Multifocal Electroretinogram Delays Reveal Local Retinal Dysfunction in Early Diabetic Retinopathy

Brad Fortune, Marilyn E. Schneck, and Anthony J. Adams

PURPOSE. To identify local retinal abnormalities in diabetic patients with and without retinopathy, by using the multifocal electroretinogram (M-ERG).

METHODS. Electroretinograms were recorded at 103 discrete retinal locations in each eye of eight persons with nonproliferative diabetic retinopathy (NPDR) and eight diabetic persons without retinopathy, using VERIS (EDI, San Mateo, CA). The amplitude and implicit time of each local (first-order) retinal response were derived and compared with normal values obtained from 16 age-matched, nondiabetic subjects. Maps of local response amplitude and implicit time were compared with fundus photographs taken at the time of testing.

RESULTS. In eyes with NPDR, the implicit times of responses from retinal sites manifesting clinical pathologic fundus lesions (e.g., microaneurysms and focal edema), were markedly delayed (e.g., up to 7 msec from normal). Responses from adjacent retinal sites that were more normal in clinical appearance were also delayed, but to a lesser extent (e.g., 2–5 msec). Smaller, yet significant local response delays were also found in eyes without retinopathy. By contrast, local response amplitudes bore no consistent relationship to fundus abnormalities in eyes with retinopathy, and amplitudes were typically normal in eyes without retinopathy.

CONCLUSIONS. The M-ERG reveals local retinal dysfunction in diabetic eyes even before retinopathy. The magnitude of delay of local ERG implicit time reflects the degree of local clinical abnormality in eyes with retinopathy. Local response delays found in some eyes without retinopathy suggest that the M-ERG detects subclinical local retinal dysfunction in diabetes. Analysis of M-ERG implicit time, independent of amplitude, improves the sensitivity of detection of local retinal dysfunction in diabetes. (Invest Ophthalmol Vis Sci. 1999;40:2638–2651)

The full-field electroretinogram (ERG) has been used as an objective tool to detect alterations of retinal function during the early stages of diabetes,1–8 to predict progression of diabetic retinopathy,4–7 and to monitor treatment effects.6 Typically measured at the cornea in the human eye, the full-field ERG is a complex potential generated by a mixture of responses from different cell types across the entire retina. Despite this complexity, an understanding of the physiological bases of the response components is emerging.9–15 Hence, the effects of diabetes on specific retinal cell types can be assessed in vivo by analyzing the individual components of the ERG.16

However, the sensitivity of the full-field ERG is limited, precisely because it reflects the activity of the entire retina. Even advanced disease, if confined to small, discrete patches, can remain undetected by the full-field ERG.17 In diabetes, the earliest clinical retinal changes are typically confined to the posterior pole, and even the more advanced lesions can be limited in extent (e.g., focal edema and capillary nonperfusion).18 Therefore, the ability to measure local ERGs in diabetes would improve objective detection of early functional alterations and assessment of local change over time. Focal electroretinography has been used to evaluate retinal function within the macula (central 3–10°) in diabetes,19,20 but, the technique is too time consuming to allow testing of more than only a few retinal areas during any one session.

In contrast, the multifocal electroretinogram (M-ERG) developed by Sutter and Tran21 and Bearse and Sutter22 enables assessment of up to hundreds of distinct retinal areas within approximately 8 minutes per eye. This technique has been applied to the study of retinitis pigmentosa,23–25 macular degeneration,22 glaucoma,26 and diabetes.27,28 Palmowski et al.27 demonstrated that in some patients with diabetes, M-ERG responses (averaged across relatively large areas of retina) were smaller in amplitude and delayed in comparison with those in normal subjects. However, they did not determine the extent to which local abnormalities were detected (versus abnormalities present throughout the retina).

The purpose of this study was to identify local retinal abnormalities in diabetic patients with and without nonproliferative diabetic retinopathy (NPDR) using the M-ERG.
Table 1. Patient Demographics

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<th>Refraction* (RE/LE)</th>
<th>Retinopathy Grade† (RE/LE)</th>
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* Spherical equivalent diopters.
† Graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol for mild NPDR. See the Methods section for details and references.

METHODS

Subjects

Thirty-two eyes of 16 diabetic patients (mean age, 48 ± 9 years) were tested and compared with a control population consisting of 16 eyes of 16 nondiabetic volunteers (mean age, 40 ± 9 years). All eyes had 20/25 or better visual acuity. Patients with visible media opacity or other history of ocular disease or surgery were excluded from the study. Diabetic patients were recruited through local endocrinology practices and a nutritional counseling and support group. Information regarding the date of onset and type of diabetes was obtained by clinical history and verified whenever possible by review of medical records. Table 1 contains further demographic details for all patients included in this study. Both groups of diabetic subjects (with and without retinopathy) showed a range of refractive errors, including some significant myopia (see Table 1). There was no significant difference in refractive error between the groups. The nondiabetic subjects showed a similar range of refractive errors (mean spherical equivalent −1.95 ± 2.39 D) that did not differ significantly from either diabetic group or all diabetic patients as one group (P ≥ 0.1 for each comparison).

The overall retinopathy grade for each eye in this study was determined according to the protocol described in the Early Treatment Diabetic Retinopathy Study (ETDRS).29 Eight diabetic patients had no ophthalmoscopically detectable retinopathy (ETDRS grade 10) in either eye at the time of testing. The remaining eight patients had mild to moderate nonproliferative diabetic retinopathy (NPDR) in both eyes. A more detailed description of the grading criteria appears in the section entitled Grading System for Local Characteristics of Retinopathy.

The nature and possible consequences of the study were explained, and informed consent was obtained from all patients before testing. All procedures were preapproved by the University of California Committee for the Protection of Human Subjects and followed the tenets of the Declaration of Helsinki.

Data Acquisition

Multifocal ERGs were recorded using the VERIS system (EDI, San Mateo, CA). Pupils were fully dilated (≥7 mm) using 1.0% tropicamide and 2.5% phenylephrine. After topical corneal anesthesia (0.5% proparacaine), a Burian Allen bipolar contact lens electrode was placed on the test eye and a ground electrode clipped to the left earlobe. Patients were positioned 33 cm from the stimulus monitor. Stimulus clarity was optimized by overrefraction, and then a final adjustment of test distance was made to maintain constant stimulus magnification (test distance, 33 cm for plano overrefraction). The stimulus was presented on a 17-inch monitor (Dotronix, Inc., New Brighton, MN), driven at a 75-Hz frame rate and consisted of an array of 103 hexagonal elements across a field subtending 44° horizontally and 40° vertically. White hexagons had a luminance of 185 to 200 cd/m², and dark frames were 1 to 2 cd/m², resulting in local contrasts of 98% to 99%. Each hexagon was temporally modulated between light and dark according to a binary m-sequence21 with a base interval of approximately 13.3 msec. Observers fixated a small gray spot in the center of the stimulus during 8-minute recording sessions (m-sequence exponent, 15). To improve fixation stability, the sessions were broken into 30-second segments with brief rest periods between each segment. Signals were amplified (gain, 10⁶), band-pass filtered (10–100 Hz), and recorded with a sampling interval of 0.83 msec (16X per video frame). Recording segments containing

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two or more amplifier-saturating artifacts were discarded and rerecorded.

Data Analysis
The amplitude and implicit time of all local (first-order) ERG responses were analyzed using a computer program (Matlab; Mathworks, Natick, MA) in a manner similar to that described by Hood and Li. Specifically, normal-response template waveforms were compiled by averaging the local responses, specific to each stimulus location, of 16 nondiabetic eyes. Left eye response arrays (7/16 nondiabetic eyes tested) were left–right reversed before averaging into the normal template array. Response arrays from the left eyes of diabetic patients were compared with a mirrored version of this average-normal right eye template array. The normal response template, specific to each stimulus location, was scaled independently in amplitude and time until the least-squares fit to the local patient response was obtained. The amplitude and implicit time of each local patient response was then derived from the scale factor for each parameter. Amplitude was calculated as the voltage difference between the first trough and the first peak of the scaled template. This is analogous to the trough-to-peak b-wave amplitude measured for photopic Ganzfeld ERGs. Implicit time was measured to the first prominent response peak of the scaled template. This method of determining response amplitude and implicit time is reliable and especially robust against response noise.

Hood and Li demonstrated that local M-ERG responses from patients with retinitis pigmentosa are more accurately fit by normal templates that are multiplicatively scaled over time (rather than additively scaled). Multiplicative scaling stretches the entire normal response along the time axis, whereas additive scaling shifts the normal response by addition of a simple latency delay. Preliminary analyses of our data showed that multiplicative time scaling provided superior fits to local ERGs in diabetic eyes as well. For short implicit time delays (<2 msec), quantitative goodness-of-fit measures indicated that the two methods were comparable. (The goodness-of-fit measure provided by this program is the “statfit” value that is the least-squares statistic minimized during the fitting routine; a statfit of 0.0 indicates a perfect fit, and a statfit of 1.0 indicates that the fitted template does no better than the mean of the data). For responses with longer delays, however, additive scaling produced much larger (poorer) statfit values (e.g., ~0.45 versus ~0.25, using the multiplicative method). Therefore, all fits were performed using multiplicative scaling. The mean (±SD) statfit value for all patient local ERG fits was 0.27 ± 0.09, and the range was 0.07 to 0.68, indicating that the template fits were accurate. In fact, the residual error of the fit was typically caused by mismatch of the late components (50 to 80 msec); reanalysis of a subset of responses with high statfit values (i.e., >0.5) using only the first 50 msec of these responses, revealed no differences for derived implicit times or amplitudes.

Grading System for Local Characteristics of Retinopathy
Stereoscopic 30° color fundus photographs were taken of seven standard fields in each eye according to the ETDRS protocol. Only standard fields 1 through 5 were included in the analyses, because the M-ERG stimulus subtended only 40° of the central retina (standard fields 6 and 7 are beyond the central 40°). A scaled transparency of the M-ERG stimulus (outline of all 103 hexagons) was overlaid onto each fundus photograph by simultaneous projection, beginning with the photograph of standard field 2 (fovea centered) in each eye. Prominent fundus features along the edges of standard field 2, such as the optic nerve and major blood vessels, were traced onto the hexagon map. These features were then used to maintain alignment between the peripheral portions of the hexagon map and photographs of the other standard fields. Retinopathy characteristics within each hexagonal local ERG test area were graded (by a masked observer) according to the ETDRS classification system as follows: The equivalent ETDRS levels for the whole fundus are also listed. Grade 1, ETDRS level 10, indicates diabetic retinopathy absent (microaneurysms [MAs] and other characteristics absent); grade 2, ETDRS level 20, MAs only (MAs definitely present, other characteristics absent); grade 3, ETDRS level 35, mild NPDR (MAs definitely present and any one or more of the following: venous loops, hemorrhage, hard exudate, or soft exudate definitely present or soft exudate, intraretinal microvascular abnormalities [IRMA], or venous beading questionably present); grade 4, ETDRS level 43, moderate NPDR (moderate to severe hemorrhages or MAs [more than standard photograph 1, equivalent to or less than standard photograph 2A], or IRMA definitely present). In addition to these ETDRS criteria for eyes, areas with moderate NPDR, we also specified local retinal areas with severe hard exudate (i.e., ≧Airlee House standard photograph 4) and/or edema as grade 4 (moderate NPDR). Fluorescein angiograms were used to evaluate the presence and extent of focal edema further in six eyes with NPDR (three patients).

The implicit time and amplitude of the M-ERG at each stimulus location for each eye were compared with the corresponding area of the fundus (photographs) to evaluate the relationship between local ERG abnormalities and clinical features of retinopathy. M-ERG responses to the three stimulus hexagons that most commonly overlapped the projection of the optic nerve head were excluded from the analysis of retinopathy grade versus M-ERG.

RESULTS
Nondiabetic Group
As expected from other M-ERG studies that assessed local response implicit time and amplitude independently, the variability of implicit time among this sample of 16 nondiabetic eyes was markedly low. The mean implicit time for all 1648 local responses (16 nondiabetic eyes × 103 local responses each) was 30.1 ± 0.84 msec. Although the overall SD of implicit time was 1.0 msec, the range of local SDs (calculated for the 16 normal response implicit times specific to each of the 103 stimulus locations) was 0.48 to 1.77 msec (median, 0.75 msec; 95th percentile, 1.36 msec).

The five values that exceeded the 95th percentile (i.e., the five most variable locations) corresponded to five adjacent stimulus hexagons in the vicinity of the blind spot. The blind-spot response is known to be the smallest and slowest within the normal response array. The residual blind-spot response is believed to result, in part, from stimulus hexagons that overlap the optic nerve head and adjacent retina and thus
produce true local ERGs. Nonlocal retinal responses, produced by light reflected or scattered from the optic nerve head, may also contribute to the residual blind-spot response. The increased interindividual variability of implicit time at the five locations mentioned earlier resulted from slight smearing of the position of the blind-spot after 16 normal response arrays were averaged. Nonetheless, the tight distribution of local M-ERG implicit time throughout most of the response array indicates that there was very low interindividual variability of this parameter.

The intraindividual variability of local response implicit time was also small. The median intraeye SD (i.e., calculated for the 103 local responses within each normal eye) was 1.15 msec (range, 0.95–1.75 msec). Implicit time varied slightly with eccentricity: For the majority of normal eyes, the responses in the center of the macula were slightly slower (~0.5–1.0 msec) than the average for that eye. The blind-spot responses were typically approximately 2 to 3 msec slower than the average within any eye. In summary, with the exception of the predictable blind-spot responses, M-ERG implicit times varied little within the normal retina.

In contrast, local response amplitudes varied to a much greater extent, both between and within normal eyes. The overall mean local response amplitude (i.e., calculated for all 1648 normal local responses) was 296 ± 75 nV. Generally, the largest amplitude responses within any normal array were found at the center of the macula, although some peripheral responses were also consistently larger than average. Local standard deviations for amplitude ranged from 57 to 98 nV (median, 71 nV).

Figure 1A shows the multifocal ERG responses associated with each of the 103 stimulus locations for a single representative normal subject. (B) The average M-ERG response implicit time (in milliseconds) for the 16 normal observers for each location. (C) The average M-ERG response amplitude (in nanovolts) for the 16 normal observers for each location. (D) The maximum implicit time observed among 16 normal eyes and responses, at each location, relative to the local average (i.e., maximum local response delay found among all normal eyes). (E) The minimum amplitude observed among all normal responses at each location, relative to the local normal average (i.e., observed/average). The typical position of the optic nerve head projection (blind spot) is shaded gray.
tive nondiabetic observer. Figures 1B and 1C show the mean of the 16 normal observers' implicit times and amplitudes, respectively, for each location. Figure 1D shows the maximum implicit time observed among 16 normal eyes and responses at each location, relative to the local average (i.e., maximum local response delay found among all normal eyes). The largest normal delays were found near the blind spot (shaded gray) because of subtle differences between the size and position of the blind spot for some persons and the average. There is only other location (other than the four near the blind spot) where a response delay greater than 2.0 msec was observed among the normal eyes—namely, the central location. Figure 1E shows the minimum amplitude observed among all normal responses at each location. These values are reported relative to the local normal average.

Expressed as a percentage of the mean, the interindividual variability of local response amplitude was approximately 10 times higher than that for implicit time (~30% versus ~3%, respectively, for most locations). It is important to note that the size of the hexagonal stimuli are scaled with retinal eccentricity to produce approximately equal amplitude responses throughout the array. The approximate stimulus scaling is illustrated, for example, in Figures 1B through 1E, which show hexagon size increasing with eccentricity. Despite the stimulus scaling, there is still some variability in the amplitude, timing, and morphology of local responses across the normal observer's array. This underscores the importance of using average-normal templates that are specific to each stimulus location, to assess most sensitively the local response abnormality in disease.

**Diabetic Group**

Figure 2A is a photographic montage of the left fundus of patient SS (upside down for ease of comparison to the re-
Figure 3. (A) Photographic montage of the right fundus of patient RB. (B) Map of multifocal ERG implicit times for this eye. The number in each hexagon is the delay (milliseconds) of the patient’s local ERG response relative to the average normal implicit time at that location. The light gray, dark gray, and black shading indicate 2 to 4 msec, 4 to 6 msec, and more than 6 msec slower than the normal implicit time, respectively. (C) Map of relative ERG amplitude for each local response in this eye. The number in each hexagon is the ratio of the patient’s local ERG response amplitude to the normal amplitude for that location (values <1.0 are reduced amplitude; 1.0, normal amplitude; and >1.0, above-normal amplitude). Light gray shading indicates a 25% to 49% reduction of local amplitude; dark gray a 50% to 75% reduction, and black a more than 75% reduction. (D) Trace array of local M-ERG responses from this patient. The fundus image (A) is printed upside down to facilitate comparison to the ERG response maps (B, C, and D), which are plotted according to the convention used for visual fields. The large circle in each panel denotes 20° eccentricity. The three smaller circles, identical in each panel, highlight the areas of interest in the fundus (areas X, Y, and Z; A) and their corresponding positions within each of the functional maps (B, C).

Response maps). Figure 2B shows the implicit time map for this eye. The number in each hexagon is the delay (in milliseconds) of the patient’s local response relative to the average normal implicit time at that location. The shading scheme indicates the magnitude of the deviation in response timing at each stimulus location. White shading means that response timing was within 2 msec of normal at that location. Light gray, dark gray, and black shading indicate 2 to 4 msec, 4 to 6 msec, and more than 6 msec from normal implicit time, respectively. All the implicit time maps shown here follow the same shading scheme. As a reminder, the overall value for implicit time SD was 1.0 msec (see nondiabetic implicit-time results shown earlier). The median local SD was 0.75 msec. Thus, although based in milliseconds the shading scheme roughly corresponds to delays of 2 to 4, 4 to 6, and more than 6 standard deviations for light gray, dark gray, and black, respectively.

In the fundus picture (Fig. 2A), two distinct focal patches of retinal edema, each outlined by a circinate pattern of exudate, are marked X and Z. Several inner retinal hemorrhages were found in the area marked Y. Figure 2B shows that the majority (87/103) of local responses in this eye were considerably delayed. Note that the three areas with the longest delays closely corresponded to the three most abnormal areas in the fundus (marked X, Y, Z). The two local responses with the longest delay (7 msec), fell precisely within the area of circinate edema marked X. The responses surrounding these were also significantly delayed, yet to a lesser extent (4–6 msec). The areas surrounding X also manifested retinopathic...
features (MAs and small dot or blot hemorrhages), which are clinically less significant than retinal thickening or hard exudate. The responses in the blind spot, corresponding to the position of the optic nerve head, were also delayed.

Figure 2C is the map of relative response amplitude for this eye. Each number is the ratio of the patient’s local response amplitude to the normal amplitude for that location (values <1.0 are reduced, 1.0 are normal, >1.0 are above-normal amplitude). Light gray shading indicates 25% to 49% reduction of local amplitude, dark gray shading indicates 50% to 75% reduction, and black shading indicates a more than 75% reduction of local amplitude. Light gray, dark gray, and black corresponded to approximately 1 to 2 SD, 2 to 3 SD, and more than 3 SD from normal, respectively. The shading scheme was based on the median local SD among normal amplitudes (the range of local standard deviations was 17% to 36% of local mean normal amplitudes, and the overall SD was 25% of the overall mean). Figure 2C shows several regions of mildly reduced amplitude in this eye (light gray shading). Some of these reduced-amplitude responses corresponded to the position of retinopathic features in the fundus (e.g., compare lower left of Fig. 2C with areas Y and Z in Fig. 2A), whereas others did not (e.g., upper left Fig. 2C). The most delayed responses in this eye (7 msec, area X, lower right quadrant of Fig. 2B) were very close to the mean normal amplitude (0.9 [90%] of normal amplitude). Figure 2D shows this observer’s M-ERG response array for the 103 locations tested.

Figure 3A is the right fundus of patient RB (upside down, as in Fig. 2A). Figures 3B and 3C are the ERG delay and amplitude maps, respectively. As with patient SS, responses with the longest delays corresponded to the retinal regions with the most advanced clinical signs. The three patches of dark gray– and black-shaded hexagons in Figure 3B, highlight responses delayed by 4 to 7 msec. Their positions corresponded very closely to the three patches of circinate edema in Figure 3A (marked X, Y, Z). Responses from the areas immediately surrounding these were also delayed but to a lesser extent (light gray, 2–4 msec). The blind spot responses were also slightly delayed beyond the normal delay for this region. Responses from ophthalmoscopically healthier retina had nor-
mal or near normal timing. Negative values indicate responses that are faster than average normal.

Figure 3C shows that responses are uniformly diminished in amplitude across the entire field, although both the delay map (Fig. 3B) and clinical picture (Fig. 3A) show only small localized areas of abnormality. Paradoxically, many of RB’s largest responses were those with the greatest delays (e.g., lower left quadrant of Fig. 3C and 3B). Moreover, most of the smallest responses (e.g., center 3C) had normal timing, reiterating the absence overlap between these two response parameters. Figure 3D shows the M-ERG response array for the 103 locations tested.

Figure 4 compares actual local ERG responses from these clinically abnormal regions (patient SS, left eye) with the normal (template) responses for each location. The ERGs shown in bold are from the clinically abnormal retinal locations marked X, Y, and Z in Figure 2A; the thin traces are the normal local responses. Also shown are responses averaged from other retinal areas in the same eye, for equal stimulus eccentricity and size, where there were no obvious retinopathic features (dashed traces). ERGs from the most (clinically) compromised areas of retina were grossly delayed throughout the entire length of the response, yet only mildly reduced in amplitude compared with normal. The ERGs from the less (clinically) affected regions were less delayed and approximately equal in amplitude. The late features of this patient’s responses were also markedly altered; specifically, the trough and peak normally present from 40 to 60 msec were absent or reduced.

Figure 5 compares ERGs from the ophthalmoscopically abnormal areas X, Y, and Z of patient RB (see Fig. 3A) with the normal (template) responses for each location. ERGs from healthier regions of equal eccentricity in the same eye are also shown for comparison. As with the results presented in Figure 4, ERGs from ophthalmoscopically abnormal areas were delayed throughout the response compared with normal but were approximately equal in amplitude to (or even larger than) the ERGs from the less affected retina. The late response features (i.e., the second trough and peak, 40–60 msec) of RB’s clinically abnormal retina (areas X, Y, and Z) were also diminished or absent compared with those of average normal local responses from the same retinal locations.

The ERGs shown in Figures 4 and 5 further demonstrate the value of analyzing local response implicit time as an index of local retinal health in diabetic retinopathy. The amplitudes of the ERGs in these eyes with diabetic retinopathy were reduced relative to normal across most of the fundus, but local amplitude changes did not indicate (or correspond well to) ophthalmoscopic abnormalities. The amplitudes of responses from clinically abnormal areas were not necessarily smaller than those from unaffected retinal areas (in fact, they were sometimes larger). In contrast, both ophthalmoscopically normal and abnormal areas within these eyes produced ERGs that
were delayed relative to normal, but the response delays were much longer (≥4 msec) for the more abnormal areas. This was consistently observed in the other eyes with NPDR as well.

Local response abnormalities were also present in diabetic eyes with little or even no retinopathy. As in the eyes with moderate NPDR, retinal areas with delayed ERG responses were often unrelated to areas with decreased amplitude responses. Figure 6A shows the delay map and Figure 6B the amplitude map for a patient with mild NPDR (JS, left eye). Comparison of the delay and amplitude maps shows poor correspondence between the two measures of response abnormality. The smallest amplitude responses, found at the center of the macula, had normal timing, whereas the largest amplitude responses (0.9–1.0, far left), were significantly delayed (2–4 msec). The actual ERG traces for each stimulus location are shown in 6C.

Figures 7A, 7B, and 7C present a similar comparison for a patient with no retinopathy (GM, left eye). Delayed responses are concentrated in the lower right quadrant (7A), including three responses prolonged by more than 2.0 msec from normal average (light gray). The single shaded hexagon on the left of the delay map corresponds to the position of the blind spot. The amplitude map (Fig. 7B) shows that this patient’s responses were all larger (~1.6 times) than the average normal amplitude. Consequently, the entire amplitude map is shaded white. There was little correspondence, between the position of the delayed responses and the position of the relatively smaller responses within this eye. This discrepancy between the delay and amplitude maps was found for most of our patients with and without retinopathy. In the patients without retinopathy (example in Fig. 7), it was most common to find a normal amplitude map but a delay map showing one or more focal areas of delayed responses.

The association between local retinopathy grade and local ERG amplitude and implicit time was examined for all retinal locations in all diabetic eyes. Figure 8 shows the distribution of M-ERG response parameters relative to their normal local averages, grouped by local retinopathy grade (see Grading System in the Methods section). By plotting the response measures relative to normal local averages, the inherent variability due to field location was minimized. Data from the 1600 retinal locations for normal eyes were also represented (blind spot responses are excluded from this pool, because locations containing substantial portions of the optic nerve head in the diabetes groups were not given a retinopathy score). A small number (<5%) of peripheral hexagon locations from eyes with NPDR were not sufficiently well focused in the photographs to be graded reliably. Thus, the ERG responses at such locations were also excluded.

Figure 8 shows that 90% of the M-ERG implicit times in the normal group fell within ±0.84 msec (one time bin) of the normal average. In fact, 60% of the normal M-ERG implicit times (956/1600) were equal to the normal local average (i.e., 0.0 msec delay), thus exaggerating the apparent positive skew. It is apparent from Figure 8 that increased local retinopathy severity was associated with increased delay of implicit time.
Median delay of local implicit time increased with each successive retinopathy grade. The median for each group was greater than the 75th percentile of the normal distribution. Even fundus locations without any retinopathy (grade 1), from eyes without any NPDR or eyes with NPDR elsewhere, showed a modest increase in implicit time compared with normal nondiabetic eyes.

Figure 8 (bottom) compares local response amplitudes compared with normal local averages for regions of varying retinopathy grade. No association between local M-ERG amplitude and retinopathy grade was apparent. There appeared to be only a mild reduction in local response amplitude across all levels of retinopathy.

Figure 9 shows the number of local responses in each eye that had an abnormal implicit time (top) and an abnormal amplitude (bottom). Responses were considered abnormal if they were beyond 2 local SD from the normal local mean. Data were compared for nondiabetic eyes, diabetic eyes without retinopathy, and diabetic eyes with NPDR. Figure 9 (bottom) shows that local ERG amplitudes in eyes of diabetic patients with or without NPDR were rarely reduced beyond the normal range of variability. The mean number of responses with reduced amplitudes for eyes without retinopathy was 5.6 and for eyes with NPDR was 8.7. In contrast, Figure 9 (top) shows that a large number of local ERGs were delayed beyond the normal range for diabetic subjects, both with and without NPDR. There were more eyes with many abnormally delayed responses in the NPDR group than in the group without retinopathy. Among eyes with NPDR and those without retinopathy, the mean number of responses with abnormal implicit times was 61 and 25, respectively. The fairly even distribution of ordinal values in Figure 9 (top) suggests that local ERG implicit time delays occurred as much, or more often than global abnormalities in both patient populations. Global disease effects (e.g., diabetic cataract or reduced ocular blood flow) would tend to produce abnormal responses in (nearly) all retinal locations.

**DISCUSSION**

The results demonstrate that M-ERG implicit time analysis is a highly sensitive method of assessment of local retinal function in diabetes. The range of local ERG implicit times observed for the normal eyes in this study was very narrow, consistent with the findings of other M-ERG studies. Consequently, local ERG delays as small as 2.5 msec may be regarded as representing significant local retinal dysfunction in diabetic eyes. In eyes with NPDR, delays of local responses were greater and were found throughout more of the retina than in eyes without retinopathy. Response delays were progressively worse toward the center of discrete ophthalmoscopic lesions in the retinopathic eyes (see, for example, Figs. 2 and 3). Local ERGs delayed by 4 msec or longer were found only in, or
immediately adjacent to diabetic retinal lesions. Smaller, but significant local response delays were found in eyes without retinopathy (and in the normal-appearing areas surrounding diabetic retinal lesions in eyes with retinopathy) suggesting that the implicit time analysis revealed subclinical, local retinal dysfunction in these areas.

In contrast, local ERG amplitudes were more variable than implicit times between normal eyes (~10 times) and within normal eyes (~5 times). It is widely recognized that there is a higher degree of interindividual variability of amplitudes compared with implicit times for other electrophysiological responses as well (e.g., visual-evoked potentials and full-field ERGs). The results of this study, and other M-ERG studies, for example references 23, 25, and 33, suggest that this is also true for local ERGs throughout the retina. Amplitude variability within diabetic eyes with or without retinopathy was also large, but no greater than that for the nondiabetic eyes. Large interindividual variability of local ERG amplitude diminishes the usefulness of this parameter for detection of local retinal abnormalities in early diabetes. Perhaps as a result of relatively larger variability, local ERG amplitudes did not relate as well as local implicit times nor did they relate to ophthalmoscopic signs of retinopathy. In fact, it was common to find severely delayed ERGs for the most unhealthy patches within an eye, which were actually among those with the largest response amplitudes within that eye.

![Figure 8: Distributions of local ERG implicit time delays (top) and relative amplitudes (bottom) grouped by retinopathy grade at each fundus location.](image)

The leftmost column represents the group of 1600 local ERGs in nondiabetic eyes. Second from left are 1600 local ERGs from eyes in the group of eight diabetic patients with no evidence of diabetic retinopathy in either eye (no NPDR; therefore, all locations are grade 1, no retinopathy). The remaining four columns are comprised of responses from locations grouped by fundus grades from the 16 eyes of eight patients with NPDR. Thus, for all NPDR eyes, any fundus location with a grade of 1 (no retinopathy present), regardless of which eye or patient it belongs to, is grouped into the third column; and similarly, for more advanced signs of NPDR (grades 2 to 4). In each case, the three hexagons/locations in each eye that contained the projection of the optic nerve were not included. Seventy-six peripheral fundus locations (<5%) in the NPDR group could not be graded because of poor photographic resolution. Box plots indicate quartiles (25th and 75th percentiles), middle hash is the group median, and whiskers show 5th and 95th percentiles of each distribution.
Recently, Palmowski et al. 27 reported that implicit times of M-ERGs, averaged across the whole retina, were significantly delayed in some diabetic eyes without retinopathy. Whole-field response delays were greater in magnitude and more prevalent among their group of eyes with NPDR. For comparison with their results, we summed the local M-ERG responses in a similar manner and found significant global implicit time delays for both groups of eyes, with and without retinopathy (data not shown). Although comparable only in a general sense, M-ERG delays were consistent with the results from other ERG studies of diabetes, which found implicit time delays for full-field photopic ERGs, using either flash 7,8,28 or 30-Hz flicker 5,8,28 stimuli.

It has been demonstrated that decreased stimulus contrast or luminance affects M-ERG amplitude to a much greater extent than implicit time. 25,45,35 Thus, it is very unlikely that decreased effective stimulus contrast and/or luminance within patches of retinal edema, for example, were solely responsible for the alterations of local ERGs observed here—namely, long implicit time delays with relative preservation of response amplitudes. Rather, these timing changes appear to represent neural response or conduction delays perhaps secondary to compromised local metabolism and/or blood flow. Even the early features of the diabetic responses (first trough and peak, or a- and b-wave analogues, respectively) appeared to be delayed. This suggests that the generators of early response components may be functionally compromised within these retinal regions. The initial negative and positive voltage deflections of the M-ERG have been shown to behave much like the components of the photopic, full-field flash ERG and are likely to be generated by the same retinal elements. 51 Based on this parallel, it is possible that some of the response timing changes observed here represent compromised function in the outer retina (cone photoreceptors) and/or middle retina (cone bipolar cells, Müller cells) secondary to diabetes. Palmowski et al. 27 described abnormalities in the second-order response component of the M-ERG (a measure of interactions between consecutive responses), which suggest that the M-ERG also detects inner-retinal (possibly amacrine cell) dysfunction in early diabetes. The abnormalities noted here for the late components of first-order responses (40–60 msec) are consistent with the second-order abnormalities reported by Palmowski et al. 27 in that nonlinear effects (interactions between responses to successive stimulus frames) are known to contribute to the shape of these late first-order features under these stimulus conditions.

The presence of significant local response delays in eyes without clinically evident retinopathy suggests that such M-ERG changes may provide a very early indicator of local retinal dysfunction in diabetes. Observing these patients longitudinally will help determine whether abnormal M-ERG responses (timing delays, in particular) predict development and/or progression of retinopathy in discrete retinal locations. It is possible that such early local ERG changes, found in the absence of retinal vascular findings, are caused by early diabetic choroidal lesions. 56 However, it should be noted that profound retinal hypoxia has been measured in diabetic cats without angiographic evidence of retinal capillary dropout or choroidal perfusion deficits. 57 Retinal hypoxia is thought to be a major stimulus leading to increased expression of vascular endothelial growth factor and vascular permeability factor (VEGF/VPF), 48,60 although increased glucose concentration alone may be sufficiently damaging. 40–42 In turn, increased expression of VEGF/VPF is likely to be a critical factor in the development of even the earliest retinal vascular lesions in NPDR. 43–47 In fact, local breakdown of the blood–retinal barrier has been associated with increased immunoreactivity for VEGF/VPF in the early stages of experimental diabetic retinopathy. 48,49 as well as in diabetic human eyes in patients in whom fellow eyes had no evidence of retinopathy. 55

Taken together, these results suggest that the M-ERG may serve to monitor local metabolic conditions that lead to (or are related to) the development of diabetic retinal vascular lesions such as breakdown of the blood–retinal barrier. Use of the M-ERG may also improve objective follow-up of treatment interventions. Indeed, one recent study 28 used the M-ERG to document local changes in retinal function after focal laser treatment for diabetic macular edema, which did not appear in the Ganzfeld, 30-Hz flicker ERG.

In summary, we believe that the results presented here are the first to demonstrate that implicit time delays of multifocal ERGs reveal abnormal local retinal function in diabetes corresponding to local, discrete retinopathic lesions. The M-ERG is
easily obtained in a clinical setting, and provides a very sensitive, objective assessment of local retinal health in diabetes.

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