Mapping of Retinal Function in Diabetic Retinopathy Using the Multifocal Electroretinogram

Anja M. Palmowski,* Erich E. Sutter,* Marcus A. Bearse, Jr.,* and Wayne Fung†

**Purpose.** To investigate focal abnormalities in the electroretinogram (ERG) signal in diabetic patients, with and without retinopathy, using a multifocal ERG.

**Methods.** Sixteen patients with diabetes mellitus, 8 of whom had diabetic retinopathy (mean duration of diabetes: 18.5 years) and 19 approximately age-matched healthy volunteers underwent multifocal ERG testing. One hundred three retinal locations within the central 50° were stimulated concurrently, according to a pseudorandom m-sequence. Response components were extracted for each stimulated retinal location.

**Results.** In diabetic patients with retinopathy, the overall amplitudes were reduced ($P < 0.01$), and peak implicit times were increased ($P < 0.01$) in the first-order component (mean flash response) and in the first slice of the second-order component (local two flash interaction). In addition, local reductions of amplitude could be seen in the first- and second-order components. In patients without retinopathy, only amplitudes of the second-order component were reduced ($P < 0.01$). Another salient difference was observed in a special feature of the second-order component that was reduced in diabetic patients, with and without retinopathy ($P < 0.05$).

**Conclusions.** Second-order components depend on nonlinear dynamics. Thus our findings indicate changes in the nonlinear dynamics of a fast-gain control in diabetic patients, presumably located in the inner retina. This suggests that early functional changes of the inner retina are evident in diabetic patients before impairment of the outer retina is observed. Multifocal nonlinear analysis permits the detection of subclinical diabetic retinopathy and offers the advantage of topographic mapping of retinal dysfunction. Invest Ophthalmol Vis Sci. 1997; 38:2586-2596.

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Diabetic retinopathy is a major cause of visual disability among working-aged people in industrialized countries. At present, prevention and treatment consist mainly of constant blood sugar control and timely retinal laser photocoagulation. The mechanism of action of photocoagulation is not entirely clear yet, but it is believed that destroying ischemic retinal areas decreases the production of angiogenic factors and thereby reduces neovascularization and its complications. However when avascular retinal areas occur, they signify that a “point of no return” has already been reached in the course of diabetic retinopathy.

Alternative treatment options aiming at an earlier, less destructive therapy of diabetic retinopathy are therefore needed. Results of current research, using animal models, aimed at the reduction of basement membrane thickening and the prevention of early vascular changes of retinopathy, seem promising. In humans, early treatment of diabetic retinopathy with sorbinil has been beneficial: Patients with less retinopathy at the beginning of the study showed improvement in their foveal ERG measurements after 1 year of treatment with sorbinil. Patients in whom foveal ERGs did not show improvement had more advanced retinopathy at the beginning of the study.

To know when to initiate treatment of diabetic retinopathy and monitor its effects, new tests are needed to detect subclinical retinal changes. The con-
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FIGURE 1. The stimulus array that was presented on a 32- × 22-cm computer screen consisted of 103 hexagons, independently alternating between black and white. It covered the central 50° of the retina. A small fixation cross is presented in the center.

Conventional ERG can be affected in early diabetes mellitus. However, small areas of retinal dysfunction may go undetected in the recording of the overall retinal response, because when large stimuli are used, small abnormalities will not affect responses evoked from a retina that is predominantly normal.

The recently developed Visual Evoked Response Imaging System (EDI, San Mateo, CA) enables simultaneous ERG testing of multiple small retinal areas and allows a fast, objective evaluation of retinal function, using a multifocal technique. The Visual Evoked Response Imaging System has been shown to detect small areas of retinal dysfunction. The purpose of this study was to explore the efficacy of the multifocal ERG in detecting and localizing dysfunctional retinal areas in diabetes.

METHODS

We studied 16 patients with diabetes mellitus (5 women, 11 men; ages 32 to 74 years; mean age, 53 years; duration of diabetes, 0.5 to 45 years; mean duration, 18.5 years) and 19 healthy volunteers (13 women, 6 men; ages 29 to 60 years; mean age, 40 years). Of the diabetic patients, 15 had insulin-dependent and 1 non–insulin-dependent diabetes mellitus (duration of disease, 0.5 years). The tenets of the Declaration of Helsinki were followed. On giving their informed consent, all subjects underwent ERG testing, as described later. Thereafter, an ophthalmic examination with a slit lamp and ophthalmoscope was performed on all patients. Eyes with clouding of the media or previous surgery were excluded from the analysis.

The visual stimulus (Fig. 1) consisted of 103 hexagons displayed on a monochrome monitor driven at a rate of 75 frames/second (interframe interval 13.33 msec). A grounded cone was attached to the front of the monitor to eliminate artifacts caused by electromagnetic radiation from the cathode ray tube. The stimulus array was presented on a 32-cm × 22-cm screen and stimulated the central 50° of the retina. Hexagon size was scaled with eccentricity to evoke focal responses of approximately the same amplitude per stimulus element in the response arrays of normal subjects.

The hexagons flickered concurrently between black and white, according to a complete cycle of a binary m-sequence. A different starting point within the same m-sequence cycle rendered the individual hexagonal elements uncorrelated.

In most experiments, we used a binary m-sequence of 2 steps. Here, a step in the m-sequence corresponded to one video frame. Within this m-sequence, all possible temporal black-and-white combinations of 15 stimulus frames take place once. Black-and-white stimulus frames occur the same number of times, regardless of the sequence length. At any time, approximately 50% of the stimulus elements displayed are white and 50% are black.

In this study, the luminance was 200 cd/m² in white hexagons and 1 cd/m² or less in black hexagons. This resulted in a contrast of ~99%. Luminance of the surrounding screen area and of the fixation cross was 100 cd/m².

Pupils were maximally dilated with tropicamide and phenylephrine hydrochloride. A ground electrode was attached to the center of the forehead. Retinal activity was recorded monocularly with a Burian–Allen bipolar contact lens electrode, which was inserted after the cornea was anesthetized with proparacaine hydrochloride. With the electrode in place, subjects were refracted for best visual acuity at the stimulus viewing distance. The resulting acuity was 20/50 or better in all patients and control subjects. The viewing distance was adjusted to compensate for changes in the retinal image size caused by the large corrective lenses that were placed 3 cm from the cornea. The contralateral eye was occluded with light pressure to help suppress blinking.

Retinal signals were bandpass filtered (10 to 300 Hz), amplified (gain = 100,000; amplifier: Grass Neurodata Acquisition System, model 12; Quincy, MA), and recorded at 16 samples per display frame (sampling interval 0.83 msec). Two recordings were obtained from each eye per stimulus condition. Each record consisted of responses to a full 8-minute cycle of binary m-sequence stimulation. For subject comfort, each record was collected in 16 segments, each ~30 seconds long. Between segments, subjects were allowed to rest a few seconds. The first 1000 msec of
FOCAL amplitudes

A. 1st order
B. 2nd order, first slice
C. 2nd order, second slice

possible combinations of successive stimuli

A bright stimulus
black stimulus
50% black stimulus
50% white stimulus

mean amplitude per flash
resulting response amplitude

FIGURE 2. Computation of response components: (A) first-order, (B) first slice of the second order, and (C) second slice of the second order. The left column represents the focal stimuli that generate a response component (right column). The schematic representation of the response amplitudes is simplified, in that the response amplitude generated by a black frame is represented by 0.

each m-sequence segment duplicated the last portion of the segment that preceded in the m-sequence cycle. The first half of this overlapping data was discarded to avoid onset transients. For data processing, the second half of the overlapping segment was joined with tapered splices to avoid discontinuities.\(^8\)\(^14\) The quality of the recording was controlled by monitoring the raw signal. Contaminated segments were discarded and rerecorded. The results of two 8-minute recordings were averaged to improve the signal-to-noise ratio. An artifact elimination technique described by Sutter and Tran was applied once.\(^8\) With m-sequence stimulation, local first and higher order response components can be derived by means of a single cross-correlation between the m-sequence and the response.\(^12\)\(^13\)\(^15\)

We examined the first- and second-order components of the ERG. For each stimulus area, the first-order component is the difference between the mean response to all white frames in the sequence and the mean response to all black frames (Fig. 2A). It can be described as the mean local response to all the flashes occurring in a stimulus cycle.

The second-order response component represents the temporal interaction between two focal flashes (white frames) separated by an integral number of stimulus base intervals (steps in the m-sequence). For example, the first slice of the second-order component represents the interaction between two consecutive focal flashes (white frames one base interval apart), whereas the second slice of the second order represents the interaction between two focal flashes two stimulus base intervals apart. The second-order response component is computed as shown in Figures 2B and 2C. For each stimulus element, the first slice of the second-order component is calculated as the difference between the average response after two successive m-sequence steps of the same luminance and those after transitions in luminance, regardless of the direction. The second slice of the second-order response component is computed in the same way, but in this case, the two m-sequence steps are two stimulus base intervals apart (Fig. 2C). For statistical analysis, a two-tailed Student's \(t\)-test was applied.

RESULTS

On ophthalmoscopic examination, eight diabetic patients showed no clinical signs of retinopathy, whereas the remaining eight had nonproliferative diabetic retinopathy (NPDR). We included 33 eyes of control subjects, 14 eyes of diabetic patients without retinopathy, and 14 eyes of diabetic patients with NPDR in our analysis. Only 1 eye was included in subjects who did not agree to testing of both eyes (\(n = 5\), control group), or in subjects in whom the other eye had to be excluded because of clouding of the media (\(n = 2\)) or because of previous surgery (\(n = 2\)). The \(t\)-test results showed no difference between the results obtained from subjects in whom only one eye was analyzed and those obtained from subjects in whom both eyes were analyzed. Therefore, these results were included in the overall data analysis.

First-Order Component

Figure 3 shows the response arrays of the first-order component (mean focal flash response) of a normal subject (A), a diabetic patient without clinically apparent retinopathy (B), a patient with early NPDR (C), and a patient with late NPDR (D). In the arrays, normal responses are approximately equal in height, because they are not yet divided by the solid angle subtended by the area of the corresponding stimulus element. Marked in gray are the central response and the area of the blind spot. The amplitude density (amplitude per unit of retinal area) of the first-order response component showed a prominent peak in the foveae, where receptor densities are highest. The area...
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FIGURE 3. Response arrays of the first-order component (~flash response) of the left eye of (A) a healthy volunteer, (B) a diabetic patient without clinically apparent retinopathy, (C) a diabetic patient with early, nonproliferative diabetic retinopathy, and (D) a diabetic patient with late nonproliferative diabetic retinopathy. The area of highest response amplitude per stimulated retinal area in the center and the dip in amplitude in the area of the blind spot are marked in gray.

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The mean amplitude was 10.1 ± 2.7 nV per degree squared. In patients with NPDR, amplitudes were reduced (mean = 3.8 ± 2.1 nV per degree squared, \( P < 0.01 \)) and latencies of the first trough (mean = 24.5 ± 1.3 msec) and the following peak (mean 32.4 ± 2.5 msec) were increased (\( P < 0.01 \)). Diabetic patients without retinopathy showed only a significant decrease in amplitude (mean = 7.5 ± 2.7 nV per degree squared, \( P < 0.01 \)). The latencies of the first trough (mean = 22.3 ± 0.5 msec) and the following peak (mean = 28.8 ± 1.7 msec) did not differ from those in the control subjects.

A major difference could be observed in the waveforms of the first slice of the second-order response component as illustrated in Figure 6. Panel A depicts areas of approximately equal eccentricities whose responses were averaged to obtain the six waveforms that are compared between a control subject and two diabetic patients in panel B. These response averages are obtained from the subjects shown in Figures 3 and 5, panels A, B, and C. For better comparison, the waveforms are normalized to have equal root mean square amplitudes. The gray bar highlights a feature at 38.33 to 40 msec (~42 to 45 msec in the central 5°) that was markedly reduced in amplitude in the diabetic patients with NPDR.

There are essentially two ways in which this feature can arise. The first possibility is that it is an integral
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Two main response features. Thus, this experiment demonstrates that the second feature is related to a response in the following interval.

The stimulus base interval is defined as the separation between m-sequence steps. Accordingly, each increase in the base interval produces a corresponding decrease in the number of m-sequence steps possible within a given recording period. This generally results in a reduced signal-to-noise ratio. A base interval of 26.67 msec provided an acceptable signal-to-noise ratio within a recording time of 16 minutes while sufficiently separating the feature. We therefore tested a subgroup of our subjects, namely 9 control subjects and 11 diabetic patients (6 with NPDR), using this base interval. The resulting normalized first slice of the second-order waveforms obtained from the same

part of the response to the second flash shown in Figure 2B. The second possibility is that this feature is a contribution from the flash response in the following base interval. How this contribution might be generated will be explained in the Discussion section. To determine which of the two interpretations is correct, we need only to vary the base interval used in the m-sequence stimulation. If an increase in the base interval results in a similar increase in the latency of this feature, then it must be associated with the response in the following interval.

Figure 7 shows the ring averages of a normal subject for stimulus base intervals of 13, 26, 39, and 53 msec. The brackets at the top of each column depict one base interval. They commence at a peak at ~30 msec that is marked by a dashed line. The region containing the feature is indicated in gray. Increasing the stimulus base interval indeed produced a corresponding increase in the separation between the two

FIGURE 5. Response array of the first slice of the second-order component of (A) a healthy volunteer, (B) a diabetic patient without clinically apparent retinopathy, (C) a diabetic patient with early nonproliferative diabetic retinopathy, and (D) a diabetic patient with late nonproliferative diabetic retinopathy. (Same subjects as in Figure 3).

FIGURE 6. First slice of the second-order response component with a stimulus base interval of ~13.33 msec. (A) Areas of approximately equal eccentricities whose responses, having similar waveforms, were averaged to achieve a better signal-to-noise ratio. (B) Response averages, derived from rings shown in (A) in a control subject, in a diabetic patient without retinopathy, and in a diabetic patient with retinopathy. A feature found in control subjects at a latency of ~40 msec is marked in gray. It was greatly reduced or absent throughout the retina in nonproliferative diabetic retinopathy. The responses were normalized to have equal root mean square amplitudes.
Second order, first slice

Subjects as in Figure 6 is shown in Figure 8. We estimated the amplitude of this feature by calculating the scalar product over the interval of 42 to 66 msec. Each waveform was multiplied point by point with its corresponding template (average of control subjects, normalized to a root mean square of 1). Increasing the base interval to 26.67 msec revealed that the feature (marked in gray) is not only absent or reduced in amplitude in diabetic patients with retinopathy (bottom) but also, albeit to a lesser degree, in patients without retinopathy (middle; $P < 0.05$).

This feature can only exist in the presence of a nonlinear effect that lasts over at least two base periods (see Fig. 9). Such effects were found in all control subjects and in all diabetic patients. This is evident from the presence of a response of the second slice of the second order (Figs. 2C) in all subjects. We thus conclude that the disappearance of this feature is not related to a shortening in the duration of a nonlinear effect, but rather to a change in the nonlinear dynamics (see Discussion).

DISCUSSION

There is good evidence that the first-order response components originate predominantly in the outer retina. Sutter and Tran showed that under photopic conditions, the decrease of the first-order response component with eccentricity follows approximately that of retinal cone density. In addition, when all the focal responses of the multifocal cone ERG are averaged together, the response bears a strong similarity to the full-field flash ERG. Flash ERG responses originate predominantly in the outer 70% of the retina.

The reduced overall amplitudes and the delayed latencies in the first-order response component observed in our diabetic patients with NPDR may thus indicate some impairment of outer retinal function in diabetes.

Although the second-order component of the multifocal ERG also contains outer retinal contributions, it appears to have substantial contributions from sources in the inner retina and also from the optic nerve head. Recently, it was reported that a response originating from nerve fibers near the location of the optic nerve head can be extracted from the second-order component of the multifocal ERG. In addition, these second-order contributions appear to be selectively affected in optic atrophy and in early glaucoma, consistent with their generation by ganglion cell axons. In our study, amplitudes of the second-
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Normal

Diabetic, no clinically apparent retinopathy

Diabetic, NPDR

FIGURE 8. First slice of the second-order response recorded with a stimulus base interval of ~26.67 msec for the same subjects as in Figure 6. The responses were normalized to have equal root mean square amplitudes.

order response component were significantly reduced in diabetic patients with and without retinopathy. This suggests that retinal processing is becoming more linear in diabetes under the rapid-flicker stimulation used in our experiments.

A difference between the waveforms obtained from control subjects and diabetic patients, which was found in a feature of the first slice of the second-order response waveform, supports this hypothesis. This feature was greatly reduced or absent throughout the retinas of patients with and without diabetic retinopathy. By varying the base interval of stimulation, we demonstrated that this feature is related to the response to flashes in the following base interval.

To understand the mechanism responsible for this contribution, we must extend our considerations regarding the computation of Figure 2B to the following base interval. In the following base interval, a flash occurs exactly 50% of the time for each one of the four stimulus configurations shown. In the derivation of the kernel slice, this response is added exactly the same number of times as it is subtracted. The lack of complete cancellation must be caused by effects on the response from preceding flashes.

In Figure 9, we consider the response to this flash (shown in the rightmost frame) for each of the four possible stimulus configurations of Figure 2B. The bar graphs on the far right again symbolize the response amplitude for each stimulus configuration. The gray portion of the bar represents the reduction in amplitude (A1) caused by an adaptive mechanism. We see that there can be no contribution from this flash to the kernel slice if the reductions resulting from the two preceding flashes are additive—that is, if the reductions A1 and A2 add up to the reduction A3 produced by the preceding flash pair. The example of Figure 9 illustrates the present case, in which the reduction A3 caused by the paired flashes is larger than A1 + A2. In this case, the net response produced by the flash in the following base interval has a positive amplitude—that is, it has the same polarity as the preceding second-order waveform (compare Figs. 7 and 8).

We see from Figure 9 that, in principle, the disappearance of this feature could be related to a reduction in the duration of the effect of previous flashes on the response. If the duration of the gain reduction becomes less than two base intervals, then the flashes in the left column of the figure no longer have an effect and the feature disappears. However, this explanation can be ruled out by the presence of a response in the second slice of the second-order kernel, which represents interactions of flashes over two base intervals in control subjects and in diabetic patients.

The reduced amplitudes in the first slice of the second order, the reduction of the feature described above in diabetic patients, and the ultimate disappearance of these components in diabetic retinopathy must therefore be caused by changes in the dynamics of adaptive mechanisms. We hypothesize that the affected adaptive mechanisms, which may include a local decrease in sensitivity, are embodied in neurons of the inner retinal layer.

Our observations are consistent with previous suggestions that early diabetes causes dysfunction within the inner retina before the photoreceptors are affected. In a 3-year follow-up study on diabetic patients with insulin-dependent diabetes mellitus type 1 without angiographic changes of retinopathy, no changes in photoreceptor function were seen as opposed to impairment of the middle...
retinal layers (ganglion cells and preganglion cells). 28

Other ERG studies of patients with diabetes mellitus have yielded inconsistent results. Arden et al 29 reported reduced amplitudes in the pattern ERG of diabetic patients only in the presence of cotton–wool spots and angiographic evidence of capillary nonperfusion. Nesher et al also found reduced amplitudes in the pattern ERG of diabetic patients with retinopathy, 30 whereas others report normal or even supernormal amplitudes in the flash ERG of diabetic patients with NPDR in whom pattern ERG amplitudes were normal. 31 Wanger and Persson could not find any flash ERG or pattern ERG changes that would distinguish between the presence or absence of retinopathy in diabetic patients. 32

Reductions in the amplitudes of oscillatory potentials have also been reported in diabetic retinopathy. 6, 7, 33–36 However, others did not find any such changes. 37 Yet other authors have cautioned that oscillatory potentials have a high intra- and intersubject variability. 29, 37

Oscillatory potentials are best elicited with a conditioning flash followed by a test flash approximately 30 seconds later. 38 It has been shown that it is possible with the multifocal technique to detect oscillatory potentials and to plot their topography, 29 but only when the stimulation rate is significantly reduced. Because slower stimulation rates lead to significantly longer recording times to achieve adequate signal-to-noise ratios, it was not possible to study the topography of oscillatory potentials in our patients.

In conclusion, our results show that the multifocal ERG can detect early impairment of retinal function in patients with diabetes, even when retinopathy is not clinically apparent. Early changes occur in the waveform of the second-order response component and point toward impaired adaptive mechanisms, presumably located in the inner retina. Reduced amplitudes and delayed responses in the first-order component indicative of outer retinal involvement are found in NPDR and in individual patients without diabetic retinopathy. Future research with larger sample sizes will help to determine whether the changes we observe in the second-order multifocal ERG have clinical applications in diabetes.

**Key Words**

diabetes, diabetic retinopathy, electrophysiology, human, multifocal electroretinogram
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References


