Temporal interactive response is resistant to cloudy ocular media in the slow flash double-stimulation multifocal electroretinogram

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Abstract

Purpose

To examine the influence of cloudy media on the slow flash double-stimulation multifocal electroretinogram (mfERG).

Methods

Slow flash double-stimulation mfERG responses were measured from twenty-six subjects with normal ocular health under normal and light scattering conditions (induced using acryclic sheets) (Experiment 1) and another nine cataract patients before and after cataract surgery (Experiment 2). The amplitudes and implicit times of the first (M^1) and second (M^2) stimulation were compared under normal and light scattering conditions in Experiment 1; and they were compared pre- and post-cataract surgery in Experiment 2.

Results

Compared to control conditions (normal and post-cataract surgery), the M^1 amplitude in the central region was significantly reduced in light scattering conditions (acrylic sheets and pre-cataract surgery); the M^2 amplitude and both M^1 and M^2 implicit times of all regions examined were moderately affected in pre-cataract surgery. The M^1/M^2 amplitude ratio and implicit time ratio were virtually unaffected in cloudy media for either central or mid-peripheral regions.

Conclusion

Cloudy media affects the mfERG amplitude and implicit time in the slow flash double-stimulation, but does not affect the response ratio (i.e. M^1/M^2 amplitude ratio and implicit time ratio) between the two stimulations. This suggests that the ratio analysis can be applied in patients with mild to moderately cloudy ocular media to evaluate the functional integrity of the retina.

Introduction

The multifocal electroretinogram (mfERG) examines the regional response of the retina in only a few minutes by using fast flickering stimulation.[1] It can detect a variety of retinal disorders such as glaucoma [2], diabetic retinopathy [3], and retinitis pigmentosa [4, 5]. However, certain ocular disorders such as diabetic retinopathy are associated with the development of cataract.[6] A number of studies have shown that the mfERG response is adversely influenced under light scattering conditions.[7-9] This would be expected to restrict the use of the standard mfERG in clinical situations, most especially for the eye diseases common in geriatric populations.

The presence of cloudy ocular media degrades the spatial detail presented in the retinal image of the mfERG stimulus. Vision examination involving temporal vision may be an alternative way of assessing retinal function behind media opacities. Critical flicker frequency, which is a psychophysical measurement of the ability to resolve flickering stimuli as separate entities, is reported to be less likely to be influenced by the presence of media opacities.[10] It is affected by the presence of retinal diseases such as retinitis pigmentosa,[11] glaucoma[12, 13] and diabetic retinopathy.[14]

MfERG measurement also involves temporal interactive responses between consecutive stimulations.[15] Chan, Siu, Yap and Brown[8] found that the conventional macular mfERG response for fast flickering stimulation shows only 30% reduction with increasing levels of

scattering even when visual acuity reduces from 6/6 to 6/18 (3 times reduction). This suggests that the mfERG might be resistant to media opacities to a certain extent.

Our laboratory has recently developed a slow flash double-stimulation mfERG paradigm, which emphasizes the temporal interactive response between two consecutive flashes. This new protocol can detect early functional loss before signs of diabetic retinopathy are present[16] and before inner retinal defects such as glaucoma are evident.[17] In view of the fact that temporal visual function is relatively resistant to ocular media opacities and mfERG response examines the temporal interactive response of the retina, the present study aims to investigate the influence of light scattering (induced by a number of acrylic sheets) and by cataract, on the slow flash double-stimulation mfERG.

Methods

Experiment 1: Effect of external light scattering on mfERG response

Subjects

Twenty-six subjects aged from 42 to 61 years (mean = 51 ± 5; median = 52 years) were recruited from the Optometry Clinic of The Hong Kong Polytechnic University. All received an eye examination including refraction, biomicroscopy and fundus examination. Those with history of epilepsy, any known systemic diseases or ocular diseases, were excluded from the study. The research procedure followed the tenets of the Declaration of Helsinki, and was approved by the Human Ethics Committee of The Hong Kong Polytechnic University. Informed written consent was obtained from the subjects before the start of the study.

Multifocal ERG stimulation

The stimulus pattern consisted of 103 hexagons scaled with eccentricity (stretch factor: 10.46), and was presented on a 22" color liquid crystal display (Model: VX2260wm, ViewSonic, China). The stimulation was produced by the Visual Evoked Response Image System (VERIS) (Version 5.0.9, Electro Diagnostic Imaging Inc., San Mateo, CA, USA). The hexagonal pattern subtended 40° horizontally and 34° vertically at the working distance of 40 cm. The slow flash double-stimulation mfERG paradigm consisted of 5 video frames in each cycle and was initiated with two frames of multifocal flash (i.e., M^1 and M^2) and was followed

by 3 dark frames (i.e., “OOO”);[16] the frame rate was 75 Hz (Figure 1a(i)). For the first two frames with multifocal flash, each hexagon was temporally modulated between bright and dark, and the two multifocal flash frames were manipulated by two independent pseudorandom binary m-sequence (2^{12}). The luminance of the white and dark hexagons of the multifocal flash was set at 171 cd/m^2 and 1 cd/m^2 respectively. The dark frame was also set to 1 cd/m^2 and the background luminance of the monitor was 109 cd/m^2.

Multifocal ERG recording

The pupil of the tested eye was dilated with 2 drops of 1% Tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) to at least 6 mm before recording. A Dawson-Trick-Litzkow (DTL) electrode was placed at the inferior cornea of the tested eye as an active electrode. Gold cup surface electrodes were placed 10 mm from the ipsilateral outer canthus and on the forehead as reference and ground respectively. The signal was amplified 20,000 times and the bandpass was from 10 to 100 Hz (Model: ICP511C, Grass Teletactor, Warwick, RI, USA). The total recording time was 4 minutes and 32 seconds, which was divided into 16 slightly overlapping segments for recording. The responses for the first (M^1) and second multifocal flash (M^2) were recorded separately as two time slices by the software. The signal was monitored in real-time using the VERIS system and any response segment contaminated with artifacts such as blinks or eye movements were rejected and re-measured immediately.

The mfERG examination was performed under normal (no light scattering) and light scattering conditions. The order of condition was randomized to each subject. For the normal condition, the refractive error of the tested eye was corrected for the working distance using 35mm diameter spectacle trial lenses; the untested eye was occluded during the examination. For the light scattering condition, three acrylic sheets were interposed between the lens and the display. This sheet caused a reduction of acuity by 0.20 logMAR (two lines on the Early Treatment Diabetic Retinopathy Study (EDTRS) chart used).

Experiment 2: Effect of cataract on mfERG responses

Subjects

In order to compare the above stimulation protocol to a clinical situation, nine cataract patients aged from 59 to 77 years (mean = 69 ± 7 years, median = 69 years) were recruited from a private cataract clinic. All had received a detailed eye examination, and were free of eye disease apart from cataract. Patients with systemic diseases and history of epilepsy were excluded. Subjects received a mfERG examination on the day of cataract surgery and 14 days after the surgery. The same surgeon performed the cataract surgery with phacoemulsification for all patients. The pre-operative visual acuity of the subjects ranged from 6/9.5 to 6/60 (mean = 0.44 ± 0.26 logMAR, ~ 6/15) and the post-operative visual acuity was from 6/6 to 6/15 (mean = 0.12 ± 0.12 logMAR, ~ 6/7.5).

Multifocal ERG recording

The mfERG stimulus consisted of 37 hexagons scaled with eccentricity (stretch factor: 13.18) (Figure 1a(ii)). The number of hexagons was reduced compared to Experiment 1 in order to improve the signal-to-noise ratio of each local region, although this reduces the resolution of the response topography. The hexagonal pattern also subtended 41° horizontally and 44° vertically at the working distance of 33 cm. The stimulus was presented and manipulated in the same way as in Experiment 1. The mfERG recording followed the procedure used in Experiment 1.

Analysis

Experiment 1

The mfERG responses were pooled into 6 rings for analysis (Figure 1b(i)). Only the first order kernel of the first (M^1) and second (M^2) multifocal flash responses were analyzed. The amplitude was obtained by using peak-to-peak measurement and the implicit time was measured from the onset of stimulus to the response peak. The amplitude and implicit time ratios of M^1 to M^2 stimulations were also calculated and compared between normal and light scattering conditions.

Statistical Packages for the Social Sciences (SPSS 15.0, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Two-way repeated-measures ANOVA was performed using scatter/no scatter and eccentricity (Ring number) as factors. Bonferroni post hoc tests were performed to evaluate changes in response. These analyses were performed for mfERG amplitude and implicit time, and for M^1/M^2 response ratio.. The level of significance was set at 0.05.

Experiment 2

The analytical method was the same as for Experiment 1, except that the local mfERG responses were pooled into 4 concentric rings for analysis (Figure 1b(ii)).

Results

Experiment 1

Figure 2 shows the typical mfERG waveforms of the first (M^1) and second (M^2) multifocal flash frame in this paradigm recorded from one of the subjects under normal and light scattering conditions. The response was made up of a negative trough and then a positive peak. The M^1 amplitude was significantly reduced under the light scattering condition compared to the normal condition for ring 1 ($p < 0.001$) but not for the other regions (Figures 2a and 3a). In contrast, the M^2 amplitudes (Figures 2b and 3b), and both M^1 and M^2 implicit times (data not shown) of all regions were virtually the same under both conditions. The M^1/M^2 amplitude ratios showed no significant change, except for ring 6 ($p < 0.01$) (Figure 3c). In addition, the M^1/M^2 implicit time ratios showed no significant change for any region examined (data not shown).

Experiment 2.

Figure 4 shows the typical response waveforms of the M^1 and M^2 stimulations before and after cataract surgery from one of the cataract subjects. For all regions, the waveforms of both M^1 and M^2 stimulations consisted of a negative trough followed by positive peak. Quantitatively, both the M^1 and M^2 amplitudes of all regions examined were consistently increased after cataract surgery but the relative increment in amplitude decreased from central

to peripheral region (Figures 5a and 5b). Only the increment of M^1 amplitude at rings 1 and 2 was statistically significant (all $p < 0.05$). In contrast, both the M^1 and M^2 implicit times of all regions examined were delayed after the cataract surgery by about 1 ms (Figures 5d and 5e) but only the change of M^1 implicit time for rings 3 and 4 (all $p < 0.05$) (Figure 5d) and M^2 implicit time at ring 3 ($p < 0.05$) (Figure 5e) were statistically significant. Interestingly, both amplitude and implicit time ratios of M^1 to M^2 response remained almost unchanged after surgery (Figure 5c and 5f).

Discussion

The central M^1 response amplitude was affected by light scattering (ring 1) and by the presence of cataract (rings 1 and 2) (Figure 3a and 5a). The M^1 response of the slow flash double-stimulation mfERG and the first order kernel of conventional mfERG are very similar in terms of the mathematical derivation of the response. Both protocols represent the averaged response to a bright stimulus. However, the late portion of the slow double-stimulation paradigm consists of less overlap of the response associated with subsequent flashes, owing to the presence of three dark frames at the end of each cycle. Previous studies using conventional mfERG stimulation showed that the central mfERG response amplitude was affected by cataract[18, 19] and light scattering.[8] Our findings for the M^1 response are consistent with previous studies showing that the central mfERG response amplitude is influenced by light scatter, including that produced by cataract.

Both the M^1 and M^2 implicit times of all regions were generally delayed after cataract surgery in this study, especially the paracentral M^1 implicit time (at rings 3 and 4) and M^2 implicit time (at ring 3) being significantly increased (Figure 5c), but has not been reported in previous studies with conventional mfERG with simulated media opacities[7-9] or after cataract surgery.[18-20] The discrepancy may be caused by the differences in the mfERG stimulation paradigm. Moreover, previous studies only studied subjects with visual acuity reduced to 6/18, but the present study included some cataract patients with visual acuity as low

as 6/60. The implicit time of the mfERG responses was reported to be lengthened with increasing stimulus contrast levels.[21] We speculate that increased M^1 and M^2 implicit times after cataract surgery are probably related to the increased retinal image contrast after surgery.

The M^2 response was moderately increased in amplitude and lengthened in implicit time after cataract surgery (but only the IT increase for ring 3 was statistically significant (Figure 5e)). The M^1 and M^2 responses were manipulated with two independent binary m-sequences. The M^1 response only involves a direct response to the M^1 stimulation, and the M^2 response also involves a direct response to the M^2 stimulation and the temporal interaction with preceding flashes in the M^1 stimulation.[16, 17] The cloudy media (for both the external light scattering condition and cataract) would reduce the light intensity of the stimulus projected onto the retina and so produce weaker response. This was reflected by the reduction of M^1 and M^2 response amplitudes (Figure 5a and 5b), although no statistical change in M^2 response was shown (Figure 5e). This also illustrates that the change of both M^1 and M^2 responses can be purely influenced by optical effects, and these changes may be due to factors other than retinal diseases.[16, 17]

We found that the amplitude and implicit time ratios of M^1 to M^2 response (i.e. M^1/M^2 amplitude ratio and M^1/M^2 implicit time ratio), representing the temporal interaction between two consecutive stimulations from M^1 and M^2, were generally unaffected under conditions of light scattering and cataract (Figure 3c, 5c and 5f). Since the luminance of the first two

multifocal flashes in the slow double-stimulation paradigm is the same, the cloudy ocular media affects the strength of both stimuli equally. The M^2 response changes proportionally, according to the strength of the M^1 response. The response ratio represents the adaptive change of the retina after the preceding stimuli (i.e. the first multifocal stimulations), which should be influenced by the functional recovery of retina. Critical flicker frequency (CFF), which measures the temporal processing mechanism of the visual system, has also been reported to be resistant to artificially cloudy media[10] and cataract.[22-24] However, CFF is strongly affected by different retinal diseases[11-14] and the concurrence of media opacities and retinal disorders.[25] It has been suggested that the flicker response related to visual temporal interaction is less likely to be degraded by the media opacities.[10] Hence, the ratio analysis of the first (M^1) and second (M^2) stimulation of the mfERG response, which is based on temporal processing mechanisms in the retina and reflects the functional integrity of the retina, is unaffected by the presence of cloudy media.

In conclusion, this study has shown that the amplitude and implicit time of the responses from both stimulations of this special paradigm were affected by the presence of cataract or light scattering. On the other hand, the amplitude and implicit time ratios between M^1 and M^2 responses were nearly resistant to cataract. Together with our recent studies,[16, 17] these suggest that the ratio analysis in the slow flash double-stimulation mfERG measurement may be applied to detection of retinal diseases, even in the case of moderately cloudy ocular media.

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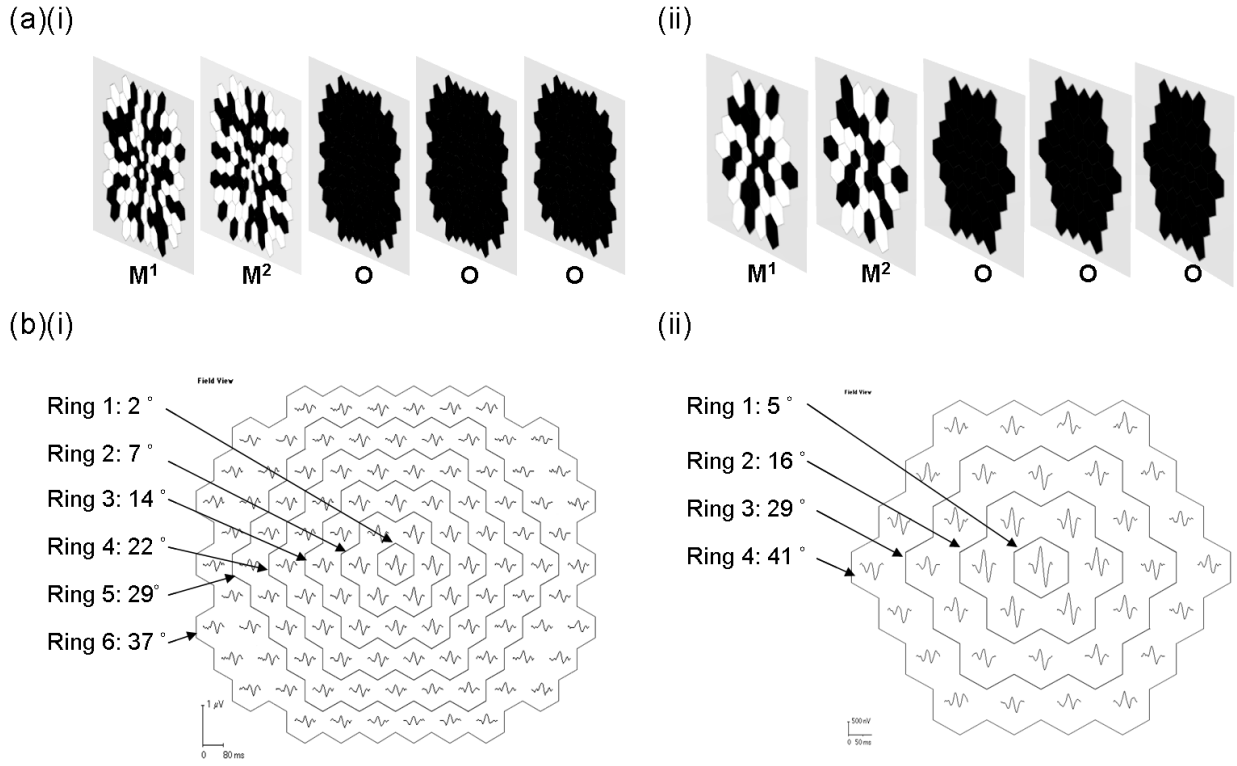

Figure 1. (a) Stimulus sequence of the video frames in the slow double-stimulation mfERG paradigm (M^1M^2OOO). The hexagonal array consisted of (i) 103 and (ii) 37 scaled hexagons for simulated media opacities (Experiment 1) and cataract patients (Experiment 2) respectively. Both of the stimulation sequences were initiated with the two independent frames of multifocal flash (i.e. namely, M^1 and M^2 for the first and second multifocal flash, respectively) following by three dark frames (i.e. namely as 'O' in the diagram) in each cycle. (b) The local responses were pooled into different regions using concentric rings. The 103 and 37 local mfERG responses, as in Experiment 1 and 2 respectively, were grouped into (i) 6 and (ii) 4 concentric rings. The horizontal eccentricity boundary of each ring is indicated.

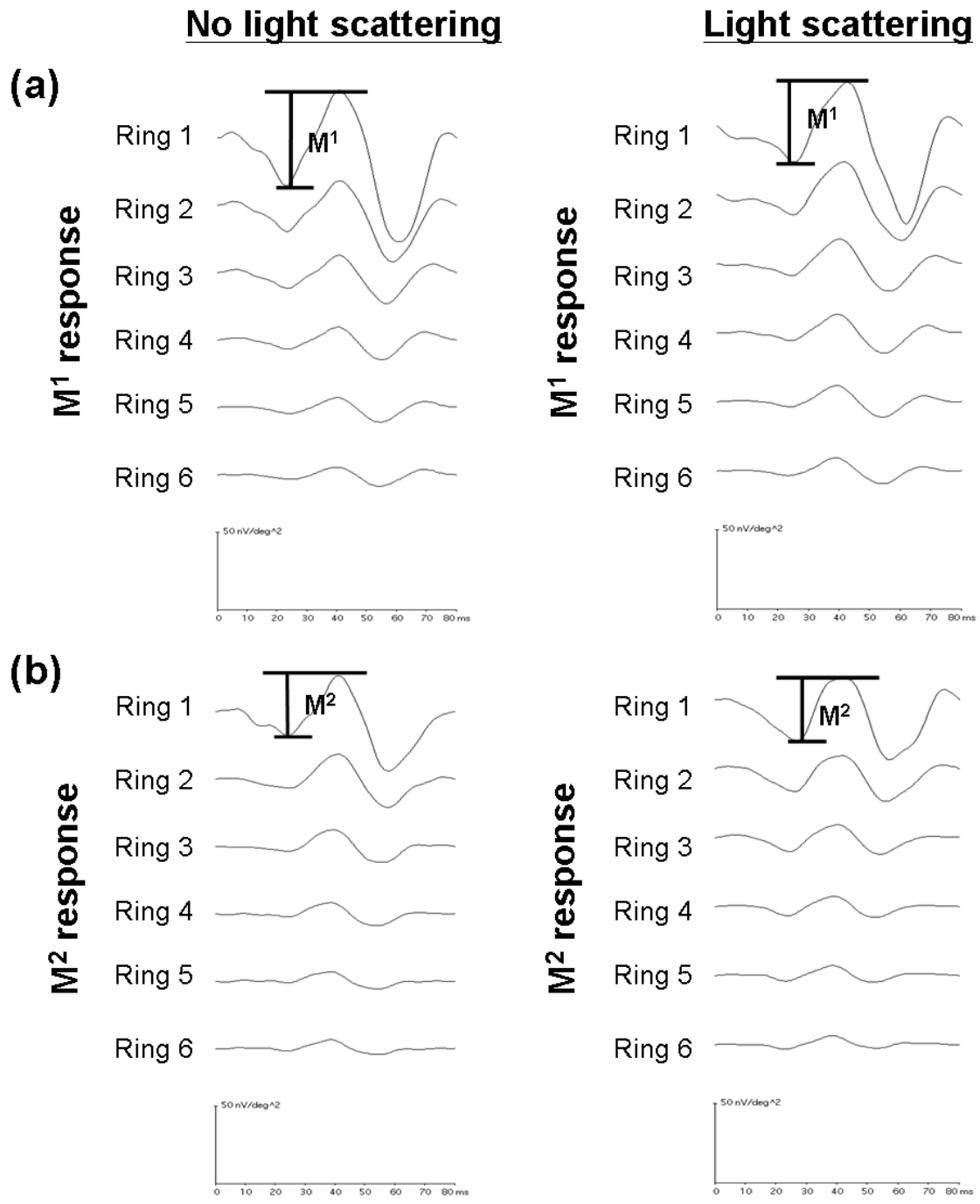

Figure 2. The typical waveforms measured from one subject under normal (no light scattering) (left) and light scattering (right) conditions for the first (M^1) (a) and second (M^2) (b) multifocal flash frame for rings 1-6 (see Figure 1(b)(i)).

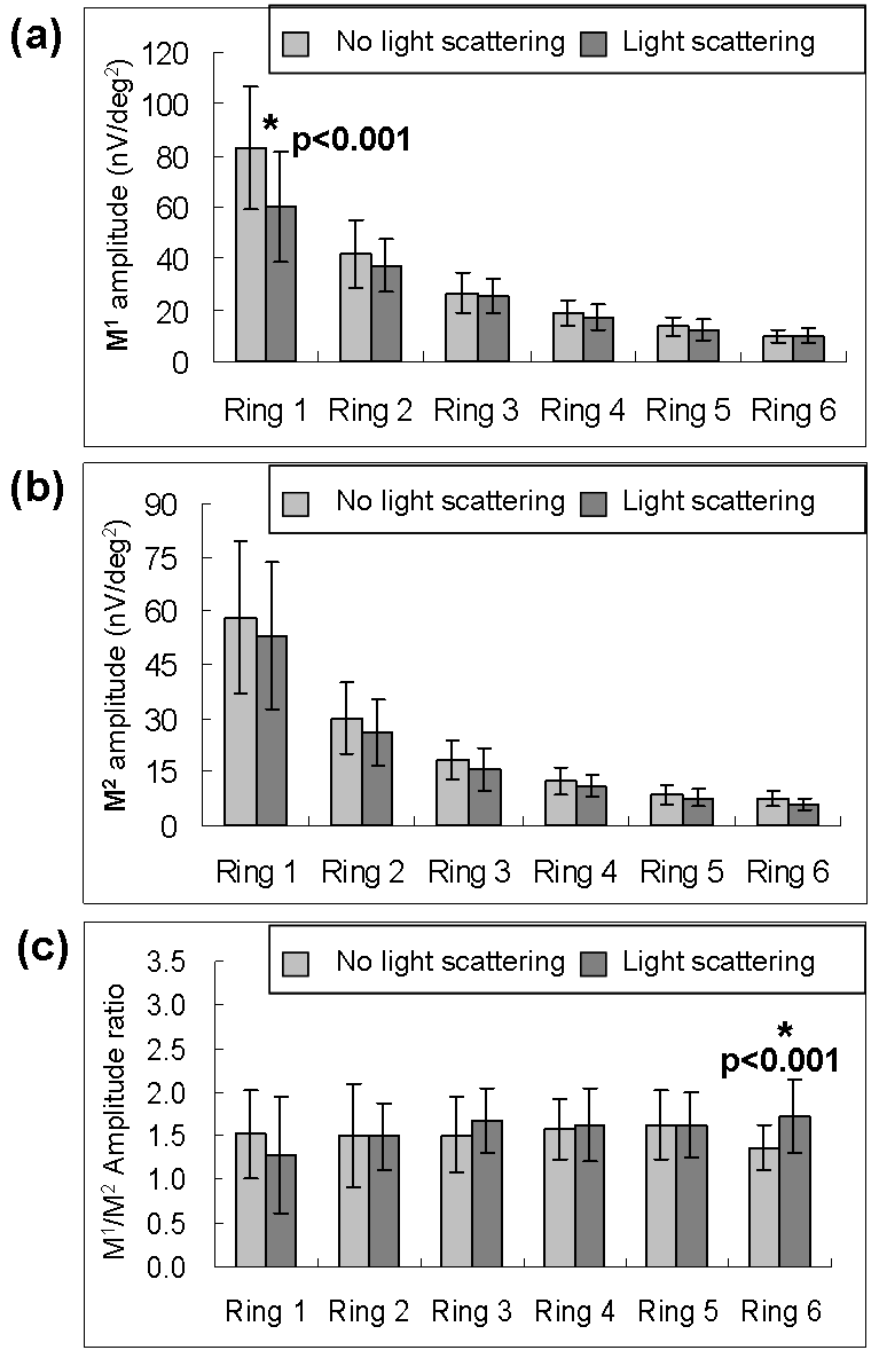

Figure 3. Averaged response for the M^1 amplitude (a), M^2 amplitude (b) and M^1/M^2 amplitude ratio (c) for different rings under normal conditions (light grey bar) and light scattering conditions (dark grey bar). The error bars are ± SD. * Significance levels are Bonferroni corrected.

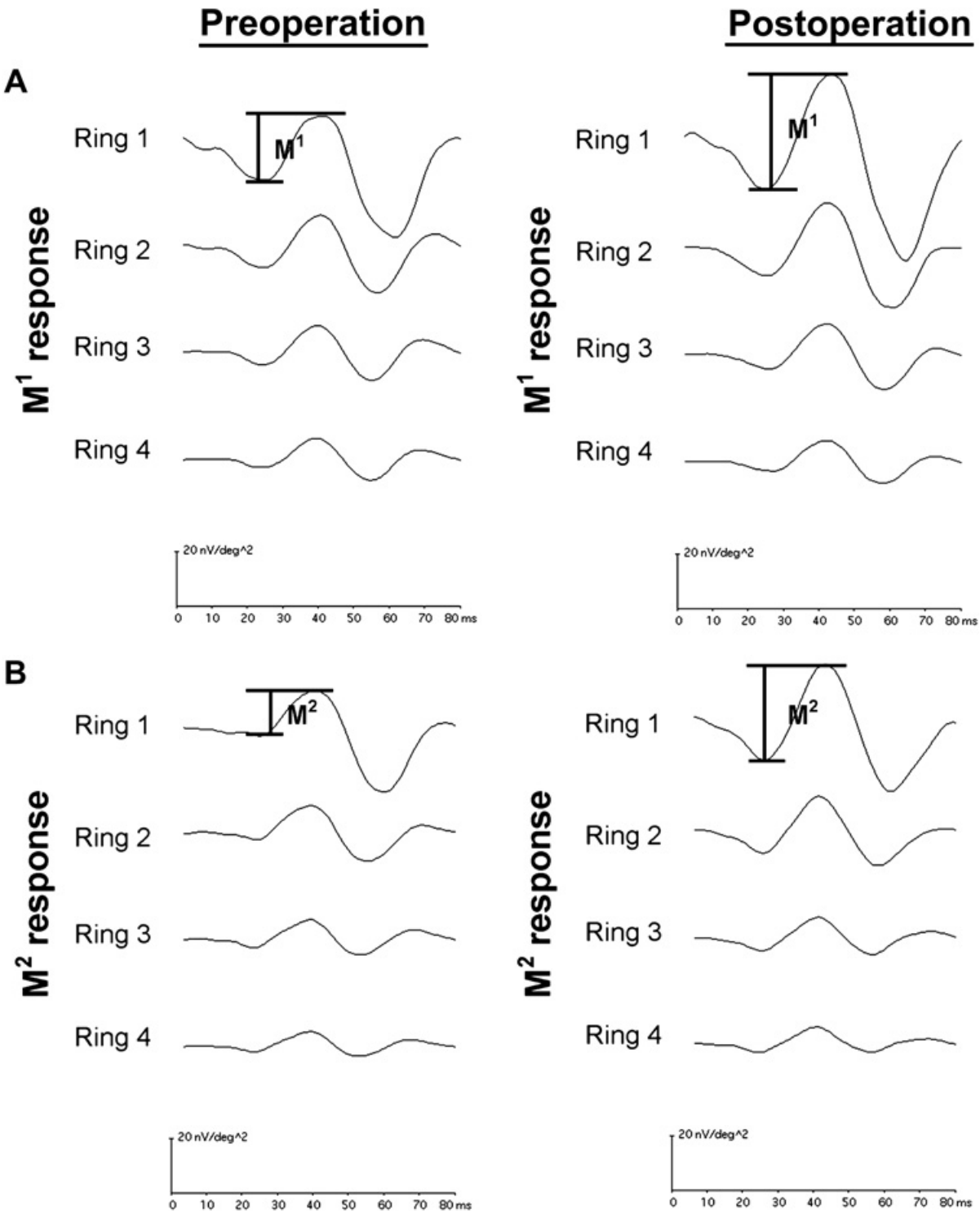

Figure 4. The typical M^1 (a) and M^2 (b) waveforms measured from one cataract subject before (left) and 2 weeks after (right) cataract surgery for rings 1-4 (see Figure 1(b)(ii)).

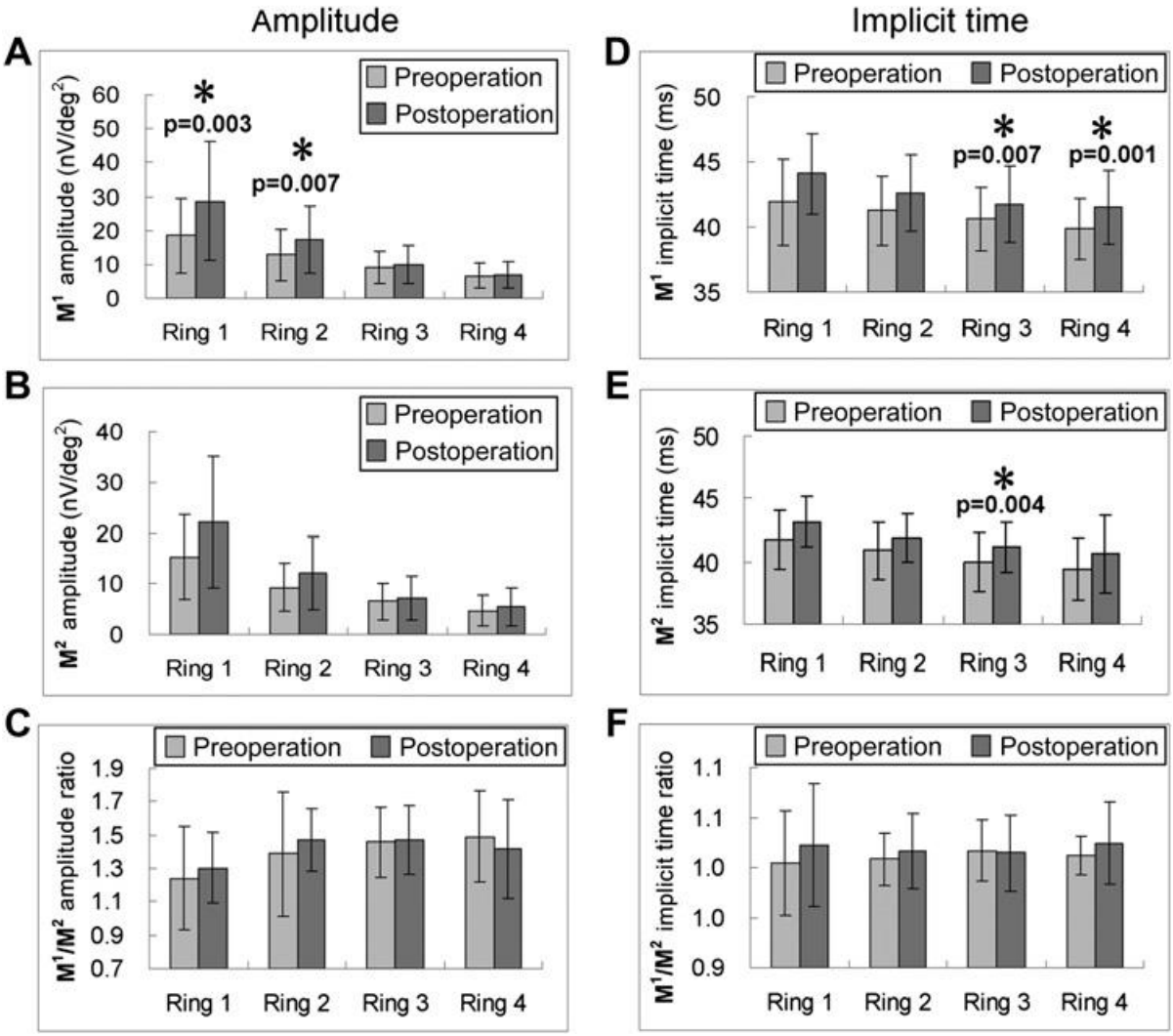

Figure 5. The averaged M^1 amplitude (a), M^2 amplitude (b), M^1/M^2 amplitude ratio (c), M^1 implicit time (d), M^2 implicit time (e), and M^1/M^2 implicit time ratio (f) for different rings pre- (light grey bar) and post- (dark grey bar) cataract surgery. The error bars are ± SD. * Significance levels are Bonferroni corrected.