Dynamics of sensitivity regulation in primate outer retina: The horizontal cell network

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The goal of these experiments was to define the time course and degree of cone adaptation in primate outer retina by use of probe stimuli upon temporally modulated backgrounds. Recordings were obtained from primate horizontal cells. Test probes were either a low-amplitude, high-frequency sinusoid superimposed on a slowly modulated background or small test pulses superimposed on backgrounds of various frequencies. The amplitude of the test response was modulated by the background, indicating sensitivity regulation. Results were consistent with gain controls which, at 1000 td, required ~10-20 ms to completion. These mechanisms could also account for some of the distortions of horizontal cell responses to sinusoids and pulses. Modulation of test responsivity occurred at low background contrasts, suggesting no threshold change in light level must be exceeded to evoke sensitivity regulation. As retinal illuminance increased from darkness, sensitivity regulation was evident at 10-20 td.

Keywords: horizontal cells, macaque, adaptation, outer retina, cone

Introduction

The first stages in the regulation of visual sensitivity occur in outer retina. Recording from horizontal cells provides a means of monitoring adaptation at this locus. Horizontal cells engage in triadic synaptic contacts with the cone receptors and bipolar dendrites. Because adaptation seen in horizontal cells is both cone specific and spatially local (Lee, Dacey, Smith, & Pokorny, 1999), it is likely to be dependent on processes within the cone or its synaptic triad and thus to provide a measure of adaptation at the receptor level. There are two horizontal cell types in primate retina, the H1 cell, which receives input only from the middle- and long-wavelength cones, and the H2 cell, which sums inputs from all three cell types, although input from the short-wavelength cone is dominant (Dacey, Lee, Stafford, Smith, & Pokorny, 1996). We have recently provided a description (Smith, Pokorny, Lee, & Dacey, 2001) of primate horizontal cell (H1) responsivity at different adaptation levels. Adaptation became evident above ~10 td and was accompanied by changes in response dynamics. Cell responses could be well captured by a model composed of sets of first- and second-order filters. Outer retinal adaptation in the primate was found to extend to lower levels of retinal illuminance than expected from measurements on isolated cones (Schneeweiss & Schnapf, 1999) but fell short of Weber's law at the low- to mid-photopic levels tested. In another report (Lee et al., 1999), we introduced a method of testing horizontal cell responsivity on modulated backgrounds. A low-amplitude sinusoidal test probe was superimposed on a high-contrast modulated background, which we termed the vehicle wave.

Here we further explore H1 responses to test probes on modulated backgrounds. We show evidence that the results are robust with changes of contrast and frequency, and we confirm the steady-state measurements as to the extent of adaptation in the low- to mid-photopic range (Smith et al., 2001). In the second part of this work, we are specifically concerned with the time course of the gain changes that occur. Measurements from human psychophysics indicate that most gain changes following an abrupt alteration in light level are complete within a short time, of the order of tens of milliseconds (Crawford, 1947; Hayhoe & Wenderoth, 1991). This time constant presumably reflects a combination of the time courses of outer retinal and of more proximal mechanisms. The time course of gain controls in the primate outer retina is undetermined. It has been suggested that all outer retinal
receptor adaptation in primate is instantaneous, due to some form of response compression (Makous, 1997). On the other hand, if feed-forward or feed-back mechanisms with temporal filtering are involved, sensitivity control must exhibit a finite time course. One method of probing the time course of adaptation might be to manipulate vehicle wave frequency. This has been attempted in psychophysical experiments (e.g., Boynton, Sturr, & Ikeda, 1961; Hood & Graham, 1998) by measuring pulse thresholds on modulated backgrounds. Modulation of psychophysical sensitivity occurs up to more than 30 Hz. In the physiological measurements, with slowly modulated vehicle waves, it was feasible to employ a high-frequency sinusoid as a test, but with high-frequency vehicle waves, sinusoidal tests become impractical. It is more convenient to use individual test pulses delivered at different phases during the vehicle cycle, as in the human psychophysical tests. The pulse-on-vehicle protocol has been applied less frequently in physiological experiments, although Lankheet, Wezel, Prickaerts, and van der Grind (1993b) used this paradigm to study adaptation in cat horizontal cells. Here we describe responses of H1 horizontal cells to incremental and decremental pulses presented on vehicle waves of different frequencies. We also analyze response waveform to sinusoids and incremental and decremental pulses.

**Methods**

A detailed description of the physiological preparation and generation of stimuli is provided elsewhere (Smith et al., 2001). Briefly, eyes of macaques were prepared for in vitro recording by dissecting the retina together with pigment epithelium and choroid and maintaining it under standard in vitro conditions. Intracellular penetrations were achieved under direct visual control with high-resistance micropipettes. Recordings were obtained between 30-50 deg of eccentricity.

**Visual Stimulation**

Light from three light-emitting diodes (LEDs) was combined with dichroic mirrors and focused near the objective lens of the microscope. This provided a uniform field in the plane of the retina. The dominant wavelengths in the plane of the retina were 460, 554, and 638 nm. The LEDs were driven with a frequency-modulated train of 250 µs pulses providing a linear relation between light output and input voltage. Calibration of retinal illuminance levels is described in Smith et al. (2001). The three LEDs were initially set equal in luminance for the CIE 10° observer after correction for the absence of the ocular media; they were then checked and adjusted by flicker photometry on individual parasol cells. Quantal normally incident on the retina were ~250000 quanta/µ²/sec per LED. Estimated troland levels were equivalent to 500 td per LED, after correction for the Stiles-Crawford effect, which is substantial because peripheral cones orient toward the pupil. Data acquisition was usually performed with a sampling rate of 1250 Hz. Data were averaged over 32 or 64 cycles of the vehicle wave.

**Results**

**Responsivity on Modulated Backgrounds**

We usually employed sinusoidal waveforms as they are more amenable to linear analysis. Stimulus waveforms are shown in Figure 1A and responses of an H1 cell are shown in Figure 1B. The vehicle wave was a 0.61 Hz high-contrast sinusoid (mean illuminance 830 td, 100% modulation) and the test wave 32 cycles of a 19.5 Hz sinusoid (mean illuminance 170 td, 75% modulation). The combined mean illuminance was 1000 td after adding the two waves with a vehicle wave contrast of 82.5%. Test wave contrast \( (L_{MaxTest} - L_{MinTest})/(L_{MaxTest} + L_{MinTest} + L_{Vehicle}) \) varied with vehicle wave phase, with a minimum of 13.1% at the peak of the vehicle wave and a maximum of 75% in the trough. Responses to the combined waveform and to the vehicle wave alone are shown in the upper traces of Figure 1B. In this and subsequent figures, the horizontal line represents resting membrane potential recorded immediately before data acquisition; its value is given in the legend. The test wave response varied with the vehicle wave phase. We isolated the test response by subtracting the vehicle response from the combined response to give the difference wave in the lower trace, in which the modulation of the test response is apparent. For the test response to be a meaningful index of responsivity, it is necessary that the test and vehicle responses do not interact (i.e., the vehicle response was not affected by the superimposed test). In Figure 1C are plotted Fourier spectra of responses to the vehicle and to the vehicle plus test, and the vector difference between them (i.e., the spectrum of the difference wave). For vehicle plus test, there were peaks centered around the 32nd harmonic and multiples thereof, which derived from the test response. Below the 32nd harmonic, the two spectra are very similar; they superimpose at low frequencies. For the cell sample \( (n = 39) \), addition of the test to the vehicle gave a higher first harmonic amplitude by 6.3% on average (SD 7.5%) and delayed response phase by 0.44° (SD 1.2°). This suggests the test plus vehicle wave response was a sum of vehicle and test harmonic components, and that the subtraction procedure adequately isolated the test wave response.
Figure 1. H1 cell responses to sinusoidal vehicle and test wave stimuli. A. Top two traces show the vehicle + test wave stimulus and the vehicle wave alone; bottom trace shows the test wave stimulus after subtraction from the vehicle wave. Vehicle wave was 0.61 Hz, 82.5% contrast; test wave was 19.5 Hz, contrast variable. Mean illuminance was ~1000 td and stimulus size ~1 mm, 32 stimulus repetitions. B. H1 cell response to stimuli shown in A. Averaged responses to combined waveforms (top trace) and to the vehicle wave alone (middle trace) were measured. The vehicle wave response was subtracted from the test response to give the difference wave (bottom trace). Resting membrane potential (horizontal line) was -31.2 mV. C. Fourier spectra of responses. Spectra of responses to the vehicle wave alone and to the vehicle + test wave are similar except near the 32nd harmonic and multiples thereof. The spectrum of the difference wave is also shown. D. Upper plot shows the first-harmonic response amplitude of 32 test wave cycles as a function of vehicle wave phase (•). Data have been fitted with Equation 1 (solid line; fit parameters were \( R_0 \), 5.88 mV, \( B \), 0.0082, \( \phi_{\text{delay}} \), -9.9°). Also shown is contrast gain (○), calculated by relating test amplitude to the instantaneous vehicle level. Contrast gain is not constant, indicating Weber's law does not hold. Fitted curve is derived from Equation 1. The lower plot shows test response phase as a function of vehicle wave phase (○). It is modulated synchronously with response amplitude. The amplitude fit curve has been scaled and inverted to describe the phase data (solid line).

The test wave response was divided into 32 one-cycle segments and the amplitude and phase of the first-harmonic response extracted. These are plotted as response amplitude as a function of vehicle wave phase in Figure 1D (•, upper plot). The solid line indicates the least-squares fit of Equation

\[
R = \frac{R_0}{L \cdot B \left( C \sin(V_{\text{phase}} + \phi_{\text{delay}}) + 1 \right) + 1}
\]

where \( R \) is the response amplitude, \( L \) the mean retinal illuminance, \( C \) is vehicle contrast, and \( V_{\text{phase}} \) is vehicle phase. Free parameters are, \( R_0 \), which represents the response expected in the dark-adapted state, \( B \), which determines the degree to which modulation of the test response (adaptation) takes place, and \( \phi_{\text{delay}} \), which is a phase lag term. A satisfactory fit was obtained. Fit parameters are included in the figure legend. Test response phase is also plotted (lower plot, Figure 1D) and was modulated by \(~10^\circ\) during the vehicle wave. This was comparable to the difference in response phase at these luminance levels found in steady-state measurements (Smith et al., 2001). To test if the modulation in phase followed the same time course as the modulation in amplitude, the fitted curve from the amplitude data was...
Table 1. Summary of Fit Parameters for Different Conditions

<table>
<thead>
<tr>
<th>Total cell sample; n = 39</th>
<th>B</th>
<th>Response vs. intensity</th>
<th>Delay (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.61 Hz vehicle 19.5 Hz test</td>
<td>0.0030±0.0022</td>
<td>-0.61±0.17</td>
<td>44±16</td>
</tr>
<tr>
<td>Frequencies:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85% Contrast vehicle, 1000 td; n = 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.61 Hz Veh. 19.5 Hz Test</td>
<td>0.0019±0.0009</td>
<td>-0.47±0.11</td>
<td>49±12</td>
</tr>
<tr>
<td>1.22 Hz Veh. 19.5 Hz Test</td>
<td>0.0017±0.0003</td>
<td>-0.46±0.10</td>
<td>39±18</td>
</tr>
<tr>
<td>0.61 Hz Veh. 9.76 Hz Test.</td>
<td>0.0024±0.0011</td>
<td>-0.54±0.10</td>
<td>46±12</td>
</tr>
<tr>
<td>Steady state, 19.5 Hz Test</td>
<td>0.0013±0.0003</td>
<td>-0.42±0.10</td>
<td>-5.2±3</td>
</tr>
<tr>
<td>Luminance contrasts:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.61 Hz vehicle, 19.5 Hz Test 1000 td; n = 13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85%</td>
<td>0.0027±0.0013</td>
<td>-0.54±0.12</td>
<td>40±16</td>
</tr>
<tr>
<td>60%</td>
<td>0.0026±0.0023</td>
<td>-0.61±0.13</td>
<td>43±18</td>
</tr>
<tr>
<td>35%</td>
<td>0.0024±0.0028</td>
<td>-0.59±0.15</td>
<td>39±12</td>
</tr>
<tr>
<td>20%</td>
<td>0.0034±0.0053</td>
<td>-0.62±0.18</td>
<td>40±23</td>
</tr>
</tbody>
</table>

Values shown are means and SDs, values of response versus intensity. Slope show minor variation between the different groups. This was due to inter-animal variability of unknown origin.

Values shown are means and SDs, values of response versus intensity. Slope show minor variation between the different groups. This was due to inter-animal variability of unknown origin.

scaled and inverted and can be seen to provide a satisfactory description of the phase data.

Modulation of the test response did not yield constant contrast gain (mV/% contrast), as shown in Figure 1D (O, upper plot). Contrast gain could be legitimately calculated from the data because the linear range of H1 cells is extensive, with no evidence of saturation to sinusoidal stimuli (Smith et al., 2001). Response amplitude was divided by the instantaneous contrast during each test wave cycle. The variation in contrast gain indicates that Weber's law is not achieved in horizontal cell sensitivity regulation.

All 39 H1 cells tested with this protocol yielded similar results and Equation 1 provided a satisfactory fit to the data, except for one neuron where a greater degree of adaptation occurred than permitted by Equation 1. A second saturation stage captured this cell's behavior. For other cells, this stage did not improve fits.

Fit parameters B and φdelay are summarized in Table 1, in which cells are grouped according to the sets of experimental manipulations described in later sections. R0 varied from cell to cell and was not related to the other parameters. Mean phase lag (φdelay) was 9.68° (σ = 3.17°; n = 39), corresponding to a mean delay of 44.1 ms. This delay term represents a combination of two factors.

Firstly, from Bode plots as shown in Smith et al. (2001), responses at 19.5 Hz are delayed in phase relative to the stimulus by 180-210°, which is equivalent to a lag of ~25-30 ms. Secondly, a delay due to time course of operation of gain controls may be present. The mean delay term, φdelay, was 44.1 ms, and thus there may be an additional delay component.

The mean value of B was 0.0030 with some inter-cell variability (σ = 0.0022; n=39). For comparison with data obtained in other experiments (Smith et al., 2001), this value can be recalculated in terms of the slope of the relation between log test responsivity and log retinal illuminance. Mean slope around 1000 td was -0.61 (σ = 0.17). This corresponds to a 5.9-fold change in gain for a log unit change in illuminance, which falls short of Weber’s law. The slope is similar to that derived from the steady-state measurements (-0.65 to -0.7), and to that derived from experiments on cat horizontal cells (-0.64) (Lankheet, Wezel, & van der Grind, 1991a) at 150 cd/m². These values of mean slope are also included in Table 1.

For the fit parameters to be physiologically meaningful, they must be robust with variation in vehicle and test wave frequency. We therefore made measurements with a test-wave frequency of 9.76 Hz, and also tested a vehicle-wave frequency of 1.22 Hz. These control measurements are shown for one cell in Figure 2. Figure 2A (upper panel) shows the standard condition (0.61 Hz vehicle, 19.5 Hz test), and Figure 2B the slower test frequency (9.75 Hz). The test response was then larger (R0 increased) but the fit parameters B and φdelay...
Figure 2. H1 cell responses to varying vehicle and test wave frequencies. Fit parameters remained largely unaffected. Vehicle contrast was 85% (32 repetitions; mean illuminance ~1000 td; ~1 mm field). A-D. Top panels show test response amplitudes (solid lines) plotted as in Figure 1D and fitted with Equation 1. Fit parameters: A. $R_0 = 1.04$ mV; $B = 0.00091; \phi_{delay} = -13.1^\circ$. B. $R_0 = 1.18$ mV; $B = 0.00119; \phi_{delay} = -12.6^\circ$. C. $R_0 = 1.05$ mV; $B = 0.00069; \phi_{delay} = -20.8^\circ$. D. $R_0 = 1.60$ mV; $B = 0.00140; \phi_{delay} = 0.3^\circ$.

Bottom panels show data replotted as log test response amplitude as a function of instantaneous retinal illuminance during each test cycle. Hysteresis is due to the delay term and the plots have a curvilinear shape. An increase in vehicle frequency gave greater hysteresis, which was not seen in the steady-state condition. The sequence of test responses through the vehicle wave is indicated by the arrows.

remained similar. In Figure 2C, vehicle wave frequency has been doubled; fit parameters remained similar, $\phi_{delay}$ remained similar in time but doubled in terms of phase angle. Figure 2D shows responsivity in a steady-state condition, for which data were obtained by setting mean illuminance to correspond to different levels in the vehicle wave and then by testing responsivity following a brief (300 ms) adaptation period. The abscissa is expressed as equivalent vehicle phase. Again, fit parameters remained similar except that $\phi_{delay}$ was zero. Similar results were obtained with six other cells tested with this set of stimuli and average values are included in Table 1. This analysis showed that modulation of responsivity is robust with modification of test and vehicle frequency.

The lower panels of Figure 2 show an alternative way of plotting the results. Log test responsivity (mV/test td) and fitted curves are plotted against instantaneous log illuminance during the vehicle wave; the arrows indicate the direction of the vehicle wave. The hysteresis is due to the delay, $\phi_{delay}$ and is similar when vehicle wave frequency was the same (Figure 2A and 2B), and approximately doubled when vehicle wave frequency was doubled (Figure 2C), and was zero in the steady-state condition. Data and curves were not linear; the slope was shallower at lower illuminances; the course of this relation is pursued further in experiments described below.

These results are consistent with a gain control mechanism that was complete within at most a few tens of milliseconds. Under certain circumstances (e.g., during recovery from bleaching), additional, slower gain changes might be expected. To test if slower components were present at 1000 td, we used a square vehicle wave. Data and analysis are shown in Figure 3. Response traces as a function of vehicle phase are shown in Figure 3A, in the format used in Figure 1. The first-harmonic amplitudes showed no indication of a slower adaptation component (Figure 3B). Within 1-2 cycles of the test, its response amplitude stabilized, accompanied by a change in phase. The Fourier spectra in Figure 3C show that no interaction of vehicle and test wave was present. Recordings from 11 further cells gave a similar result.

This suggests that adaptation processes with a time constant in the range of seconds are not present. Although adaptation with a very slow time course, of the
Figure 3. Use of a square vehicle wave does not reveal any slower adaptation components. A. Response of an H1 cell to a square-wave vehicle (0.61 Hz) at 71% contrast (19.5 Hz test). Resting membrane potential (horizontal lines) -57.7 mV. B. Plots of first-harmonic response amplitude (top plot) and phase (bottom plot) as a function of vehicle-wave phase (●). Amplitude reached a steady level within 2-3 test cycles following the change in luminance. C. Fourier spectra of responses showed little difference between vehicle wave alone and combined vehicle + test wave conditions.

Vehicle Wave Contrast

Vehicle contrast was varied to test the generality of Equation 1. The goal was to test if the degree of activation of gain controls was dependent on contrast; we reasoned that if that were the case, the parameter $B$ in Equation 1 should be dependent on vehicle wave contrast. Figure 4 shows test response amplitude as a function of vehicle phase, and fitted curves for two H1 cells at four vehicle contrasts (20%, 34%, 60%, and 85%) at 1000 td. Modulation of test response occurred even at the lowest vehicle contrast tested. Fit parameters were unaffected by vehicle contrast (see Table 1 for mean values). For the sample of 13 cells tested, an analysis of variance for parameters $B$ and $\phi_{\text{delay}}$ showed no significant effect of contrast ($B$, $F=1.31; p > .2$; $\phi_{\text{delay}}$, $F=1.13, p > .2$). This would indicate that there was, for example, no threshold change in mean illuminance, which must be exceeded before changes in responsivity occur. Although responses at low contrasts are usually thought to be dominated by linear mechanisms, clearly adaptation processes are activated.

Effect of Alteration in Mean Illuminance

To investigate adaptation at lower illuminance levels, we inserted neutral density filters into the light path. This reduced not only mean illuminance but also the modulation amplitudes of vehicle and test waves. Results for two representative cells for modulation at 1000, 100, and 10 td are shown in Figure 5A and 5B. In the upper panels, amplitude responsivity (expressed as millivolts of response per test troland) to the test is plotted as a function of vehicle wave phase. Modulation of responsivity at 1000 td was similar to that shown previously (Figures 1, 2, and 4). At 100 td, modulation of amplitude responsivity of the test was smaller than at 1000 td, while at 10 td, little modulation of test responsivity was apparent although the data were noisy.
due to the low amplitude of the test wave. Equation 1 was used to fit all three luminance levels simultaneously. The fitted curves provide an adequate description of the data, considering that data at different illumination levels were collected up to one hour apart.

In the lower panels of Figure 5A and 5B, the data have been replotted as a function of instantaneous retinal illuminance of the vehicle waves (cf. the lower panels of Figure 2). The sets of data acquired at the different levels concatenate (as expected with a single set of fit parameters). Although some inter-cell variability was apparent (the cell in Figure 5B attained a steeper slope around 1000 td compared with the cell in Figure 5A), both cells showed similar behavior. Figure 5C shows the mean fitted curves from 15 cells tested, with the dashed lines indicating ±1 SD (the delay term has been omitted for clarity). Responsivies from Smith et al. (Figure 9B, 19.5 Hz data) are plotted as symbols for comparison and fall within the range of the current measurements. These data thus confirm the conclusion of Smith et al. that sensitivity regulation for the H1 cell is initiated when retinal illuminance rises above ~10 td.

**Time Course of Adaptation - Harmonic Distortion of the Vehicle Wave**

The aim of the experiments in this and the subsequent sections was to determine whether the sensitivity regulation observed was instantaneous or due to a process with a finite time course. In this section, we analyze harmonic distortion of responses to high-contrast vehicle waves. These are illustrated in Figure 6 for four frequencies: 0.61 (a), 4.88 (b), 9.76 (c), and 30.3 Hz (d) at 100%, 50%, and 25% contrast. The solid lines show the data, and the dashed lines (which are sometimes hidden) show the fit of Equation 2 below. The horizontal lines through each curve represent the resting membrane potential level measured just before the response. At 0.61 Hz, the hyperpolarizing response to peak illumination was compressed and there was an accelerating depolarization at the minimum of the stimulus sinusoid. This distortion was also apparent at 4.88 Hz, and in addition the hyperpolarizing slope of the response was steeper than the depolarizing slope. At 9.76 Hz, this distortion was less, but at higher frequencies (30.3 Hz shown in the figure), the depolarizing slope is seen to be steeper than the hyperpolarizing slope. Harmonic distortion was less apparent at lower contrast.

Cat horizontal cells exhibit similar harmonic distortion of responses, which was modeled as a multiplicative gain control with delay (Lankheet, Wezel, & Grind, 1991b). We tested this hypothesis. Responses were recorded at 11 frequencies from 0.61 to 39.0 Hz (4 of which are shown in Figure 6) at 3 contrasts (25, 50 and 100%). For each frequency, f, the response waveforms, R(t), as a function of time were fitted simultaneously for all contrasts by Equation 2.

$$R(t) = -\frac{L.A(C \sin(2\pi ft + \phi_{response}) + 1)}{L.H(C \sin(2\pi ft + \phi_{response} + \phi_{delay}) + 1) + 1} + B$$

where $\sin(2\pi ft)$ is the stimulus sinusoid, L mean illuminance and C is vehicle contrast. Free parameters are A (a scaling constant), $\phi_{response}$ (response phase), $H$ which governs response distortion, $\phi_{delay}$, a delay term and B, an offset representing the dark adapted membrane potential. The fitted curves provided a good description of the data at all contrasts below 15 Hz, but some discrepancies became apparent at higher frequencies, as seen in the 30.3 Hz data in Figure 6.

Similar data were obtained from five other cells (not shown). For frequencies less than 9.76 Hz, mean values of $H$ were 0.00162 ($\sigma$ 0.00016, n = 6), and $\phi_{delay}$ had a
mean value of 15.2 ms (σ 1.7 ms, n = 6). The low-frequency response distortions are thus consistent with operation of a gain control with a delay of 10-15 ms. To further check consistency with the previous analysis, each cell was also tested at a mean retinal illuminance of 100 and 10 td. At 100 td, the mean value of $H$ became 0.32 and the mean delay term was 14 ms. At 10 td, little response distortion is apparent. This behavior is consistent with that from the earlier analysis.

At low frequencies (<15 Hz), the scaling factor A and $\phi_{\text{response}}$ showed a similar relation to frequency as first-harmonic response amplitude and phase obtained by Fourier analysis of responses as described in the previous report (Smith et al., 2001). $B$ was consistent with steady-state membrane potential measurements. However, in order for the response distortion to reverse at frequencies above 15 Hz, $\phi_{\text{delay}}$ must reverse in sign. At these frequencies, response amplitude and shape were less well described by Equation 2, in that response shape could not be captured simultaneously at all contrasts. Gain controls with delay do account for the distortions observed, but we could not put together a plausible set of filters which could simultaneously account for response distortions at all frequencies.

**Modulation of Pulse Responses by Vehicle Waves of Different Frequencies**

The goal of this set of experiments was to analyze modulation of test pulse responses by a background vehicle at a range of vehicle frequencies. A further advantage of using test pulses is that their phase relative to the vehicle is fixed so that any ambiguity as to timing (as in the case of test sinusoid latencies) can be resolved.

Incremental or decremental test pulses (9 ms) were delivered at 8 phases of the vehicle wave. Pulse responses with vehicle wave frequencies of 1.22, 4.88, 9.76, and 30.3 Hz were recorded, as well as under steady-state conditions. For the 1.22 Hz condition, a test pulse was
delivered in every vehicle wave cycle and at the higher frequencies on every 2nd, 3rd, and 5th cycle, respectively. Figure 7A-7C shows responses for an H1 cell obtained with incremental pulses for two phases (90 and 270 deg), chosen to demonstrate the pulse responsivity modulation by the vehicle wave. Figure 7D-7F shows an equivalent set of data for decremental pulses. At 1.22 Hz, the shape of the pulse response was readily seen superimposed on the vehicle, but it was less obvious at higher frequencies. To isolate the pulse response, we adopted the same procedure as with the sinusoidal tests; the response to the vehicle alone was subtracted from the combined vehicle plus test, and these responses are shown in the bottom panels. The responses to the two phases are plotted superimposed on the same time scale relative to the beginning of the pulse.

The response to the pulse was modulated in amplitude by the vehicle wave for both incremental and decremental pulses. At 9.76 and 30.3 Hz, there was less modulation of pulse amplitude than at 1.22 Hz, although there was some distortion of response shape at 30.3 Hz. The peak pulse response amplitudes were measured and are plotted as a function of the phase at which the pulses were presented in Figure 8A. Modulation of the test pulse response was apparent. Amplitude of decremental pulses was larger and was more deeply modulated than for incremental pulses. There was a decrease in modulation of the pulse response at 9.76 Hz, and a phase delay of the modulation was seen. We fit the modulation of pulse responses using Equation 1 (solid lines, Figure 8A) except at 30.3 Hz. Response distortions at this frequency may make pulse amplitude measurements unreliable, and we did not attempt to fit these data.

The three fit parameters at the different frequencies are plotted in Figure 8B. \( R_0 \) was independent of frequency, \( B \) (which determines the degree to which modulation of the test response takes place) decreases at 9.76 Hz, and \( \phi_{\text{delay}} \) (the phase lag term) increased with frequency with a slope corresponding to a delay of 5 ms for incremental and 8 ms for decremental stimuli. Two other cells for which complete data sets were obtained behaved in a similar manner, and partial data from five further cells confirmed these findings (not shown).

These results were consistent with those using sinusoidal probes, in that at low frequencies they provided similar degrees of modulation of the test stimulus (\( B \)). The delay term (5-8 ms) was comparable to the delay estimated (10-15 ms) with the sine test after subtraction of the 19.5 Hz phase lag component. However, the difference in amplitude between the incremental and decremental pulses is not inherent in Equation 1, and we now examine whether the difference is due to operation of sensitivity regulation within the 9-ms pulse duration.

**Responses to Incremental and Decremental Pulses**

The difference in response amplitude to incremental and decremental pulses might result from a static nonlinearity (i.e., response compression or from a rapid sensitivity control). We measured cell responses to attempt to distinguish between these possibilities. Incremental and decremental pulses of 1, 2, 4, 8, 16, 32, and 64 ms in duration at 25%, 50%, and 90% Weber contrast were tested on a 1000-td background. Figure 9a shows typical responses to incremental and decremental, 90% contrast, pulses of 1 ms and 64 ms in length. The 1-ms responses were similar in size and shape but

Figure 6. Responses of an H1 cell to sinusoids at frequencies of 0.61 (A), 4.88 (B), 9.76 (C), and 30.3 Hz (D), and at three contrasts (100%, 50%, and 25%); mean illuminance, 1000 td. Responses are shown by the solid black line, which can obscure model fits (dashed line). Horizontal lines indicate the resting potential at 1000 td (-62.5 mV). Characteristic response distortions were observed, which are well captured by Equation 2 at frequencies up to 15 Hz.
Figure 7. Responses of an H1 cell to incremental pulses superimposed on 50% contrast vehicle wave frequencies of 1.22 (A), 9.76 (B), and 30.3 Hz (C). Pulse duration was 9 msec; pulse contrast was 25% relative to the mean level. Pulses were presented at 8 phases of which two are shown (90° and 270°, top and middle traces, respectively). Pulses were presented every cycle at 1.22 Hz, every second cycle at 9.76 Hz, and every fifth cycle at 30.3 Hz. Resting membrane potential (horizontal lines) -55.4 mV. The pulse response was clearly seen at 1.22 Hz but difficult to distinguish at 9.76 and 30.3 Hz. To isolate the pulse response, the response to the vehicle alone was subtracted from the combined response (bottom traces). These have been superimposed temporally to demonstrate that the time course of the pulse responses was not affected by the vehicle wave except at 30.3 Hz. Amplitude of the pulse response is modulated by the vehicle at all frequencies. Average of 32 presentations. D and F. Equivalent set of records using decremental pulses.
inverted. The 64-ms responses differed in both size and shape. This difference in shape constrains possible mechanisms. It would not be expected that a difference in shape could result from response compression. We tested this using the simple models sketched in Figure 9b and 9c. In both models, an initial filter was defined by the 1-ms pulse response, which is taken from the impulse response from the model in Smith et al. (2001). In the instantaneous model, a saturating nonlinearity follows (model 1). There are three free parameters, an amplitude scaling term, a half-saturation constant and a term setting the steady membrane level. The difference in shape also constrains possible models. We tried various arrangements of filters for a divisive model and that shown in Figure 9b yielded the most satisfactory results. A feed-forward signal is derived before the initial filter, which passes through a low-pass filter to provide a divisive gain control (model 2). There are four free parameters; an amplitude scaling term, a half-saturation constant, the time constant of the feed-forward filter, and its number of stages.

The 64-ms pulse responses were used to test between the alternative models, which were fitted to the data using a least-squares criterion. Figure 10A replots the cell’s response to incremental and decremental 64 ms, 90% contrast pulses (Ο). Superimposed on the data (solid lines) are best fits of the two models. Model 1 predicts the difference in amplitude between the incremental and decremental pulse but not the difference in shape (upper traces); as expected, no instantaneous nonlinearity can predict such a difference in shape. Model 2 provides a more satisfactory description of the data (lower traces).

The parameters generated by the fit in Figure 10A also predicted responses to other durations and contrasts. Figure 10B shows 64-ms pulse responses and fits at 50% contrast. There is less difference in amplitude and shape than at 90% contrast. Responses are predicted by the model. Figure 10C compares peak amplitude of the response as a function of pulse duration and contrast with the model 2 predictions. The main features of the data are captured. Four other cells were studied with this protocol; the difference in response shape with

![Figure 8](image_url)

Figure 8. Peak amplitude of the pulse response and model fits for the cell shown in Figure 7. A. Peak pulse response amplitude plotted against vehicle wave phase (●) for the steady-state condition and for vehicle wave frequencies of 1.22, 4.88, 9.76, and 30.3 Hz. Incremental and decremental pulse responses are shown in the upper and lower plots, respectively. Responses to decremental pulses are larger than those to incremental pulses and more deeply modulated. The degree of modulation on steady backgrounds and 1.22 Hz was similar for both pulse directions, but decreased by ~10% at 4.88 Hz and ~30% at 9.76 Hz. Data were fitted with Equation 1 (solid lines) for all conditions except 30.3 Hz. B. Fit parameters as a function of vehicle wave frequency (● increment pulses; O decrement pulses). \( R^2 \) was frequency independent. \( B (0.67) \) was in the range expected from earlier sections. The delay term is equivalent to a lag of 5 ms (incremental pulses) and 8 msec (decremental pulses).
Discussion

Functional Considerations

Several features of the current results suggest that sensitivity regulation in outer retina has a finite time course rather than being based on an instantaneous nonlinearity, such as response compression (Makous, 1997). These are (1) the change in phase of the test response during the vehicle wave (Figure 1); (2) the response distortions to low-frequency sinusoids (Figure 6); (3) data obtained with pulse test probes on a sinusoidal background (Figures 7-8); and (4) the difference in waveform of the response to incremental and decremental pulses (Figures 9-10). We use equations to describe these results, which are consistent with a multiplicative gain control, in which sensitivity regulation at 1000 td appears to lag the temporal response by ca. 10 ms. The cone specificity and spatial localization of this mechanism (Lee et al., 1999) suggest it has its locus before summation of cone signals in the horizontal cell, either in the cone itself or in the cone-bipolar-horizontal cell synaptic triad.

This description neglects certain features, such as the illumination-dependent changes in time constants describing the temporal response of the cell (Smith et al., 2001). Nevertheless, the analysis is consistent with outer retinal gain controls being rapid but not instantaneous.

Time constants of further gain control mechanisms in inner retina (and beyond) will also contribute to psychophysical measures of the time course of adaptation. At the retinal ganglion cell level, cells of both magnocellular (MC) and parvocellular (PC) pathways adapt to a step change in illuminance within a few tens of milliseconds (Yeh, Lee, & Kremers, 1996). Adaptation of PC-pathway cells to a change in chromaticity has a much longer time course, which was attributed to the physiological counterpart of “second-site” effects (Yeh et al., 1996).

Our data indicate that outer retinal adaptation falls short of Weber behavior below 1000 td. Adaptation of M/L-cone opponent cells of the PC-pathway (Lee, Pokorny, Smith, Martin, & Valberg, 1990; Purpura, Tranchina, Kaplan, & Shapley, 1990) also falls short of Weber's law and it is possible that light adaptation in this pathway reflects primarily outer retinal adaptation. Psychophysical thresholds for detection of chromatic modulation at low photopic levels also fall short of Weber behavior (Swanson, Ueno, Smith, & Pokorny, 1987), consistent with a physiological substrate for this task in the PC-pathway. On the other hand, with luminance sinusoids, cells of the MC-pathway show Weber behavior at low temporal frequencies (Lee et al., 1990). The change in temporal response as a function of retinal illuminance resembles that observed in human psychophysical detection measurements (Lee et al., 1990).
Figure 10. Responses of the H1 cell shown in Figure 9 to 64 msec pulses at different pulse contrasts. A and B. Responses to incremental (downward inflected) and decremental (upward inflected) pulses (●). Solid lines show the model's fit to the data. Top traces in A are responses to 90% contrast pulses fitted with model 1 shown schematically in Figure 9B. Bottom traces in A are the same data fitted with model 2 from Figure 9C. Model 1 with an instantaneous nonlinearity could not predict the change in shape with the two pulse directions; a difference in shape requires a gain control mechanism with a time constant (model 2). Traces in B showed responses to the same pulse duration but at 50% contrast. The data were well fitted by parameters generated by model 2. C. Peak response for incremental (closed symbols) and decremental (open symbols) pulses as a function of contrast and duration has been plotted and compared with the model predictions (solid lines). The model captured the main features of the data.

It is thus likely that there are additional gain controls in inner retina for the MC-pathway.

The degree of adaptation observed in the current experiments was similar to that found in the steady-state measurements (Smith et al., 2001) and was comparable to that observed by Valeton and van Norren (1983) in their mass recordings of outer retinal activity; it exceeds that observed in primate cone outer segments (Schneeweiss & Schnapf, 1999). This discrepancy was further discussed in the previous paper (Smith et al., 2001).

Presentation of pulses on modulated backgrounds as in Figure 7 has been used in a psychophysical context (Boynton et al., 1961; Hood & Graham, 1998). Psychophysically, modulation of threshold occurs at least to 30 Hz. The evidence presented here suggests that there may be modulation of cone responsivity up to 30 Hz. It is not certain how far further sensitivity regulation may occur within bipolar cells. Furthermore, inner retinal mechanisms may modify the outer retinal signal to make the relation to psychophysical data substantially more complex. These mechanisms include the presence of both on and off channels, rectification because spike rates cannot be less than zero, and response saturation (B.B. Lee, L. Rüttiger, H. Sun, unpublished observations).

Comparison to Other Physiological Results

Most physiological data from mammalian horizontal cells have been derived from the cat. Some of these experiments were similar to those we report here (Lankheet, Przybyszewski, & Grind, 1993a; Lankheet et al., 1993b). These authors recorded responses to light pulses upon a modulated background and many features of our data resemble their results. These authors also considered various models of horizontal cells to account for distortions in the responses to sinusoids and other features of their data. One of their alternatives was the feed-forward gain control. They note that feedback gain controls do not predict a responsivity versus illuminance slope greater than 0.5. We used an equivalent formulation because we found that feedback controls tend to be too slow to account for our results. However, the pathway by which such a feed-forward mechanism could be physiologically realized is unclear, and alternative formulations may be possible.
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References


