Adaptation Responses in Early Age-Related Maculopathy

Beatrix Feigl, Brian Brown, Jan Lovie-Kitchin, and Peter Swann

PURPOSE. To investigate the global-flash multifocal electroretinogram (mfERG) in early age-related maculopathy (ARM).

METHODS. Thirty-two eyes from 20 healthy control subjects and 12 age-matched subjects with early ARM were investigated with the conventional and the global-flash mfERG. Early ARM subjects were graded according to an international grading system. The conventional mfERG consisted of 103 hexagons flickering according to a pseudorandom m-sequence. The global-flash mfERG paradigm used four frames starting with the conventional m-sequence stimulation, followed by a dark frame, a global flash, and another dark frame. The responses include a direct response (DR) and a later induced component (IC). The first-order kernel peak-to-trough response densities of the conventional mfERG (N1P1), the global-flash DR and IC, and the implicit times of the conventional P1, global-flash DR, and IC peak were analyzed after averaging the results into five groups according to five field locations: a central area and four quadrants.

RESULTS. There was a significant reduction of the global-flash mfERG DR response density (P ≤ 0.05) in the early ARM group compared with the control group. Neither the IC response density nor DR and IC implicit times were significantly impaired. However, the superior retina showed longer implicit times than did the inferior retina for the DR in the early ARM group. There was no significant correlation between funduscopic features and the central averaged responses of the global-flash mfERG (for the DR response density: r = -0.19, P = 0.3, or for the DR implicit time: r = -0.18, P = 0.5). None of the conventional mfERG parameters was significantly different between the two groups.

CONCLUSIONS. The global-flash mfERG detects deficits in early ARM before the conventional mfERG. Retinal ischemia may play a role in producing function impairment in ARM. (Invest Ophthalmol Vis Sci. 2005;46:4722–4727) DOI:10.1167/iovs.05-0795

Age-related maculopathy (ARM) is a condition involving different tissues of the eye: the choroidal,1–3 the retinal pigment epithelium (RPE), and Bruch’s membrane4–6 and the neurosensory retina.7–11 It is still not known where the primary defect lies, but it is clear that there are close interactions between these layers, and damage to one has consequences for the others. Reduced ocular blood flow has been demonstrated in early ARM4–5,12 but whether this causes ischemia and dysfunction of the adjacent tissues is still to be determined.3,5,13–15 However, the oxygen demand in the neurosensory retina is different in different layers.16 The outer and inner plexiform layers, where there is high synaptic activity, have higher oxygen consumption than do other retinal layers.16 It can be hypothesized that the outer and inner plexiform retinal layers are preferentially affected by ischemia in early ARM.

Cone adaptation is impaired in the early course of ARM, as shown by psychophysical tests.17,18 An objective technique to measure the adaptation function of the retinal layers has been developed with the multifocal electroretinogram (mfERG).19–21 The first-order kernel of the conventional fast-flicker mfERG is thought to reflect photoreceptor and mainly ON/OFF bipolar cell contributions22 and thus the function of retinal layers nearer to the choroid. With the conventional mfERG, conflicting findings of significant impairment23–26 or no impairment27–30 have been reported. Amplitudes or peak implicit times31 have been reported. However, reduced second-order kernels reflecting nonlinear contributions32,33 have been demonstrated in ARM.10 However, second-order kernels are small and have poor signal-to-noise ratios. The global-flash mfERG is a relatively new paradigm and is thought to detect nonlinear contributions with a better signal-to-noise ratio.25,26 It has been successfully applied in diabetics,29,30 glaucoma,31,32 and hydrochloroquine retinopathy.53 Given that there is reduced choroidal1 and central retinal artery perfusion25,54 and possible ischemia in early ARM that targets retinal postreceptoral nonlinearities first,55,56 we expect that this paradigm may be specifically advantageous. We hypothesize that the global-flash mfERG27,28,30,51 better reflects impairment of adaptation responses than the conventional mfERG in early ARM.

SUBJECTS AND METHODS

We investigated 32 eyes of 32 subjects. Of those, 20 subjects were in the healthy control group (aged between 58 and 78 years; mean, 70 ± 6 years) and were age-matched with the 12 subjects of the early ARM group (aged between 66 and 80 years; mean, 74 ± 4 years). High-contrast visual acuity was assessed with the Bailey-Lovie charts36 and was equal to or better than 6/9.5 in the healthy group and better than 6/15 in the early ARM group. Table 1 shows the characteristics and grading results of the ARM subjects. Early ARM was defined by the presence of drusen (soft distinct and indistinct) and/or RPE abnormalities (hyper- and hypopigmentation) based on photographic grading and the grids of the Age-Related Eye Disease Study Group37 performed by two experienced observers (PS, masked; BF, not masked). Advanced cataract was excluded by using templates from the Age-Related Eye Disease Study Group (AREDS).58 The tenets of the Declaration of Helsinki were followed, and informed consent was obtained from every subject.

Two different protocols were randomly presented and applied as will be described in detail later, with a multifocal ERG system (VERIS Science, 5.1.5X; EDI Inc., San Mateo, CA). The pupils were maximally

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dilated with 0.5% tropicamide and 2.5% phenylephrine. Retinal signals were recorded with DTL electrodes, and two recording files for each paradigm were collected and averaged together in most of the subjects (in 25 subjects for the conventional paradigm and in 22 subjects for the global-flash paradigm). Fixation was controlled using the eye refractor camera provided with the system. Retinal signals were band-pass filtered from 10 to 300 Hz, amplified 100,000 times, and sampled every 0.83 ms. The first-order kernels were analyzed after two iterations of artifact removal and spatial averaging (with 17% of the response of its neighbors). We measured the peak-to-trough response densities and peak implicit time for each paradigm. Given that there is nasal-temporal asymmetry of amplitudes and variation in central waveform shapes,\textsuperscript{27,30,31} we chose to divide our analyzed data into five groups (central field, temporal superior, temporal inferior, nasal superior, and nasal inferior fields) shown in Figure 1. The records of left eyes were mirror imaged to right eyes so that appropriate parts of the retina were meet there.\textsuperscript{40}

![Figure 1](image1.png)

**Figure 1.** The five-group–averaging method used for analysis is shown in grayscale.

A repeated-measures ANOVA (SPSS, ver. 11.5; SPSS, Chicago, IL) was applied to determine whether there was a location effect and/or group by location interaction. In case of a significant group by location interaction, a paired t-test was applied for further statistical analysis within this group. For correlation between the mfERG response density and implicit time values and funduscopic grading results, Spearman rank correlations were performed; the control group with no ARM changes was ranked 0, ARM subjects with AREDS level L1-LIa,b were ranked 1, and ARM subjects with AREDS levels LIIc, LIIia-c were ranked 2. $P \leq 0.05$ was considered to be statistically significant.

**Conventional Fast-Flicker, Cone-Mediated mf-ERG**

The hexagons flickered at a 75-Hz frame rate, according to a pseudorandom binary m-sequence (2$^{15}$−1) with a luminance of 199 cd/m$^2$ for the white hexagons and $<3$ cd/m$^2$ for the black hexagons, and a surround luminance of 100 cd/m$^2$ was chosen. Recordings were divided into 16 segments resulting in a total recording time of approximately 7 minutes per eye.

**Global-Flash, Cone-Mediated mfERG**

In the global-flash paradigm each m-sequence step comprised four frames (2$^{15}$−1). In frame one the first-order response was examined for a stimulus flash flickering between black and white (100 cd/m$^2$ for the white hexagons and $<3$ cd/m$^2$ for the black hexagons) as determined by the binary m-sequence. Frame 2 consisted of a dark frame ($<3$ cd/m$^2$), frame 3 of a light (global flash) frame (200 cd/m$^2$), and finally frame 4 consisted of another dark frame ($<3$ cd/m$^2$; Fig. 2).\textsuperscript{28,30}

We chose a protocol similar to that suggested by Shimada et al.\textsuperscript{28} but kept a constant surround of $77$ cd/m$^2$, as this condition was better tolerated by our subjects, rather than a surround that flashed or was kept dark according to the inserted frames.\textsuperscript{28} We analyzed thepeak-to-trough response densities and peak implicit times of the early direct component (DR) and the later induced component (IC)\textsuperscript{50} as shown in Figure 3.

**Table 1.** Characteristics of Subjects with Early ARM

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>VA</th>
<th>Grading Results (AREDS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>6/7.5</td>
<td>RPE abnormalities with pigment present and depigmentation at least questionable in the central or inner subfield (LIIc3)</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>6/15</td>
<td>Drusen size $\geq$63 $\mu$m and total area $&gt;375$ $\mu$m (LIIib)</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>6/12</td>
<td>Drusen sizes $\geq$63 $\mu$m and $\leq$125 $\mu$m and total area $\geq$125 $\mu$m (LIIa + LIIb)</td>
</tr>
<tr>
<td>4</td>
<td>79</td>
<td>6/15\textsuperscript{2}</td>
<td>Drusen size $\geq$63 $\mu$m and total area $&gt;372$ $\mu$m (LIIib)</td>
</tr>
<tr>
<td>5</td>
<td>76</td>
<td>6/7.5\textsuperscript{2}</td>
<td>RPE abnormalities with increased pigment and depigmentation at least questionable in the central or inner subfield (LIIc3 + LIIib)</td>
</tr>
<tr>
<td>6</td>
<td>74</td>
<td>6/15\textsuperscript{2}</td>
<td>Drusen size $\geq$65 $\mu$m and total area $&gt;650$ $\mu$m, RPE abnormalities with increased pigment, and depigmentation at least questionable (LIIc 3 + LIIib)</td>
</tr>
<tr>
<td>7</td>
<td>73</td>
<td>6/15</td>
<td>Drusen sizes $\geq$65 $\mu$m and total area $&gt;125$ $\mu$m (LIIa)</td>
</tr>
<tr>
<td>8</td>
<td>72</td>
<td>6/12</td>
<td>Drusen size $\geq$125 $\mu$m (LIIib)</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>6/15</td>
<td>Drusen size $\geq$63 $\mu$m and total area $&gt;372$ $\mu$m (LIIib)</td>
</tr>
<tr>
<td>10</td>
<td>77</td>
<td>6/9.5\textsuperscript{1}</td>
<td>Drusen size $&lt;65$ $\mu$m and total area $&lt;125$ $\mu$m (LII)</td>
</tr>
<tr>
<td>11</td>
<td>71</td>
<td>6/15</td>
<td>Drusen sizes $\geq$65 $\mu$m and $\leq$125 $\mu$m (LIIb)</td>
</tr>
<tr>
<td>12</td>
<td>76</td>
<td>6/12\textsuperscript{5}</td>
<td>Drusen size $\geq$65 $\mu$m and total area $&gt;125$ $\mu$m (LIIa + LIIb)</td>
</tr>
</tbody>
</table>

Superscript numbers after visual acuity denote additional letters read correctly (+) beyond the scored line, or errors made in the same or preceding line (−).

![Figure 2](image2.png)

**Figure 2.** The global-flash stimulation paradigm.
induced response (right, from 50 to 80 ms) of the right eye of a healthy control subject; this asymmetry has been demonstrated by Shimada et al.30 In Figure 4B, the topographic distribution of the scalar product response density with templates obtained from rings is demonstrated for the same control subject. In general, the DR tended to be larger centrally than the IC.30

RESULTS

The first-order kernel results for N1P1, DR, and IC response densities and peak implicit times for the five averaged group responses for each protocol are shown in Table 2.

The conventional mfERG was recorded in 20 control subjects and 12 with ARM, and the global mfERG was recorded in 19 control subjects and 11 with ARM. There were significantly reduced averaged response densities of the DR of the global-flash mfERG for the early ARM subjects compared with the age-matched control group ($F_{(1,28)} = 4.1, P \leq 0.05$). However, the averaged IC response density was not significantly different between the two groups ($F_{(1,28)} = 0.1, P = 0.7$). Figure 5 shows the data points for each subject for the response densities of the DR (crosses for each of the control subjects and squares for the ARM subjects) with lower peak-to-rough response densities on average compared to the control group.

Figure 6 shows the group averages of the five group analyses in two subjects with early ARM (subjects 2 and 12) compared with those of an age-matched healthy control subject. The DRs are reduced for the subjects with early ARM.

There was a significant location effect for the DR ($F_{(4,25)} = 19.1, P < 0.01$) and for the IC response densities ($F_{(4,25)} = 8.7, P < 0.01$), showing higher response densities for the central group compared to the other groups for the ARM subjects and the control subjects (Table 2).

There were no significant differences in the global-flash DR implicit times ($F_{(1,28)} = 0.5, P = 0.5$) and IC implicit times ($F_{(1,28)} = 0.0, P = 0.9$) between the two groups. However, there was a significant group by location interaction for the ARM group with significantly longer DR implicit times ($F_{(4,25)} = 6.3, P < 0.01$) for the inferior fields (superior retina) compared to the superior fields (inferior retina). This effect was not evident for the IC implicit times which were significantly

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**Figure 3.** The overall global-flash mfERG response is composed of a DR and a later IC. Response densities (trough to peak) and implicit times (time from onset of stimulus to peak of the DR and IC) were analyzed for each of these components.

**Figure 4.** (A) The trace array from the right eye in a control subject with normal features of the DR (from 0 to 50 ms, left) and the IC (from 50 ms to 80 ms, right) demonstrated a typical nasal temporal asymmetry with higher responses temporally. (B) The three-dimensional scalar product response density plots showed a large distinct DR peak and a smoother smaller IC peak.
The N1P1, DR, and IC response densities are expressed in nanovolts per square degree ± SEM and the peak implicit times in milliseconds ± SEM for P1, DR, and IC for the conventional and global flash mfERG.

* Significant at $P < 0.05$.
† Significant group by location interaction at $P < 0.01$.

The conventional mfERG did not show a significant difference in the N1P1 response densities ($F(1,45) = 2.8, P = 0.1$) or the P1 implicit times ($F(1,30) = 0.9, P = 0.4$) between the control group and the early ARM group. However, there was a significant location effect for N1P1 response densities ($F(4,27) = 72.7, P < 0.01$) which were significantly higher and longer, respectively, centrally compared to the surrounding peripheral areas. This phenomenon has been previously described in healthy subjects and is based on cone topography.\(^{19,41–44}\)

We correlated the central funduscopic features with N1P1 response densities and P1 implicit times (Spearman rank correlations). There was no significant correlation between funduscopic features and the central averaged responses of the global-flash mfERG, for the DR response density ($r = -0.19, P = 0.3$), or for the DR implicit time ($r = -0.19, P = 0.3$).

The results for the response densities of the DR for each subject (crosses for control subjects and squares for ARM subjects) are shown in this panel.

**Discussion**

On average, we found significantly reduced response densities of the DR with the global-flash paradigm in early ARM subjects compared with an age-matched control group. DR peak implicit times were significantly longer in the superior retina (inferior fields) than in the inferior retina (superior fields) in the ARM group. The conventional fast-flicker mfERG was not significantly impaired, suggesting that the DR of the global-flash mfERG is affected first in early ARM. We did not expect a correlation between drusen and RPE changes and the global-flash mfERG is affected first in early ARM subjects. The new paradigm is thought to target more nonlinear contributions from layers farther from the choroid.

**Figure 5.** The results for the response densities of the DR for each subject (crosses for control subjects and squares for ARM subjects) are shown in this panel.

**Figure 6.** The five-group–averaged responses of a control subject (left) and two subjects with early ARM (middle and right) with reduced DR densities are shown (arrow indicates the peak of the DR).
The DR is thought to be reduced if recovery from the preceding global flash is impaired or if light adaptation is impaired because of retinal desensitization. Although it is thought to be similar to the conventional fast-flicker response, it was much smaller centrally and slightly slower in the surrounding quadrants (Table 2). This difference may be caused by more complex dynamics of adaptation mechanisms, given that it is also influenced by the preceding global flash. We hypothesize that DR may better reflect adaptational responses and nonlinear contributions from postreceptorial layers than the conventional paradigm. Given that reduced DRs have been interpreted as early indicators of ischemic disease in diabetes, our findings suggest that ischemia may also play a role in the functional results in our patients with ARM. It is known that there is reduced ocular blood flow in early ARM. The choroid supplies the retina up to a depth of 130 μm (this includes parts of the inner nuclear layer). Thus, our results may reflect ischemia in layers farther from the choroid, such as the inner nuclear and inner plexiform layers. In addition, this region is the watershed zone between two sources of blood supply: choroid and central retinal artery and may be even more susceptible to ischemia. It has been shown that particularly the inner plexiform layer has high oxygen demands.

Although this need for an abundant oxygen supply is also evident in the outer plexiform layer, our finding of a normal mfERG suggests the ischemic insult to be less there, perhaps because of the proximity of this part of the retina to the choroid, which may provide better resistance against ischemic conditions. Retinal defects within the inner plexiform layer in ARM may be further supported by findings of reduced S-cone pathway sensitivity in early ARM and may be even more susceptible to ischemia. It has been shown that particularly the inner plexiform layer has high oxygen demands.

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In contrast to some other studies, we did not find a significant difference with the conventional mfERG between the control and the early ARM groups. This mfERG result may be explained by the different methodologies used and/or the different ARM stages investigated in these studies. However, when we analyzed our results by a concentric six-ring averaging method and investigated a younger group of healthy subjects. Thus, we cannot compare our results and possible topographical differences between younger and older subjects. However, we found longer implicit times on average in the superior retina (inferior field) than in the inferior retina (superior field) for the DR in the early ARM subjects. The differences between the hemifields found with the conventional mfERG have been suggested to be due to aging. Tzekov et al. have demonstrated that the superior retinal responses decreased faster than the inferior retinal responses with aging, and there is also lower mean blood flow in the superior retina. Remulla et al. found that delayed implicit times measured with the foveal ERG are associated with prolonged choroidal perfusion. Given that ARM may be considered an accelerated age-related process and there is also reduced blood flow with increasing age-related changes, our findings in the superior retina may have reflected their finding.

We applied a new mfERG method in early ARM and suggest that the DR of the global-flash mfERG detects reduced adaptational responses earlier than the conventional mfERG. As with the conventional mfERG, it takes less than 10 minutes and is easily applied. Our findings suggest that there may be damage in early ARM, targeting postreceptorial sites first, which are responsible for complex adaptational responses as evoked by the global-flash mfERG. Whether ischemia is the cause of these functional changes should be investigated in a longitudinal prospective study with a larger sample size. Ideally, the mfERG would be performed together with ocular blood flow measures.

References


