Focal cone electroretinograms in dominant retinitis pigmentosa with reduced penetrance

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Focal electroretinograms (ERGs) from the green and red cone systems in combination were elicited with a method of rod silent substitution from three young patients with dominant retinitis pigmentosa with reduced penetrance. With this method, two alternating lights of different wavelength, matched in brightness for the rods, were presented to the same retinal area. The brighter light for the green and red cones was designated as the stimulus increment (ls) and the dimmer light as the background (lb). Foveal cone ERGs were normal in amplitude and b-wave implicit time, and midperipheral cone ERGs were minimally reduced in amplitude but markedly delayed in b-wave implicit time. Minimal reductions in amplitude with marked delays in implicit time could be recorded from normal subjects in the midperiphery when the brightness of lb was reduced more than the brightness of ls while still maintaining rod silent substitution; this was not observed either when the brightnesses of ls and lb were proportionately diminished or when the area of lb and ls was decreased. These findings support the idea that the abnormal midperipheral cone ERGs observed in young patients with this type of retinitis pigmentosa are due in part to a decreased state of adaptation of the cone system and that a reduced amount of visual pigment in the photoreceptors or reduced numbers of photoreceptors cannot, by themselves, be responsible for these abnormal cone ERGs.

Key words: retinitis pigmentosa, retinal degeneration, retina, electroretinogram, cone, rod, fovea, light adaptation, dark adaptation

Previous studies have shown that full-field cone electroretinograms (ERGs) from young patients with dominant retinitis pigmentosa with reduced penetrance could be normal or nearly normal in amplitude at a time when cone ERG b-wave implicit times were very delayed. These ERGs were recorded from patients who had normal visual acuities and full central fields. Since the full-field cone ERG is generated primarily by extrafoveal cones, the possibility was raised that the cone ERGs seen in this disease were due to an abnormality in extrafoveal cone function.

The present investigation was done to evaluate ERGs generated by foveal and midperipheral cones in patients with the early stages of dominant retinitis pigmentosa with reduced penetrance. Effort was also made to simulate in normal subjects changes in focal...
cone ERGs comparable to those recorded in these patients.

**Methods**

A three-channel maxwellian-view optical system (Fig. 1) was used to elicit ERGs from the green and red cone systems in combination with a method of rod silent substitution. Two channels were used to present to the same retinal area two lights of different wavelength (10 nm half bandwidth), alternating at 5 Hz, that were matched in brightness for the rods but mismatched in brightness for the green and red cones in combination; the third channel was used to present a dim red fixation light subtending 20°. The rod match was determined psychophysically by nulling flicker at scotopic levels for normal subjects and confirmed by calculation based on the scotopic luminosity function. The brighter light (20 msec duration) for the green and red cones in combination was designated as the stimulus increment (I_s) and the dimmer light (180 msec duration) as the background (I_b). This method of rod silent substitution was chosen so that cone ERGs, unmodified by any rod contribution, could be measured at low levels of cone adaptation. In addition, testing could be done at low frequencies to separate the b-wave of one response from the a-wave of the next response. It was also possible to vary the brightness of I_s or I_b relative to the green and red cones by varying their respective wavelengths while still maintaining lights matched in brightness for the rods.

Initial studies were done with I_s at 556 nm (1700 photopic trolands) and I_b at 500 nm (120 photopic trolands); these lights were chosen to maximize the brightness of I_s relative to the brightness of I_b for the green and red cone systems in combination. Under these test conditions, flicker could not be seen by a rod monochromat or a blue cone (π_3) monochromat, even when the visual angle of the test spot subtended 48° (the maximum visual angle permitted by this optical system). To establish that the focal cone ERGs were, in fact, generated by localized areas of retina under these test conditions, ERGs were evaluated in normal subjects with a 3.75° diameter test spot centered on the foveola and with test annuli of different inner and outer diameters concentric with the foveola. Pilot studies indicated that no response could be de-
tected with the 3.75° spot positioned beyond an eccentricity of 6°; therefore annuli were used to stimulate enough cones at a given eccentricity from the foveola to generate responses large enough for evaluation of waveforms. Amplitudes of the responses to the spot centered on the foveola and annuli concentric with the foveola of varying inner-outer diameter (4.5° to 7.5°, 9° to 12.5°, and 38.3° to 40.1°). Osterberg's plot of cone density vs. eccentricity from the foveola (hatched line) is shown for comparison.

After it was established that the cone ERGs obtained under these test conditions were generated by localized regions of the retina (see Results), foveal cone ERGs were monitored in normal subjects and patients with dominant retinitis pigmentosa with reduced penetrance with a 3.75° test spot centered on the foveola. Midperipheral cone ERGs were monitored with a test annulus of 38° inner diameter and 44° outer diameter concentric with the foveola. This annulus had an inner diameter that fell anterior to the optic disc and an outer diameter that fell just within the outside limits permitted by the optical system.

Patients and normal subjects were seated in an electrically shielded cage, and the eye to be tested was topically anesthetized with 0.5% proparacaine hydrochloride. The patient was brought into maxwellian-view by an adjustable bite-board. ERGs were recorded with a Burian-Allen double electrode contact lens with a ground lead on the forehead, differentially amplified at a gain of 10,000 (3 db down at 1 Hz and 100 Hz), attenuated at 60 and 120 Hz with notch filters, tuned to 18 Hz by a bandpass filter (Q = 1.5), summed (n ≥ 256) by a signal-averaging computer (4 msec time constant), displayed on an oscilloscope, and photographed. An automatic, bipolar artifact reject mode in the averager was employed to exclude responses with deviations greater than 10 µV (presumably generated by eye movements) during the analysis interval. Response amplitudes were measured from the trough of the cornea negative a-wave to either the peak of the cornea positive b-wave or the major cornea positive deflection. Implicit times were measured from onset of I_s to either the peak of the b-wave or the major cornea positive deflection. At least three responses to a given test condition were obtained from normal subjects and patients with disease. Responses from a normal subject or a patient varied within ±7% of the mean in amplitude and ±1% of the mean in implicit time for a given test condition on repeat trials.

Three patients with dominant retinitis pigmentosa with reduced penetrance were tested. Patient 1 (P_1) is V-36 in family 631 in the files of the data bank of the Berman-Gund Laboratory for the Study of Retinal Degenerations; Patient 2 (P_2) is VI-92 in family 1562; and Patient 3 (P_3) is VI-90 in family 1562. Family histories of these patients have been described. P_1, a 17-year-old boy, had 20/20 visual acuity, visual fields constricted to the 30° to 40° isopter with the 1-4 white test light in the Goldmann perimeter, and slight attenuation of the retinal vessels with a granular appearance to the midperiphery. He showed no bone spicule pigmentation. P_2, a 15-year-old boy, had 20/30 visual acuity, visual fields constricted to the 30° isopter with the I-4 white test light in the Goldmann perimeter, and slight attenuation of the retinal vessels with a granular appearance to the midperiphery. He showed no bone spicule pigmentation. P_3, a 20-year-old woman, had 20/25 visual acuity, visual fields constricted to the 15° isopter with the I-4 white test light, and full visual fields with the V-4 white test light.
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Results

Fig. 2 illustrates that cone ERGs elicited from the green and red cone systems in combination by a spot and annuli in normal subjects showed a sharp decline in amplitude per square degree of retinal area stimulated for increasing eccentricities from the fovea.

Osterberg's plot of cone density vs. eccentricity from the fovea corresponds closely with these data; this comparison substantiates that ERGs obtained under these test conditions are, in fact, focal cone ERGs from localized regions of the retina.

Fig. 3 shows focal ERG responses generated by the green and red cone systems in combination from the fovea (left column) and midperiphery (right column) for a representative normal subject and three patients with dominant retinitis pigmentosa with reduced penetrance. Response amplitudes from these patients were normal in the fovea (normal range, 0.21 to 0.81 μV) and reduced from 20% to 50% below normal in the midperiphery.

Fig. 4. Focal ERGs from the green and red cone systems in combination for a normal subject in response to an annulus concentric with the fovea presented in rod silent substitution. First row (reference normal tracings): 38° to 44° diameter annulus with Is at 556 nm (1260 photopic trolands) and Ib at 548 nm (830 photopic trolands); second row: annulus as above with 0.3 ND (left) and 0.7 ND (right) before Is and Ib; third row: Is and Ib same as first row with 38° to 43° diameter annulus (left) and 38° to 42° diameter annulus (right); fourth row: same annulus as first row with Is at 556 nm (54 photopic trolands) and Ib at 500 nm (3.8 photopic trolands) (left) or with Is at 556 nm (17 photopic trolands) and Ib at 500 nm (1.2 photopic trolands) (right). Vertical lines from b-wave peaks of reference traces have been extended through lower traces. See legend to Fig. 3 for calibration markers.
periphery (normal range, 1.0 to 1.7 μV). Response b-wave implicit times were normal in the fovea and were delayed 7 to 14 msec in the midperiphery (for normal ranges see Fig. 3, vertical bars). When test spots were used so that amplitudes in the fovea were matched to those in the midperiphery in a given patient (i.e., by increasing the diameter of the central spot), all three patients showed slower responses in the midperiphery than in the fovea; this was not observed in our normal subjects.

An effort was made to simulate in normal subjects the ERGs recorded from the midperiphery of these patients. Neutral density (ND) filters interposed between the test annulus and the eye (simulation of decreased amount of visual pigment) resulted in a delay in b-wave implicit time of about 2 msec for a 25% reduction in amplitude and 4 msec for a 50% reduction in amplitude. Reduction in the area of the test annulus (simulation of decreased number of photoreceptors) resulted in a delay in implicit time of about 1 msec for a 25% reduction in amplitude and 2 msec for a 50% reduction. In both instances, the delays in implicit time for these amplitude reductions were considerably less than those delays observed for the same amplitude reductions in the patients with retinitis pigmentosa. Disproportionate reduction in the brightness of I_s relative to the brightness of I_b for the green and red cones while still maintaining rod silent substitution could not be done by shortening the wavelength of I_b, due to the fact that the wavelength of I_b (i.e., 500 nm) corresponded with the peak of the scotopic luminosity function.

Studies were also done in normal subjects with I_s unchanged in wavelength (i.e., 556 nm) and I_b changed from 500 nm to 548 nm, while still maintaining rod silent substitution. A normal response from the midperiphery under these conditions is illustrated in Fig. 4, top row, and was used as reference. When the brightnesses of I_s and I_b were proportionately diminished (Fig. 4, second row) or the area of I_s and I_b was decreased (Fig. 4, third row), reductions in amplitude with minimal if any delays in cone b-wave implicit time were observed. When the brightness of I_b was reduced more than the brightness of I_s for the green and red cones in combination by shortening the wavelength of I_b to 500 nm and interposing neutral density filters between the test annulus and the eye (while still maintaining rod silent substitution), an 11 msec delay in implicit time for a 25% reduction in amplitude (bottom left trace) and a 20 msec delay in implicit time for a 50% reduction in amplitude (bottom right trace) were recorded.

Discussion

This study shows that regional differences exist in the temporal aspects of the cone ERG in young patients with dominant retinitis pigmentosa with reduced penetrance. Under these test conditions, focal ERGs generated by the green and red cone systems in combination were normal in b-wave implicit time in the fovea but markedly delayed in implicit time in the midperiphery. Implicit times were slower in the midperiphery than in the fovea for a given patient, even when responses in the fovea and midperiphery were matched in amplitude (i.e., comparable numbers of cones were stimulated in these respective areas). These findings support the idea that the abnormal full-field cone ERGs seen in the early stages of dominant retinitis pigmentosa with reduced penetrance are due to an abnormal extrafoveal cone contribution to the response.

An effort was made to simulate in normal subjects midperipheral cone ERGs that were slightly reduced in amplitude and very delayed in b-wave implicit time. Slight reductions with marked delays were not observed in normals when ND filters were placed between the test annulus and the eye or when the area of the test annulus was reduced. These findings suggest that reduced visual pigment in the photoreceptors or reduced numbers of photoreceptors cannot, by themselves, be responsible for these abnormal focal cone ERGs.

A previous study of the full-field cone ERG in a young patient with dominant stationary night blindness (of the Nougaret type) with a
normal cone ERG but no detectable rod ERG showed that, for a given stimulus intensity, the dark-adapted cone ERG response was slower in b-wave implicit time than the cone ERG response obtained in the presence of background adaptation, even though response amplitudes were comparable under these two conditions. Decreases in stimulus intensity resulted in slower and smaller cone ERGs. Neither a decrease in background adaptation nor a decrease in stimulus intensity alone led to full-field cone ERGs in the Nougaret nyctalope that simulated those ERGs seen in patients with dominant retinitis pigmentosa with reduced penetrance. These findings raised the possibility that some combination of decreased background adaptation with decreased stimulus intensity could result in a marked delay in cone ERG b-wave implicit time with only a slight reduction in cone ERG amplitude.

The method of rod silent substitution permitted separation of green and red cone function in combination in normal subjects under mesopic conditions without a rod contribution to the response. When the brightness of the background (I_b) was lowered disproportionately relative to the brightness of the stimulus increment (I_s) while still maintaining rod silent substitution, slight reductions in cone ERG amplitudes with marked delays in cone ERG b-wave implicit times were recorded from the midperiphery of normal subjects. These delays in implicit time for given reductions in amplitude (i.e., Fig. 4, bottom traces compared with top traces) were comparable to or even exceeded those delays seen in the abnormal ERGs of young patients with dominant retinitis pigmentosa with reduced penetrance (i.e., Fig. 3, right column, lower 3 traces compared with top trace). These findings support the idea that abnormal cone ERGs in the midperiphery in the early stages of dominant retinitis pigmentosa with reduced penetrance are due in part to a lower-than-normal level of adaptation of the cone system.

REFERENCES