Physiology of L- and M-cone inputs to H1 horizontal cells in the primate retina

Dennis M. Dacey

Department of Biological Structure and the Regional Primate Research Center, University of Washington, Seattle, Washington 98195-7420

Lisa C. Diller and Jan Verweij

Department of Biological Structure, University of Washington, Seattle, Washington 98195-7420

David R. Williams

Center for Visual Science, University of Rochester, Rochester, New York 14627-0270

Received July 19, 1999; accepted August 17, 1999; revised manuscript received September 16, 1999

In the primate retina, H1 horizontal cells form an electrically coupled network and receive convergent input from long- (L-) and middle- (M-) wavelength-sensitive cones. Using an *in vitro* preparation of the intact retina to record the light-evoked voltage responses of H1 cells, we systematically varied the L- and M-cone stimulus contrast and measured the relative L- and M-cone input strength for 137 cells across 33 retinas from three Old World species (*Macaca nemestrina, M. fascicularis*, and *Papio anubis*). We found that the L- and the M-cone inputs were summed by the H1 cell in proportion to the stimulus cone contrast, which yielded a measure of what we term L- and M-cone contrast gain. The proportion of L-cone contrast gain was highly variable, ranging from 25% to 90% [mean ± standard deviation, (60 ± 14)%]. This variability was accounted for by retinal location within an individual, with the temporal retina showing a consistently higher percentage of L-cone gain, and by large overall variation across individuals, with the mean percentage of L-cone gain ranging from 32% to 80%. We hypothesize that the relative L- and M-cone contrast gain is determined simply by the relative number of L and M cones in the H1 cell's receptive field and that the variability in L- and M-cone contrast gain reflects a corresponding variability in the mosaic of L and M cones.
[S0740-3232(00)00403-8]

OCIS codes: 330.0330, 330.1720, 330.4270, 330.5310, 330.5380.

1. INTRODUCTION

In trichromatic primates, signals from the long-wavelength-sensitive (L) and the middle-wavelength-sensitive (M) cones are combined at the first synapse with bipolar and horizontal cell interneurons, representing the earliest stage in the formation of a luminance channel in the visual pathway. The precise relationship, however, among the spatial distribution of the two cone types, neural events at the cone synapse, and spectral coding remains unclear. The human photopic luminosity function is well modeled as the weighted sum of L- and M-cone signals,[1] but the relative L- and M-cone contribution to the luminosity function varies greatly across individuals (with a mean L/M cone ratio of ~2:1). This has long been interpreted as reflecting large individual variation in the relative numbers of L and M cones.[2–4] Two recent findings strongly support this view. First, identification of the L and the M cones in the eyes of two human subjects confirms large individual variation in the cone ratio[5]; and, second, the cone weightings predicted from a physiological measure of the eye's spectral sensitivity for these two subjects agreed with the observed cone ratios.[6] Thus individual variation in spectral sensitivity is determined directly by a corresponding variation in the L- and the M-cone ratios.

What do these results obtained from the human retina suggest about retinal processing of a luminance signal? The simplest hypothesis is that retinal neurons that combine L- and M-cone input do so simply in proportion to the relative number of the two cone types in their spatial receptive fields. This question has not been addressed directly at the level of single-cell physiology. Luminance-coding ganglion cells in the macaque monkey show a spectral sensitivity that matches the human photopic luminosity function,[7,8] a result that is indicative of a 2:1 L- to M-cone ratio. By contrast, overall electroretinogram (ERG) measurements of the spectral sensitivity of macaque eyes suggest a relatively low mean L- to M-cone ratio of approximately 1:1.[3] It is not clear whether this disagreement reflects differences in the methods used for estimating the cone weighting or whether it reflects a real difference in the way that cone signals are weighted at different points in the retinal circuitry.

The present study is part of a series of experiments conducted with the goal of more directly measuring the relative weights of L- and M-cone inputs to single neurons at different points in the pathway from cones to ganglion cells. Here we focus on L- and M-cone inputs to the H1 horizontal cell type. The H1 cell is a key interneuron for understanding L- and M-cone signal pathways; it is im-

plicated in creating the receptive field surround for both the red–green color opponent and the nonopponent luminance pathway ganglion cells that project via the dorsal lateral geniculate nucleus to the primary visual cortex.[9] Several features of this interneuron make it ideal for characterizing how L- and M-cone signals are combined in the outer retina. H1 cells form an electrically coupled network, summing input from hundreds of L and M cones across a large receptive field.[10] H1 cells lack measurable short-wavelength-sensitive- (S-) cone input and appear to avoid or nearly avoid all contact with S cones.[11,12] Rod input to the H1 cell has been characterized so that it is possible to use a stimulus protocol that effectively eliminates any rod contribution to the H1 cell light response.[10] Finally, it is possible to make stable, long-lasting intracellular recordings from morphologically identified H1 cells by use of an *in vitro* preparation of the intact retina.[11]

We found that, for a large sample of H1 cells, the ratio of L- to M-cone physiological gain varied widely. This variation can be accounted for by a consistent nasal-to-temporal increase in the L-cone contribution within single retinas and by a large variation across individual retinas in the mean L- to M-cone gain ratios. We show elsewhere that the variable physiological weights of L- and M-cone inputs to H1 cells are strongly correlated with the relative L- and M-cone photopigment mRNA content measured in the same piece of tissue,[13] which supports the conclusion that the physiological L- to M-cone ratio is determined directly by the relative number of L and M cones in the H1 cell's spatial receptive field.

2. METHODS

A. *In vitro* Preparation and Histology

H1 horizontal cells were targeted for recording by use of a previously described[10,11] *in vitro* preparation of the intact retina. Eyes from two macaque species, *M. nemestrina* ($n = 21$) and *M. fascicularis* ($n = 7$), and from a baboon, *Papio anubis* ($n = 5$), were obtained through the Tissue Distribution Program at the Regional Primate Research Center at the University of Washington and were removed under deep barbiturate anesthesia prior to euthanasia. Retinas were dissected free of the vitreous and the sclera in an oxygenated culture medium (Ames' medium, Sigma) and were placed flat, vitreal surface up, in a superfusion chamber mounted on the stage of a light microscope. H1 cell nuclei were identified at the outer border of the inner nuclear layer after superfusion of the fluorescent nuclear stain 4,6 diamidino-2-phenylindole (DAPI) (10 μM). For combined intracellular recording and staining, microelectrodes were filled with a solution of 2–3% Neurobiotin (Vector Laboratories, Burlingame, California) and 1–2% pyranine (Molecular Probes, Eugene, Oregon) in 1M KCl. Electrical impedances ranged from 180 to 300 MΩ. Pyranine fluorescence in the electrode and DAPI in cells were viewed together under episcopic illumination with the same filter combination. Cell body penetration was confirmed by iontophoresis of pyranine.

After recording from a cell, the cell was iontophoretically injected with Neurobiotin (+0.1–0.2 nA; ~15 min).

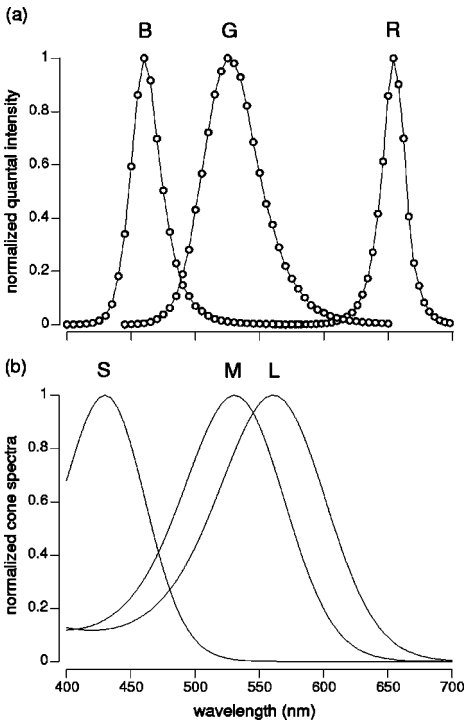

Fig. 1. (a) Normalized quantal intensity of the red (R), green (G), and blue (B) LED's as a function of wavelength, measured in the plane of the retina. (b) Predicted L-, M-, and S-cone spectral sensitivities (modified from Baylor *et al.*[16]).

After the experiment, retinas were dissected from the choroid and were fixed in 4% paraformaldehyde (0.1M, phosphate buffer, pH 7.4) for ~2 h and were then placed in a buffered solution of 0.1% Triton X-100 (Sigma) containing avidin–biotin–horseradish-peroxidase complex (Vector Laboratories, Burlingame, California) for 5 h or overnight. Retinas were rinsed for 2 h, and horseradish-peroxidase histochemistry was performed with the use of diaminobenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland) as the chromogen. Retinas were subsequently mounted on slides in a water-based solution of polyvinyl alcohol and glycerol.

B. Light Stimulation

Horizontal cell light responses were recorded with a light-emitting-diode- (LED-) based stimulator.[14,15] Light sources were red, green, and blue LED's (with peak wavelengths of 656, 525, and 460 nm, respectively) mounted on a small optical bench above the microscope such that the light path was projected through the camera port as a homogeneous field with a diameter of ~3 mm in the plane of the retina. The spectral output (referred to here as quantal intensity) of the three LED's was measured in the plane of the retina at 2 or 5 nm intervals by use of a fiber-optic probe coupled to a spectroradiometer (Gamma Scientific, San Diego, California) and is shown in Fig. 1(a). The irradiance of the stimuli is expressed as log quanta per square millimeter per second.

To quantify the relative strengths of the L- and the M-cone inputs to an H1 cell our goal was to systematically vary the L- and the M-cone contrasts in the light stimulus while keeping all three cones in a similar state of adaptation. To do this we first measured the spectral intensity

of the three LED's and predicted their effectiveness for each cone type. We set the mean level for each LED to produce the same mean quantal catch for each cone type while sinusoidally modulating the LED intensities to produce a particular modulation depth for the L and the M cones.

To calculate the efficiency of the LED's for the three cone types in our preparation we had to choose some measure of the spectral sensitivity of the L, M, and S cones. Possible choices involved the psychophysically derived human cone fundamentals,[1] photopigment spectra and their polynomial fits,[17] or electrophysiologically determined spectral sensitivities of single macaque cones.[16,18] The polynomial fit calculated by Baylor *et al.*[16] for the macaque cone spectral sensitivities was chosen since this fit required the fewest assumptions. For example, there is now strong evidence that the macaque and the human do not have identical L- and M-cone spectra[3]; furthermore, if we had chosen the human fundamentals, we would have had to make a correction for the lack of preretinal absorption in our *in vitro* preparation. We also rejected photopigment nomograms, as we would have had to assume that macaque pigments had a similar shape, and we would have had to choose a wavelength of peak absorption.

We adjusted the polynomial fit given by Baylor *et al.*[16] to correct for a slightly larger effect of photopigment self-screening in our preparation. In the study conducted by Baylor *et al.*, single cones were illuminated from a direction perpendicular to the main axis of the outer segment[16]; the effect of pigment self-screening in the data of that study was therefore negligible. In the intact eye the outer segments of the cones are directed at the center of the pupil; when the retina is laid flat in the recording chamber the photoreceptors are tilted relative to the path of the light stimulus. We assumed that, based on the geometry of the eye, the angles of the outer segments are roughly equal to their retinal eccentricity (in degrees). We recorded from H1 cells located in the retinal periphery at eccentricities of 25–80 deg. Given a diameter for the L-, M-, and S-cone outer segments of ~3 μm,[16] the path length of the light through the cone outer segment in our preparation was 3–7 μm. We also assumed that the pigment axial density for all three cones at their respective peak wavelengths was 0.016 μm^{-1}, which implies that all three cones have equal quantal efficiency at their peak wavelengths. Given a mean path length of 5.5 μm, the added pigment density in our preparation is only 0.04. Thus the estimated spectral sensitivity of the cones in our preparation, $s'(\lambda)$, was described by the formula

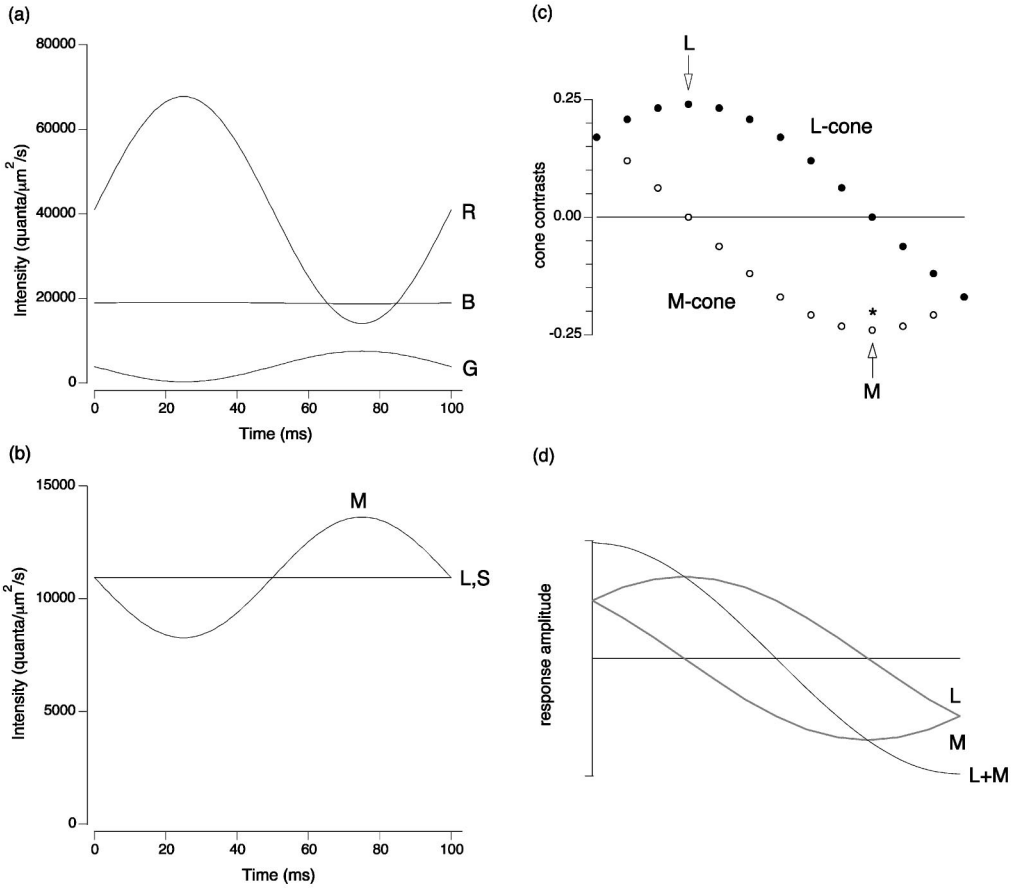

Fig. 2. (a) Example of a cone-isolating stimulus. Mean intensities of the red (R), green (G), and blue (B) LED's were adjusted to produce equal mean quantal catches in each cone type, whereas the amplitudes of the LED's were adjusted to modulate only M cones. (b) Predicted response of the three cone types to the stimulus shown in (a). Only M cones are modulated while L and S cones remain silent. (c) Predicted L-cone (●) and M-cone (○) contrasts for the 13 conditions in our stimulus paradigm. Arrows indicate cone contrasts for the L- and the M-cone-isolating conditions. The cone contrast of the M-cone-isolating condition described in (a) and (b) is indicated by an asterisk. (d) Traces denoted by L and M are the predicted L- and M-cone contrasts shown in (c). Trace denoted by L + M is the predicted response of an H1 cell that receives additive input from L and M cones.

$$s'(\lambda) = 1 - 10^{-0.04s(\lambda)}, \quad (1)$$

where s is the corresponding polynomial given by Baylor *et al.* and λ is wavelength (in nanometers). Our cone spectral sensitivity curves differ only slightly from the polynomials of Baylor *et al.* [Fig. 1(b)].

We calculated the relative efficiency of each of our LED's for each of the three cone types (P_C), using the equation

$$P_C = \frac{\sum_{\text{all}\lambda} [P_i(\lambda) \times s_C'(\lambda)]}{\sum_{\text{all}\lambda} P_i(\lambda)}, \quad (2)$$

where $P_i(\lambda)$ is the quantal intensity of the LED at wavelength λ and $s_C'(\lambda)$ is the spectral sensitivity of the cone type. We designed a stimulus paradigm to keep all three cone types in an equal adaptive state while varying the modulation depth of one cone type. Assuming that all three cone types are in an equal adaptive state if they all have the same quantal catch, the mean intensities for the three LED's were chosen to produce an equal quantal catch in each cone type. The stimulus paradigm was constructed so that we could modulate only L and M cones at 9.7 Hz while keeping the S cones silent. The contrast for each cone type, defined as the amplitude divided by the mean quantal catch for each photopigment, was varied between stimuli. Figure 2 provides a graphical representation of the stimulus. In this example M cones are modulated at 25% contrast while the L and the S cones are kept silent. The mean and modulation intensities of each LED are shown in Fig. 2(a), and the predicted mean and modulation for each cone type are shown in Fig. 2(b).

Using similar calculations, we designed a paradigm in which the L- and the M-cone contrasts were systematically varied over 13 stimulus conditions, as shown in Fig. 2(c). The L- and the M-cone contrasts are initially equal and in phase. The M-cone contrast is then decreased while the L-cone contrast is increased until M-cone contrast is zero [L-cone isolation; Fig. 2(c), arrow labeled L]. M-cone contrast then reverses phase and increases, and the L-cone contrast decreases until the M-cone-isolating point is reached [Fig. 2(c), arrow labeled M]. Finally, the two cone contrasts come back into phase.

C. Data Collection and Analysis

For each condition in the stimulus paradigm, data was averaged over at least 3 s, and the stimulus paradigm was repeated several times on each cell. To measure the L- and the M-cone input strength a model was fitted to the family of voltage responses initiated by the stimulus paradigm illustrated in Figs. 2(c) and 2(d). For cells that sum L- and M-cone input, there is a phase reversal in the voltage response around the point at which the L- and the M-cone inputs are opposite in sign, and a response minimum is attained (see Fig. 4 below). The amplitudes of the first harmonic component of the response having the approximate phase of the starting stimulus were assigned a positive value, while those with a near-180-deg phase shift were assigned a negative value. This modified response amplitude was fitted with a model that simply added the inputs from the two cones:

$$\text{Response amplitude} = W_L L_c + W_M M_c, \quad (3)$$

where W_L and W_M are the strengths of the L- and the M-cone inputs and L_c and M_c are the contrasts presented to the L and the M cones.

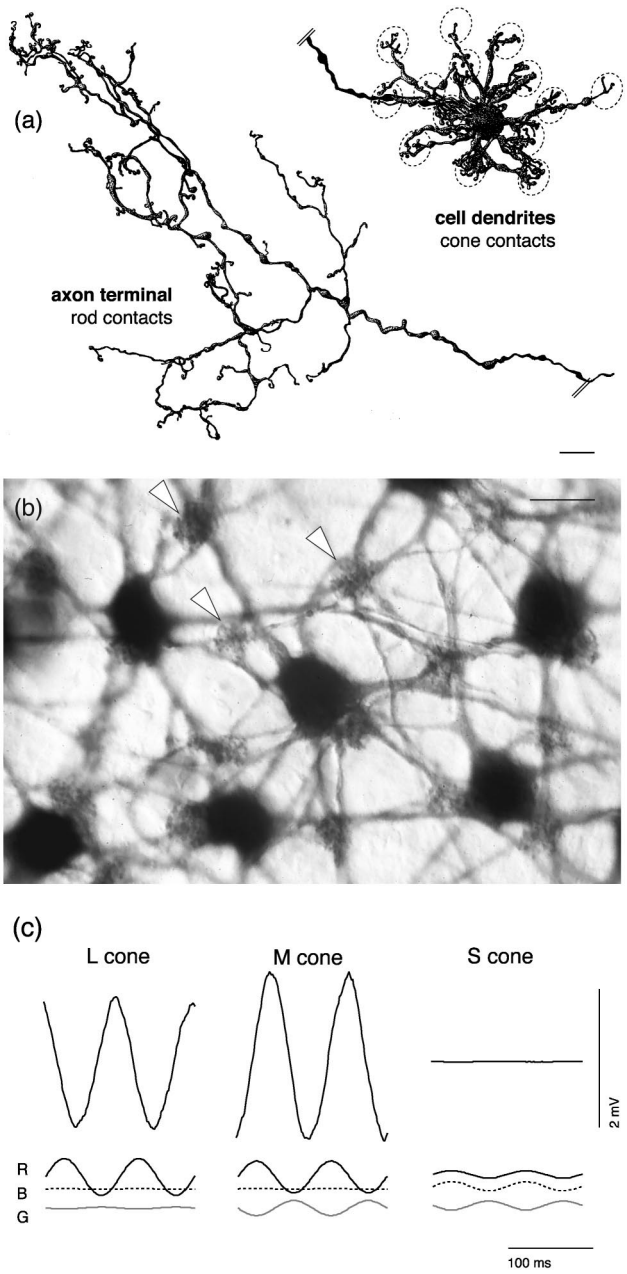

Fig. 3. (a) Camera lucida tracing of a Golgi-impregnated macaque H1 cell showing the cell body and axon terminal (entire axon not shown). Dotted circles are drawn around cone-contacting dendritic terminals. The cell was located 13 mm from the fovea. (b) Photomicrograph of a flat-mounted macaque retina showing a patch of the H1 cell mosaic revealed by intracellular injection of neurobiotin. Arrowheads indicate three cone pedicles demarcated by H1 cell dendritic terminals. Scale bars in (a) and (b), 10 μm. (c) Responses (upper traces) of a macaque H1 cell to L-, M-, and S-cone-isolating stimuli (lower traces) as described in Fig. 2(a).

3. RESULTS

A. Identification of H1 Horizontal Cells and Their Cone Inputs

To confidently measure the relative strengths of L- and M-cone inputs to the H1 horizontal cell it was first necessary (1) to establish that all the recordings were taken from identified H1 cells, (2) to confirm a consistent lack of measurable S-cone input to the H1 cell network, and (3) to ensure that a potential input from rod photoreceptors does not affect the cone input measurements.

All the recorded horizontal cells were identified *in vitro* by intracellular injection of pyranine. H1 cells were reliably distinguished from other cell types of the outer retina by their distinctive dendritic morphology[11,19] [Fig. 3(a)]. Intracellular injection of neurobiotin was also used to demonstrate the morphology of these cells by horseradish-peroxidase histochemistry after tissue fixation.[11] Neurobiotin apparently passes through gap junctions to neighboring H1 cells,[20] revealing a distinct network of H1 cell bodies and dendrites [Fig. 3(b)]. Fingerlike dendritic terminals innervate and clearly demarcate the majority of L- and M-cone pedicles[11,12] [Fig. 3(b), arrowheads] while avoiding significant contact with the sparsely distributed S-cone pedicles.

The lack of anatomical connection to S cones is reflected in the lack of a voltage response to stimuli that modulate S cones in isolation (see Section 2). Figure 3(c) shows the voltage response of one H1 cell to 2 cycles of L-, M-, and S-cone-isolating modulation. No response to the S-cone modulation could be measured; L- and M-cone-isolating modulations gave large responses of differing amplitude.

A relatively weak rod input can be recorded at the H1 cell body, and this input arises via the rod–cone gap junction.[10] This rod signal is difficult to demonstrate and requires a significant period of dark adaptation, stimuli of low temporal frequency, and retinal illuminances in the low mesopic range. For the cone-modulating stimuli used in the present study, the retina was not dark adapted; retinal illuminance (~2000 trolands) and stimulus temporal frequency (10 Hz) fell outside the response range of the measured rod signal in H1 cells.[10] We are confident, therefore, that all the voltage responses reported here reflect input from L and M cones only and that they can be used to determine the relative contrast gains of input from the two cone types.

B. Responses of H1 Horizontal Cells

The response of an H1 cell to a stimulus series that systematically varied the percentage and the sign of the L- and the M-cone contrast was well fitted by a linear model (see Section 2) in which the strength of each of the cone inputs is given by the cone contrast and in which the two inputs are added by the H1 cell (Fig. 4). One cycle of the voltage response of an H1 cell to the stimulus series is shown in Fig. 4(a). Response maxima occur at the extremes of the series in which total $L + M$ cone contrast is highest. For this cell the response minimum, and a 180-deg phase reversal, occurs at the point at which L- and M-cone contrast are approximately equal but opposite in sign, which indicates that the strength of the L- and the M-cone inputs are nearly equal. This can also be appreciated in the response amplitudes to the L- and the M-cone-isolating conditions. The first harmonic amplitude for each response is plotted along with the model fit to the data in Fig. 4(b). The fit is excellent, and the ratio of L- and M-cone contrast gain derived from the point at which the curve fit to the data gives zero amplitude response is 1.27 [$L/(L + M) = 0.56$].

One hundred thirty-seven H1 cells were sampled from a total of thirty-three animals of three species (see Section 2). The results were similar for all three species and are not further distinguished, although, given the small sample from two groups (*M. fascicularis* and *P. anubis*), species-specific differences cannot be ruled out. The cone contrast gain ratios varied over a broad range (Fig. 5). The proportion of L-cone contrast gain varied from a low of 0.25 to a high of 0.9 with a mean of 0.60, indicating an L- to M-cone gain ratio of 1.5. To determine to what degree, if any, the reliability of our recordings and/or the physiological quality of the *in vitro* retinal preparation contributed to this variability, we ran repeated trials of the stimulus protocol. Contrast gains were found to be extremely reliable. For example, 14 runs for a single

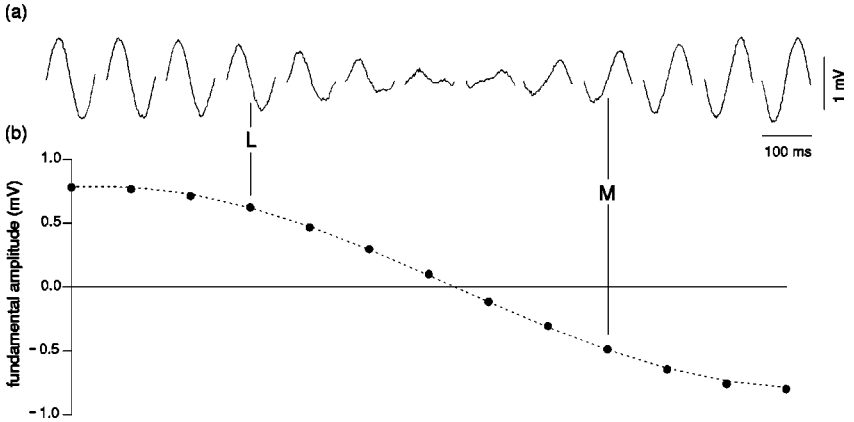

Fig. 4. (a) Responses of an H1 horizontal cell to the 13 conditions of our stimulus paradigm. Each trace is the average of at least 3 s of data. (b) Fundamental amplitude of the responses shown in (a). The lines joining the traces in (a) with the data points in (b) indicate responses to L- and M-cone-isolating stimuli. The dotted curve drawn through the data points is the fit of the model used to determine the relative L- and M-cone contrast gains. This cell had an $L/(L + M)$ gain of 0.56 ($L/M = 1.27$).

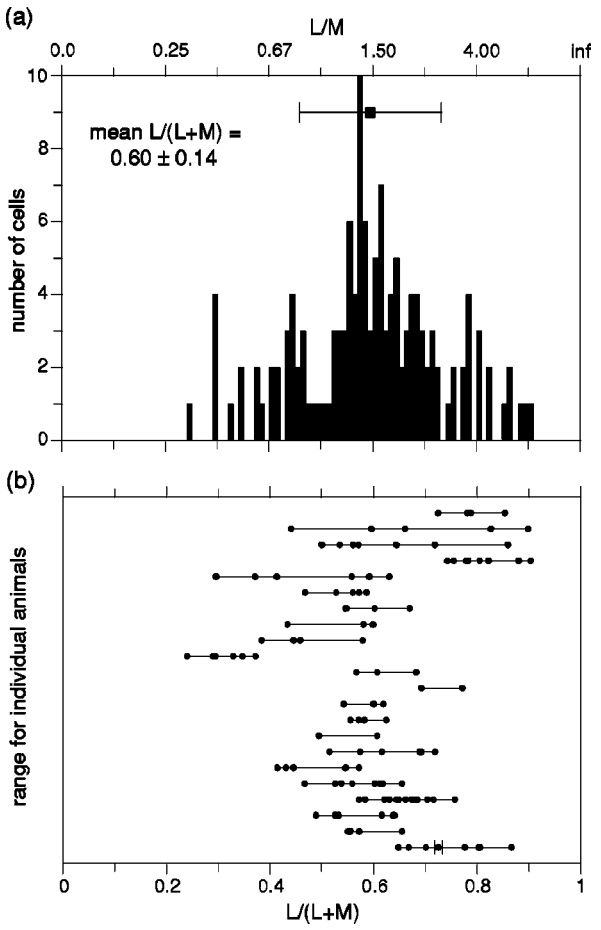

Fig. 5. (a) Histogram of the $L/(L + M)$ ratio for all 137 H1 horizontal cells. The $L/(L + M)$ value is given on the bottom axis, whereas the corresponding L/M value is given on the top axis. The histogram was generated with a bin size of 0.01 along the $L/(L + M)$ axis. There was a large variability in the relative strength of L- and M-cone signal strength, with a mean of $L/(L + M) = 0.60$. (b) The range of L- and M-cone signal strength in 22 animals (123 cells), demonstrating a large variation in the relative strength of L- and M-cone signals in H1 cells among the animals. Error bars [standard deviation (SD), 0.0073] for measurements of the $L/(L + M)$ ratio in one cell are included for the animal whose data are shown at the bottom of the plot.

cell [Fig. 5(b)] gave a mean ±1 SD $L/(L + M)$ value of 0.72±007. The SD's for all the cells were similarly small, ranging from 0.5% to 1.5% of the mean. In addition, we found that contrast gains measured at a particular retinal location at the beginning of a recording session were the same as those measured for essentially the same retinal location near the end of a recording session, up to 12 h later. We conclude that the variability in the L- versus M-cone contrast gains cannot be accounted for by any progressive changes in the physiological state of the retina or the reliability of the cells' response to the stimulus.

Variation in the L- versus M-cone gains was, however, accounted for by a combination of small shifts as a function of retinal location together with larger shifts across individuals. This feature is clear when the data in Fig. 5(a) are replotted to show the range in $L/(L + M)$ for each individual [with at least two locations sampled; Fig. 5(b)]. Within an individual the proportion of L-cone gain can vary by ~10% around the mean (SD's ranged from 2% to 18%). However, across individuals this variation can be much greater. At the extremes were individuals with mean ±1 SD L-cone gain proportions of (32 ± 4)% and (81 ± 5)%. Thus, despite the variability within a given retina, different individuals may show no overlap in the proportion of L-cone gain.

The variability in the proportion of L-cone gain within a retina was not random but showed a systematic nasotemporal asymmetry. All the cells were recorded in the retinal periphery, ranging in eccentricity from approximately 5–15 mm (25–75 deg of visual angle). We mapped the H1 cells belonging to the nasal, temporal, superior, and inferior retinal quadrants (Fig. 6). The sample size and the eccentricity range were similar for nasal, superior, and temporal retina; only a few cells were located in the inferior quadrant and are not further considered. For any given retina, the temporal quadrant tended to have the highest L-cone gain. We quantified this by comparing the proportion of L-cone gain across quadrants for each retina in which the temporal quadrant was sampled. The temporal values were then normalized to 1.0, and the data from the other quadrants were plotted relative to the temporal retina (Fig. 6, inset). The means ±1 SD for 13 retinas showed a 17% reduction (22 cells) in the nasal quadrant and a 10% reduction (17 cells) in the superior quadrant as compared with the temporal quadrant (27 cells).

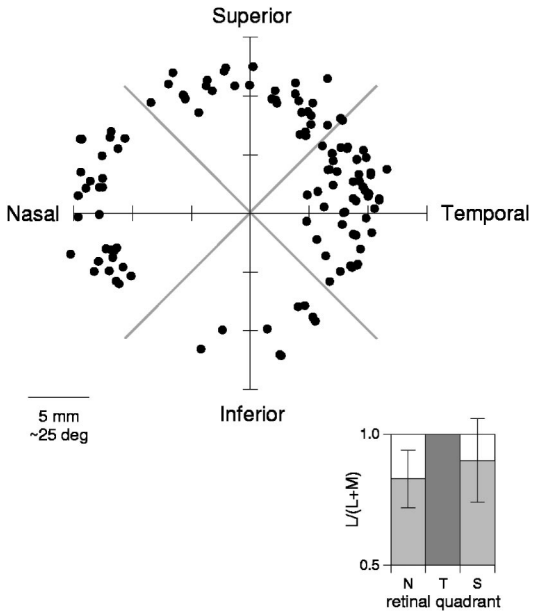

Fig. 6. Retinal location of 125 H1 cells from 25 retinas, showing the nasal–temporal and the superior–inferior meridians. Diagonal lines bisecting the meridians indicated the four retinal quadrants. The majority of cells from which recordings were made were located in the far periphery. The inset shows the relative $L/(L + M)$ ratio for a subset of 66 cells from 13 retinas, plotted as a function of retinal quadrant (nasal: $n = 22$; temporal: $n = 27$; superior: $n = 17$). Only retinas with sampled temporal cells were included, and the data were normalized to the temporal quadrant. The $L/(L + M)$ ratio of nasal cells and superior cells was 86% and 90%, respectively, relative to that of temporal cells. Error bars are SD = 0.11 for nasal cells and SD = 0.16 for superior cells. The inferior quadrant was omitted because the subset included only two inferior cells.

4. DISCUSSION

The relative strengths of L- and M-cone inputs to an H1 horizontal cell were well characterized by the sum of the cone inputs weighted by the stimulus contrast presented to each cone type (e.g., Fig. 4). Because the stimuli were designed to produce a constant mean quantal catch for all the cones, it is unlikely that the relative strengths of L- and M-cone inputs to a given H1 cell reflect any significant differences in the relative sensitivities (adaptation states) of either cone type. The parsimonious hypothesis that would account for these results is that the L- and the M-cone contrast gain measured for any H1 cell is simply given by the relative number of L and M cones that provide the input to the cell's receptive field. This conclusion fits with the well-established anatomical picture (Fig. 3) showing that the H1 cell network contacts all the L and the M cones indiscriminately and with an apparently equal number of synaptic contacts.[11,19] Similarly, in the cones themselves, there is no evidence either from synaptic numbers[21] or from physiological recordings[22] to suggest that the two cone types might be associated with different signal gains.

Further evidence that the relative cone number predicts physiological gain at the H1 cell has recently been shown by comparison of the H1 cell L- versus M-cone contrast gain with the L- versus M-cone photopigment mRNA content measured in the same piece of retina.[13] This was accomplished by production of an H1 cell recording in the *in vitro* retina and subsequent removal of a small region of retina, a few millimeters in diameter, that included the recorded H1 cell. The tissue was then subjected to analysis of cone opsin mRNA content. The relative L- and M-cone opsin mRNA values and physiological gain values were strongly correlated.[13] H1 cell cone contrast gains and mRNA content also showed similar variability with retinal location and across individuals. Both measures showed greater L-cone weight in the temporal retina relative to the nasal retina.[13,23] Finally, both the physiological cone signal and the opsin message showed a similar broad variability across individuals, with a mean L/M ratio of 1.5. Other studies have suggested that the L- to M-cone pigment gene ratios are given by the relative number of L and M cones in the tissue sample.[23] Thus the correspondence between the physiological gain and mRNA content—two extremely different measurements—is very likely due to relative cone numbers.

If we accept that the L- and the M-cone contrast gains for the H1 cells and the pigment gene measures made by Deeb *et al.*[13] reflect relative cone numbers, then the overall picture of the L- and the M-cone mosaic for the macaque appears very similar, but perhaps not identical, to the emerging picture of the human cone mosaic. In the human the L- to M-cone ratio is also variable both across individuals[5] and as a function of retinal location.[23] Setting variability aside, one finds that L cones dominate in the macaque, with a mean L- to M-cone ratio of 1.5. In the human a large body of diverse evidence, from psychophysical measurements,[4] ERG flicker photometry,[3] and pigment gene content,[23] suggests that the average L- to M-cone ratio for the normal human retina lies between 1.5 and 2.

Despite the overall similarity, a slight increase in L-cone percentage in the human retina when compared with the macaque retina may be a real species difference. ERG flicker photometric measures of spectral sensitivity and L- to M-cone ratios in large human and macaque samples consistently yield results indicating that the macaque has a more balanced L- to M-cone ratio than does the human.[3] Identification of L- and M-cone pigment by microspectrophotometry in a sample of individual macaque cones,[24] or directly from small patches of retina,[25,26] also suggests a reduced L- to M-cone ratio for the macaque. Nevertheless, it is becoming clear that data from macaque and human populations overlap extensively and that the population mean is a poor predictor of the cone ratios for an individual.

The correspondence between the L- and the M-cone signal gain at the H1 cell and the cone opsin mRNA measurements[13] suggests that the relative number of L and M cones at any given point in the cone array is preserved in a postreceptoral neural signal. L- and M-cone input strength measured for other retinal cell types in the macaque also suggests that the relative number of L and M cones in a neuron's receptive field determines that cell's spectral sensitivity. For example, parasol ganglion cells, whose axons project to the magnocellular layers of the lateral geniculate nucleus, also show variability in the weighting of L- and M-cone input but on average have a spectral sensitivity that reflects the overall spectral sensitivity of the eye.[7,27] We have recently made a comparison of the strengths of L- and M-cone inputs to horizontal cells, bipolar cells, and ganglion cells that have overlapping spatial receptive fields and that thereby share input from the same portion of the cone mosaic. The results show that cells that receive input from the same retinal location have nearly identical L- and M-cone contrast gain ratios.[28,29] This picture is in agreement with the recent finding that, in two human subjects, the relative strengths of L- and M-cone contributions predicted from ERG-derived spectral sensitivity curves closely matched the L- and the M-cone ratios imaged directly in the subjects' eyes.[6] These results argue against the idea that postreceptoral signal processing can adjust the relative weights of L- and M-cone signals to produce a luminance code of fixed spectral sensitivity.[8,27] The data support the conclusion that at the single-cell level the spectral sensitivity of a luminance signal that emerges from the retina is simply given by the relative number of L and M cones and, further, that this number can vary greatly both across individuals and as a function of retinal location.

REFERENCES

1. V. C. Smith and J. Pokorny, "Spectral sensitivity of the foveal cone photopigments between 400 and 500 nm," Vision Res. **15**, 161–171 (1975).
2. W. A. Rushton and H. D. Baker, "Red–green sensitivity in normal vision," Vision Res. **4**, 75–85 (1964).
3. G. H. Jacobs and J. F. Deegan II, "Spectral sensitivity of macaque monkeys measured with ERG flicker photometry," Visual Neurosci. **14**, 921–928 (1997).
4. P. Lennie, J. Pokorny, and V. C. Smith, "Luminance," J. Opt. Soc. Am. A **10**, 1283–1293 (1993).

5. A. Roorda and D. R. Williams, "The arrangement of the three cone classes in the living human eye," Nature **397**, 520–522 (1999).
6. D. H. Brainard, A. Roorda, Y. Yamauchi, J. B. Calderone, A. Metha, M. Neitz, J. Neitz, D. R. Williams, and G. H. Jacobs, "Functional consequences of the relative numbers of L and M cones," J. Opt. Soc. Am. A **17**, 607–614 (2000).
7. B. B. Lee, P. R. Martin, and A. Valberg, "The physiological basis of heterochromatic flicker photometry demonstrated in the ganglion cells of the macaque retina," J. Physiol. (London) **404**, 323–347 (1988).
8. R. Shapley, "Parallel neural pathways and visual function," in *The Cognitive Neurosciences*, M. S. Gazzaniga, E. Bizzi, I. B. Black, C. Blakemore, L. Cosmides, S. M. Kosslyn, J. E. LeDoux, J. A. Movshon, S. Pinker, M. I. Posner, P. Rakic, D. L. Schacter, J. Tooby, and E. Tulving, eds. (MIT Press, Cambridge, Mass., 1995).
9. D. M. Dacey, "Primate retina: cell types, circuits and color opponency," in *Progress in Retinal and Eye Research*, N. N. Osborne and G. J. Chader, eds. (Pergamon, Oxford, UK, 1999), Vol. 18, pp. 737–763.
10. J. Verweij, D. M. Dacey, B. B. Peterson, and S. L. Buck, "Sensitivity and dynamics of rod signals in H1 horizontal cells of the macaque monkey retina," Vision Res. **39**, 3662–3672 (1999).
11. D. M. Dacey, B. B. Lee, D. K. Stafford, J. Pokorny, and V. C. Smith, "Horizontal cells of the primate retina: cone specificity without spectral opponency," Science **271**, 656–659 (1996).
12. A. K. Goodchild, T. L. Chan, and U. Grünert, "Horizontal cell connections with short-wavelength-sensitive cones in macaque monkey retina," Visual Neurosci. **13**, 833–845 (1996).
13. S. S. Deeb, L. C. Diller, D. R. Williams, and D. M. Dacey, "Interindividual and topographical variation of L:M cone ratios in monkey retinas," J. Opt. Soc. Am. A **17**, 538–544 (2000).
14. W. H. Swanson, T. Ueno, V. C. Smith, and J. Pokorny, "Temporal modulation sensitivity and pulse-detection thresholds for chromatic and luminance perturbations," J. Opt. Soc. Am. A **4**, 1992–2005 (1987).
15. D. M. Dacey and B. B. Lee, "The blue-ON opponent pathway in primate retina originates from a distinct bistratified ganglion cell type," Nature **367**, 731–735 (1994).
16. D. A. Baylor, B. J. Nunn, and J. L. Schnapf, "Spectral sensitivity of cones of the monkey *Macaca fascicularis*," J. Physiol. (London) **390**, 145–160 (1987).
17. S. M. Dawis, "Polynomial expressions of pigment nomograms," Vision Res. **21**, 1427–1430 (1981).
18. T. D. Lamb, "Photoreceptor spectral sensitivities: common shape in the long-wavelength region," Vision Res. **35**, 3083–3091 (1995).
19. H. Wässle, B. B. Boycott, and J. Röhrenbeck, "Horizontal cells in the monkey retina: cone connections and dendritic network," Eur. J. Neurosci. **1**, 421–435 (1989).
20. D. I. Vaney, "Patterns of neuronal coupling in the retina," in *Progress in Retinal and Eye Research*, N. N. Osborne and G. J. Chader, eds. (Pergamon, Oxford, UK, 1994), Vol. 13, pp. 301–355.
21. D. J. Calkins, "Synaptic organization of cone pathways in the primate retina," in *Color Vision: from Genes to Perception*, K. R. Gegenfurtner and L. T. Sharpe, eds. (Cambridge U. Press, New York, 1999), pp. 163–180.
22. D. M. Schneeweis and J. L. Schnapf, "The photovoltage of macaque cone photoreceptors: adaptation, noise, and kinetics," J. Neurosci. **19**, 1203–1216 (1999).
23. S. A. Hagstrom, J. Neitz, and M. Neitz, "Variations in cone populations for red–green color vision examined by analysis of mRNA," NeuroReport **9**, 1963–1967 (1998).
24. J. Bowmaker, "Visual pigments and color vision in primates," in *From Pigments to Perception*, A. Valberg and B. B. Lee, eds. (Plenum, New York, 1991), pp. 1–10.
25. J. D. Mollon and J. K. Bowmaker, "The spatial arrangement of cones in the primate fovea," Nature **360**, 677–679 (1992).
26. O. S. Packer, D. R. Williams, and D. G. Bensinger, "Photopigment transmittance imaging of the primate photoreceptor mosaic," J. Neurosci. **16**, 2251–2260 (1996).
27. B. B. Lee, "Receptor inputs to primate ganglion cells," in *Color Vision: from Genes to Perception*, K. R. Gegenfurtner and L. T. Sharpe, eds. (Cambridge U. Press, New York, 1999), pp. 203–218.
28. L. C. Diller, J. Verweij, D. R. Williams, and D. M. Dacey, "L and M cone inputs to peripheral parasol and midget ganglion cells in primate retina," Invest. Ophthalmol. Visual Sci. Suppl. **40**, S817 (1999).
29. O. S. Packer, L. C. Diller, B. B. Lee, and D. M. Dacey, "Diffuse cone bipolar cells in macaque monkey retina are spatially opponent," Invest. Ophthalmol. Visual Sci. Suppl. **40**, S790 (1999).