S Cone Contributions to the Magnocellular Visual Pathway in Macaque Monkey

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Summary

The magnocellular visual pathway is believed to receive input from long (L) and middle (M), but not short (S), wavelength-sensitive cones. Recording from neurons in magnocellular layers of lateral geniculate nucleus (LGN) in macaque monkeys, we found that magnocellular neurons were unequivocally responsive to S cone-isolating stimuli. A quantitative analysis suggests that S cones provided about 10% of the input to these cells, on average, while L:M ratios were far more variable. S cone signals influenced responses with the same sign as L and M cone inputs (i.e., no color opponency). Magnocellular afferent recordings following inactivation of primary visual cortex demonstrated that S cone signals were feedforward in nature and did not arise from cortical feedback to LGN.

Introduction

The primate retina introduces three parallel pathways to the early visual system by differentially combining inputs from L, M, and S cones (see Rodieck, 1998). Two pathways compare photon catches between cone types, conferring some specificity to stimulus wavelength. Putatively, parvocellular neurons signal the difference between L and M cone excitation (red/green opponency), whereas koniocellular cells compare S and L + M cone signals (blue/yellow opponency). A third channel, the magnocellular pathway, is believed to sum L and M cone contributions, making it very sensitive to luminance contrast (e.g., Merigan and Maunsell, 1993). Arising from parasol retinal ganglion cells, the magnocellular pathway is relayed by the two ventral layers of LGN to layers 4Cτ and 6 of primary visual cortex.

The commonly held view, based primarily on retinal electrophysiology (Dacey and Lee, 1994; Lee et al., 1988) and psychophysical flicker detection (e.g. Eisner and Macleod, 1980), is that S cones do not contribute to the magnocellular pathway (see Dobkins, 2000; Martin, 1998). Earlier work directly recording from LGN could not provide definitive answers regarding the existence of magnocellular S cone signals (e.g., Wiesel and Hubel, 1966; Padmos and Norren, 1975; Schiller and Malpeli, 1978; Creutzfeldt et al., 1979; Nothdurft and Lee, 1982). These studies characterized the responses as being spectrally broadband; but without explicit S cone-isolating stimuli, the considerable overlap in cone spectral sensitivities (Schnapf et al., 1988) confounds interpretation of the data. Derrington et al. (1984), by varying their chromatic stimuli systematically in opponent color space, did find results suggestive of S cone input to magnocellular LGN. Less direct measures examining higher-order phenomena like luminance perception, to which the magnocellular pathway may contribute, have produced conflicting results (Drum, 1983; Stockman et al., 1993a; Eisner and Macleod, 1980; Verdon and Adams, 1987).

Recordings from visual area MT also provide insight regarding magnocellular cone contributions since the bulk of MT’s input is magnocellular in origin (Maunsell et al., 1990; see Livingstone and Hubel, 1987, 1988; Yabuta et al., 2001). It was initially reported that area MT receives little or no S cone input (Gegenfurtner et al., 1994), but a recent study found clear S cone-initiated responses. Granting the paucity of direct evidence supporting (or for that matter, opposing) S cone input to the magnocellular pathway, a koniocellular route is not unreasonable. But, a more parsimonious explanation would be that the S cone signals observed in MT are carried by magnocellular neurons.

The purpose of our study is to determine whether such a signal exists by recording directly from magnocellular layers of LGN while presenting cone-isolating gratings. We show that S cones contribute significantly to the magnocellular pathway, accounting for about one-tenth of the total input from all three cone types. Our results are consistent with anatomical data about the proportions and spatial distributions of cone types in macaque retina. We also show that magnocellular S cone responses are not from cortico-geniculate feedback, finding undiminished responses when recording from LGN afferents in layer 4Cτ of inactivated cortex. Finally, we show that S cone signals are not color opponent but, rather, sum with L and M cone input. These results provide evidence that the S cone signals do not reach the central visual system solely via koniocellular input but are carried by the magnocellular pathway as well.

Results

S Cone Signals to Magnocellular Neurons

Figure 1A compares contrast response curves for a magnocellular neuron obtained with achromatic (L + M + S, or black and white) and S cone-isolating drifting gratings. The neuron’s S cone contrast sensitivity was 9% that of L + M + S, and at maximum achievable S cone contrast (89%) the response was 37% of saturation (see Experimental Procedures). Figure 1B shows normalized responses for the 15 magnocellular neurons from which we obtained complete S and achromatic contrast response functions, averaged across cells. For these cells, mean S cone contrast sensitivity was 5%
Figure 1. Contrast Response Curves Obtained from Magnocellular LGN with Achromatic (L + M + S) and S Cone-Isolating Stimuli
Responses to achromatic (open circles, L + M + S) or S cone-isolating (open diamonds, S) drifting gratings are plotted against stimulus contrast. Curves were fitted using the model described in Experimental Procedures, and semisaturation parameters derived from these fits were used to estimate contrast sensitivity. In (A), an example of a single magnocellular neuron. The calculation of S cone $C_{eq}$ (the equivalent achromatic contrast, used in Figure 2) is illustrated by the dotted line connecting the highest-contrast S cone response to the equivalent response on the L + M + S curve and specifying the L + M + S contrast (abscissa) for that point. In (B), data from 15 magnocellular single units is shown, with each data point (± standard error) representing normalized first harmonic response, averaged across all cells.

that of L + M + S, with maximum response reaching 25% of saturation, showing that magnocellular neurons clearly respond to S cone-initiated input.

We recorded from a total of 57 single units in magnocellular LGN and found significant S cone input across the population: mean spike rates in response to S cone-isolating stimuli at the maximum achievable cone contrast were significantly above spontaneous firing rates (Wilcoxon paired-sample test, $p < 0.001$; see Experimental Procedures). Figure 2A summarizes the distribution of individual magnocellular responses to S cone-isolating drifting gratings at 89% cone contrast. S input is expressed in terms of equivalent achromatic contrast ($C_{eq}$), which is the contrast of an achromatic stimulus evoking the same average response as the S cone-isolating stimulus in the same cell (see Figure 1A). Shown are the 49 out of 57 neurons whose S cone responses averaged over repeated trials were greater than two standard deviations above baseline. This was our criterion for selecting reliable, stationary data for further analysis (see Experimental Procedures). The mean value of S cone $C_{eq}$ for these cells was 8.3% ($±$ 5.7%, SD); i.e., the S cone-initiated signal at maximum cone contrast was equal, on average, to a magnocellular neuron seeing a black and white grating at 8% contrast.

Neurons throughout primary visual cortex are responsive to S cone-isolating stimuli (e.g., Cottaris and DeVelaer, 1998; Lennie et al., 1990; Conway, 2001). Since the majority of synapses in LGN are feedback synapses from primary visual cortex (see Sherman and Koch, 1986), we addressed the possibility that S input detected in LGN was actually the result of cortical feedback to magnocellular layers instead of retinal feedforward input. By superfusing cortex with muscimol (Chapman et al., 1991), we inactivated neurons in all layers including layer 6, the principal source of corticogeniculate-projecting neurons (Fitzpatrick et al., 1994). “Silencing” cortical neurons then allowed us to record from the much smaller magnocellular afferent spikes in layer 4C$_{a}$, representing geniculate responses without cortical feedback (see Experimental Procedures).

Figure 2B shows the distribution of S cone input to geniculate afferents, with all stimulus parameters and analysis procedures identical to the experiments described in Figure 2A. The S input to magno afferents was highly significant (Wilcoxon paired-sample test, $p < 0.001$, $n = 23$). 17 out of 23 sites met our criterion for stable recordings, and the mean S cone equivalent contrast for these sites, 10.7% ($±$ 4.0%, SD), was similar to that found in magno LGN. This suggests that magno S cone signals are primarily feedforward in nature and are not a result of cortex returning koniocellular or parvocellular input back to magno LGN. Also, finding S input to magno afferents in 4C$_{a}$ precludes interpreting S cone signals seen directly in magno LGN as being koniocellular or parvocellular recordings; koniocellular neurons do lie between and within magnocellular LGN layers (see Hendry and Reid, 2000), but only magno afferents project to layer 4C$_{a}$ (e.g., Blasdel and Lund, 1983; Merigan and Maunsell, 1993).

We checked the calibration of our S cone-isolating stimuli in multiple ways (see Experimental Procedures),

Figure 2. Distribution of S Cone Signals in Magnocellular Neurons
Histograms showing the strength of S cone input to (A) single units recorded from magnocellular layers of LGN and (B) magnocellular afferent sites in layer 4C$_{a}$ of silenced primary visual cortex. Stimuli were S cone-isolating gratings at maximum cone contrast (89%). In both panels, S cone input is expressed as the contrast of an achromatic grating needed to elicit the same response seen with the S cone-isolating grating (the equivalent contrast, $C_{eq}$; see Figure 1)
but given the uncertainties introduced by using human foveal cone fundamentals for monkey experiments at different eccentricities (since no fundamentals exist for the macaque that take into account preretinal filtering), the possibility remained that a stimulus designed to activate only S cones could be exciting rods or L and M cones. To test for this, a very bright yellow light (>250 cd/m², >300 scotopic cd/m²) was superimposed on the screen along with the achromatic and S cone stimuli. Such light levels are more than adequate to saturate rods (Aguilar and Stiles, 1954; Hood and Finkelstein, 1986). If the nominal S cone-isolating stimuli were driving L and M cones instead, then preferentially adapting the L and M cones would decrease sensitivity to achromatic and S cone stimuli equally (Wandell et al., 1999; Seidemann et al., 1999; Dougherty et al., 1999).

Figures 3A–3B show the effects of yellow light adaptation on the responses of a representative magnocellular neuron. For this neuron, contrast sensitivity to achromatic gratings decreased in the presence of yellow adapting light by a factor of 6.6 (Figure 3A; sensitivity reduction factor = Cₑadapted/Cₑnonadapted, with larger factors indicating greater sensitivity reduction under yellow adapting illumination and a factor of one corresponding to no change; see Experimental Procedures). This decrease was almost 7-fold greater than the decrease seen for S cone-isolating gratings (factor of 0.96) under identical yellow illumination (Figure 3B). Normalized and averaged responses from 11 single units used for this control (Figures 3C–3D) show that L/M/rod adaptation reduced contrast sensitivity by a factor that was about 7.5-fold greater for achromatic than for S cone-isolating stimuli. Contrast response functions for individual cone types also demonstrated the substantial difference in the yellow adapting light’s effect on L and M sensitivity (Figures 3E–3F) versus S (Figure 3G; L, M, and S curves from same cell). Across the population of magnocellular neurons we studied under yellow light adaptation (11 cells), as well as for three multunit recording sites in magnocellular layers of LGN, achromatic contrast sensitivity decreased by a factor of no less than 5 and up to 18, whereas S cone contrast sensitivity never fell by a factor of more than 2.5 (Figure 3H).

To further explore this control, we examined how yellow illumination affects S and L + M signals at matched response strengths by nulling the S cone response at maximum cone contrast with a superimposed L + M signal of opposite phase. If the response to S cone-isolating gratings was actually the result of L + M intrusion, then the effect of the yellow adapting light at the null (where the putative artifactual L + M signal driven by the stimulus peak equals the true L + M signal of the trough) should cause roughly the same decrease in both L + M-initiated responses, thus maintaining the null. However, if a true S cone signal was nulled by an opposite-phase L + M signal, then adding the adapting light should selectively decrease the L + M response, making the null impossible at that L + M contrast; this is the result we observed in each of nine cells we tested (example in Figure 3I). Also, while the relative first harmonic phase of the responses to drifting gratings shifted by about 180° at higher L + M contrasts, reflecting the dominance of the (L + M)-driven part of the response, there was no phase shift with the yellow light (Figure 3J), indicating that the response continued to be driven more by the S cone stimulus. Taken together with the contrast sensitivity controls, these results imply that the responses of magnocellular neurons to S cone-isolating stimuli cannot be explained by errant excitation of other cone types or rods.

### Proportion of Input from L, M, and S Cones

Most of our data were collected in the form of neuronal responses to cone-isolating drifting gratings at the maximum cone contrasts achievable by our monitor, which presented the problem of calculating relative cone strengths given that the stimuli were shown at very different contrasts (22% contrast for L cones, 27% for M, and 89% for S). Running every stimulus at the lowest common contrast was not feasible for this study, because magnocellular neurons do not respond significantly to an S cone contrast of 22% (e.g., Figure 1B). So we modeled the neuron as a linear-nonlinear cascade (Figure 4), showing that under cone-isolating conditions, one can estimate the weight of input from each cone type as the ratio of its equivalent achromatic contrast (Cₑ) to the cone contrast of the stimulus (see Experimental Procedures).

Figure 5 shows the relationships between L, M, and S cone contributions to 47 magnocellular LGN neurons (same LGN neurons as in Figure 2A, minus two cells having variable L cone data). With cone weights normalized, constraining their sum to be unity, each weight expresses the proportion of that cone’s input to the neuron. Figure 5A plots the input of L versus M cones, demonstrating an anticorrelation, which is expected if the S cone input is small and stays relatively constant across the population of cells. Comparing the weights of L versus S and M versus S (Figures 5B and 5C) shows that the S cone input remained at roughly 0.1, independent of the L:M ratio. Figure 5D summarizes the LGN magnocellular data. The mean L, M, and S cone inputs were 0.37 (±0.17, SD), 0.54 (±0.15, SD), and 0.09 (±0.04, SD), respectively. Thus, each magnocellular neuron received the bulk of its drive from L and M cones, with about 10% of total input coming from S cones.

For every neuron described above, achromatic gratings were used to determine its optimal temporal and spatial frequency parameters before running color experiments using these same parameters (generally ranging from 6–10 Hz and from 0.4–0.8 cycles per degree [cpd], respectively). The mean L:M ratio acquired with these stimuli was 0.69 (lower than estimates of 1.0–1.5 determined by other methods, e.g., Dobkins et al., 2000; Roorda et al., 2001). However, we found that the mean L:M ratio actually exhibited spatial frequency dependence. For 15 cells (Figure 5E), responses to both optimal spatial frequency cone-isolating gratings as well as gratings at lower frequencies (0.1–0.3 cpd) were collected. Cone weights extracted from the lower frequency trials gave a higher mean L:M ratio of 1.30. Figure 5F shows the optimal frequency cone weights (L:M = 0.78) of the cells used in Figure 5E, verifying that our sample was not biased toward cells with unduly high L:M ratios. The mean L cone weight was significantly different between the two groups (Wilcoxon paired-
Figure 3. L, M, and Rod Photoreceptor Adaptation Controls

(A) Achromatic contrast responses of a representative single unit under bright yellow illumination (crosses) had a contrast sensitivity 6.6 times lower than under normal, non-adapted conditions (open circles).

(B) S cone-initiated responses of the same unit under identical yellow illumination (crosses) had essentially the same contrast sensitivity as under normal illumination (open diamonds).

(C–D) Normalized and averaged achromatic contrast responses (±SEM) for 11 single units under the adapting light were 8.9 times less sensitive than under nonadapted conditions (C), while S cone contrast sensitivity of the same neurons (D) dropped by a factor of only 1.2 (same symbols as in [A] and [B]).

(E–G) Sensitivity reductions for a single neuron obtained with L, M, and S cone-isolating stimuli in adapted (crosses) and nonadapted (open squares, triangles, diamonds, respectively) states, were by factors of 5.0, 16.0, and 1.1, respectively.

(H) Sensitivity reduction factors for responses to S cone-isolating gratings versus achromatic gratings for 11 single units (closed circles) and three multiunit recording sites in magno LGN (open circles). Higher numbers indicate greater sensitivity reduction under yellow adapting illumination, with one being no change.

(I) Nulling experiment under normal (closed diamonds) and yellow (open triangles) illumination. The neuron’s response to S cone gratings under normal illumination was nulled by roughly 4% L/M contrast of opposite phase, but the adapted state showed no null in the contrast range tested, indicating greater adaptation of the (L/M)-initiated signal.

(J) Relative phases for the first harmonic response in different adaptation states (same symbols as in [I]). The 180° phase shift at the null indicates that the L + M part of the response began to dominate. No phase shift was seen in the adapted state.
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Sample test, \( p = 0.013 \), as was the mean M cone weight (Wilcoxon, \( p = 0.022 \)). The average S cone weight did not change significantly (mean S cone weight was 0.08 for low spatial frequencies and 0.09 for optimal; Wilcoxon, \( p > 0.30 \)).

The shift in the L:M ratio was not due to M or S cone response degradation at suboptimal spatial frequencies, as first harmonic responses before cone weight calculation and normalization tended to be higher for L cone-isolating stimuli at lower spatial frequencies, although this trend was not statistically significant for our sample of 15 cells (Wilcoxon, \( p = 0.067 \)). The M and S cone responses did not significantly change (Wilcoxon, \( p > 0.30 \) for M, \( p > 0.40 \) for S). The fact that S cone responses and cone weights were essentially unchanged at very low spatial frequencies also argues strongly against the possibility that chromatic aberration caused artifactual L or M cone responses to our S cone-isolating stimuli.

S Cone Input Exhibits No Color Opponency

We used reverse-correlation experiments to determine whether the S cone input to a given magnocellular LGN neuron had the same or opposite sign as the L and M cone input (i.e., whether S cone signals showed any color opponency). Spatiotemporal receptive field (STRF) maps were obtained using Hartley basis functions (Rin-
Figure 6. Receptive Field Profiles Obtained with Achromatic (L/H, M/H, S) and Cone-Isolating Stimuli

Spatiotemporal receptive field profiles for (A) a magno ON-center and (B) a magno OFF-center LGN neuron. Panel sections to the far right are receptive field (RF) maps obtained with L/H, M/H, and S stimuli shown at peak latency. These maps were scaled and translated so that the maximum value is +1 (red, ON) and the minimum is −1 (blue, OFF). The boxed traces to the left, obtained with cone-isolating stimuli (L, M, and S), show the time course of the RF for each cone type averaged over a group of pixels representing the center of the cell’s RF. Horizontal axis is temporal latency (τ, in ms); vertical axis is the spike-triggered average cone contrast where +1 and −1 are the maximum and minimum achievable cone excitation for each cone-isolating stimulus, and 0 is the mean excitation (gray background). Thus, positive values indicate the cell fired, on average, after increased cone excitation (ON), and negative values indicate the cell fired, on average, after decreases in cone excitation (OFF). For comparison, (C) shows data from a blue-ON/yellow-OFF koniocellular neuron recorded in layer K3 of LGN.

gach et al., 1997), which are sinewave gratings of various spatial frequencies, orientations, and spatial phases. Figure 6A is an example of a magnocellular ON-center neuron’s receptive field profile. The map to the far right of the panel was acquired with achromatic stimuli and shows the receptive field 40 ms before a spike (which was near the peak latency for this neuron). STRFs were also obtained with cone-isolating stimuli at maximum achievable cone contrast. The three traces on the left hand side of the panel show the time course for the receptive field center of the neuron’s responses to L, M, and S cone-initiated signals. All three cone types showed an ON-center response and therefore no color opponency.

Figure 6B is an example of a magno OFF-center neuron’s receptive field profile. Again, the S cone signal had the same sign (OFF) as both L and M cone signals. We obtained complete L, M, S, and achromatic maps for 8 magnocellular LGN neurons and in no unit did we encounter opponent S cone signals. The time courses of L, M, and S cone STRFs for each cell also had similar kinetics, as measured by the time to zero of responses (the zero crossing between the peak and the opposite-signed “rebound;” see Chander and Chichilnisky, 2001). Zero crossing times were not significantly different between L/S, M/S, and L/M pairs (Wilcoxon paired-sample test, p > 0.05). This serves as another control for rod intrusion in our S cone responses, since rods exhibit significantly slower temporal response functions (Lee et al., 1997).

Figure 6C shows responses of a koniocellular blue/yellow color-opponent cell recorded in LGN, demonstrating that the identical response signs seen for each cone type in the previous figures were not due to artifacts or errors in our methods and that color opponency could be unambiguously determined if present. We also probed for color opponency with drifting gratings, examining the relative phase of a cell’s first harmonic response to L, M, and S cone-isolating gratings. In-phase responses (clustering around 0° phase difference) imply that the 3 cone types are driven by the same part of their respective cone-isolating gratings (either the peak, “ON,” or the trough, “OFF”), whereas out-of-phase responses (shifted toward 180° relative phase) indicate color opponency. The phase differences seen for 49 L/S, M/S, and L/M pairs (Figures 7A–7C) all confirmed that magnocellular neurons did not receive cone-opponent input. For comparison, Figure 7D shows the distribution of relative phase between L and M cone-initiated responses in 20 parvocellular neurons encountered as
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Thus, there is a plausible anatomical pathway for S cone signals to find their way to magnocellular neurons in LGN. The alternate hypothesis, that diffuse bipolars (and, by extension, parasol cells) go out of their way to avoid S cones, is supported by the conclusions of Dacey and Lee (1994). Their intracellular recordings from morphologically identified parasol cells in an in vitro preparation revealed no S cone input using cone-isolating stimuli. The discrepancy with our own study is difficult to reconcile. We cannot attribute our findings to koniocellular encroachment on our magnocellular recordings since we found no color opponency to magnocellular neurons. Weingrings did find S cone signals in afferent projections to 4C/H9251, Histograms showing the absolute value of the first harmonic phase which are only from magnocellular neurons. Residual L, M, and rod responses to our S cone stimuli are unlikely, based on the yellow light adaptation experiments. Unless there is something radically different about the retina’s response properties in vivo versus in vitro, and short of positing a hitherto unknown ganglion cell type that relays S cone signals to magnocellular neurons, the only likely explanation we can advance is that the S cone contrasts of their stimuli were not high enough to reliably detect a response. They constructed their stimuli with three light-emitting diodes, and although the maximum S cone contrast was not reported, we infer that it was likely lower than the 85% contrast reported in a later paper (Dacey et al., 1996) since the blue diode used in the former had a dominant wavelength of 470 nm, longer than the 445 nm reported in the latter report. Red and green diodes had the same peak emission in both studies.

The wide variation of L and M cone weights seen in our population of magnocellular neurons can also be explained by assuming that a parasol cell collects all cone inputs available to it. Unlike S cones, L and M cones in the macaque and human retina are patchy in spatial distribution and tend to aggregate into clusters of the same cone type (Roorda et al., 2001; Roorda and Williams, 1999, Packer et al., 1996). Dacey and colleagues (2000), recording from H1 cells (which are thought to form the surround of parasol cell receptive fields), found that variations in relative L and M cone responses could be accounted for by differences in L:M ratio in the retinal region from which they were recording. On a macroscopic level, Brainard et al. (2000) showed that flicker electroretinogram variation between individuals reflected the difference in each person’s total L:M ratio. Thus, depending on what part of the cone mosaic the parasol cell is sampling from, one can imagine highly variable L:M ratios for single ganglion cells as well. In light of this, our relative cone weight data are consistent with the notion of random wiring for parasol cells.

The spatial frequency dependence of L and M cone weights was somewhat surprising. L cone weights were less than M cone weights at higher spatial frequencies, differing from published reports of relatively stronger L cone input (e.g., Roorda et al., 2001), whereas at lower frequencies we did find L greater than M, as expected. At the spatial frequencies used in our study (less than 1.0 cpd), it is unlikely that chromatic aberration could account for the observed trends (Marimont and Wandell, 1994). It is possible, given variations in cone weights between animals, as well as wide cell-to-cell variations.
in a single animal, that we somehow recorded from a biased subset of magno cells. Alternatively, our results suggest either that L and M cones do not cluster in the same way or that there is a more complex relationship between cone clusters in the retina and relative cone inputs to parasol ganglion cells (e.g., differences in gap-junctional coupling for different cone types). Cortical feedback to LGN could complicate the interpretation, since the cells used in our spatial frequency experiments were recorded in LGN with active cortical projections (not afferent recordings), and Johnson et al. (2001) showed that different color directions have different spatial frequency tuning curves in primary visual cortex. This is an unresolved issue that requires further study.

It is worth noting that the greater L cone weights we found with lower spatial frequencies seem at odds with Wiesel and Hubel's (1966) characterization of "type IV" cells in which large spots of red or white light suppressed the maintained firing rates of these cells. However, due to differences between our experimental paradigm and theirs (e.g., they examined suppression of maintained discharge with a constant red light, not the amplitude of response modulation to a stimulus that sinusoidally drives L cones), it may not be valid to compare the two data sets. Also, Hubel and Wiesel report that type IV cells are a smaller subset of the magnocellular population, and we may not have encountered a sizable number of them in our set of 15 cells used for spatial frequency experiments.

S Cone Contributions to Luminance Perception
Luminance is precisely defined in terms of the CIE spectral sensitivity curve \( V_o \), which is the standard for physical photometry. However, the term has become loosely identified in the literature with any number of paradigms that describe additive spectral-luminosity functions, as well as their possible neural bases. In particular, the Judd-modified \( V_o \), which can be faithfully modeled as the weighted sum of inputs from only L and M cones (to reflect the cone contributions inferred from a number of psychophysical measures like heterochromatic flicker photometry [HFP] and minimal distinct border tests [MDB]), has often been used to represent the spectral sensitivity of a distinct postreceptoral pathway (reviewed in Lennie et al., 1993) called the luminance channel, thought to have as its physiological substrate the magnocellular pathway (e.g., Lee et al., 1988; Lennie et al., 1993). High temporal resolution and lack of L/M color opponency certainly reflect a strong magnocellular contribution to luminance perception. And \( V_o \)-like spectral sensitivity curves have been obtained in remarkably close agreement with the psychophysics by using HFP and MDB stimuli in studies of macaque parasol ganglion cells (Lee et al., 1989; Kaiser et al., 1990), further cementing the notion that the magnocellular pathway and the luminance channel are essentially equivalent.

The evidence for S cone input to this psychophysical construct of luminance is equivocal and seems to depend on exactly how spectral sensitivity is measured (Lennie et al., 1993), possibly because different methods used to probe spectral sensitivity recruit separate neural pathways. Early studies found no S cone contribution using flicker photometry (Eisner and MacLeod, 1980). Soon afterwards, flicker sensitivity to yellow targets (L + M) was shown to differ from that of white targets (L + M + S), implying S cone input to the spectral luminosity function (Drum, 1983). Stockman et al. (1993a) demonstrated that S cones do respond to and relay rapid flicker by showing that S cone flicker superimposed on a slightly faster L or M cone flicker produced visible beats. Finally, a number of papers described S cone signals detected in the luminance pathway as inverted in sign (i.e., opponent) with respect to L + M signals (e.g., Lee and Stromeyer, 1988; Vos et al., 1990; Teufel and Wohrnhahn, 2000).

How do we reconcile the psychophysics with our own physiological measurements? Since humans have a zone of roughly 100 microns in central retina with zero S cone density whereas macaques do not (Bumsted and Hendrickson, 1999), the relative weakness of S cone contributions to the spectral-luminosity function may be due to a foveal bias in human psychophysical tests. Or, perhaps flicker techniques like HFP are not well suited to detect the S cone signal seen with cone-isolating gratings. As for the sign inversion, one possibility is that the kinetics of cone responses are different at higher temporal frequencies (as in HFP methods); however, our own reverse correlation studies revealed no substantial temporal response differences or inversions. Yet another possibility is that stimuli of widely varying cone contrasts for L/M versus S cones might produce a relative phase shift in the S cone response, perceptually appearing as a sign inversion. However, even the divergent cone contrasts used in this study did not produce effectively opponent S cone signals.

Although it is not the intent and is beyond the scope of this paper to tackle a revised definition of luminance or the existence of a luminance channel, the fact that the S cone signals we see in magnocellular neurons are different from the S cone contribution inferred psychophysically calls into question the strong-form interpretation of exclusive association between the magnocellular pathway and luminance. S cone opponency is the hallmark of the pathway originating in small bistratified cells of the retina (Dacey and Lee, 1994; Chichilnisky and Baylor, 1999), which project to the intercalated koniocellular layers of LGN (see Hendry and Reid, 2000). Parvocellular neurons might carry S opponent signals as well (Klug et al., 1993). Are these channels somehow feeding into the magnocellular-dominated luminance channel? The anatomical convergence of parallel pathways within visual cortex is extensive (e.g., Yabuta et al., 2001; Sawai-tari and Callaway, 2000; Callaway, 1998). Ultimately, there is a lot of brain between magnocellular LGN and perception, and we can only say that magnocellular neurons do not completely reflect the properties ascribed to the luminance function.

S Cone Input to Motion Pathways
Indirect evidence for S cone input to the magnocellular pathway comes from psychophysical and physiological studies of motion perception. Since the magno pathway is thought to carry signals used in motion computations by virtue of strong connections to area MT, it seems that S cone signals in the magno pathway should be found in MT as well. Of course, S input seen in MT is
not sufficient to prove S input to magnocellular neurons. Convergence of other visual streams could contribute S cone signals to motion areas, and this logic plagues the interpretation of S input seen in a number of psycho-physical studies (e.g., Cavanagh and Anstis, 1991; Chichilnisky et al., 1993; Dougherty et al., 1999).

Our results dovetail with recent recordings from macaque MT (Seidemann et al., 1999). Using cone-isolating drifting gratings and recording from (mostly) multunit sites, Seidemann et al. demonstrated robust S cone input that was strikingly similar to our own measurements in magnocellular LGN. Most of their recording sites showed some responses to S cone-isolating stimuli, and they found that MT neurons were only about one-tenth as sensitive, on average, to S cone-initiated signals as they were to luminance input. Our magnocellular data could, therefore, account for most of the S cone input measured in MT. Interestingly, Seidemann and colleagues propose a koniocellular origin for their S input, although this seems to be based primarily on the commonly held view that S cones do not contribute to the magnocellular pathway. New evidence (Barberini et al., 2001, Soc. Neurosci. Abstr.) suggests another possibility, indicating that S cone input to MT may sum together with L and M cone input and, therefore, might not be part of a color opponent pathway. This is consistent with our own findings in LGN, and taken together with the Seidemann et al. (1999) study, provides evidence for the hypothesis that S cone signals are relayed to area MT primarily via the magnocellular pathway.

Koniocellular neurons may contribute as well. Our results are agnostic on the point, and there is certainly anatomical evidence of koniocellular convergence via cortical layer 4A projections to thick stripes of V2, which subsequently project to MT (Levitt et al., 1994). Also, Seidemann et al. documented a fair amount of variability in the S cone contribution, which leaves open the possibility of a heterogeneous distribution of S cone signals arriving at MT. But the total magnocellular input likely overwhelms any koniocellular or parvocellular contribution, so the S cone input we observed in magnocellular neurons should manifest itself quite strongly in MT. There is no need to invoke other pathways or novel retinal or cortical circuitry for constructing nonopponent S input to MT.

**Experimental Procedures**

**Animal Preparation**

We used 5 juvenile macaque monkeys (three M. mulatta, two M. radiata) in this study. Each animal was initially tranquilized with ketamine, tracheotomized, and placed in a stereotaxic apparatus. Anesthesia was maintained with sufentanil citrate during surgery and recording (6–12 μg/kg/hr, i.v.). Dexamethasone (0.5 mg/kg i.m.) was administered every 48 hr to reduce brain swelling. We made a small craniotomy over LGN, reflected dura, positioned an electrode, and sealed the craniotomy with warm agar and wax. After surgery, paralysis was induced with pancuronium bromide (0.1–0.2 mg/kg/hr, i.v.). Eyes were dilated with 1% atropine and corneas protected with gas-permeable contact lenses. External lenses refracted the eyes (with optimal refraction determined by a neuron’s ability to resolve fine spatial frequencies). EEG, EKG, SpO2, heart rate, and body temperature were monitored continuously to judge the animal’s health and to maintain proper anesthesia levels. All procedures were approved by the Salk Institute Animal Care and Use Committee.

For cortical inactivation experiments, we made a large (~8 × 5 mm) craniotomy posterior to lunate sulcus, above the operculum, and behind the V1/V2 border (parafocal representation on striate cortex), and reflected dura. A 7 × 5 mm piece of Gelfoam was placed on the pial surface, leaving a small space for the electrode to make a tangential penetration. We covered the Gelfoam with bone wax, and a tube was run through its center to administer the potent GABAγ agonist muscimol (Sigma-Aldrich, St. Louis, MO). The craniotomy was sealed with warm agar. Control experiments showed that all cortical layers below the muscimol-saturated Gelfoam patch were inactivated. Usually within an hour of starting muscimol perfusion (50 mM in 0.9% sterile saline, 0.1–0.2 mL/hr), layer 6 was silenced.

**Visual Stimuli and Display Calibration**

Stimuli were generated by a Silicon Graphics O2 computer, 24-bit color, using custom software (PEP, Dario Ringach) and were shown on an SGI GDM-17E21 CRT display at 100 Hz refresh rate. We confirmed additivity of the red, green, and blue guns, and linearized gun intensities (i.e., the output intensity of each gun was made to vary linearly with frame buffer value). The spectral power distributions of monitor phosphors were obtained with a PhotoResearch PR 650 spectroradiometer, and cone-isolating directions were calculated as in Wandell (1995) using the Stockman cone fundamentals (Stockman et al., 1993b). The monitor was recalibrated often, usually after every one or two recording sessions.

Stimuli were sinusoidal gratings, either in the form of a circular patch of drifting grating (typically of radius 1–2”) or, in the case of reverse correlation experiments, a square frame (2–5” on a side), shown at a distance of 100 cm from the animal. All stimuli were presented on a constant gray background (each linearized gun at half-maximal intensity), of mean luminance ~28 cd/m² and CIE color coordinates x ~ 0.29, y ~ 0.27. Cone-isolating stimuli passed through the background gray between peak and trough.

As an end-to-end check of our calibration methods, we constructed cone-isolating stimuli for three sets of glass filters whose transmissions roughly mimicked the spectral absorptions of the three cone types, and a photodiode was used to measure the power of these stimuli through each filter set (Chichilnisky and Baylor, 1999). In the case of S cone isolation, the stimulus isolated the model S cones 30 times better than model M cones and 70 times better than model L cones.

Finally, we performed a physiological check of cone-isolation with the L/M cone and rod adaptation control (e.g., Wandell et al., 1999) in which light from a slide projector was passed through a Kodak Wratten 15 filter (whose transmission spectrum appears yellow and strongly drives L and M cones while leaving S cones near mean excitation) onto the display. The gray background plus yellow illumination had a luminance of ~350 cd/m² (~310 scotopic cd/m²). We allowed the animal to adapt to a new state of illumination for 3–4 min prior to recording visual responses.

**Data Collection and Histology**

For LGN recordings we used epoxy-lute-insulated tungsten electrodes of 3 Mohm impedance (measured at 1 kHz; Frederick Haer and Co., Brunswick, ME). Stimuli were amplified, then sorted using custom software that allowed for defining multiple voltage-time constraints to select waveforms of precise shape.

We advanced through LGN until we reached magnocellular neurons with receptive fields of ~5–15’ eccentricity, where the primate retina has fairly low and uniform macular pigment density (Snodderly et al., 1984). After isolating a single unit, we ran a series of experiments with drifting achromatic gratings, obtaining spatial frequency, temporal frequency, and contrast response functions to optimize stimulus parameters. Cone-isolating experiments were run using these spatial and temporal frequencies. We presented L, M, and S cone-isolating drifting gratings in random order for 4 s each at the maximum cone contrast achievable by our monitor for each cone-isolating direction. We typically averaged over 3–4 repeats of these stimulus sets. After every third grating, we inserted a blank trial to acquire a measure of spontaneous firing rate.

Reverse correlation experiments were done using a low-pass subset of the two-dimensional Hartley basis functions (Ringach et al.,...
1997). Image sequences came from a set of orthonormal sine waves (generated by the cas function) of different orientations, phases, and spatial frequencies, the latter bounded by a defined value that we generally chose to be twice the highest spatial frequency to which the neuron responded (obtained from the spatial frequency tuning curves). During visual stimulation, an image was drawn randomly from this subset of images at every alternate screen refresh (every 20 ms), creating a rapid sequence of images shown for a total of 10–20 min. This procedure was repeated for all cone-isolating stimuli. Receptive fields were reconstructed by cross-correlating the image sequence with the cell’s spike train.

We also recorded from multunit sites, substantially relaxing the spike-sorting criteria to efficiently obtain responses from a number of magnocellular neurons at once. Full contrast response functions (achromatic and S cone) were recorded from five sites, with three sites exhibiting highly stationary and reliable responses across repeated contrast experiments. These were used, along with the set of 11 single units described in the Results section, for the yellow adapting light controls.

For afferent recordings in silenced cortex (methods described in detail by Chapman et al., 1991), long tangential penetrations were made in V1. Sharp, low-impedance electrodes (1–2 Mohm at 1 kHz; Frederick Haer and Co.) were advanced through cortex until we encountered the afferent hash characteristic of layer 4C. Presumably, these were signals summed across terminal branches of single magnocellular geniculate axons in 4C and not fibers of passage going to superficial layers, since layers 4B and 5 (through which axons to superficial layers must pass) were always relatively silent with no isolatable spikes. We may rule out the possibility that these were axons from intrinsic horizontal connections because a large patch of surrounding cortex was inactivated. Also, the response properties of these afferents (high contrast sensitivity, no color opponency, short response latency) were similar to the single units recorded directly in magnocellular LGN.

After recording, small marking lesions (3.5 μA for 3.5 s, electrode tip negative) were made in LGN and cortex to reconstruct electrode tracks and assign units to layers. Units that could not be unambiguously assigned to a particular layer were discarded from analyses. At the end of the experiment, the animal was given a lethal dose of Nembutal and perfused through the heart (0.9% saline in phosphate buffer solution, followed by 4% paraformaldehyde, 10% and 20% sucrose). The brain was blocked, sunk in 30% sucrose, and sections (10–20 min) required to obtain L and S contrast response functions and the responses to cone-isolating stimuli (sensitivity reduction factor which equals 0.4, SD) was close to the model’s assumption.

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