Evidence From Human Electroretinogram A and Off Responses That Color Processing Occurs in the Cones

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Purpose. To investigate two apparent anomalies of the human electroretinogram: the “on” and “off” components of the cone based PIII are unequally sized, and transitions from red to green, which are electroretinographically silent, yield reverse transitions (green to red) in which a-waves develop.

Methods. Ganzfeld electroretinograms were obtained with intense 100 msec flickering flashes from red and green light-emitting diodes. Such stimuli light-adapt the retina, and the responses are caused by the excitation of long and medium wavelength cones.

Results. In the 10-20 msec after the beginning of a flash (black to green or black to red) the beginning of rapid receptor-generated a-wave is seen. Ten to twenty milliseconds after the end of the flash, the beginning of a rapid positive-going off response, also derived from receptors can be seen. If the retina is stimulated by the abrupt change from one wavelength of light to another (eg, from “green” to “red”), at times > 20 msec after the change there are always slow changes in potential (presumably caused by postsynaptic activity) regardless of the relative intensities of red and green. However, if the two light intensities are adjusted appropriately, 10-20 msec after the transition from green to red no electroretinographic a-wave (or off response) develops—the transition is “silent.” When the transition reverses (changes back from red to green), an a-wave occurs. In the same way if a red-to-green transition is made silent by altering the relative light intensities, the green-to-red reversal evokes an a-wave. This occurs for numerous pairs of red and green intensities. Rod intrusion or minor electroretinogram components do not explain this result. The relative red:green intensity in two color-anomalous subjects is different to that in three normal subjects. The rule for a silent transition is that the decrease in excitation in one cone type should be twice the increase in excitation in the second cone type.

Conclusions. The most likely cause is a reduction in the amplitude of cone receptor potentials 20-50 msec after the onset of the stimulus, caused by a sign-reversing feedback mechanism such as that described in amphibians. This implies that the chromatic signals for color vision required by theorists are partly generated in the cones. Invest Ophthalmol Vis Sci 1993;34:2079–2091.
for this rests (in humans) on the spectral effectiveness of various wavelengths in eliciting either flicker or off responses,\textsuperscript{2,3} or, in the case of LED, which can only be used at two fixed wavelengths, in the separation of cone from rod responses by progressively light-adapting the retina by altering the flash duration, interflash interval and intensity.\textsuperscript{1} The a-wave of the ERG can be generated by both rods and cones, but prolonged flashes, delivered as a slow flicker, light-adapt the retina so greatly that the a-waves also are evoked solely from cones. Furthermore, the initial part of the a-wave and the off response is believed to be generated by the receptors and not by postsynaptic activity. This was demonstrated in humans for rod a-waves\textsuperscript{4,5} and for cones has generally been accepted as a result of experiments on the primate with penetrating microelectrodes and current source density and principal component analysis,\textsuperscript{6-8} which confirmed earlier accounts\textsuperscript{9-11} of the common genesis of the a- and off responses. The in vitro responses of single isolated primate cones have been described in detail elsewhere.\textsuperscript{12-15} The membrane current changes caused by light affect the internal ionic composition and thus the transduction processes themselves. This feedback leads to complex response waveforms, but the entire process is linear in that increments and decrements of light produce changes in outer limb dark current, which are opposite in sign, but similar in magnitude. Such experiments provide a basis for interpreting the human ERG a-wave and off responses.

Although in recordings from single cells or by extracellular microelectrodes located within focally illuminated light-adapted primate retina, the a-wave and off response are nearly mirror images of one another.\textsuperscript{2-3,6-11} In the human corneal ERG it was found in a previous paper\textsuperscript{1} that even for the earliest changes caused by light, both the amplitude and the rate of change of voltage of the a-waves was larger than the corresponding values for the off responses. In general terms, this observation implies either that there may be additional components that contribute to the a- or off response (or to both), or else that the cone receptor response declines during a prolonged stimulus. This problem is further investigated here, which however is concerned not with prolonged flashes superimposed on a dark background, but with the ERG evoked by the change from one wavelength of light to another, and the intensities under which these changes evoked no rapid voltage changes.

The responses of the eye to changes in the wavelength of stimulating light have been investigated many times, usually to measure spectral sensitivity. If the b-wave is used as an index, the spectral sensitivity may show complexities, which may reflect postsynaptic interactions.\textsuperscript{14-16} These are absent if the index is the a-wave, isolated by slow sinusoidal flicker.\textsuperscript{2} Such experiments have been used as an evidence that the a-wave is evoked by receptors. In psychophysical experiments the method of “silent substitution” has been employed to analyze the effect of change in wavelength. The underlying idea is that when the wavelength of light falling on a photoreceptor is changed abruptly, the intensity may also be modified so that no change in excitation occurs (see \textsuperscript{17} for a review). Silent substitution occurs trivially if only one receptor type (eg, rods) is present, but is of interest when several types of receptor are stimulated at one time. In almost all experimental work, complete silent substitution cannot be realized. In experiments on the ERG, with intense flickering light, rod responses are reduced to a point where they can be neglected even when the flicker has 100% contrast and a 50% duty cycle.\textsuperscript{1} Therefore, when, as in the experiments below, color shifts are the effective stimulus, the luminance contrast is still less than in the study by Spileers\textsuperscript{1} and it is most unlikely that rod-generated responses could be detected. Thus color shift ERG are generated by the differential excitation of the two common classes of cone, long wavelength and medium wavelength (LW and MW). In mass recordings from many photoreceptors, a condition may be obtained in which a change in wavelength and light intensity is arranged in such a way that the increase in the electrical response of one class of cone matches the decreased electrical response in the other class so the transition is "silent." However, there may still be responses in neurons further "upstream," because no allowance has been made for subsequent chromatic data processing. Only two accounts have appeared in which the electroretinogram (ERG) has been used as an index in experiments on “silent substitution.”\textsuperscript{18,19} In both, it was found impossible to obtain a “color shift” without evoking some ERG (and the current results confirm these findings), but at the time of those experiments, the contribution of the photoreceptors to the ERG was not fully understood. Accordingly, there have been no prior attempts to analyze cone receptor potentials in humans by silent substitution. Our experiments are limited to the voltage changes that occur in the 10-20 msec following a change in stimulus. Both logic and the experimental results just cited indicate that the earliest voltage changes in the ERG we record can be ascribed with some confidence to the cones themselves. The later electrical responses in the ERG reflect other stages in processing, and are more difficult to analyze: thus our results need not be related to work on flicker ERG at higher repetition rates.\textsuperscript{14,16,20} The simplest explanation of our findings is that soon after the onset of the cone receptor potential its amplitude is diminished by approximately one half and this reduction continues unchanged until after the offset of the stimulus. Before such a conclusion can be accepted, it is necessary.
to demonstrate the possibility of making measurements on the small ERGs evoked by color shift, and to perform control experiments to demonstrate that rod currents or minor components of the ERG are not influencing the recordings, as described in Results.

METHODS

Photostimulator

For our experiments, we used the equipment described in detail elsewhere. The stimulator consisted of a segment cut from a plastic dome 4.5 inches in diameter, subtending over 160 degrees of visual angle and lined with 368 LED placed as closely together as possible. The construction automatically ensured that all the LEDs pointed toward the center of the dome, which could be made the anterior nodal point of the subject’s eye. This in practice ensured a “Ganzfeld” stimulus, and the LED, of a type chosen for maximum light output (Stanley “superbright”), produced sufficient retinal illumination to strongly light-adapt the subjects’ eyes and to evoke very large ERG. The LED were connected in series-parallel configurations. They were driven by simple pulse generators. The light intensity was continuously variable, controlled by a 10-turn potentiometer. The light intensity in the pupil plane, varied from 0 to 14,000 mW • m^-2 for green light, (550 ± 5 nm light, and 85,000 mW • m^-2 red, 660 ± 5 nm (to half power).

Calibrations and Calculation of Effective Light Intensities: A calibrated radiometer (Tektronix J16 [Tektronix, Beaverton, OR]) photodiode was placed in the position of the subject’s pupil occupied during the experiments. To determine the rate of change of light, and the duration of brief pulses, a rapidly acting photodiode was placed in the position of the subject’s pupil, and connected to an amplifier and oscilloscope (system band pass > 10 MHz). The light output to prolonged flashes was maintained without any initial transient overshoot. When the intensities were adjusted appropriately, transitions from green to red light could be achieved without any transient change of photocell output being visible. The flash intensities were calculated as previously described in terms of quanta incident per unit area of retina. In brief, to calculate the effectiveness of the LED light in stimulating long wavelength and medium wavelength cones, we assumed that the relative spectral distribution of light in the LED is as specified by the makers, and that the spectral variation of absorption of light in LW and MW cones is as described by other researchers. Then the relative numbers of quanta absorbed in LW and MW cones for equal energy outputs of the green and red LED can be calculated, as follows: a quantum correction is made to the relative spectral energy output of the LED, and the value at each wavelength is multiplied by the relative amplitude of the relative sensitivity of the appropriate cone mechanism at that wavelength. The resulting values are totaled for wavelengths from 510 to 710 nm, at intervals of 10 nm. The result is shown in Table 1. Further details of the calculations are given in the Appendix to the article by Spileers.

Subjects: The experiments were performed on three subjects without color vision abnormality, and also on two subjects with deuteranomalous trichromasy. The procedures were carried out in compliance with the local ethical requirements, which include obtaining informed consent and the tenets of the Declaration of Helsinki.

Recording Techniques: ERG were obtained with pupils dilated by tropicamide 1%. The corneal electrode was a gold foil, and Ag-AgCl earth and reference electrodes were placed on the forehead and ipsilateral temple. Recordings were made using a clinical evoked potential system (M60, Medelec, Woking, Surrey, UK). Between 100 and 200 responses were averaged to achieve a high signal to noise ratio. Responses were elicited in pseudorandom, predefined sequences.

RESULTS

Figure 1 shows responses evoked by red–green and green–red transitions. The trace begins where a red light step ends and a green light step begins. One hundred milliseconds later, the colors reverse, and the red light reappears as the green is extinguished. The onset of the highest green intensity, bottom trace, 1100 mW • m^-2, left side of record, evokes a cone-negative on response, followed by a brief cornea-positive deflection. In preliminary experiments the intensity of either the red or green epochs was still further reduced to make sure that the on responses shown in

| Table 1. Relative Quanta Absorbed by MWC and LWC for Equal Energy Outputs of Red and Green LEDs (No Significant Bleaching) |
|-----------------|-----------------|-----------------|-----------------|
| MWC | Green LED | Red LED |
| LWC | Green LED | Red LED |
| 0.5611 | 0.0103 | 1.00 | 0.1543 |
Figure 1. Responses to alternating red and green light steps are shown for different intensities of green light. In the first half of the trace, row 4, there is a downward, negative-going a-wave showing that the increase in receptor potential caused by the green light stimulus is larger that the simultaneous decrease in receptor response caused by the end of the red step. In row 1, the green light is weaker, so at the beginning of the trace the positive off response to red can be seen. In the second half of the traces, the reverse transitions occur, causing (row 4) a positive-going off response, and (row 1) a negative a-wave. Note in row 2, there is electrical silence soon after the red to green transition, and in row 3, there is silence (second half of trace) at the green to red transition. The hatching shows the window in which measurements were made. Light intensity measurements made in the pupil plane.

Figure 1 progressively increased in amplitude to become waveforms such as those commonly observed for bright flashes on a zero background (see illustrations in 1). Thus, this part of the record in Figure 1 shows the well-known a- and b-waves of the photopic ERG. As stated before, this article is only concerned with the initial changes, 10–20 msec after the stimulus changes. This time interval is shown in the figures by hatching. In the upper trace, the intensity of the green light step has been reduced (100 mW·m^-2) and the initial response inverts (becomes cornea positive-going). From the previous results 1 we know this represents an off response. The third trace with an intermediate green light intensity (300 mW·m^-2) shows a considerably smaller a-wave, and 195 mW·m^-2 the change in voltage between 10 and 20 msec after the trigger is approximately zero.

At times >20, <110 msec after green-on, there are no pairs of lights that when interchanged lead to a voltage null, but at such times complex activity, driven by postsynaptic events, must certainly occur and analysis of that fraction of the ERG (which is what has been attempted in experiments on flicker and the ERG) is beyond the scope of this article.

At the end of the green step (after 100 msec) the red light is turned on, and the green is turned off. These changes both occur during a period too brief to be measured precisely (see Methods). The top trace of Figure 1 shows a prominent a-wave and b-wave (on response) associated with the green-to-red transition. Increasing the green intensity (second trace, green intensity 195 mW·m^-2) first decreases the a-wave and with still further increase (bottom trace) the response to the green–red transition becomes a prominent off response.

It can be seen that the time window chosen is appropriate for making measurements that describe an early voltage null. For all these traces shown the averager was triggered at the red–green transition, and we did control experiments to prove the waveforms recorded were unaffected by changing the trigger point to the green–red transition.

Figure 2A and B shows a series of recordings where the intensity of either the red-light step or the green light was increased, while keeping the other stimulus constant. In Figure 2A, top trace, the red light was weakest (1900 mW·m^-2). The transition from red to green at the beginning of the trace evokes an a-wave: the green light is stronger than the red, in terms of evoking a-waves. Increasing red intensity (lowest trace) changes the response at the first transition to an off response: now, the red is more effective than the green. The intermediate setting (center trace) presents an almost silent (zero amplitude) ERG in the period 10–20 msec. It is immediately obvious that at the reverse transition (green to red) shown at the right of the center trace, a quite different response is seen: at this intermediate setting of red light there is a prominent a-wave. A null can be achieved for the green to red transition, if the relative light intensities are altered: this is shown in the upper row—the weakest red intensity causes a null. Thus, if the relative red and green light intensities are adjusted so that at a red–green transition, the relative excitation of retinal mechanisms is unchanged, the relative excitations appear to have altered 100 msec later, when the green–red transition occurs.

Figure 2B shows responses to three different green light intensities, with a different constant intensity of red. The top trace, with the strongest green light, produces an a- and b-wave at the red-to-green transition, and an off response at the green-to-red transition. For the weakest green light (lowest trace) the situation is reversed, as expected. In between, it appears that a green intensity of 745 mW·m^-2 causes an a-wave at both transitions. Figures 1 and 2 show...
Silent Substitution in Human ERG

FIGURE 2. (A) Responses to alternating red and green light steps. The green light intensity is constant, whereas the red light intensity increases from 1900 to 14036 mW·m⁻². Note that the silent substitution in the first half of the trace occurs in the middle row. The green–red transition however nulls in the upper row. (B) Responses to alternating red and green light steps. Now the red light intensity is kept constant, while the green light intensity changes. Again it is clear that it is impossible to obtain a silent ERG for both transitions for a single pair of intensities of red and green light. In the center row, there are a-waves at both transitions. Hatching indicates window for measurements.

representative recordings, and provide evidence from which the feasibility of making measurements to determine intensities at which “silent substitutions” occur may be judged.

Minor components of the ERG, the proximal negative response, and the m-wave are vitreous-negative for both "on" and "off." The proximal negative response amplitude is greatest when the stimulus is localized on the retina. The small degree of inhomogeneity of retinal illumination normally present in the stimulator might excite a proximal negative response, and account for records such as that seen in Figure 2B, middle row. The experiments represented in Figure 2 were therefore repeated with additional diffusers placed between the LED and the eye. These completely removed any spatial inhomogeneity in the stimulus and reduced the absolute intensity. The relative intensities of red and green were however unchanged. The relative intensities of red and green for silent substitution remained the same, and it remained impossible to obtain a zero voltage change in the 10–20 msec period after both transitions (not illustrated).

Experiments with a Rod-Suppressing Background: Red-to-green and green-to-red transitions will not be equivalent if green light preferentially evokes responses with a rod contribution. The reason is that the rod receptor potential has a rapid onset but a very slow return to the baseline, so off responses are entirely cone generated, and only rod a-waves would be evoked by the green light. Such an occurrence might be thought to explain the asymmetry between green-to-red and red-to-green seen in Figures 1 and 2, despite the high light intensities used and the 5 Hz rate of reversal. To analyze this point we used a steady rod-suppressing green background, on which the transitions were superimposed. The effect of this background is shown in Figure 3A, for isolated flashes. In this experiment note that without a background, the 15 mW·m⁻² intensity green light flash evokes a clear slow rod b-wave that is greatly reduced by the background. The higher level of green light (400 mW·m⁻²), also evokes a large rod a- and b-wave. The background reduces the amplitude, but leaves a small, fast ERG, characteristic of cone responses. Figure 3B shows red–green transitions, with and without the same background, and using both weaker and stronger green steps. With the dimmer green stimuli, the background changes the waveform of the responses, but for higher intensities of flickering light, which themselves light-adapt rods and suppress scotopic activity, the background has no effect (eg, compare row 4, Figure 3B, left and right columns). In summary, for higher intensities of flickering light, the ability to obtain “silent substitution” with a single combination of red and green persists unchanged with a rod-suppressing background.

By carefully adjusting the relative intensity of the red and green stimulus it is possible to define a number of conditions in which the transition from red to green causes a zero change of voltage 10–20 msec...
The results confirm that the red:green intensities are different for the two transitions. For R:G the ratio is 32.66 ± 12.38, and for G:R 14.19 ± 4.14.

The significance of the result becomes clearer if the relative excitation of the MW and LW cones are considered separately (see Methods). Table 3A and B, shows the calculated absorptions at which transitions from red to green, and vice versa, produced no change in the ERG trace, from 10–20 msec after the transition.

There is a large range in the differences of excitation of long and medium wavelength cones. However, if the ratio of excitation before and after a transition from red to green is considered, apart from one anomalous result (starred in Table 3A) there is a fair degree of consistency. It appears that the condition for no voltage change in the specified period is that LW cones absorptions decrease to one quarter and MW cones absorptions double. This occurs over a large range.
TABLE 2. Stimulus Intensities (mW·m⁻² at Pupil) that Produce a Zero Voltage from 10 to 20 msec after a Red–Green or a Green–Red Transition

<table>
<thead>
<tr>
<th>Subject</th>
<th>Red to Green</th>
<th>Green to Red</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Red Intensity</td>
<td>Green Intensity</td>
</tr>
<tr>
<td>Subject 1</td>
<td>3021</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>5072</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td>8470</td>
<td>255</td>
</tr>
<tr>
<td>Subject 2</td>
<td>5833</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>17493</td>
<td>675</td>
</tr>
<tr>
<td></td>
<td>23333</td>
<td>1017</td>
</tr>
<tr>
<td>Subject 3</td>
<td>4195</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>3724</td>
<td>92</td>
</tr>
</tbody>
</table>

Results were obtained from three normal subjects.

range of intensities, and the calculated ratios do not seem to change with the absolute light intensity, at least within the range investigated. The calculations show that negligible bleaching of cone photopigment would occur with the stimulating light. If during the 100 msec of exposure, a significant proportion of the pigment in MW or LW cones was bleached, the red-green ratios should change systematically.

Table 3B shows similar calculations for the reverse transition from green to red: the ratios are again very similar over a wide range of light intensities, but the mean values are different: the LW cones absorptions double and the MW cones absorptions drop to a quarter. Note that there seems to be a reciprocal relationship between the changes of R–G and G–R.

Before considering the significance of this, it is necessary to analyze the relationship between response amplitude during the 10–20 msec after a transition, and the change in stimulus intensity from that which produces a silent substitution. Qualitatively, it is easy to make a prediction. Supposing at one transition (R–G) there is electrical silence. In any single experiment, the ratios of R–G must be identical at the reverse transition (G–R), and Table 3B predicts that at the reverse transition, there will be an “on” response. This is exactly what was found (eg, Figure 2A, center trace). The reverse is also true for G–R transitions, which produce an electrical silence: on responses should occur at the reverse, and they do (Figure 1, 3rd trace and 2A top trace). We noted that the amplitudes of these responses were roughly similar for all the experiments in Table 2. This suggested a quantitative analysis would be possible. To simplify matters, experiments were performed in which the red light intensity or the green light intensity was held constant, and the intensity of the other color varied. Figure 4 shows the results in one subject. The abscissa represents the numbers of additional quanta absorbed in both the LW and MW cones, expressed as a ratio of those required for a silent transition. Note the scale goes negative: for these results, the sign of the response also alters—that is, an “on” was replaced by an “off.” Figure 5 shows the results for the opposite transition, green to red. Although there are a few anomalous results, there is evidently a good linear relationship. The regression coefficients are $r = 0.75$, $DF = 29$ and the slope of the regression line is significantly different from 0 ($P < 0.001$) for each graph.

One of the authors is color defective, deuteranomalous, and requires four times more green light (or less red light) for a silent substitution than do other sub-

TABLE 3. Relative Absorptions in LW and MW Cones Required to Produce Zero ERG Voltage 10 to 20 msec After a Color Transition

<table>
<thead>
<tr>
<th>Before Change</th>
<th>After Change</th>
<th>Ratio Before:After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long WV Cones</td>
<td>Medium WV Cones</td>
<td>Long WV Cones</td>
</tr>
<tr>
<td>Red to Green</td>
<td>466</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>783</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>1507</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>2699</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>3603</td>
<td>241</td>
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<tr>
<td></td>
<td>647</td>
<td>43</td>
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<td></td>
<td>1443</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>575</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>783</td>
<td>52</td>
</tr>
<tr>
<td>Mean</td>
<td>0.24</td>
<td>1.98</td>
</tr>
<tr>
<td>SD</td>
<td>0.11</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Green to red

| Red to Green  | 783 | 52 | 595 | 334 | 1.32 | 0.16 |
|              | 900 | 60 | 464 | 260 | 1.94 | 0.23 |
|              | 3603 | 241 | 1354 | 760 | 2.66 | 0.32 |
|              | 231 | 15 | 87 | 49 | 2.67 | 0.52 |
|              | 2880 | 192 | 1440 | 808 | 2.00 | 0.24 |
|              | 1443 | 96 | 982 | 551 | 1.47 | 0.17 |
|              | 783 | 52 | 378 | 212 | 2.07 | 0.25 |
|              | 991 | 66 | 293 | 164 | 3.38 | 0.40 |
| Mean         | 2.19 | 0.26 |
| SD           | 0.64 | 0.08 |
subjects. This phenomenon has been previously reported (see 26 for a review). If this is allowed for, the same relationship between additional quanta absorbed and ERG voltage is seen for this subject, though, because he requires more green light, only off responses could be obtained within the limits of the instrumentation (Fig. 6A). However, the same type of relationship between change in light intensity and ERG amplitude occurs (Fig. 6B). A second person with less severe color defects gave similar responses to those shown in Figure 6, although in his case, some on responses could be obtained. It is clear that the relationship between the amplitude of on and off responses and change in light intensity is similar and linear, over the range investigated: doubling the additional number of quanta absorbed doubles the amplitude of the response.

Experiments with flashes of varying duration were carried out, to discover whether the intensities at which voltage changes are zero (from 10-20 msec after a transition) are affected by the flash duration. Figure 7 shows that alternating red–green and green–
red transitions of 50, 100, and 200 msec (with equal periods of red and green light in each case) require the same relative intensities to produce a "silent substitution."

With flashes shorter than 50 msec, the b-waves of one transition occur during the a- or off waves of the next. The ERG waveform changes because each b-wave is terminated by the succeeding a-wave. In addition the inflection on the a-wave which signals the beginning of the b-wave is delayed, so the time-to-peak of the a-wave increases. Therefore, analysis of the traces becomes problematic. It appears that the rules for silent substitution of the initial parts of the a- and rapid positive-going off waves described above still hold: no pairs of light intensities ever cause a null of the b-waves, though a range of intensities gives a minimum b-wave.

DISCUSSION

No experiment on the human ERG can provide conclusive evidence of the source of generation of the voltages recorded. However, in light-adapted eyes the ratio of a-waves to off responses reaches a constant value for a range of different intensities and rates of stimulation, a finding consistent with the hypothesis that a single process is responsible for both waves. Microelectrode recordings from primate retinas, including current density analysis and principal component analysis also lead to such conclusions, and confirm earlier reports that both waves originate in the cones. The first effect of light must be exerted on the photoreceptors, and by confining measurements to the earliest voltage changes in the ERG, it is possible to assume that the results obtained refer to recep-
FIGURE 7. Alternating red and green light steps with different durations (50, 100, 200 msec). Top 3 transitions are for red to green. Between 10 and 20 msec after the transition, the maximum voltage change is 3 μV. The light intensities are red: 22883 mW·m⁻² and green: 1017 mW·m⁻² corresponding to a decrease in LW cones' relative absorptions of 3531 to 1017, with a concurrent increase in MW cones' absorptions of 236 to 571. The lower 3 traces are for the reverse transitions, green to red, and the light intensities have been readjusted to obtain less than 4 μV in the 10–20 msec period after the flash. For green the intensity is 1500 mW·m⁻² and for red 17903 mW·m⁻² corresponding to an increase in LW cones' relative absorptions from 1500 to 2762 and a corresponding decrease in MW cones from 842 to 236 (these results are not in table 3). Note that the later activity varies with flash length, but that from 10–20 msec an approximate null occurs at the same pairs of light intensities, for all step durations.

The results exclude rod contributions. “Blue” (SW cones') b-waves have been studied and are “rod-like.”27,28,29 Very different to those shown in the figures, but the waveform of the relatively infrequently encountered blue cone receptor potential is the same as that of the other cone classes.12,13 Any small contribution blue cones might make to a- and off responses such as those shown in Figures 1, 2, and 3, could not account for the findings on quantitative grounds. Thus in Figure 2A, the green to red transition toward the end of the center trace causes a large on response, which must be due partly to long-wavelength cone activation. Reducing the red light intensity by less than 50% as in the top trace will cause a trivial difference in the direct light adaptation of SW cones, but it transforms the ERG (upper trace, left) at the red–green transition. Therefore the change in the relative light intensity must affect the ERG through its effect on the medium wavelength cones. Thus to a first approximation, the result is due to altering the excitation of LW and MW cones.

If on and off responses develop at different rates, this would explain the results, but all evidence is against this suggestion. Thus, the 10–90% current change in single primate cells13 in response to a 1 sec step of light delivering 2350 photons um⁻² (13 Fig. 3B) occurs at the rate of 73 pA/sec at “on” and 71.4 at “off,” which is nearly identical.

The other explanation is that the amplitude of on and off responses are unequal. For this to be true for every flash in a prolonged sequence, the receptor photocurrents would have to decline during the time (>20 msec and <50 msec) when the electrical activity of receptors is masked by the other ERG components.

Reduction in amplitude of a response may also imply a change in sensitivity. During intense step stimuli,15 primate cone sensitivity may decrease, at first rapidly, and then to a plateau level after about 1 sec. This form of light adaptation was only seen when intense flashes were given on a zero background and the magnitude of loss of sensitivity was dependent on light intensity. In our experiments with red–green alternation, the overall change in intensity for each receptor...
was less than in the article cited, none of the responses approached saturation, and the effects we observe are not dependent on the absolute level of light. Schnapf et al.13 (Fig. 8) found no changes in sensitivity in such circumstances in isolated single cones.

The linearity demonstrated in Figures 4, 5 and 6 argues a simple relationship between response amplitude and light intensity, so our findings imply that 20 msec after the beginning of a light step a decline in the amplitude of the cone photoresponse begins and after about 50 msec this decline reaches a new steady level. If the sensitivity is defined as μV/photon, there is a decline in sensitivity. When the step of light terminates, sensitivity recovers after a similar delay. This is quite different to the sensitivity changes in single isolated cones previously described.13

A Model for Modifications of the Cone Photoresponses

Figure 8 is a diagram showing what we have observed and a possible explanation. Although it has been reported6,7,9,10 that monkey cone receptor potentials are maintained, others11,15 have demonstrated a pronounced biphasic nature of the flash response, and have shown that the step response can be predicted from this with the assumption of linear superposition, and these last findings are incorporated into Figure 8.

The upper rows show the stimulus, the alternation of red and green. The next row shows the changes in membrane voltage or current of a MW cone during the “green” half of the red-green alternation. There is a net increase in hyperpolarization: note the response in the diagram never saturates. There is a peak to the response, due to the mechanism suggested by,13 a rapid loss in intracellular calcium. At the end of the light step, the current change is in the reverse sense and temporarily goes beyond the dark level. Note, there is a delay of about 10 msec between the flash onset and offset and the beginning of the change in membrane current. The hatching shows the period during which the experiments represented by Table 2 took place, no changes in potential were seen.

The third row of Figure 8 shows the presumed time course of the modification (reduction) in receptor response in our experiments. It occurs with a delay of approximately 20 msec, and is maximal by 50 msec after the flash. At the end of the stimulus, the restoration is presumed to possess the same time course. When this process operates on the photoresponse, the changes in membrane current are transformed as shown in the 4th row. The onset is scarcely changed, but the offset grows much smaller. The diagram shows what happens to an MW cone, but at its “on” transition, there will be a corresponding “off” for a LW cone. The argument for larger “on” response than “off” also holds for the LW cone. That is, at the time of the increase of red light, the “on” of the LW cone will necessarily be bigger and the “off” of the MW cone will necessarily be smaller: so silent substitution cannot occur, unless the light intensities are readjusted. This explanation shows why the increase and decrease of the light intensities required at the two transitions is symmetrical for the two cone classes. If the cones are operating in their linear range, the ratio between the two intensities of red and green light which achieve silent substitution at one transition should be constant as the intensities alter. The inverse of the same ratio describes the light intensities required at the other transition: the proposed mechanism operates equally on both cone classes, and the amplitude is approximately reduced by 50%.

The question as to the mechanisms involved cannot be directly investigated with the ERG but the simplest hypothesis is that feedback from H-cells30,31 depolarizes the inner portions of the cones and reduces the radial longitudinal current. Such a feedback has hitherto been seen only in amphibian and reptilian retinas, but the anatomic substrate exists in humans.32,33 The delays in the feedback are such that only the falling phase of the cone impulse response is reduced.30 Thus, the measurements of the a-wave made in this article would be unaffected by the feedback mechanism.

FIGURE 8. Explanation of results with red–green and green–red transitions. The changes in retinal illumination are indicated in the first row of the diagram. The hatched time-lines show where silent substitution measurements were made. The waveform of the isolated green cone is shown, based on Schnapf et al.13 Note the symmetrical overshoot at onset and offset of the green light. The postulated delayed inhibitory feedback is indicated: the lower trace shows how the receptor potential is thereby modified, and the “on” and “off” responses become asymmetric.
The Origin of 'Chromatic' Responses

When psychophysical responses, or ganglion cell discharges, or ERG b-waves are recorded with rapid red-green flicker, a ratio of red:green intensities is found at which minimal responses can be seen. If the wavelengths are changed, the spectral sensitivity of the mechanism can be investigated.\textsuperscript{14,15,20} For the b-wave and for ganglion cell discharges, such experiments provide evidence of chromatic processing. For the PIII component of the ERG in primate retina with a stimulus consisting of slow temporal sinusoidal color change, the spectral sensitivity of PIII under these circumstances is that of the luminosity function, \(V_\lambda\text{lambda}\), but under such conditions the sharp transients we measure would be much reduced or absent. Table 3 shows that for the rapid transients, the relationship between intensity of the two wavelengths for silent substitution is more complex. Our work has been restricted to two colors only, but the interaction we have detected is subtractive, rather than additive. Such subtractive interactions are a constant feature of theories of color vision.\textsuperscript{34,35} Some indications of them have been seen in the b-wave of the ERG,\textsuperscript{15} even when sinusoidal flicker is used. Our results suggest that subtraction occurs in the receptor itself, and is in the radial longitudinal current generated by light. Because the current is maximal in darkness, the interaction causes an increase in radial current. Horizontal cell feedback is the most likely cause of this change, which has not been seen in experiments when a single cell was isolated and illuminated. Our results also suggest that the reason why color discrimination depends on abrupt spatial or temporal changes lies at least partly in the cones themselves.

Key Words
human ERG, a-wave, off response, color mechanisms, silent substitution

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References
Silent Substitution in Human ERG