
Postreceptoral adaptation abnormalities in early age-related maculopathy

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Abstract

Age-related maculopathy (ARM) has become the major cause of blindness in the Western World. Currently its pathogenesis and primary site of functional damage is not fully understood but ischemia is believed to play a major role. Early detection and precise monitoring of progression of ARM are main goals of current research due to lack of sufficient treatment options, especially in the dry, atrophic form of this disease. We applied the multifocal electroretinogram (mfERG) that can detect any local functional deficit objectively in the central retina. We recorded two paradigms in early ARM patients, the fast flicker and the slow flash paradigm which both represent fast adaptation processes of the proximal retina but under differing photopic conditions and stimulation rates. By subtracting the waveform responses we extracted a late component in the difference waveform that was significantly reduced in the early ARM group compared to a healthy control group ($p \leq 0.05$). We propose that this multifocal nonlinear analysis permits the detection of adaptive deficits and provides topographic mapping of retinal dysfunction in early ARM. The difference waveform component we extracted with this novel approach might indicate early functional loss in ARM caused by ischemia in postreceptoral layers such as bipolar cells and inner plexiform regions.

Keywords: Macula, Multifocal electroretinogram, Retinal bipolar cell, Adaptation, Ischemia-hypoxia

Introduction

Age-related maculopathy (ARM) is a blinding disease of unclear pathogenesis. Many theories regarding its aetiology exist including a genetic predisposition (Klein et al., 2005) together with environmental risk factors (Bird, 2003). Ischemia caused by reduced choroidal blood flow (Pauleikhoff et al., 1990; Grunwald et al., 2005) has been suggested to play a major role in ARM (Cimbalas et al., 2004; Arden et al., 2005; Provis et al., 2005; Feigl et al., 2006).

Currently it is not possible to differentiate between the retinal layers that are first affected by ARM, objectively and *in vivo*. Hence, objective functional methods to investigate pathophysiological changes in ARM are needed. These should allow determination of the primary site of functional deficits, monitoring of progression and, ideally, detection of impairment of function before morphological changes are apparent. The multifocal electroretinogram (mfERG) is a candidate objective functional test method because it reflects fast retinal adaptation dynamics located at postreceptoral layers (Hood, 2000), where effects of ARM are most likely to be apparent (Phipps et al., 2003; Feigl et al., 2006).

It can detect functional impairment due to hypoxia (Pavlidis et al., 2005) and reveals functional deficits in other ischemic disease such as diabetic retinopathy before retinal disease is visible ophthalmoscopically (Han et al., 2004).

The mfERG uses an array of black and white hexagonal patches for stimulation. The stimulation sequence for each patch consists of long series of flashes controlled in a pseudorandom manner, by a binary m-sequence. This m-sequence stimulation mode is presented at a rate, which produces a first order kernel waveform response (mean focal flash response) at a high temporal adaptation level that requires fast interaction of retinal neurons from the entire retina (Sutter & Tran, 1992; Hood, 2000). The output waveform also contains superimposed response components or higher order kernel-series in its descending part of the waveform (Sutter, 2000; Bearnse et al., 2004). However, these are challenging to derive by conventional mfERG methods given they are small in amplitude and have poor signal to noise ratio (Palmowski et al., 2001).

We apply a new mfERG method for measuring higher order nonlinearities by using two stimulation modes, the standard fast flicker and slow flash mfERG. The slow flash response, like the fast flicker response, is generated predominantly by postreceptoral ON and OFF bipolar cells (Hood et al., 2002) but reflects less nonlinear processing effects (Bearnse et al., 2004). Our hypothesis was that subtraction of the first order slow flash mfERG waveform from first order fast flicker mfERG waveform enhances nonlinear adaptive components in the resulting late waveform.

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In early ARM there is proven impaired adaptation under photopic conditions (Brown et al., 1986; Phipps et al., 2003) compared to a healthy group as measured with subjective tests. Previously we have shown that the direct response (DR) component of the global flash mfERG which is an early waveform response is reduced in ARM (Feigl et al., 2005). In this study we determine if the late waveform components of the fast flicker mfERG are impaired in ARM.

Materials and methods

Participants

Thirty-two eyes of 32 participants were examined. Twenty participants (aged between 58 and 77 years, mean 69 ± 5) contributed to the healthy control group and were similar in age to the 12 participants of the early ARM group (aged between 66 and 80 years, mean 74 ± 4). These participants have been previously investigated with the fast flicker mfERG paradigm using a different analysis method (Feigl et al., 2005). Visual acuity was assessed with Bailey-Lovie charts (Bailey & Lovie, 1976) and was equal to or better than 6/7.5 in the healthy group and better than 6/15 in the early ARM group. Participants with high myopia (≥ 6 diopters) were excluded. Nuclear, subcapsular, and cortical cataracts greater than grade 1 from templates of the Age-Related Eye Disease Study Group (AREDS) (Age-Related Eye Disease Study Research Group, 2001b) were excluded. We defined early ARM by the presence of drusen and/or RPE abnormalities (hyper- and hypopigmentation) based on photographs and by a grading technique using the grids of the Age-Related Eye Disease Study Group (Age-Related Eye Disease Study Research Group, 2001a) performed by two experienced observers (PS, BF) who agreed in their grading results (Table 1). The ARM participants were ranked into three groups (Table 1); Group 1: smaller drusen between $63 \mu\text{m}$ and $125 \mu\text{m}$; Group 2: larger drusen greater than $125 \mu\text{m}$ and greater area than $375 \mu\text{m}^2$; Group 3: drusen with retinal pigment epithelium abnor-

malities. One observer (PS) graded funduscopy changes without knowledge of the mfERG results.

All participants who were enrolled in the study gave written informed consent and the tenets of the Declaration of Helsinki and the requirements of the University Human Research Ethics Committee of the Queensland University of Technology were followed.

Multifocal electroretinogram

A multifocal ERG system (VERIS Science, 5.1.5X, EDI Inc, Redwood City, CA) was used for all participants, and signals were detected with DTL electrodes after dilating the pupils with 0.5% tropicamide and 2.5% phenylephrine. We collected and averaged two recording files in most of our subjects (in 26 participants for the slow flash mfERG and in 25 subjects for the fast flicker paradigm) for analysis of the fast flicker and the slow flash mfERG. For the subtraction method only one recording file from each paradigm was randomly chosen. Retinal signals were band-pass filtered between 10 and 300 Hz, amplified 100,000 times and sampled every 0.83 ms. Fast flicker and slow flash mfERGs were recorded in one session and the order of testing was chosen randomly.

Fast flicker mfERG

For the fast flicker mfERG stimulation the hexagons flickered according to a pseudorandom binary m-sequence ($2^{15} - 1$) with a luminance of 200 cd/m^2 for the white hexagons and 3 cd/m^2 for the black hexagons (average luminance 100 cd/m^2) (Fig. 1A inset). The surround was kept constant with a luminance of 100 cd/m^2 and recordings were divided into 16 segments.

Slow flash mfERG

The slow flash mfERG paradigm used a stimulation mode that was slowed down by inserting three blank frames. Each step in the binary m-sequence ($2^{13} - 1$) was four frames long (Fig. 1B inset). In the first frame each hexagonal patch had a 50% probability of

Table 1. The ARM patients' characteristics

ARM	Age	VA	Grading results (AREDS)	Ranking for correlation
1	66	6/7.5	RPE abnormalities with pigment present and depigmentation at least questionable in the central or inner subfield	3
2	76	6/15	Drusen size $\geq 63 \mu\text{m}$ and total area $> 375 \mu\text{m}^2$	2
3	73	6/12	Drusen size $\geq 63 \mu\text{m}$ and $\leq 125 \mu\text{m}$ and total area $\geq 125 \mu\text{m}^2$	1
4	79	6/15 ⁺²	Drusen size $\geq 63 \mu\text{m}$ and total area $> 372 \mu\text{m}^2$	2
5	76	6/7.5 ⁺²	RPE abnormalities with increased pigment and depigmentation at least questionable in the central or inner subfield	3
6	74	6/15 ⁺²	Drusen size $\geq 63 \mu\text{m}$ and total area $> 650 \mu\text{m}^2$	2
7	73	6/15	Drusen size $\geq 63 \mu\text{m}$ and total area $> 650 \mu\text{m}^2$ and RPE abnormalities with increased pigment and depigmentation at least questionable	3
8	72	6/12	Drusen size $\geq 125 \mu\text{m}$	2
9	80	6/15	Drusen size $\geq 63 \mu\text{m}$ and total area $> 372 \mu\text{m}^2$	2
10	77	6/9.5 ⁻¹	Drusen size $< 63 \mu\text{m}$ and total area $\leq 125 \mu\text{m}^2$	1
11	71	6/15	Drusen size $\geq 63 \mu\text{m}$ and $\leq 125 \mu\text{m}$	1
12	76	6/12 ⁺³	Drusen size $\geq 63 \mu\text{m}$ and $\leq 125 \mu\text{m}$ and total area $\geq 125 \mu\text{m}^2$	1

VA ... visual acuity [superscripts denote additional letters read correctly (+) beyond the scored line, or errors made in the same or preceding line (-)]

'Ranking for correlation' is a grading based on fundus appearance provided to permit Spearman correlation with mfERG response (see text).

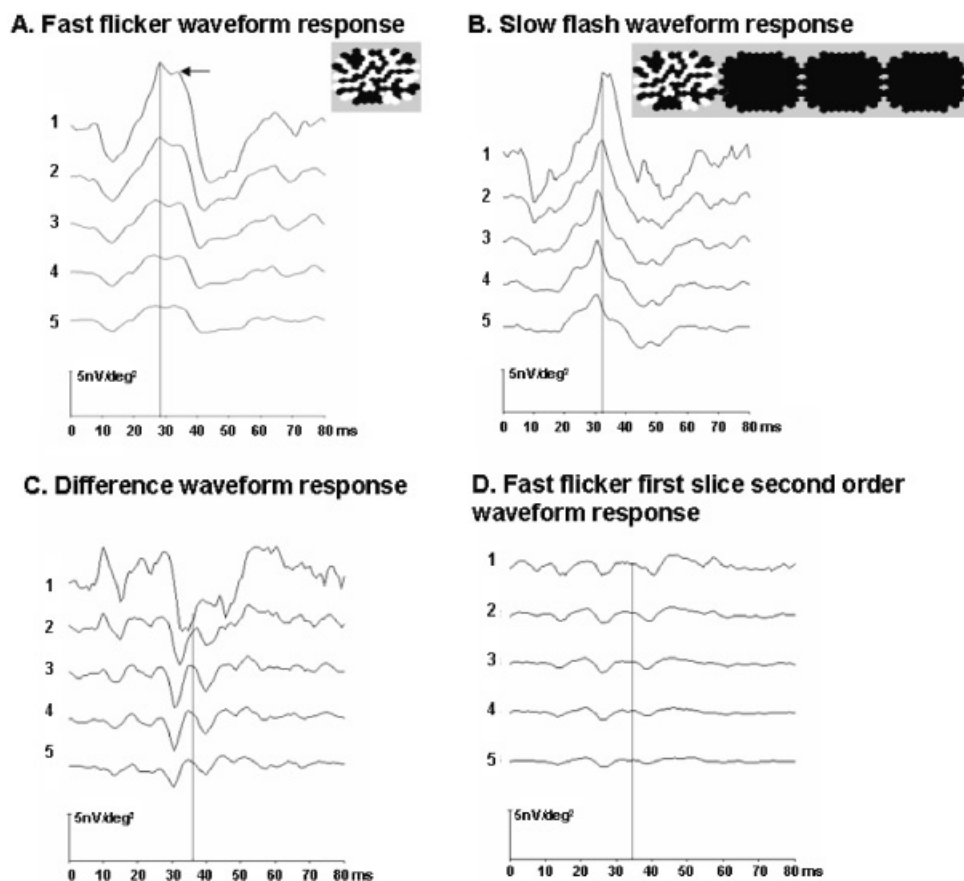


Fig. 1. The five concentric ring averages of the first order fast flicker mfERG waveform responses (1–5) demonstrate an average peak at 29 ms (vertical line) and a later shelf at about 37 ms (as indicated with arrow) most likely due to overlap of nonlinear higher order contributions. The inset (above right) indicates the one frame black and white m-sequence stimulation mode for the fast flicker mfERG (A). The five concentric ring averages for the first order slow flash mfERG has less nonlinear contributions in its descending waveform and peaks on average at 33 ms (vertical line). The inset (above left) indicates the slow flash four frame stimulation mode with a m-sequence followed by three dark frames. (B). The component resulting from the subtraction of the two first order waveforms is shown in C. This component peaks later (on average at 37.7 ms as indicated by vertical line) compared to the other paradigms. For comparison the first slice of the second order kernel of the conventional mfERG is shown in D which also reflects a nonlinear response. The averaged concentric ring second order response peaks earlier (34 ms) and is much smaller in amplitude.

flashing between white (200 cd/m^2) and black (3 cd/m^2) and the next three frames remained dark (3 cd/m^2) (average luminance 26 cd/m^2). The display surround was maintained at 52 cd/m^2 . As with the fast flicker mfERG the recordings were divided into 16 segments resulting in a total recording time of about 7 min per eye.

For all recordings, fixation was monitored using the eye refractor camera provided with VERIS 5.1. Those few-recorded segments where fixation was not satisfactory were immediately re-recorded.

Analysis

Because of the mathematical properties of the mfERG m-sequence the computation of the nonlinear kernel series (which show nonlinear dynamics of the responses of individual hexagonal patches) can be performed by means of a single cross-correlation between the binary m-sequence and the ERG response. The so-called first order kernel response can be described as the mean local response to all stimuli in a stimulus cycle. Higher order response components represent temporal interactions between focal flashes, which

are separated by two or more stimulus intervals. Higher order response components are more difficult to derive by conventional methods because of poor signal to noise ratio. This is why we concentrated on the extraction of first order responses but applied a new method for extracting higher order nonlinearities.

Recently Bearnse et al. (2004) have demonstrated nonlinear higher order contributions to the descending waveform of fast flicker first order waveform by subtracting the first slice of the second order kernel from the first order kernel. They showed that first order waveform of the slow flash mfERG was not similarly affected by nonlinearities because of increased flash intervals. In Fig. 1 we show the mfERG waveform responses as derived by the different methods to demonstrate a nonlinear effect. The waveform response of the first order fast flicker mfERG shows a shelf on its descending portion (as indicated with arrow in Fig. 1A), which is less evident in the slow flash mfERG (Fig. 1B). The mean implicit time of the first peak is at 29 ms for the fast flicker mfERG (vertical line indicates mean values averaged from the five concentric rings). The slow flash mfERG mostly avoids nonlinear contributions by increasing the interval between multifocal flashes (Sutter & Tran, 1992; Palmowski et al., 1997; Bearnse et al., 2000), which results in

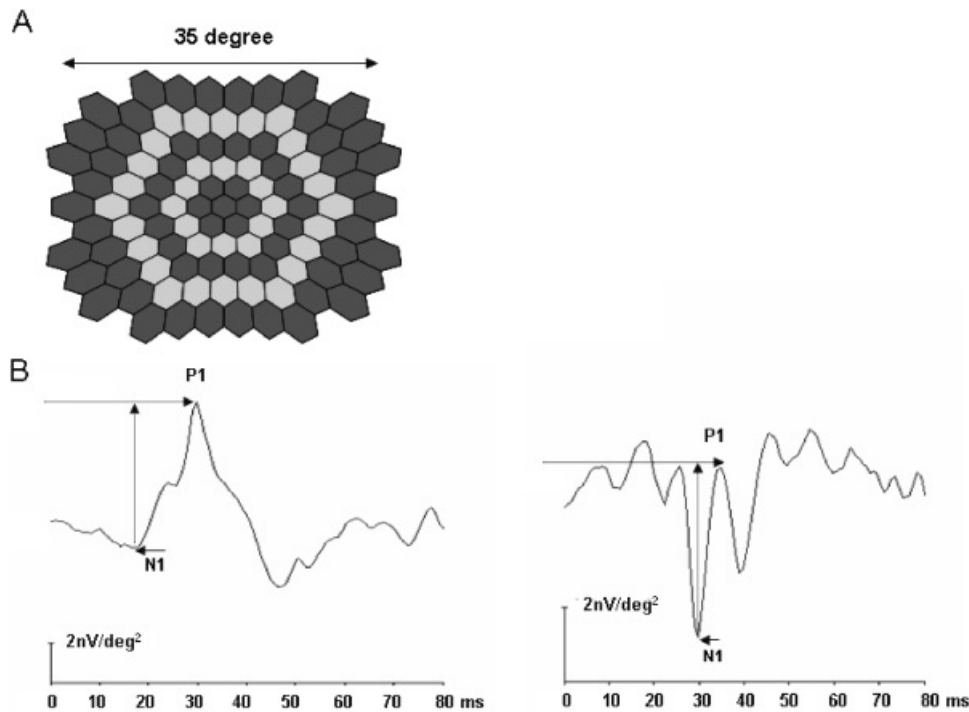


Fig. 2. The five concentric rings used for the averaging method analysis are shown in alternate gray scales on the left side (A). The trough to peak response densities and peak implicit times as they were measured for analysis of the fast flicker and slow flash mfERG data (left) and for the difference waveform mfERG (right) are shown (B).

a smoother and steeper descending waveform portion (Fig. 1B). The mean implicit time of its first peak is at 33 ms. Fig. 1C demonstrates the difference waveform that results from the subtraction of the first order slow flash waveform from of the first order fast flicker waveform. A component is evident at a mean value of 37.7 ms. For comparison, Fig 1D shows a higher order kernel waveform (first slice of the second order kernel) of the fast flicker mfERG as extracted by the VERIS software; the waveforms are much smaller in amplitude and therefore hard to distinguish from noise.

We used a five concentric ring averaging method (Fig. 2A) and analyzed the first trough to first peak N1P1 response densities and peak P1 implicit times for the fast flicker, the slow flash and the difference waveform responses as shown in Fig. 2B. We chose this analysis method based on the topography of the waveform responses that vary with eccentricity (Fig. 1). We analyzed the first order kernels after two iterations of artefact removal and spatial averaging (with 17% of the response of its six neighbors).

Changes in mfERG response densities and peak implicit data were evaluated using repeated-measures analyses of variance to establish main effects of groups, retinal locations, and their interactions. Appropriate *post-hoc* analysis was performed when significant main effects occurred. For correlation between the fundusoscopic changes and the mfERG parameters Spearman rank correlations were performed. A *p* level of ≤ 0.05 was considered to be statistically significant.

Results

The results for the N1P1 response densities and P1 peak implicit times for the averaged ring responses for the fast flicker, slow flash and difference waveform are shown in Table 2.

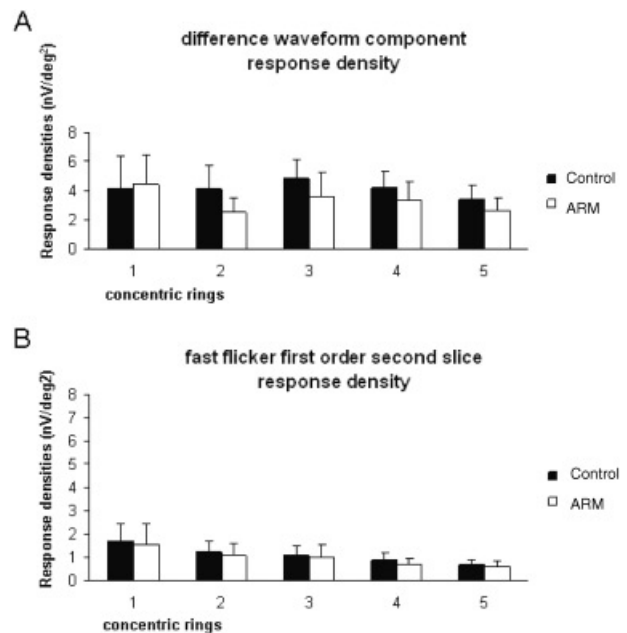


Fig. 3. The averaged ring response densities resulting from the subtraction of the first order slow flash waveform from the first order fast flicker waveform were significantly lower for rings 2–5 in the early ARM group compared to the control group. The averaged ring 2 response for the ARM group showed the lowest response compared to all other responses (A). The extracted first slice of the second order kernel of the fast flicker response (below) shows a trend of lower response densities in the ARM group compared to the healthy group; this was, however, not significant (B), even though the response was lower for all five ring responses. Error bars indicate standard deviations (SD).

Table 2. The averaged concentric ring responses from the control group and the ARM group

	Ring 1	Ring 2	Ring 3	Ring 4	Ring 5
N1P1 response density					
Fast flicker mfERG					
Control	10.1 ± 2.6	6.3 ± 1.6	4.8 ± 1.3	3.9 ± 1.1	3.3 ± 0.9
ARM	8.5 ± 3.1	5.4 ± 1.8	4.2 ± 1.2	3.4 ± 0.99	3.0 ± 0.6
Slow flash mfERG					
Control	15.1 ± 3.7	9.7 ± 2.0	8.1 ± 1.6	6.8 ± 1.4	5.5 ± 1.2
ARM	13.4 ± 3.9	8.5 ± 2.1	7.0 ± 1.2	5.9 ± 0.9	5.1 ± 0.9
Difference mfERG waveform					
Control	4.1 ± 2.2	4.1 ± 1.6	4.8 ± 1.3	4.2 ± 1.1	3.4 ± 0.9
ARM	4.4 ± 2.0	2.5 ± 0.9*	3.6 ± 1.6*	3.3 ± 1.3*	2.6 ± 0.9*
P1 peak implicit time					
Fast flicker mfERG					
Control	30.3 ± 1.8	29.1 ± 1.4	28.4 ± 1.4	28.3 ± 1.2	28.9 ± 1.5
ARM	31.0 ± 2.6	29.6 ± 1.5	28.6 ± 1.2	28.3 ± 1.0	27.7 ± 1.3
Slow flash mfERG					
Control	35.3 ± 1.4	33.6 ± 1.3	32.4 ± 1.4	31.9 ± 1.3	31.4 ± 1.3
ARM	35.3 ± 2.1	33.8 ± 0.9	32.6 ± 0.9	31.9 ± 1.0	31.3 ± 1.0
Difference mfERG waveform					
Control	39.9 ± 2.3	38.2 ± 2.0	37.4 ± 1.7	36.7 ± 1.4	36.3 ± 1.3
ARM	39.2 ± 2.1	38.3 ± 1.8	37.4 ± 1.4	36.8 ± 1.2	36.6 ± 1.6

* $p \leq 0.05$ statistically significant.

The N1P1 response density is shown in nV/deg², the P1 peak implicit times in ms ± SD.

There were no significant differences in the N1P1 response densities (fast flicker mfERG: $F_{1,30} = 2.2$, $p = 0.2$, slow flash mfERG: $F_{1,30} = 2.7$, $p = 0.1$) or P1 implicit times (fast flicker mfERG: $F_{1,30} = 0.002$, $p = 0.97$, slow flash mfERG: $F_{1,30} = 0.01$, $p = 0.91$) between the 2 groups. However, subtracting the first order kernel slow flash waveform from the first order fast flicker mfERG waveform resulted in a late component in the difference waveform that was significantly lower in trough to peak response density ($F_{1,30} = 4.1$, $p \leq 0.05$) for the early ARM group compared with the control group for rings 2–5 (Fig. 3). There was no significant location effect for the ARM group for this component ($F_{4,27} = 1.9$, $p = 0.1$) (which would indicate a preferentially affected eccentricity), although ring 2 showed the lowest averaged group response densities compared with the control group (Fig. 3A, Table 2).

Fig. 3B demonstrates the first slice of the second order kernel response of the fast flicker response. The responses are lower in amplitude for the ARM group but this was not significant ($F_{1,30} = 1.1$, $p = 0.3$).

The peak implicit times for the difference waveform component were not significantly different ($F_{1,30} = 0.02$, $p = 0.9$) between the groups (Table 2).

Fig. 4A shows the averaged five concentric rings of the difference waveform for three representative ARM patients and one healthy control (left). The resulting component (framed) is lower in response density in each of the three early ARM participants compared to the healthy participant. Fig. 4B demonstrates the corresponding fast flicker and slow flash mfERG trace arrays of these participants as well as the trace array (far right) of the late difference waveform component (note time scale from 32 to 42 ms). Slightly reduced amplitudes for the ARM patients compared with the control participant were evident for the fast flicker and slow flash mfERG each, but this was not significant for the averaged group data. In contrast the difference waveform component was reduced paracentrally as well as peripherally in these ARM patients compared to the control.

There was no significant correlation between fundus changes and the difference waveform component, either for the

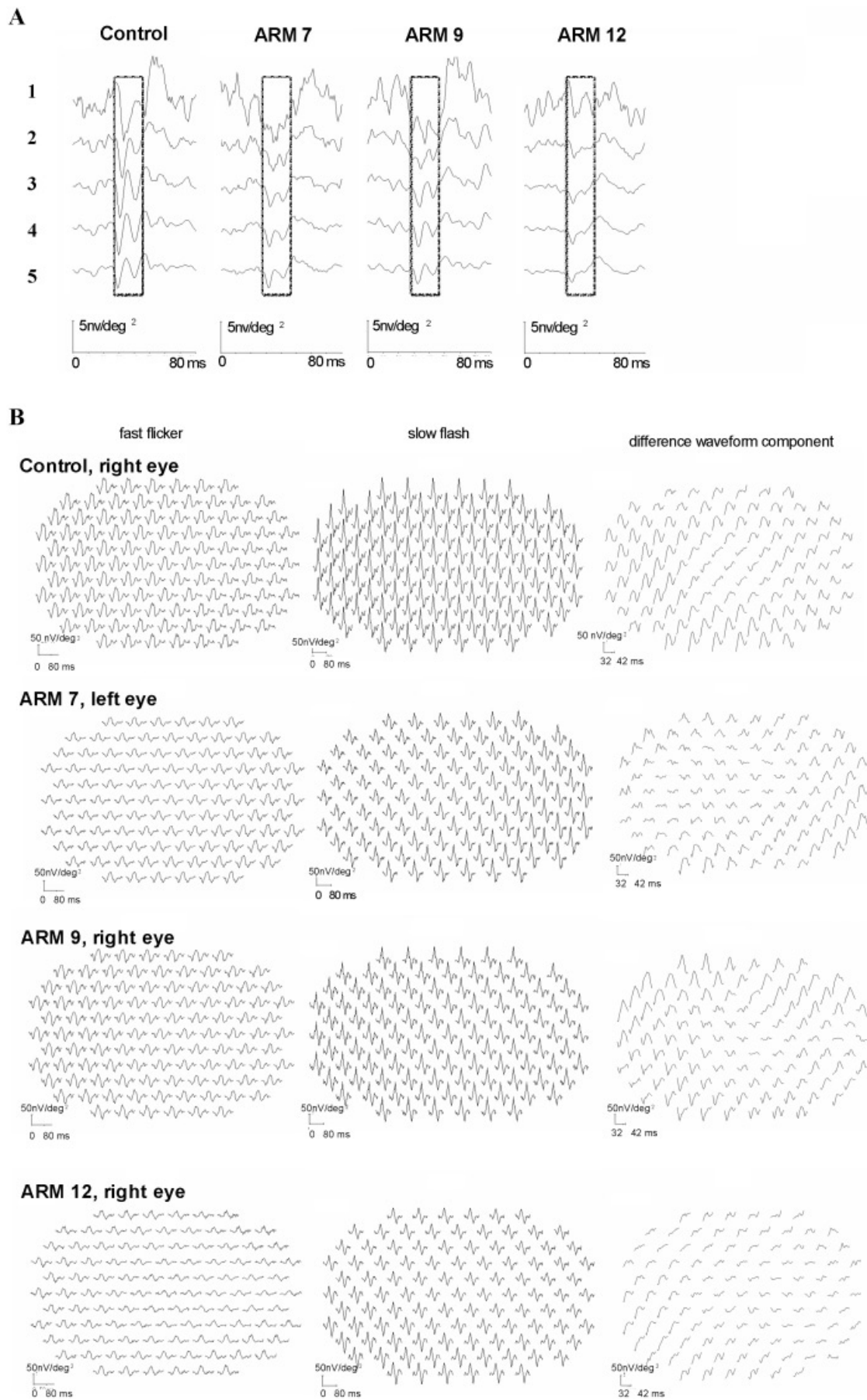
response densities or for the peak implicit times for any of the concentric rings.

Discussion

We found a significantly reduced component in the concentric ring average responses for the early ARM group compared to an age-similar control group. Neither the fast flicker nor the slow flash mfERG paradigm itself discriminated significantly between the two groups. The component we extracted was located in the later part of the difference waveform response and appeared to be less evident in the fast flicker first order waveform response. We propose that it may be dependent on the different stimulation rates but also reflects a nonlinear higher order effect resulting from different adaptive states. The test conditions we chose reflect different adaptive levels with average luminance levels of 100 cd/m² and 26 cd/m² for the fast flicker and slow flash mfERG, respectively. Our objective test results confirm subjective findings (Brown et al., 1986; Phipps et al., 2003) that ARM patients have reduced ability to adapt under photopic conditions compared to healthy controls.

We suggest various reasons why ring 1 was not significantly affected in the ARM group compared to the healthy group (Fig. 3). Firstly, ARM starts paracentrally (Sarks et al., 1988; Swann & Lovie-Kitchin, 1991) and ring 1 covered an area still less affected than the paracentral area by ARM. This is supported by the finding that ring 2 demonstrated the lowest averaged response densities compared to the other rings in the ARM group (Table 2, Fig. 3) which probably reflects the most vulnerable, paracentral zone. Secondly, contributions of nonlinearities to the waveform are better detected with increasing eccentricity (Sutter & Bearse, 1999; Fortune et al., 2002; Hood et al., 2002) than centrally as shown in our results where central responses were small in both the healthy and the ARM group (Fig. 4B). This makes non-significant differences between the two groups most likely.

Progressive fundus changes were not significantly associated with lower response densities of this late component. This is



consistent with poor correlation between fundusoscopic visible features and both, objective (Gerth et al., 2003; Feigl et al., 2004; 2005; Gerth et al., 2006) and subjective (Sunness et al., 1988) measures in early ARM.

Which retinal sources contributed to the component we extracted? Whereas the fast flicker and the slow flash mfERG mainly reflect ON and OFF bipolar cell responses, nonlinear higher order contributions are superimposed on the first order fast flicker paradigm from postreceptoral adaptative interactions (Sutter, 2000; Bearnse et al., 2004). We suggest that these become more evident with our difference waveform method. The first order kernel contains all response features that correlate with the stimulus sequence, including the effects induced by an immediately preceding stimulus to the response and the induced effects from other preceding stimulations at the same location or stimulations at neighboring locations (Sutter, 2000). The component we extracted therefore might represent an induced effect from different stimulation rates at a different adaptation level and, because of its timing, might reflect nonlinear dynamics resulting from postreceptoral interactions. As with our findings (Fig. 1A) Hood et al. (2002) have identified a shelf in the descending part of the first order fast flicker mfERG waveform (at about 35 ms), which was removed by NMDA (N-methyl-D-aspartic acid) in primates. NMDA is a glutamate agonist that depolarizes cells with NMDA receptors, which are found on some types of amacrine cells (Massay, 1990). This NMDA-sensitive component is also present in humans and is more prominent with increasing eccentricity (Hood et al., 2002). The shelf seen in our results (Fig. 1A) also becomes more prominent within the more peripheral rings and appeared to be similar in timing to our difference waveform component (Fig. 1C, Fig. 4B).

Palmowski et al. (1997) investigated higher order nonlinearities by extracting higher order kernel responses from the fast flicker mfERG in healthy persons and in patients with diabetic ischemic disease. They stated that multifocal nonlinear analysis permits the detection of subclinical diabetic retinopathy and offers the advantage of topographic mapping of retinal dysfunction in diabetes. They demonstrated a feature in the second order kernel responses, similar in timing to our component. By using a concentric ring averaging method they found a component with peak implicit times between 38–40 ms that was markedly reduced in their diabetic patients. Palmowski et al. (1997) suggested that this feature can only exist in the presence of a nonlinear effect that lasts over at least two mfERG base periods, and concluded that its reduction in their patients with ischemic disease was caused by changes in the adaptative mechanisms located in the middle/inner retinal layers (Palmowski et al., 1997). Greenstein et al. (2004) investigated nonlinearities of the fast flicker mfERG in patients with various hereditary retinal diseases such as retinitis pigmentosa and progressive cone dystrophy. Interestingly, their patients had relatively large first order responses as opposed to the expected reduced responses (Hood et al., 1998) but had impaired or non-recordable second order kernel responses. Damage proximal

to the photoreceptors involving inner plexiform cell layers has been suggested in retinitis pigmentosa (Strettoi et al., 2002). The presence of large first order responses suggests to us impaired second order negative feedback mechanisms. Similar to Palmowski et al. (1997), Greenstein et al. (2004) concluded that impairment of the higher order nonlinearities reflect an abnormality in the mechanisms of adaptation in the mid- and inner-retinal function. We have also extracted the second order kernel response from the fast flicker mfERG and found lower responses in the early ARM group which did not differ significantly from those of the healthy group (Fig. 3B). We believe that this might be mainly caused by lower amplitude responses and reduced signal to noise ratio of the higher order responses as extracted by the mfERG system (see Fig. 1D). However, we propose that the difference waveform component, which we have shown here reflects a non-linearity, similar in origin to the second order kernel response but with larger amplitudes and therefore better signal to noise ratio. We suggest that it represents an abnormal adaptation response located within the bipolar cell or inner plexiform layer.

Our results give better insight into possible retinal mechanisms involved in early ARM. We have recently proposed a model for early ARM where an ischemic insult provides an initial stress to postreceptoral, mid-retinal layers (Feigl et al., 2006). This might be mainly because of preferential vulnerability to ischemia within this region (Cringler et al., 2002), which is avascular, is distant from the choroids, and is in the watershed zone between two blood supplies, the choroidal and retinal circulations (Hayreh & Gartner, 1990). We suggest that the responses from this region are reflected by our difference waveform method. Vulnerability to ischemia in this area is also supported by our recent findings with the global flash ERG in the same ARM group where we found a significantly reduced direct response compared to the healthy group (Feigl et al., 2005). These two methods, the global mfERG and difference waveform method most likely reflect different mechanisms that are impaired in early ARM. For clinical use the difference waveform method might be more advantageous than the global flash paradigm. Performing the slow flash paradigm in addition to the usually routinely applied fast flicker protocol might be less stressful for patients due to the lower light level. The global flash protocol on the other hand utilizes interleaved high luminance flashes, which can cause greater discomfort.

In summary, we propose that the difference waveform component we extracted detects impaired fast adaptation abnormalities in the early course of ARM. It could reflect ischemic conditions that possibly trigger the genetic predisposition in ARM (Hageman et al., 2005). Although we have not confirmed ischemia with blood flow measures in our study there is considerable evidence of reduced choroidal perfusion in early ARM (Pauleikhoff et al., 1990; Chen et al., 1992; Grunwald et al., 1998; Ciulla et al., 1999; Grunwald et al. 2005). In future the new mfERG methods described previously (Feigl et al., 2005) and in this study, could serve as a clinical screening test for ischemia of postreceptoral layers even before there is ophthalmoscopic evidence of ARM.

Fig. 4. The five concentric ring averaged responses show a late reduced trough to peak difference waveform component between 36 ms to 40 ms after subtracting the first order kernel slow flash waveform from the fast flicker flash waveform in the ARM subjects compared to the normal subject (A). The fast flicker (left) and slow flash (right) mfERG trace array of a healthy control participant and for patients 7, 9, and 12 are as well as the trace array of the extracted difference waveform component (far right, note time scale is between 32 and 42 ms) are shown (B).

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