regions. Lower panel: coupling reduces the uncorrelated fluctuations and thereby improves the S/N ratio and renders low-contrast regions visible. Here, coupling was 1000 pS, which still causes neural blur narrower than the optical blur from the 2 mm pupil (panel [A]).

(C) A schematic for the cone model illustrates coupling to two neighbors (the full model included six neighbors). Photon flux at the cone outer segment (OS) controlled light-modulated conductance G_c, which in series with voltage source V and membrane conductance (2×10^9 S), generated the voltage response in the terminal. Coupling was modeled as a linear conductance (320 pS).

(D) Coupling (320 pS) improves contrast sensitivity at lower spatial frequencies by 77%. These frequencies are also best preserved by the optics of the eye. Coupling attenuates sensitivity at higher spatial frequencies (>40 cycles/degree), which are also most attenuated by optical factors. Because acuity is highest in bright light (approximately 10^4 R*·cone^{-1}·integration time^{-1}), there is some cost to coupling (top curve). But acuity is low in dim light (approximately 10^2 R*·cone^{-1}·integration time^{-1}) because of photon noise and a large pupil, so the cost of coupling is nil (bottom curve). Thus, coupling reinforces the effect of optical factors, which also preserve low-middle spatial frequencies and attenuate high frequencies. The inflection in curves for bright light arises from the different spatial scales of the functions for Gaussian blur and scatter.

(E) The improvement in the S/N ratio varies with coupling conductance approximately as the square root, from 26% at 100 pS to approximately 200% at 1000 pS. The average improvement at 320 pS was 77%.

in homogeneous patches [20]; consequently, although coupling is indiscriminate [5], it tends to be with members of the same spectral type.

Discussion

Cone-cone gap junctions have been observed in every mammalian retina where they have been looked for [21]. But whether the gap junctions actually couple the cones functionally has been doubted, especially when the sampling is extremely dense, as in primate fovea. The present recordings from pairs of adjacent cones in the ground squirrel clearly establish that mammalian cones *do* couple functionally (approximately 320 pS/pair). Furthermore, they couple even when sampling is dense because ground squirrel cones exhibit triangular packing like that in primate fovea. Consistent with this, primate cones have recently been reported to show dye-coupling [22].

We calculate that coupling of this magnitude in human fovea would cause some neural blur, but it would be narrower than the eye's optical blur (Figure 3A). Indeed, stimuli created directly on the retina (and thus bypassing the eye's optics) and detected psychophysically demonstrate precisely the blur predicted from the measured coupling conductance, approximatley 320 pS. The psychophysically measured neural blur was invariant over three log units of intensity in the middle of the cone operating range. Consistent with this, standard pharmacological manipulations applied to the ground squirrel retina in vitro did not alter coupling. Thus, although the shape of the *theoretical* optimal filter shifts toward increased pooling with lower intensity [23], we found no evidence for this with either experimental method.

The advantage of pooling cone voltages before the first synapse is that the noise intrinsic to each cone (due to fluctuation of photons, cGMP, and ion channels) is independent between cones, whereas the signal is partially shared (because of local correlations within the scene and optical blur) [24]. Consequently, coupling reduces the total noise level more than the signal, especially for signals of lower spatial frequency. This first step in neural integration of the visual signal is extremely general across species, occurring also in insect eyes where multiple photoreceptors share the identical optical image and project to the same set of interneurons [25]. Because coupling photoreceptors would provide little advantage over averaging the visual signal at some later stage if the pathway were linear [25], the advantage is probably linked to signal processing by synaptic nonlinearities, such as preferential amplification of larger voltages [26–28], and rectification [29]. Because a nonlinearity imparts different weights to equal and opposite deviations from the mean, pooling after nonlinear processing could not reduce noise from photoreceptors to the same extent. This suggests that the advantage of coupling before the first synapse is to remove noise before it would be distorted by a nonlinearity [30].

Coupling/neural blur of the magnitude shown here by electrophysiology and psychophysics can improve cone signal/noise by approximately 77%. Stronger cone coupling would give greater improvement (Figure 3E), but there would be a cost: progressive attenuation of higher spatial frequencies. Yet the power of higher frequencies in natural scenes is rather low [31]. We calculate that, viewed through a 2 mm pupil (which cuts off at approximately 60 cycles/degree), the fraction of the frequency spectrum between 40 and 60 cycles/degree is less than 5%. Filtering through the eye's optics by more than an order of magnitude reduces that proportion further. Thus, under natural conditions, the benefit of coupling foveal cones has a negligible cost.

Experimental Procedures

Electrophysiology

Slices from the ground squirrel (*Spermophilus tridecimlinatus*) retina were prepared in the light and maintained during recording as previously described [32]. Current responses were filtered with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA) at 600 Hz and digitized at 1.25 kHz. Standard methods and conventions were used for determining the junctional conductance of voltage-clamped pairs. In brief, the membrane voltages of both cones were maintained at −40 mV. The membrane of one cone was stepped to a series of voltages between −25 and 35 mV in 15 mV increments while the transjunctional current was measured in the adjacent cone. The roles of the two cones were then reversed so that current flow in the opposite direction could be measured. We did not compensate for pipette series resistance. If one assumes a worst-case pipette series resistance of 50 MΩ, junctional conductance would be underestimated by at most 10%. Although embedded in a syncytium, the membrane voltage of an individual cone was well-controlled, as evidenced by the monoexponential decline of the capacitance transient during a pulse and the sharp onset and offset of the junctional current trace in the maintained cell. A good space-clamp was not surprising because of the compact columnar shape of ground squirrel cones and the relatively small junctional conductance. Between 10% and 20% of pairs with juxtaposed ellipsoids failed to show coupling. In most cases, inspection under epifluorescence indicated that the cone pedicles and proximal inner segments were not adjacent. These pairs were excluded from the analysis.

Psychophysics

The general interferometric methods are described by Chen et al. [10], and details specific to the present observations are described by Qi [33]. Gratings from two mutually incoherent HeNe laser interferometers were superimposed on the observer's retina within a 1° test field during 2 ms flashes against a steady 3° adapting field of incoherent light (0, 10, or 100 td). The observer chose which of two flashes contained a grating. An adaptive procedure [34], adjusting stimulus contrast on each trial to optimize estimation of the stimulus contrast, yielded 92% correct responses. Each condition was tested in three or four blocks of 50 trials. The two interference gratings (same spatial frequencies) were counter-rotated from the vertical by an amount that depended on their spatial frequency, so that the illusory distortion grating they produced—the only grating the observer could detect—was always 6 cycles/degree. Stimuli were calibrated with a United Detector Technology QED 100 before each day's session.

Computations

To calculate the S/N ratio, we simulated a two-dimensional array of cones and presented either a uniform background or a sine-wave grating superimposed on a background of the same mean intensity (photons·μm^{-2}·s^{-1}). Gratings, like those for human psychophysics [35, 36], were damped with a Gaussian envelope and presented for 100 ms. The wavelength was set to excite M and L cones equally [37] (S cones are absent from the fovea) [38, 39]. The simulation included optical blur for high photopic background. This was set by a 2 mm pupil [40], modeled by a sum of 2 Gaussians [41]; for mesopic background, blur was set by a 5.7 mm pupil [40], modeled by a power function [42]. The diameter of 2 mm was chosen for the simulation because it is appropriate for high photopic backgrounds

[43] and is close to optimal [40], so it represents a lower bound on the amount of optical blur. The 5.7 mm diameter was chosen to be appropriate for mesopic backgrounds [43] where spatial acuity is much lower [40]. The blurred and attenuated stimulus was convolved with the cone aperture function [10]. The result was subsampled at the spacing of the cone inner segments (2.5 μm) [36, 44] and finally attenuated by transmittance of ocular media and macular pigment at 550 nm (reviewed by Makous [45]), the spectral sensitivity (0.65 at 550 nm) and isomerization efficiency (0.67) [37]. At this stage, the information available for each cone was a Poisson distribution of isomerizations (R*). Transduction was simulated with a simple mechanism in which the absorbed photon flux R* modulated a conductance through the equation

$$G = G_c [1 - I/(I + k_0)] \quad (3)$$

where G = light modulated conductance, G_c = maximum conductance, k_o = the intensity for half-maximal conductance, and I = intensity.

This light-modulated conductance (750 pS, $E_{rev} \approx 0$ mV) was inserted into a compartment that simulated the cone membrane (outer/inner segment, soma, axon, terminal) (Figure 3C). This was a sphere 18 μm in diameter, with Rm = 5800 Ohm-cm^2, Cm = 1×10^{-6} F/μm^2, and total a capacitance of 10 pF. At the light intensities studied (5×10^4 and 5×10^2 photons·μm^{-2}·s^{-1}, equivalent to 5×10^3 and 5×10 td; [46]), the light-modulated conductance gave a steady response between -45 and -30 mV; for the contrast studied (<0.1), signal amplitudes were 1 mV or less, so nonlinear saturation was not evident. The cones were coupled into a triangular grid (six neighbors). Because arrays larger than the period of the lowest spatial frequency tested (4 cycles/degree) gave identical results, subsequent runs used a 33×33 cone array (10-fold greater than the largest cone space constant tested). The complete simulation including light stimulus, optical blur, cone aperture, Poisson photon noise, transduction, cone compartments, and coupling was implemented in the Neuron-C simulation language. For a more detailed model of the foveal cone, see Hsu et al. [47]. Signal and noise amplitudes were measured from the central cone in the 2D array. Improvement in S/N was the ratio of noise without coupling divided by the noise with coupling. S/N improvement varied with the cutoff frequency at which the cone signal was temporally low-pass filtered because of the effect of capacitance. For a filter with a time constant of 50 ms (similar to cone transduction), coupling of 320 pS improved S/N by 1.77.

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Retinal Function: Coupling Cones Clarifies Vision

Dispatch

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Gap junctions have been shown to electrically couple cone photoreceptors: coupling blurs the image coded by cones, but this loss is offset by a decrease in noise. Electrical coupling thus improves the resolution of signals distributed across groups of cells.

The quality of the signals in our photoreceptors limits what we can and cannot see. Take two-point resolution. To tell that a distant bright speck is two thin polar bears, rather than one fat one, two conditions must be met. At least three photoreceptors must be involved: one for each of the bears, and a third to show the space between them. To see the gap, the signal in the middle photoreceptor must be noticeably different from its two neighbors' (Figure 1). It follows that our two-point resolution is limited by the spacing of our narrowest and most densely packed photoreceptors — our foveal cones. Anything that reduces the difference between the signals in neigh-boring cones reduces our ability to resolve two points. A familiar culprit is optical blur, which spreads light from a single point across several cones (Figure 1). It was surprising, therefore, to see gap junctions connecting cones in electron micrographs [1]. Gap junctions electrically couple cells, for example at electrical synapses, and will therefore impair spatial resolution by reducing the differences between signals in neighboring cones (Figure 1).

A joint study by three groups [2], published recently in *Current Biology*, has now resolved the paradox of cone gap junctions. Electrical coupling turns out to improve spatial resolution by reducing noise. The combined approaches taken by the three groups — physiology, psychophysics and modelling — provide a compelling account of the function of this neural interaction by answering three questions. How strongly do gap junctions electrically couple cones? Does coupling affect the performance of the intact cone array? And does coupling improve vision?

In this new work, the strength of electrical coupling was measured directly, by making whole cell recordings from pairs of cones in slices of ground squirrel retina, injecting current into one cell and recording the response of the other. Electrical coupling was found to be significant, and to be unaffected by neuromodulators that change the properties of other retinal circuits.

Sophisticated psychophysical tests showed that cones in the intact human retina behave as if they are coupled [2]. Electrical coupling will reduce acuity by distributing the signal from a single point to several cones. But because this coupling effect is superimposed on the broader optical point spread function of the eye's focusing system (Figure 1), it is difficult to detect the influence of cone coupling on human visual acuity. By steering coherent monochromatic laser light through a subject's pupil, one can form an interference pattern of regular stripes directly on their cone array [3]. The stripe width can be varied to values less than the diameter of a single cone to measure visual acuity.

The psychophysicists among DeVries *et al.* [2] exploited a clever trick that can be used to isolate the spatial sensitivity of cones from the effects of neural interactions higher up in the visual system [4]. When two fine interference patterns are superimposed on a subject's cone array, he or she sees a coarser pattern, which is a distortion product produced by non-linearities in the nervous system. By varying the width of the fine laser patterns and measuring the changes in the

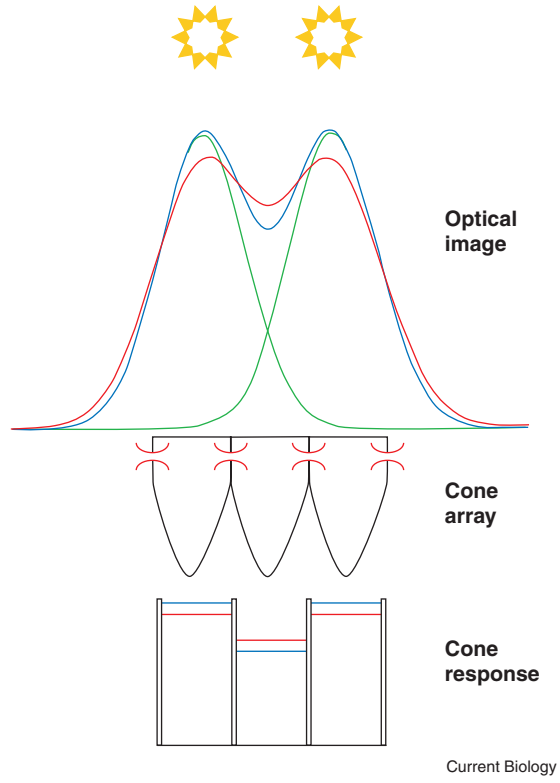

Figure 1. Cone coupling and spatial resolution.

The light from two points is blurred by the optical point spread function of the eye's optics (green), so that the resulting image (blue) is two broad peaks separated by a shallow dip. Three cones are required to resolve the two points, one for each peak and one for the dip. The dip must be deep enough to produce a signal in the central cone that is detectably different from its neighbors. Gap junctions and their effects are shown in red. Gap junctions reduce the difference between signals in neighboring cones by electrical coupling. Their effect is equivalent to a small increase in the width of the optical image and a decrease in its depth.

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visibility of the coarser distortion product, the psychophysicists deduced the spatial sensitivity of foveal cones. This fine technique showed that the central peak in cone sensitivity, corresponding to light entering a single cone, is surrounded by a ring, corresponding to signals from electrically coupled neighbors [2]. The spatial sensitivity of cones is unaffected by changes in light level, suggesting again that coupling is not modulated.

DeVries *et al.* [2] went on to formulate an electrical model of the cone array, which shows that coupling improves vision by reducing the noise level. If signals from neighboring cones are added, the noise produced by photons and ion channels tends to cancel out [5,6]. With less noise, one can resolve smaller differences in cone signal. For all but the tiniest stimuli, this improvement in resolution more than compensates for the reduction in signal differences caused by coupling. Thus cone coupling clarifies vision by judiciously blurring the image.

The electrical coupling of cones is good neural engineering. Gap junctions are simple, cheap and reliable, and the alternative fast mechanism — a chemical synapse — would inject noise into a noise reduction network. Electrical synapses are found in other networks where reliability and precision are important, including invertebrate photoreceptors [7], auditory and electrosensory systems [8], motor pattern generating circuits [9], and networks of neurons deeper in the retina [10]. But, if neurons deeper in the retina are coupled, why couple cones?

Spatial detail destroyed by cone coupling cannot be recovered later in visual processing. In principle, the eye could keep cones separate to preserve spatial detail, and reduce noise later by coupling neurons. But there are good reasons to couple cells before they synapse [6,7]. Reducing noise levels before transmission reduces the risk of saturating the synapse. Coupling also prevents synaptic non-linearities from disrupting noise reduction. Recall that coupling reduces noise by cancelling positive and negative fluctuations in neighboring cells. The non-linear input–output function of a chemical synapse skews these fluctuations by amplifying inputs of different amplitude by different amounts, and this skew invalidates the cancellation of noise [2].

The simplicity of electrical coupling should not blind us to its many advantages. In development, gap junctions could clarify the spatial distribution of morphogenetic signals to make patterning more reliable. Note here that, just as cones drive non-linear synapses, morphogens drive non-linear 'either/or' decisions of cell fate. Nor should we underestimate the role of electrical synapses in neural computation. In the salamander retina, an electrically coupled network of voltage sensitive rod inner segments can pick out the advancing edges of approaching prey [11]. Few circuit designers would choose to dispense with the electronic equivalent of electrical coupling — the resistor.

The remarkably complete analysis by DeVries *et al.* [2] of a relatively simple interaction, cone coupling, demonstrates the power of combining anatomy, physiology, psychophysics and modelling in studies of retina. Neuroanatomists reconstruct retinal circuits and identify both the sites of signaling, and their molecular mechanisms [12,13]. Physiologists use whole cell recordings to describe how these circuits process signals [2,14]. Psychophysicists have sophisticated optical systems [2,15] that can microstimulate intact retina and establish the action of circuits. Computational neuroscientists apply the powerful models that are appropriate for the level of complexity found in retina [2]. Add more attention to natural stimuli [16] and to behavior [17], and we have a potent combination. We can expect more analyses that are sufficiently complete to establish function and discover general principles of cell signaling and information processing.

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