Evaluating Macular Function Using the Focal ERG

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A stimulus consisting of 96 red LEDs mounted in the rear of a ganzfeld bowl was used to elicit focal electroretinograms (FERG) from the central 9° of the retina in human subjects. The luminance of the stimulus was driven sinusoidally at frequencies from 10–60 Hz. The temporal responsiveness and response phase lags of normal subjects and patients with retinal disease were measured. Normal subjects produced maximum amplitude FERG responses to stimuli between 30–40 Hz. Patients with retinitis pigmentosa showed a low-pass pattern of amplitude loss, with an additional frequency independent loss in sensitivity in those with poorer visual acuity. Patients with macular degeneration showed general amplitude loss associated with a relative sparing of the mid-temporal frequencies. The response phase lags in both patient groups were not significantly different from the normals. These findings point to a loss in temporal responsiveness accompanied by a secondary loss of sensitivity in these heredoretinal degenerations.


Procedures incorporating temporal variation of a light stimulus have been effective in providing reliable data on the visual system. A wide variety of cone system attributes (e.g., color discrimination, fine spatial frequency resolution, and fast flicker detection) have been assessed using psychophysical procedures. However, in evaluating the visual loss associated with diseases of the retina, the clinician is at a disadvantage when only subjective data are available. Subjective results relate to the final cortical processing of visual information, and similar psychophysically determined losses may be seen regardless of the location of the lesion site in the visual pathway. Likewise, other clinical procedures, such as ophthalmoscopy and fundus fluorescein photography, may reveal abnormalities, but do not provide direct data about the functional status of the visual system, nor do they aid in defining the physiologic nature of retinal disease processes.

Recording electrical activity derived from the eye provides a more direct measure of the functional status of the retinal elements. This electroretinographic (ERG) potential may assist in localizing retinal dysfunction, since the components which make up its characteristic waveform have been associated with specific retinal layers.1,2 However, the ERG response, as it is usually employed for clinical purposes, is a mass response evoked by a diffuse light flash, and reflects activity from large areas of retina and from mixed receptor types. As such, it is not a sensitive indicator in patients with small retinal lesions, and conversely, yields little information when large lesions are present.

In order to record ERG responses from localized retinal areas, several stimulus modifications must be made. First, a local (luminance) stimulus must be surrounded with a large illuminated background3,4 in order to prevent stray light scattered from the stimulus from exciting neighboring retinal areas. Using such a stimulus configuration, researchers have been able to map ERG amplitude as a function of retinal eccentricity and to show amplitude and phase changes associated with retinal diseases.5,6,7,8,9 The stimuli used, however, have been, for the most part, limited to brief flashes at low temporal frequencies, or to square pulses at only one or two temporal rates.

The stimulator we employed in the current work incorporates stimulus versatility similar to that used to psychophysically evaluate temporal modulation sensitivity; e.g., control of the time-varying waveform characteristics, temporal frequency, modulation depth, and mean luminance value. Using this device, we have found it possible to sample electrical events at the retinal level and perform direct comparisons with previous subjective estimates of temporal modulation thresholds. In the present study, we report our findings with normal subjects as well as the results obtained from patients with heredoretinal degenerations.

Materials and Methods

Normals

Nine subjects (eight males and one female) ranging in age from 9–35 yr, provided normative data. None had a history of ophthalmic or neurologic disease.
Patients With Retinal Disease

Nineteen patients with retinitis pigmentosa (RP) (11 males and 8 females) ranging in age from 24–51 yr and with Snellen acuities between 20/20 (6/6) and 20/60 (6/18) gave informed consent after the procedure was explained fully. Nine of the RP patients were categorized as simplex (sporadic) form of RP; four had family histories suggesting a dominant mode of transmission, and six were categorized as the autosomal recessive type. All of the patients had preserved central fields subtending at least 10° measured with a III-4 white test light in a Goldmann perimeter. Of the 19 PR patients tested, 16 had absent and 3 showed severely reduced (257, 214, and 326 μV; normal range = 411 ± 75 μV) dark adapted ERG responses to a S16 white flash from a Grass PS22 photic stimulator.

Nine eyes of eight patients (all males) with macular degeneration were also tested. They ranged in age from 14–52 yr and had visual acuities ranging between 20/30 (6/12) and 20/200 (6/60). Seven of these patients had Stargardt’s Disease and one showed an early stage of Best’s Disease.

Apparatus

The stimulus consisted of a tightly clustered array of 96 red LEDs (max λ = 630 nm) mounted in the rear of a ganzfeld bowl. A ground glass placed in front of the LEDs diffused the light so that it appeared as a uniform circle of red light subtending 9° at the eye. The LEDs were sinusoidally driven by a waveform generator (Interstate F44). Depth of modulation, temporal frequency, and average brightness could be independently varied.

Electrical Recording

Focal ERG (FERG) responses were monocularly recorded using a gold foil electrode referenced to the ipsilateral ear. The contralateral ear served as the ground. The electrical signal from the eye was amplified by a Grass P511J preamplifier (gain = 10K) using band-pass filter settings appropriate to the particular temporal frequency used. The amplified signal was summed (N = 128) on a Nicolet 1170B signal averager. Traces containing spurious potentials from eyeblinks or eye movements were rejected from the average by the artifact rejection circuitry. The averaged response was then plotted on a X-Y plotter and the amplitude measured as trough to peak deflection.

To determine the response phase shift, the driving current of the LEDs was passed through the same amplification, filtering, and averaging system used to process the ERGs. A template which accounted for electronic delays was obtained at each temporal frequency and was used to directly measure response phase lag. The stimulus-response phase differences associated with temporal frequencies from 10–60 Hz were calculated using the relationship: phase = (response lag/stimulus period) × 360°.

Procedure

After pupil dilation with 1% mydriacyl and patching the non-test eye, the subject placed his head in a ganzfeld bowl and fixated the center of the LED array. The stimulus was sinusoidally modulated at temporal frequencies ranging from 10–60 Hz around a mean luminance of 50 cd/m² and had a maximum modulation depth of 100% (i.e., 0 to 100 cd/m²). The surround luminance was maintained at 25 cd/m². This level was shown in preliminary testing to reduce activation of the peripheral retina by scattered light from the stimulus. To quantify the effect of eccentric fixation which may occur in patients with poor acuity, subjects were asked to fixate at different points of fixation on either side of the stimulus. In a second series of experiments, stimulus modulation depth was varied from 100–20% at a single temporal frequency (30 Hz).

Results

The following procedure was used to examine whether the background illumination of our stimulus was sufficient to eliminate most of the effects of scattered light. The subject’s fixation was directed to a point which located the stimulus on the optic nerve head. In a now classic experiment, Asher12 showed that stimulating the optic nerve head in the absence of a background illumination produces an ERG response as large as direct stimulation of the retina, whereas, with a background, the response was greatly reduced. In our experiments, centering the 9° LED stimulus on the optic head resulted in a 60% decrease in amplitude (in six normal subjects) compared to foveal fixation. The residual response is most likely due to the larger size of the stimulus (9°) relative to the disc area and/or any residual effects of scattered light. When the background illumination of the LED display was turned off, the recorded response grew in amplitude under both central and optic disc stimulation (Fig. 1). We conclude from this experiment that, although our stimulus may not be exclusively focal, it does primarily reflect the activity of the cones from the central 9° of the retina.

Additional evidence for this claim was provided by recording responses at eccentric fixations. Localizing the stimulus 10° temporal or 10° nasal to the fovea (with the background illuminated) resulted in an average (N = 6) amplitude decrease of 42% and 46% respectively when compared to central fixation. The responses recorded at 20° and 30° temporal to the fovea...
were decreased by 54% and 59% respectively. These data are comparable to previous reports of focal ERG changes with eccentricity. The phase of the FERG response advanced an average of 33° when the stimulus was shifted from the fovea to a position 10° temporal to the retina.

Results For Normals

Figure 2 shows focal ERG waveforms generated by a normal eye in response to sinusoidally modulated light at various temporal frequencies. At frequencies of 20 Hz or higher, the response waveform was sinusoidal at the frequency of stimulation. At the lower frequencies, waveform nonlinearities occurred. Note that the response to a 10 Hz stimulation occurs with a large 2f harmonic. Some subjects showed only a small 2f ripple riding on the 10 Hz fundamental, whereas others showed a dominant 2f harmonic.

The relationship between the mean FERG amplitude (bar length = ±1 Standard Error of the Mean) and temporal frequency (at 100% modulation) is shown in Figure 3A. Amplitude slowly increases with temporal frequency, reaching a maximum voltage at 40 Hz. At temporal frequencies above 40 Hz, response amplitude decreased markedly. The response phase vs temporal frequency function was linear for the averaged data with a slope of 8.98 ± 1.85 degrees/Hz (Fig. 3B).

The relationship between modulation depth (at 30 Hz) and FERG amplitude for normals is shown as the dashed line in Figure 4. This function is linear with a slope of 0.097 μV/% modulation for the averaged data.
from the normals. It was possible to record a measurable FERG response for all normal subjects at a modulation depth of 20%.

An analysis of the reliability of the FERG amplitude measures was also conducted. Each subject was tested twice at 30 Hz, once near the beginning of the session, and again near the end. The average variation in amplitude between the two samples was 3.1% for the nine normals; the mean amplitude of the early and late tests were not significantly different ($t = 0.38$, $P = 0.70$).

**Results For Patients With Retinitis Pigmentosa (RP)**

FERG amplitude reliability was also tested for RP patients by comparing responses to identical stimulus conditions presented in the beginning and near the end of a test session. There was an average difference of 1.1% between the two runs and these means were also not significantly different ($t = 0.58$, $P = 0.56$).

There was a general relationship between decreased visual acuity and reduced FERG amplitude as a function of temporal frequency for all RP patients. The percentage loss of FERG amplitude from the normal mean amplitude is plotted in Figure 5A. Zero loss represents normal and patient responses of equivalent amplitude, whereas 100% loss represents an absent patient FERG response. If amplitude losses were frequency independent, the resulting plot of the patient data would be a horizontal line occurring 0% amplitude loss; this is not the case for the RP patients tested. Patients with lower visual acuity showed greater amplitude losses at progressively lower temporal frequencies.

In Figure 5B, the mean phase lag for the RP patients is plotted as a function of temporal frequency. The normal mean phase lag at each frequency is indicated by the dashed line. The average phase lag for RP patients grouped by visual acuity group is represented by the symbols. The slopes of the FERG response phase lag vs temporal frequency in individual RP patients ranged between 5.6 and 15.9°/Hz. The average slope for all RP patients (10.2°/Hz) was not statistically different from the normal mean slope ($t = 1.39$, $P = ns$). However, it must be kept in mind that using a stimulus modulation depth of less than 100% might have produced different results (Fig. 4).

The FERG amplitude loss vs temporal frequency results from the RP patients were also partitioned with respect to the genetic mode of transmission. No statis-
tical difference was found among the amplitudes of various modes of transmission (ANOVA, F = 1.39, df = 2/15, P = ns). However, the patients with autosomal dominant RP showed a more variable pattern of amplitude loss as a function of temporal frequency. For example, no patient in this group had a recordable response to 60 Hz, although two patients in the group had acuities of 20/30 (6/12) or better.

Figure 4 illustrates the importance of varying modulation depth when using the focal ERG for studying cone activity in patients with RP. When 100% stimulus modulation was used, those patients with acuities ranging from 20/20 (6/6) to 20/30 (6/12) exhibited normal FERG amplitudes. Only when the modulation depth was reduced were the FERG amplitudes of those patients with good acuity abnormal. With each stepwise reduction in stimulus modulation depth, fewer RP patients achieved normal amplitude responses. Be-

results for patients with macular disease were also partitioned according to visual acuity and the percentage amplitude loss relative to the normal mean plotted for each group as a function of temporal frequency (Fig. 6A). The pattern of amplitude loss for the patients with macular degeneration is quite different from that observed in the RP patients (Fig. 6A). Amplitude losses occurred at both low and high frequencies, with relatively less loss at the intermediate (30 and 40 Hz) frequencies. This band-pass pattern of loss is maintained in patients with decreased visual acuity with an additional, frequency-independent, loss in amplitude observed. However, the patient with Best's disease (Figure
Discussion

Electrophysiologic assessment of retinal temporal sensitivity was obtained in patients where the standard, large-field, flash ERG added little information concerning the functional status of the diseased retina. The importance of a clinically applied focal ERG can be seen in the results of the current study. There was a clear separation of the patterns of frequency-specific amplitude loss between patients with retinitis pigmentosa and those with macular degeneration. Retinal patients showed a low-pass pattern of amplitude loss with increasingly lower temporal frequencies affected in patients with decreased visual acuity. Macular degeneration patients, on the other hand, showed a characteristic band-pass pattern of amplitude loss, with a frequency-independent loss overlaying this pattern in patients with lower visual acuity. These data suggest an increasing deficit in temporal processing as the disease progresses. However, this has not been a prospective study, and the course of temporal sensitivity changes in individual patients must be followed to test this hypothesis.

Is it possible that the different patterns of FERG determined temporal sensitivity loss in these patients with retinal degenerations might relate to different underlying disease process? Retinal diseases might affect receptor physiology at least three fundamental ways. First, there could be a decrease in the quantum catching ability through a loss of visual pigment, a loss of receptors, and/or a misalignment of the receptors. Previous reports have identified such abnormalities in the rods and peripheral cones of advanced RP patients. Such deficits would be expected to result in a frequency-independent amplitude loss; i.e., a simple horizontal lowering below the normal in the plots of Figures 5A and 6A. We have tested this assumption in normals by reducing the mean luminance of our stimulus and repeating FERG measures. FERG amplitude exhibited a general frequency-independent drop as luminance was decreased, but the exact pattern exhibited depended upon the luminances which were compared. Lowering the mean luminance from 187 to 93 cd/m² resulted in a sparing of the response at 10 Hz and an amplitude loss at the higher frequencies (from 20–60 Hz) which was equal at approximately 40%. On the other hand, comparing the data obtained from a 187 cd/m² condition to those from a 26 cd/m² mean luminance resulted in a small percentage loss for the low frequencies (29%), with increasing relative amplitude loss up to 30 Hz (80% loss). Above 30 Hz, the percentage amplitude loss again decreased to 40% at 50 Hz. Neither of these experimentally produced patterns of amplitude loss in normals is similar to those observed in the patient data.

Although a general frequency-independent amplitude loss can be seen in the worst acuity groups of the present data, this is not the primary deficit observed in the central cones in either group of patients. The observed sensitivity attenuation is probably related to a greater receptor loss as the diseases progress.

Second, there could be a delay in the response to visual stimuli expressed as a latency change. Such delays alone would be expected to produce only a change in the observed phase lag and not a change in response amplitude. Although we found a trend towards increased phase lag at the higher temporal frequencies in advanced macular degeneration, there was no significant difference in the slope of phase lag vs temporal frequency between the mean of either of the disease populations and the mean of the normals.

In the past, conflicting data have been presented concerning ERG response latencies in RP patients. Biersdorf used a small stimulus to elicit the focal luminance ERG and studied the amplitude and latency changes associated with macular and generalized retinal degeneration (RP). He found, as we have, that focal ERG amplitudes in both groups of patients were generally reduced. Biersdorf also found that 47% of the eyes with Stargardt’s and 31% with RP showed abnormal time-to-peak latencies. Sandberg et al. using a hand-held ophthalmoscope-stimulator to elicit focal ERGs, reported normal central cone latencies in patients with RP. The RP patient population which we examined also showed normal phase lag vs frequency slopes over a range of temporal frequencies. A major methodological difference among these three studies is the time between peak stimulus luminances (i.e., dark interval). The flash used by Biersdorf was 10 μsec in duration, with about 300 msec of dark interval. In comparison, Sandberg’s 12 msec flash had only about...
12 msec of dark interval (at 42 Hz), and our sinusoidal stimulus had virtually no dark interval. It is possible that further study of this variable may show more directly the extent to which development of the membrane potential, and hence the time-to-peak of the b-wave, may depend upon the inter-flash interval (e.g., the time-averaged level of light adaptation).

Third, there may be disease-induced changes in properties which affect temporal integration or refractory time constants. Such changes would selectively affect the higher temporal frequencies where shorter duration stimuli occur at a rapid repetition rate. In both of our disease groups, those patients with good acuity initially showed an amplitude loss at the higher temporal frequencies. In addition, RP patients with decreased visual acuity showed a deficit at increasingly lower frequencies.

Similar patterns (both low and high frequency sensitivity loss in RP patients) have been presented using psychophysical procedures.17,18 Tyler et al,19 however, have recently presented psychophysical data which show no low frequency loss in conjunction with high frequency losses similar to those we have observed. These authors proposed that this pattern of high frequency amplitude loss is due to a slowing of visual processes with a secondary decrease in the signal-to-noise ratio. The losses observed at the low temporal frequencies in both of our patient groups are problematic. It is difficult to propose a mechanism which would specifically affect low temporal frequency response amplitude. However, our findings of high frequency loss support the hypothesis of a primary deficit in temporal integration, but not latency per se, with a secondary loss in the stimulus efficiency. We must emphasize that these amplitude changes in RP are not associated with phase lag slope abnormalities over the range of frequencies tested.

In the present study, FERG modulation thresholds were obtained at a temporal frequency of 30 Hz (Fig. 4). This particular frequency was chosen because it produced large-amplitude responses and had no observable harmonic distortion in the response waveform. It was evident from the RP patients tested that, as the stimulus modulation depth decreased, the focal ERG amplitude rapidly departed from the normal value. The decrements were particularly evident in patients whose response amplitude at 100% modulation was normal. The precipitous drop in amplitude as the modulation depth decreased indicates that some aspect of cone contrast sensitivity is impaired. This finding emphasizes the need to test a number of modulation depths, rather than using a common slope based upon normal data to extrapolate to a threshold value. Studies which infer contrast thresholds based on a normal slope calculated from high contrast responses may be missing abnormalities.20 The best assay of temporal contrast sensitivity would be a complete DeLange21 function estimated using the focal ERG. We are presently collecting data on retinal temporal modulation sensitivities, while, at the same time, exploring a method for fast collection of these data using real-time response retrieval.22

In conclusion, a number of techniques for recording focal ERG responses have been presented in the literature. We have chosen an LED display for its stimulus versatility and applicability to a clinical environment. However, like many of the similar techniques described in the literature, our stimulator is limited by the examiner's inability to monitor fixation during data collection. Two methods have been recently presented which allow direct observation of a stimulus on the fundus. Sandberg et al23 have successfully employed an ophthalmoscope stimulator to present 4° targets to the retina of various patient groups. Another approach uses a laser to directly stimulate the retina. This technique has been used for perimetric studies.24,25 Although both of the latter techniques allows observation of the stimulus on the retina, neither has as yet been reported to have been modified to allow for sinusoidal modulation of the light, changing temporal frequency, or varying modulation depth of the stimulus. An ideal stimulator would incorporate both stimulus visualization and stimulus versatility.

The current data emphasize the need to examine temporal sensitivity over a range of frequencies and modulation depths. This seems especially important for early stage progressive retinal diseases, which may present with only minimal visual complaints.

Key words: focal electroretinogram, temporal responsiveness, heredoretinal degeneration

References