Central Retinal Function as Measured by the Multifocal Electroretinogram and Flicker Perimetry in Early Age-Related Macular Degeneration

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PURPOSE. To determine the retinal function in early age-related macular degeneration (AMD) assessed by the multifocal electroretinogram (mfERG) and flicker perimetry and to seek a relationship between local objective mfERG parameters and subjective flicker perimetry thresholds.

METHODS. mfERG and flicker perimetry were performed in 15 patients (15 eyes) with early AMD and 14 controls (14 eyes) of similar age. The mfERG P1 response amplitude density (nV/deg²) and P1 implicit time of the first-order kernel and the flicker thresholds of each concentric ring were analyzed. The relationship between individual mfERG responses and the corresponding individual flicker sensitivity outcomes was determined.

RESULTS. The mfERG response amplitude of the central ring (ring 1) was significantly reduced in early AMD eyes compared with the controls (P = 0.009). No significant difference in mfERG amplitude between early AMD and control eyes was detected in the other rings. The mfERG implicit time was significantly increased in the early AMD eyes but only within the central four rings of 12°. A significant reduction in flicker sensitivity was also detected in early AMD eyes but only within the central 6°. There was a significant, moderate correlation (r = −0.477; P < 0.001) between local mfERG latency and flicker sensitivity from the same tested locations within the central 6°. There was a weak correlation (r = 0.200; P = 0.014) between mfERG amplitude and flicker sensitivity.

CONCLUSIONS. Both mfERG and flicker perimetry show abnormal retinal function, but only in the very central macula, in early AMD. A novel relationship between mfERG and flicker sensitivity should enhance the clinical monitoring of disease progression.

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Age-related macular degeneration (AMD) still represents a major challenge despite advances in the treatment of neovascular AMD. New interventions are slowly being evaluated that aim to halt the progression of early stages of AMD and to preserve vision. However, the evaluation of these novel interventions is hindered by the lack of robust outcome measures of retinal function that might be used to determine trends in disease progression.

In most clinical studies on AMD, best-corrected visual acuity (BCVA) is the main clinical parameter of visual function assessed. However, eyes with early AMD have either normal BCVA or a decrease of approximately two or fewer letters. Changes in this BCVA over time are slow and do not necessarily reflect underlying changes in pathology or risk of significant vision loss. It would be helpful to have clinically applicable methods of testing macular function that show abnormal results early and worsen with worsening pathology and risk of vision loss.

We and others have previously shown that visual field sensitivity is reduced in patients with early-stage AMD, and indeed flicker perimetry is a sensitive test for detecting AMD progression. However, given the subjective nature of these psychophysical methods, an objective visual function test would also be valuable in early AMD. The multifocal electroretinogram (mfERG) offers such an objective, electrophysiological evaluation of macular function. Nevertheless, previous reports in early AMD are inconsistent. More recent studies, in contrast, have found no such significant mfERG changes in early AMD eyes. In addition, little is known of the relationship between localized subjective visual field threshold changes and objective mfERG changes.

The purpose of this study was to evaluate the mfERG functional impairment in early AMD eyes and to determine the relationship between localized flicker sensitivity changes and local mfERG parameters.

METHODS

The study was approved by the Human Ethics Committee of the Royal Victorian Eye and Ear Hospital and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants after explanation of the nature of the study.

Participants

Participants with early AMD (n = 15) were recruited from the medical retinal clinic at the Royal Victorian Eye and Ear Hospital and from private practices. Our inclusion criteria for early AMD participants were at least one eye with drusen >125 μm, pigmentary change, or both and visual acuity of 20/32 or better. Clinical status of the fellow eye was drusen or pigment changes consistent with a diagnosis of early AMD. If both eyes satisfied the inclusion criteria, the eye with the better visual acuity was designated the study eye. If the acuity and AMD status were the same in both eyes, then the left eye was chosen as the study eye.
Exclusion criteria were age ≥40 years, presence of cataract according to the Wilmer Grading system,24 glaucoma, diabetes, amblyopia, color blindness, and any other ocular, neurologic, or systemic disease, each which could compromise vision and performance of the visual function tests. Subjects with late AMD or those who were unable to sign an informed consent form were also excluded from the study. Control participants (n = 14) were unrelated family members or friends of the AMD subjects and staff and whose age range was similar to that of the AMD participants. All participants underwent a standardized questionnaire interview for demographic information, BCVA assessment using a logMAR chart, clinical eye examination, color fundus photography, autofluorescence imaging, and functional testing including the mfERG and flicker perimetry.

AMD Diagnosis

Retinal fundus photographs (50°), centered on the fovea, were taken using a non-midriatic fundus camera (CR6-45NM; Canon, Saitama, Japan). A senior retinal grader graded the fundus photographs (OptoMize PRO software; Digital Healthcare Image Management System, Digital Healthcare Ltd., Cambridge, UK) according to the International Classification and Grading System for AMD.25 Fundus autofluorescence images, obtained using an optical coherence tomography (Spectralis HRA+OCT; Heidelberg Engineering Inc., Dossenheim, Germany), were also reviewed to exclude presence of geographic atrophy.

Flicker Perimetry

The method of flicker perimetry testing has been described previously.6 In brief, flicker perimetry was performed using an automated perimeter (model M-700; Medmont International Pty Ltd., Vermont, Victoria, Australia). This is a bowl perimeter that uses light-emitting diodes (LEDs) at a maximum of 565 nm. The bowl has a background luminance of 3.2 cd·m⁻² and a maximum spot luminance of 320 cd·m⁻². The LEDs subtend 0.45° (Goldmann size III) and are arranged concentrically at various eccentricities from 1° to 50°. A macular test protocol was used that consisted of a test grid of 48 points located at 1°, 3°, 6°, and 10° from the central fixation. The test uses a ZEST fast-Bayesian threshold logic and takes 4 to 7 minutes to complete. Flickering stimuli were presented with durations of 800 ms (flicker). Fixation was checked by a blind-spot monitor, and false-positive and false-negative stimuli were presented randomly during testing. Patients who gave >20% false-positive or false-negative responses or >20% fixation losses were retested.

Multifocal Electroretinogram

The recordings were performed with a Visual Evoked Response Imaging System (VERIS Science 6; ElectroDiagnostic Imaging, Inc., Redwood City, CA). A fixation monitoring system (FMS III) was used to deliver the test stimulus. mfERGs were recorded monocularly with Dawson-Trick-Litzkow thread electrodes. Pupils were dilated with 1% tropicamide, and recording began only when the pupils were dilated to at least 7 mm. Subjects corrected for refractive errors by adjusting the refractor unit within the FMS to optimally focus the stimulus. Fixation was ensured using an infrared retinal image displayed by the FMS.

The testing stimulus consisted of 103 retinal scaled hexagons that were randomly alternated between white and black frames on an FMS microdisplay using a pseudorandom m-sequence (m = 15) at a rate of 75 Hz. The stimulus contrast was approximately 99% with the luminance of the white hexagon at 5.35 cd·s·m⁻². Background luminance was set at 200 cd·m⁻² to minimize the stray light effect. Recorded signals were band pass filtered between 10 and 100 Hz and were amplified 100,000 times (model 12; Grass NeuroData, Quincy, MA). Noise-contaminated segments, caused by blinks or small eye movements, were rejected and rerecorded.

Statistical Analysis

To determine the extent of the retinal dysfunction, mfERG responses were grouped into six concentric rings (Fig. 1A), and the flicker sensitivity was grouped into four concentric rings (Fig. 1B). mfERG responses grouped into six rings were derived from 0° to 1° (ring 1 [R1]), from 1° to 4° (R2), from 4° to 8° (R3), from 8° to 12° (R4), from 12° to 17° (R5), and from 17° to 22° (R6), respectively. The four concentric flicker perimetry rings were at 1°, 3°, 6°, and 10°, respectively. No spatial averaging was applied for the mfERG data. For mfERG, the N1-P1 amplitude density (from the first negative trough to the first positive peak) and the P1 implicit time (from stimulus onset to first positive peak) of the first-order kernel response of each ring were calculated. Mean mfERG parameters and mean flicker sensitivity, averaged in concentric rings, of subjects with early AMD and age-matched controls were compared using unpaired t-test.

To examine the relationship between mfERG parameters and flicker sensitivity, we obtained local responses within the central three rings of both tests, which were spatially matched. There were 10 flicker stimulus points that matched topographically with 10 hexagons from the mfERG stimulus matrix (Fig. 1C). Responses from only these 10 locations from 15 early AMD eyes were correlated using Spearman’s rank. Responses from all other unmatched locations were excluded from this analysis.

The differences in flicker threshold, mfERG amplitude, and latency for each tested location for early AMD eyes were expressed as standard deviation from the mean value of the control group for each respective point. To obtain a measure of the normal variation, the standard deviations of 140 measures (14 control subjects, 10 locations) for the control subject responses were calculated for flicker thresholds, mfERG amplitude, and latency at each point. The difference in flicker sensitivity and mfERG parameters of ≥2 SD was considered significant.

FIGURE 1. Configuration of concentric ring averages used in group comparisons of multifocal ERG responses (A) and flicker perimetry responses (B). Overlapping mfERG and flicker perimetry stimulus matrices show 10 flicker stimulus points that matched topographically with 10 hexagons from the mfERG stimulus matrices (C, shaded).
RESULTS

Fifteen early AMD participants (15 eyes) and 14 age-matched control subjects (14 eyes) participated in this study. A summary of demographic data of the study participants is presented in Table 1.

Representative mfERG responses from a participant with early AMD and a control participant are shown in Figure 2. The trace array plot showed a significant decrease in the mfERG amplitude at the central retina in the early AMD eye compared with the control eye. The decrease in the central retinal response of the early AMD eye was also clearly demonstrated by the 3D density plot. The P1 implicit time within the central retina was significantly increased in the early AMD eye compared with that of the normal eye.

Concentric ring averages were used to determine the extent of retinal impairment in eyes of participants with early AMD compared with control participants. The group data for mfERG N1-P1 amplitude and P1 implicit time at various retinal concentricities are shown in Figure 3. On average, mfERG response amplitude densities of the central ring (R1) in early AMD eyes were significantly reduced compared with the control eyes (51.73 ± 23.19 vs. 75.95 ± 22.89 nV/deg²; P = 0.009). Nonsignificant differences in mfERG amplitude were found between early AMD and control eyes in all the other remaining rings (R2-R6).

There was a significant increase in the mean implicit time in participants with early AMD compared with control participants, but only within the central four rings (Fig. 3B), as follows: R1 (31.61 ± 2.26 vs. 29.82 ± 1.85 ms; P = 0.028), R2 (31.22 ± 1.81 vs. 28.99 ± 1.70 ms; P = 0.002), R3 (30.06 ± 2.28 vs. 28.33 ± 1.53 ms; P = 0.018), and R4 (30.17 ± 1.89 vs. 28.51 ± 1.88 ms; P = 0.026). The differences in P1 implicit time between early AMD and control eyes at R5 and R6 were not significant.

The mean flicker sensitivity of the early AMD eyes was significantly reduced within the central three rings compared with the control eyes (Fig. 3C); R1 (19.20 ± 3.70 vs. 23.95 ± 3.65 dB; P = 0.002), R2 (19.83 ± 3.74 vs. 23.09 ± 3.22 dB; P = 0.018), and R3 (20.76 ± 2.03 vs. 22.58 ± 2.61 dB; P = 0.045). There was no significant difference in the mean flicker sensitivity of the early AMD eyes and control eyes at 10° (R4; P = 0.323).

Overall, the mfERG and the flicker perimetry findings showed some agreement that central retinal function is reduced in early AMD. However, the mfERG P1 implicit time appears to be more sensitive than the flicker sensitivity in detecting areas of reduced retinal function. A significant delayed P1 latency was detected within the central 12°, whereas

### Table 1. Summary of Demographic and Clinical Findings of Participants

<table>
<thead>
<tr>
<th></th>
<th>Early AMD (n = 15 eyes)</th>
<th>Controls (n = 14 eyes)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.47 ± 8.34</td>
<td>65.21 ± 13.00</td>
<td>0.950</td>
</tr>
<tr>
<td>Sex, F:M</td>
<td>13:2</td>
<td>8:6</td>
<td>0.080</td>
</tr>
<tr>
<td>LogMAR VA</td>
<td>−0.01 ± 0.12</td>
<td>−0.06 ± 0.12</td>
<td>0.251</td>
</tr>
</tbody>
</table>

Age and LogMAR values are mean ± SD.

**Figure 2.** Representative mfERG responses of a control participant and a participant with early AMD. Trace array, N1-P1 response density amplitude plot, and P1 latency plot for the control eye are shown on the left (A, C, E, respectively); those for the early AMD eye are shown on the right (B, D, F, respectively). Note the reduction in the central peak response amplitude (D) and the central area of increased latency (F) in the early AMD compared with the normal eye. All plots are in field view.
A significant decrease in flicker sensitivity was detected only within the central 6°.

The degree of agreement between the objective mfERG and subjective flicker visual fields can be seen qualitatively in Figure 4, which shows the change in mfERG latency (Fig. 4A) and amplitude (Fig. 4B) plotted against the change in flicker threshold for each test point.

In Figure 4A, each point was plotted according to its SD change in latency (ms) and its change in flicker sensitivity. Most locations in early AMD eyes returned both normal timing and sensitivity (74.0%). The points with significantly delayed mfERG P1 implicit times fell above the horizontal line in Figure 4A. Most of these points with delayed latency showed significant or close to significant reductions in flicker sensitivity. The reverse was not strictly true; some points with significant reductions in flicker sensitivity showed ERG latencies well within the normal range. However, a significant moderate correlation ($r = 0.477; P \leq 0.001$) was observed between changes in mfERG latency and flicker sensitivity.

Unlike delays in implicit time, amplitude loss did not appear to correlate as well with losses in flicker sensitivity (Fig. 4B). Most locations in early AMD eyes returned both normal amplitude and normal sensitivity (79.3%). Of the points with reduced sensitivity, the vast majority (82.6%) had amplitude losses within the normal range. A significant but weak corre-
Significant and marked reductions in flicker sensitivity were closely matched with the regions in which significant delays in mfERG P1 latency were recorded. Morphologically visible changes of drusen did not closely match regions returning significant delays in mfERG latency and flicker sensitivity (A). A significant, but weak correlation \( r = 0.200; P = 0.014 \) was observed between changes in mfERG amplitude and flicker sensitivity (B). Symbols represent AMD subjects.

**FIGURE 4.** Change in ERG latency (A) and amplitude (B) was plotted against the decrease in flicker threshold for each test point. Parameters expressed in standard deviation from the mean of the controls. A significant moderate correlation \( r = -0.477; P < 0.001 \) was observed between changes in mfERG latency and flicker sensitivity (A). A significant, but weak correlation \( r = 0.200; P = 0.014 \) was observed between changes in mfERG amplitude and flicker sensitivity (B). Symbols represent AMD subjects.

**FIGURE 5.** mfERG stimulus matrix (A) consisting of 103 hexagons. Flicker visual field test grid with a total of 48 points, as indicated in (B). Plots show the standard deviation from normal of the mfERG latency (C) and flicker visual field thresholds (D) of the right eye of a 71-year-old male subject with early AMD. Gray: regions of functional defect. Note the sensitivity reduction at position “1” correlated with the area of significant delays in mfERG P1 implicit time. Regions with drusen did not closely match regions of retinal dysfunction.
reductions in flicker sensitivity or delays in mfERG implicit time (Figs. 5, 6).

**DISCUSSION**

There is a lack of robust and reliable functional outcome measures that can be used to monitor and detect early functional deficits in early AMD. In this study, we have shown a significant reduction in mfERG amplitude of central responses in participants with early AMD and significantly delayed implicit times. We have also confirmed previous findings of significant losses in flicker sensitivity within the maculae of early AMD eyes compared with those of age-matched controls. These localized functional abnormalities were apparent in the absence of a decrease in logMAR visual acuity. In addition, a novel significant correlation was found between local flicker sensitivity and mfERG responses, particularly the mfERG implicit time. We therefore believe that the mfERG technique, when analyzed as described here, is a useful objective technique for detecting early functional abnormalities in early AMD. As such, this study has laid the foundation for future studies in mfERG to prospectively follow these central functional deficits over time and to evaluate mfERG as a potential tool in monitoring the progression of early AMD.

Our findings confirm the results of some previous studies, but not others, that mfERG amplitude is reduced and implicit time is increased in patients with early AMD. One possible reason for the nonsignificant differences found by some studies may be related to the method by which the mfERG data were analyzed in these studies. Spatial averaging and group averaging are known to conceal individual abnormal responses. Nonsignificant differences found in other studies could have been influenced by masking the localized abnormalities through spatial averaging (ratio = 6) and extensive group averaging of mfERG responses into two groups: the central 15° and the peripheral 15°. We believe spatial averaging and extensive group averaging are likely to mask significant alterations in central retinal function in early AMD.

Our findings of significant differences in mfERG results between the early AMD and control groups may also be related to our multifocal recording protocol. The intensity of the white hexagonal stimulus used in our study was set to 5.33 cd · s · m⁻², which is much brighter than the intensity used in all previous studies. Our fixation monitoring system (FMS III) enabled accurate real-time monitoring of fixation and unreliable segments to be discarded and rerecorded.

A few studies have examined the relationship between mfERG and psychophysical measures, such as contrast sensitivity, saturated and desaturated Panel D-15 tests, and static perimetry. However, to date, no study has investigated quantitative comparisons between subjective flicker perimetry and mfERG outcomes in early AMD. This study compared outcomes between mfERG with flicker perimetry, which our laboratory has shown exposes deeper foveal deficits in early AMD than static perimetry. We have shown regions in which significantly reduced flicker sensitivities correlated well with areas of abnormal mfERG responses, particularly P1 implicit time.

The lack of strong agreement between the mfERG parameters and flicker sensitivity may be attributed to differences in the nature of each test. Flicker visual fields represent a threshold profile, whereas mfERG yields a suprathreshold response profile. The stimulus size of the flicker perimetry spot is smaller than each respective stimulus hexagon of the mfERG. Testing by subjective visual fields will also be influenced by subjective factors such as response time and concentration. Greater concentration is required in subjective flicker perimetry than in objective mfERG because not only must subjects maintain stable fixation in subjective flicker perimetry, they must also pay attention to the peripheral field to provide reliable responses.

The novel findings of significant delays in mfERG P1 implicit time at similar locations in which marked reductions in flicker sensitivity were observed support the use of mfERG in the early detection of AMD.
sensitivity were found suggests that monitoring implicit time may have a role in following the progression of early AMD because we have found flicker perimeter useful in this regard (data not shown). Both may also provide useful outcome measures for determining the efficacy of novel treatments for early AMD. Work at our laboratory is under way to prospectively follow mfERG changes over time.

We do not believe the increase in mfERG implicit time observed in this study was due to the stray light effect for the following reasons. First, the retinal area with an increase in mfERG P1 implicit time was very similar to the retinal area of abnormal flicker sensitivity (Figs. 5, 6). Second, the drusen were too much smaller than the hexagonal element, particularly in the outer rings, to produce a significant stray light effect. Third, the background luminance level was increased to minimize the effect of stray light on the recordings. Fourth, we examined the P1 responses around the blind spot and found no significant difference in the implicit time between the high-stimulus intensity of 5.33 cd·s·m⁻² with a background luminance of 200 cd·m⁻² and the low-stimulus intensity of 2.67 cd·s·m⁻² with a background luminance of 100 cd·m⁻² (data not shown).

A criticism of similar studies that have found significant abnormal mfERG outcomes in their cohorts with early AMD has been the difference in age between the early AMD and the control groups because of the possibility that the significant mfERG differences could have been influenced by the effect of age rather than the disease itself.22 It is well known that there is a progressive decrease in N1-P1 amplitude (10.5% per decade) and a slight increase in P1 implicit time (1.0% per decade) with age.23 One of the strengths, therefore, of this study is that the ages of our early AMD subjects (65.5 ± 8.5 years) and control participants (65.2 ± 13.0 years) were well matched (P = 0.950). Thus the significant differences in mfERG outcomes found in this study did not occur as a result of age differences. In addition, the novel fixation monitoring system (FMS III) enabled accurate monitoring of fixation during ERG recording; this was not a feature of other mfERG recording protocols.

The limitations of our study include, first, the relative small sample size. Our two cohorts were, however, well matched for age, and significant results were obtained. Second, our flicker visual field testing system lacked a fundus tracking system. As such, the ability to check fixation relied on checking the blind spot and the rates of false-positive and false-negative results. Nevertheless, we have previously shown that flicker perimeter in our laboratory is highly reproducible.6 To date, unfortunately, no perimeter incorporates an accurate tracking system along with a flickering target.

In conclusion, we found that eyes with early AMD showed a significant reduction in mean foveal mfERG amplitude and an increase in mean mfERG implicit time in the central 12° compared with control eyes. Retinal areas of individual eyes with significant reductions in flicker sensitivity (within the central 6 degrees) correlated well with regions of prolonged mfERG implicit time. Aside from these eccentricities, the results between AMD subjects and controls were comparable. These findings suggest that mfERG and flicker perimeter may have a role in assessing early AMD, may be useful in monitoring the progression of disease over time, and should be evaluated in future prospective longitudinal studies. Both flicker perimeter and mfERG may prove useful functional biomarkers in the quantitative assessment of localized retinal function in early AMD, which can be followed over time to assess disease progression and may provide a useful outcome measure with which to assess the efficacy of novel interventions.

References


