Retinal Function in Normal and Diabetic Eyes Mapped with the Slow Flash Multifocal Electroretinogram

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PURPOSE. It has been suggested that late components of the standard multifocal electroretinogram (mfERG) are preferentially affected by diabetes mellitus. The slow-flash (sf)-mfERG stimulates with flashes separated by dark periods, facilitating interpretation of late first-order response components compared with standard multifocal stimulation. Retinal function and response component changes were examined using the sf-mfERG in diabetic subjects with and without diabetic retinopathy.

METHODS. Eighteen control subjects, 12 diabetic patients without retinopathy and 17 diabetic patients with nonproliferative diabetic retinopathy (NPDR), were tested monocularly. A total of 103 areas of the central 45° were stimulated by pseudorandom 100-cd/m² flashes separated by at least 53.3 ms. Major components and the amplitude of the first-order sf-mfERGs were examined. Each subject’s N1, P1, and N2 implicit times (ITs) and scalar product amplitudes (SPs) were measured at all 103 retinal locations and converted into z-scores based on the control values. Abnormalities were defined as z-scores greater than 2.33 (P < 0.01).

RESULTS. Local functional abnormalities were found in both the diabetic patients with NPDR and in those without retinopathy. In both groups of diabetic patients, most abnormalities occurred more frequently in the inferior retina. Later components (P1 and N2) of the local sf-mfERGs were not preferentially affected by diabetes. The local SP and P1 IT measures distinguished the subject groups better than N1 IT and N2 IT.

CONCLUSIONS. Local functional retinal abnormalities in diabetic persons with or without NPDR can be detected and mapped by the sf-mfERG. Diabetes and NPDR do not, however, preferentially affect the late P1 and N2 response components. (Invest Ophthalmol Vis Sci. 2004;45:296–304) DOI:10.1167/iovs.03-0424

Eye complications resulting from diabetes mellitus are the major cause of irreversible loss of sight in working-age people in the United States.1 Damage to the retina (retinopathy) from microvascular anomalies is primarily responsible for this. Ideally, an objective test would detect retinal dysfunction before the appearance of diabetic retinopathy, thereby identifying individuals and retinal locations at risk for development of retinopathy. Such a test could also be used by researchers and clinicians to evaluate the efficacy of candidate therapies for the prevention or treatment of diabetic retinopathy.2–4

The multifocal electroretinogram (mfERG) is an objective technique for mapping retinal function.3–12 mfERG studies that have analyzed responses averaged over retinal areas or summed over the entire stimulated field have shown reduced amplitudes and/or response delays in diabetes.14–18 Localized mfERG abnormalities have also been reported in diabetic patients with retinopathy, both in retinal regions corresponding to diabetic retinopathy and areas without it.19–24 In addition, localized mfERG delays occur in some diabetic eyes that do not have evidence of retinopathy.19,22

Most of the previous mfERG studies of diabetes have used standard multifocal stimulation, which presents pseudorandom flashes with a minimum interflash interval of 13.3 ms.14,17–22 This stimulation mode presents focal flashes before the retinal response evoked by the preceding flash has fully developed. Because of this, higher order effects are superimposed on the waveform of the typically studied standard first-order mfERG kernel.15,13,14,23,24 This makes problematic the measurement and interpretation of late components of the standard first-order mfERG waveform. Furthermore, the effectiveness of the standard mfERG in diabetes may be limited by the superposition of higher order effects, because results obtained using a template-stretching technique to estimate implicit times suggest that the late waveform components of the first-order kernel may be preferentially affected by diabetes (that is, more significantly delayed than the earliest components).19,20,22,25,26

Increasing the minimum interval between multifocal flashes can allow the local retinal responses to develop and decay more completely before the next flash occurs, thereby avoiding the superimposition of higher order effects on the later waveform components.14,25,27–29 In the current study, we used such a multifocal stimulus and referred to the technique and the retinal responses it evokes as the slow-flash (sf)mfERG to distinguish it from the standard multifocal technique.

Greenstein et al.20 used an sf-mfERG stimulus to study five eyes of diabetic patients with clinically significant macular edema as part of a larger study and found widespread abnormalities, particularly response delays. However, they did not examine diabetic patients without retinopathy and diabetic patients with milder types of retinopathy. Kurtenbach et al.15 used an sf-mfERG stimulus in a study of patients with type I diabetes without retinopathy, examining the small multifocal oscillatory potentials (mfOPs) in retinal signals band-pass filtered 100 to 1000 Hz and also found some significant delays. More recently, Onozu and Yamamoto29 used a modified sf-mfERG stimulus to study the first-order kernel and mfOPs recorded in diabetic patients with preproliferative diabetic retinopathy and reported significant delays and amplitude reductions. In the latter two studies, responses were averaged over retinal quadrants and/or rings concentric with the fovea before they were analyzed, rather than analyzed locally.15,29

In the present study, we examined retinal function in diabetic patients free of retinopathy and diabetic patients with early nonproliferative diabetic retinopathy (NPDR) by measuring the major sf-mfERG waveform features of 103 local responses and mapping response abnormalities. The stimulus we used, in combination with 10 to 300 Hz band-pass filtering,
rather than high-pass filtering, provided retinal responses that were robust enough to measure at 103 retinal locations.

**METHODS**

**Subjects**

One eye of each subject was examined in this study. Eighteen control subjects (46.7 ± 9.9 years of age; mean ± SD), 12 diabetic patients without retinopathy (49.3 ± 14.1 years of age) and 17 diabetic patients with early NPDR (51.6 ± 7.5 years of age) were tested. Of the diabetic patients without retinopathy, three (25%) had type I and nine (75%) had type II diabetes. Four of the diabetic patients with NPDR (24%) had type I diabetes and 13 (76%) had type II. Thirteen of the subjects with NPDR had only mild retinopathy (microaneurysms and dot hemorrhages), and the remaining four with type II diabetes had edema confined to a midperipheral area smaller than a single stimulated patch of the retina. All subjects had corrected visual acuity of 20/25 or better. Subjects with moderate or worse cataracts, those with other diagnosed or suspected ocular complications, and those with high (≥6.0 D) myopia were not included in the study.

In addition to dilated ophthalmic examinations, the diabetic subjects had fundus photographs taken within 1 month of sf-mfERG recording. The photographs were examined and graded according to Early Treatment Diabetic Retinopathy Study (ETDRS) criteria by a retinal expert at the University of California Berkeley School of Optometry who was masked to the sf-mfERG results. Informed consent was obtained from all subjects after the experimental procedures were described to them. Approval of this research was obtained from our institutional human subjects experimentation committee, and the tenets of the Declaration of Helsinki were observed.

**Visual Stimulation**

A visual evoked response imaging system (VERIS 4.3 system with a refractor/camera; EDI Inc., San Mateo, CA) was used to present an array (diameter, ~45°) of 103 hexagons scaled with eccentricity (scaling factor = 10.46) to the central retina. We used an sf-mfERG stimulus in which the shortest possible interval between focal flashes was 53.5 ms (four video frames). That is, each step in the binary m-sequence used to drive the luminance of the hexagons was four frames long (Fig. 1). In the first frame, each patch had an equal probability of flashing at a luminance of 100 cd/m² or remaining dark (< 2 cd/m²). In the next three frames, all hexagons remained dark. The display surrounding the hexagonal array and a central fixation cross were maintained at 50 cd/m². Ambient room illumination was approximately 50 cd/m².

**Response Acquisition and Initial Processing**

The pupil of the tested eye was dilated to its maximum with 1% tropicamide and 2.5% phenylephrine hydrochloride. Retinal potentials were recorded with a bipolar Burian-Allen contact lens electrode inserted after the cornea was anesthetized with 0.5% proparacaine, and a ground electrode was attached to the right earlobe. The fellow eye was occluded with light pressure to prevent blinking. Stability of fixation and the contact lens electrode position were monitored during recording with the refractor/camera. Retinal signals were band-pass filtered 10 to 300 Hz, amplified 100,000 times, and sampled every 0.833 ms. A recording was approximately 7.5 minutes long (213 m-sequence steps), separated into 16 segments for subject comfort. If loss of fixation was observed or significant artifacts occurred, the affected segment was discarded and replaced. The first 80 ms of the first-order kernel were analyzed after one iteration of artifact removal and spatial averaging of each response with one sixth of its immediate neighbors. Descriptions of the subsequent data analyses are provided in the following sections.

**RESULTS**

**Second-Order Contributions to Standard mfERG Waveforms**

To confirm that higher order (fast adaptive) interactions contribute to the late portion of the commonly studied standard first-order mfERG waveform, and thereby to justify the use of the sf-mfERG stimulus, we recorded the standard mfERG from the right eye of one of our control subjects, a 46-year-old man. Standard conditions (flash and surround luminances of 200 and 100 cd/m², respectively, and a 75-Hz frame rate) and a fully dilated pupil were used.

The minimum interflash interval of standard mfERG stimulation is 13.3 ms, and so the first-order mfERG waveform can contain contributions from second-order effects at 13.3-ms intervals (one m-sequence step).10,11,25,24 The approximate waveform of the largest second-order contribution can be visualized by plotting the first slice of the second-order mfERG kernel, delayed by 13.3 ms relative to the first-order kernel. Figure 2 shows results obtained from retinal patches in two locations: the foveal center and at approximately 19° eccentricity in the temporal retina. The second-order contribution in the retinal center is delayed relative to that in the periphery (compare K2 shifted, Figs. 2A, 2B).30 Because of this, we find that the higher order contributions to the main features of the standard first-order mfERG waveform tend to be larger in the peripheral retina than in the center. This has important implications for the interpretation of standard mfERG waveforms.

To approximate the standard first-order mfERG waveforms as they would appear without the second-order contributions, the shifted second-order kernels were subtracted from them. The resultant difference waveforms (K1–K2 shifted) were plotted (Figs. 2C, 2D; open circles), along with the original first-order waveforms (K1; black traces). In the central retina (Fig. 2C), the original and difference waveforms diverged slightly at approximately 45 ms, producing a small change in N2 implicit time. However, in the peripheral location (Fig. 2D), the original and difference waveforms began to diverge near the peak of P1 at approximately 27 ms, the shape of N2 was very different, and N2 implicit time was decreased by 5.8 ms.

![Image](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932922/ on 11/21/2018)
Unfortunately, subtraction of the shifted first slice of the second-order kernel from the first-order waveform only partially removes the higher order contributions to the standard mfERG, because small contributions from other second- and third-order kernel slices remain. Even with these limitations, it is clear that the fast flicker rate used to evoke the standard mfERG affected the late (P1 and N2) components of the first-order kernel, and these effects were not the same in the central and peripheral retina. In contrast, as Figures 2E–2H show, the first-order sf-mfERG kernel was not similarly affected, because there was a minimum of an additional 40 ms between focal flashes.

**sf-mfERG Arrays**

In all the following figures, response arrays and maps of response measurements were plotted as left eyes in retinal view. Right eye data were reflected to appear in left eye orientations. Figure 3 shows example response arrays recorded from a control subject (A), a diabetic patient without retinopathy (B), and a diabetic patient with NPDR (C). The gray area in each
array represents the approximate location of the optic disc. Individual sf-mfERGs are replotted in larger form to the right of each array for easier comparison of waveform differences. The quality of these recordings is representative of the samples.

As previously reported, a small but consistent nasal–temporal asymmetry in waveform is present in control eyes: the temporal response waveforms, on the right half of the array, are slightly larger and contain relatively more high-frequency wavelets at corresponding eccentricities. Some differences in waveform can be seen in the array of sf-mfERGs recorded from the diabetic eye without retinopathy, including a reduction in the second negativity (N2) in the inferior retina compared with normal. In the eye with NPDR, there was an extensive central area where the sf-mfERGs were reduced in amplitude, including a small temporal area where edema and hard exudate were observed (the arrow indicates

![Image](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932922/)
the single response at this location, which is replotted on the right).

**Mapping Retinal Abnormalities**

To analyze the local sf-mfERGs, we first calculated, for the control subjects, the means and standard deviations of four response measures at each of the 103 stimulated locations. The four response measures we examined were N1 implicit time (IT), P1 IT, N2 IT, and response amplitude, measured using the scalar product (SP) method. ITs were measured using the cross-correlation method provided in the evoked response imaging system software (VERIS 4.3; EDI). SPs were calculated over the 80-ms postflash epoch, using 105 local waveform templates computed from the control eyes. Analysis of several locations indicated that the distributions of control values did not differ significantly from a normal distribution, permitting the use of a z-score (the difference, in standard deviation units, between a value and the mean control value) to quantify each of the local response measurements.

Representative examples of N2 IT z-score maps, plotted as left eyes, are shown in Figure 4. In these maps, patches with z-scores between 2.33 and 3.00, indicating abnormal N2 delays at a significance level of 0.01 > P ≥ 0.0013, are shaded gray. Patches with z-scores greater than or equal to 3.00 (P < 0.0013) are black. In this control subject (Fig. 4A) there were no significant N2 delays, although a delay of 2.1 occurred near the optic disc. On average, approximately one abnormal location is expected to occur in each eye by chance alone when a criterion z-score of 2.33 (P = 0.01) is used (0.01 × 103 test locations = 1.03 abnormal locations). The map of the diabetic patient without retinopathy (Fig. 4B) shows a fairly large area of delay (z-scores from 3.8 to 7.3) in the inferior nasal retina, although fundus examination and photographs were normal. Furthermore, except in the central retina, most of this subject’s z-scores are positive, indicating that most of the N2 ITs were delayed relative to the control mean. Figure 4C shows extensive regions of N2 IT abnormality in a diabetic eye with NPDR, although this retina had only a few scattered microaneurysms and a patch of edema smaller than a stimulus hexagon in the inferior temporal retina where the large group of black hexagons is located. In this eye, all but one N2 z-scores were positive, indicating diffuse delay of this sf-mfERG component.

**Frequencies of sf-mfERG Abnormalities**

Maps summarizing the frequencies of the four types of sf-mfERG waveform abnormalities were constructed by calculating the proportion of eyes with significant abnormalities (P ≤ 0.01) at each of the 103 tested retinal locations. Figure 5 shows maps for the control subjects, diabetic patients without retinopathy, and diabetic patients with NPDR (left, middle and right columns, respectively) constructed using the N1 IT, P1 IT, N2 IT, and SP abnormality frequencies (top to bottom rows, respectively). The darkness of each hexagon in these maps indicates the percentage of eyes that were classified as abnormal at each tested retinal location (white represents 0%, black represents 40% or greater). The number below and to the right of each map is the maximum percentage of abnormality (the value at the darkest location in each map). Below and to the left of each map is the number of locations (Nhex) where at least one eye was abnormal (i.e., the number of nonwhite patches of 103 total).

As Figure 5 indicates, the number of affected retinal locations and the frequencies of abnormality were greater in the diabetic patients than in the control subjects and were greatest in the diabetic patients with NPDR. In the control group, the three IT measures produced no location with more than one abnormal eye (maximum 5.6%), and there were no abnormal SPs. As shown earlier, an average of 1.03 locations per eye can be expected to be abnormal by chance. Therefore, on average, we can expect a total of approximately 18.5 abnormalities (1.03 locations/eye × 18 eyes) to be due to chance in the control group. Variation of control group values around the expected value is probably due to the restricted size of the sample (n = 18). In the diabetic patients without retinopathy (Fig. 5, center column), P1 IT and N2 IT abnormalities were most frequent in the inferior and peripheral retina, and SP abnormality was most frequent in the inferior nasal retina. In contrast, N1 IT abnormalities were more common in the superior retina. The retinal patterns of abnormality in the diabetic

![Z-scores for N2 Implicit Times](image)

**FIGURE 4.** Representative examples of N2 IT z-score maps for left eyes. Retinal locations are shaded gray where z-scores fall between 2.33 and 3.00, indicating abnormal N2 delays at a significance level of 0.01 > P ≥ 0.0013. Locations with z-scores greater than or equal to 3.00 (P < 0.0013) are black. A map obtained from a control eye is shown in (A). Map obtained from (B) a diabetic without retinopathy shows a fairly large area of extreme delay in the inferior nasal retina and (C) from a diabetic eye with NPDR shows extensive regions of significant N2 IT delays, although this retina had only a few scattered microaneurysms and a small patch of edema in the inferior temporal retina where the large group of black hexagons is located.
patients with NPDR were similar to those of the diabetic patients without retinopathy, although greater in frequency and wider in affected areas. The frequency maps provide information about the common patterns of abnormality by superimposing the retinal maps of individual subjects. Thus, these maps do not provide information about the incidence of abnormalities within individual eyes.

To characterize the results in terms of the average frequency of each type of response abnormality per eye, the median number of abnormal ($P < 0.01$) locations per eye was calculated for each subject group and abnormality type (Fig. 6A). The control eye data (C) have median SP, P1 IT, and N2 IT abnormalities of zero and a median of one N1 IT abnormality. Diabetic eyes without retinopathy (D) have the following me-
and retinopathic eyes. To do this, an eye was classified as abnormal if three or more of its sf-mfERGs were abnormal (that is, three or more local z-scores greater than 2.33 for the IT measures or less than −2.33 for SP). Assuming that the local responses are independent (which may be an oversimplification in diabetic eyes), the probability that an eye will be classified as abnormal due to chance is 0.085 (8.5%). Figure 6B shows the classification results for control subjects (white bars), diabetic patients without retinopathy (gray bars), and those with NPDR (black bars). This analysis reveals that, whereas N1 IT and N2 IT classified from 50% to 88.2% of the eyes in the diabetic groups as abnormal, 33.5% of control eyes were also classified as abnormal by N1 IT and 16.7% by N2 IT. The SP and P1 IT measures discriminated the subject groups better than N1 IT and N2 IT. The SP measure classified as abnormal 15% of the control subjects, 16.2% of the diabetic patients without retinopathy, and 41.2% of diabetic patients with NPDR. P1 IT classified 5.6% (one eye) of the control subjects, 25% of the diabetic patients without retinopathy, and 52.9% of the eyes with NPDR as abnormal.

Finally, to determine whether different types of sf-mfERG abnormality tend to occur within the same retinas, an eye was classified as abnormal only if it had multiple (two or more) abnormalities detected by each of at least three of the response measures (for example, two SP, four P1 IT, and three N2 IT abnormalities). This criterion (labeled “Combo” in Fig. 6B) with a probability of 0.066 produces slightly better results than those obtained with either the SP or P1 IT measures alone. The eyes of none of the control subjects, 25% of the diabetic patients without retinopathy, and 64.7% of the those with NPDR were classified as abnormal by this criterion. This indicates that both diabetes and NPDR often affect multiple components of the sf-mfERG waveforms.

**DISCUSSION**

We examined different types of sf-mfERG abnormalities using two approaches: measurement of 103 individual local responses and the classification of eyes as abnormal. The results demonstrate that functional abnormalities of the retina occur in individuals with diabetes mellitus, both in those without retinopathy and those with early NPDR. Consequently, the sf-mfERG stimulus used is appropriate for the study and measurement of diabetic retinal complications in the early stages. The ability to measure and map local retinal dysfunction clearly has advantages over analysis of grouped responses. When nasal–temporal differences in response waveforms exist, as they do in the normal sf-mfERG, combining responses within rings concentric with the fovea is of dubious value. Furthermore, grouping of any kind can average normal and abnormal responses together, especially in diabetic retinopathy, in which gross structural abnormalities are local. Analysis of the 103 local sf-mfERGs revealed the presence of significantly abnormal function covering more than 20% of the tested retina in 13 (44.8%) of the 29 diabetic eyes. These extensive abnormalities were in 11 (64.7%) of the retinopathic eyes and in 2 (16.7%) of the eyes that had no evidence of diabetic retinopathy in fundus photographs. These findings are consistent with those reported in recent studies that used different methods (the standard mfERG stimulus and a template-stretching technique to measure local amplitudes and implicit times).19,22,25

In the present study, construction of frequency maps revealed that response abnormalities have similar retinal distributions in diabetic patients with NPDR and in those without retinopathy, although the severity is greater in eyes with retinopathy. Specifically, in both groups of diabetic patients, the inferior retina is more frequently affected than the superior retina in three (P1 IT, N2 IT, and SP) of the four local sf-mfERG measures. To our knowledge, this is a new finding. These results suggest that retinal function is compromised and that the inferior retina is more susceptible to damage, even before the appearance of retinopathy. Greater susceptibility of the inferior compared with the superior retina may be related to the inferior retina’s lower vasodilator reserve and a potential for associated increased risk for ischemic damage.32,33
Classifying eyes as abnormal is a possible step toward identifying eyes at risk for development or progression of diabetic retinopathy. Our four local sf-mfERG measures differed in their ability to classify eyes as abnormal. Although N1 IT and N2 IT classified high percentages of the eyes in both diabetic groups as abnormal, they also classified several of the control eyes as abnormal. In contrast, whereas the P1 IT and SP measurements classified few or no control eyes as abnormal, they classified fewer diabetic eyes as abnormal. The criterion combining three different types of response abnormality produced good separation of the three subject groups and reasonably high frequencies of abnormality, which suggests that different types of response abnormalities often occur within a diabetic retina. Issues of optimal criteria, sensitivity, and specificity can only be resolved, however, with larger study samples.

We suspected that the late response components of the sf-mfERG might be preferentially affected in diabetes. Ranked by the median number of abnormal local implicit times per eye, N1 is most affected by diabetes, N2 is moderately affected, and P1 is least affected. That N1 IT is most frequently abnormal in diabetic patients is a surprising and previously unreported finding, for either the standard or sf-mfERG. This result must be considered, however, along with the fact that this response component was very small, relatively more affected by noise, and (as a result) abnormal in some of the control eyes. In any case, the results do not clearly establish that the late (P1 and N2) components of the sf-mfERG are preferentially affected in diabetes.

What pathologic changes underlie the observed response delays and amplitude reductions? It has been suggested that retinal hypoxia may be responsible for areas of implicit time abnormalities.19–26 In diabetic eyes and retinal regions without retinopathy, these delayed areas could be associated with early or undetected perfusion defects associated with choroidal capillary degeneration.3,34–36 Amplitude (SP) reduction may indicate an advanced condition where portions of the retina within the abnormal stimulated patches do not generate sf-mfERGs. Our results suggest that diabetes has a nonspecific effect on the retinal layers contributing to the different response components we examined. In support of this, reports using non-multifocal ERG techniques have demonstrated photoreceptor and postreceptor abnormalities in diabetic patients with various levels of retinopathy.3,57–39 However, our response measurements may not discriminate contributions from different retinal layers, because the cellular generators of the standard mfERG and the sf-mfERG are predominantly ON and OFF bipolar cells.31,40–42

In summary, the sf-mfERG is a promising tool for the study of localized retinal dysfunction in diabetes. It may become useful in the evaluation of new pharmacological treatments of retinopathy that are currently under development.2,4 The sensitivity and specificity of this technique may be increased by refining the way the response components are measured (by measuring response delay with a stretching method, for example) and/or digitally filtering the responses before measurement to reduce noise.25 We are currently evaluating whether sf-mfERG abnormalities correlate with diabetic retinopathy and are working on improving the measurement techniques.

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References


