The Hong Kong Polytechnic University

School of Optometry

Effects of light scattering on the multifocal electroretinogram (mfERG)

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Thesis Title: Effects of light scattering on the multifocal electroretinogram (mfERG)

CERTIFICATE OF ORIGINALITY

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Abstract

Introduction

The multifocal electroretinogram (mfERG) was developed by Sutter & Trans in 1992 and has been used for the objective examination of numerous retinal diseases such as age-related macular degeneration, diabetic retinopathy and retinitis pigmentosa. Patients who suffer from these eye diseases are normally elderly and they may have a certain degree of age-related change in the crystalline lens which would cause light scattering. Forward light scattering reduces the stimulus contrast and backward light scattering reduces the stimulus luminance. Both affect the mfERG. Therefore, the effect of light scattering on the mfERG is of interest.

Objectives

- To study the effects of forward light scattering on mfERG topography.
- To study the effects of different degrees of nuclear cataract on mfERG topography.
- To compare mfERG topography before and after cataract surgery.
- To study the effects of aging on mfERG topography.

Methods

There are four experiments in this study.

In Experiment 1, a liquid crystal diffuser (L-C-D) was used to produce different degrees of forward light scattering. The mfERG was recorded in thirty young subjects under different degrees of forward light scattering produced by the liquid crystal diffuser.

In Experiment 2, the mfERG was recorded from thirty elderly subjects, ten with very mild, ten with mild, and ten with moderate nuclear cataract. Their mfERG topographies were compared.

In Experiment 3, the mfERG was recorded from ten elderly subjects (10 eyes) with nuclear cataract of grade five (Lens Opacities Classification System III) before and after cataract surgery.

In Experiment 4, the mfERG were recorded in three groups of subjects: 1) eighteen young subjects (age 18-24 years), 2) eighteen elderly subjects (aged 60–70 years) with intraocular lens (IOL), and 3) eighteen elderly subjects (aged 75-85 years) with IOL.

Results

In Experiment 1, we found that the amplitudes of P1 from the central retina decreased, but the amplitudes of P1 in the mid peripheral retina increased with the increase of forward light scattering. N1 latency, P1 latency, and N1 amplitude were not affected by forward light scattering. In Experiment 2 and 3, we found that cataract can significantly decrease the N1 and P1 amplitudes from the central retina,

but N1 and P1 amplitudes from the mid peripheral retina did not change significantly.

In Experiment 3, we found that both N1 latency and P1 latency did not change significantly after cataract surgery. In Experiment 4, by comparing the mfERG responses in young subjects to elderly subjects with IOL, we found that N1 and P1 amplitudes from central to mid peripheral retina (0° to 43.8° diameter) did not change significantly with increasing age. However, N1 latency from central to peripheral retina increased significantly after the age of 70 years.

Conclusions

Forward light scattering can affect mfERG topography. As cataract can affect the topography of the mfERG, caution should be taken when interpreting the mfERG topography in subjects who have some degree of cataract. We suggest that each laboratory should establish a norm for different degrees of cataract for clinical diagnosis. Since our results showed that elderly subjects with IOL have similar response amplitude to young subjects, age-related decline of the mfERG response amplitude shown in previous studies are likely to be due to optical rather than neural factors.

Publication arising from the thesis

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Abbreviations

AHCPR	Agency for Health Care Policy and Research
APB	L-2-amino-4-phosphonobutyric acid
ARM	Age-related macular degeneration
C	Cortical opacities
cCSNB	complete type Congenital Stationary Night Blindness
cGMP	Cyclic-GMP
CRT	Cathode-ray tube
CS	Contrast sensitivity
DTL electrode	Dawson, Trick, and Litzkow electrode
ERG	Electroretinogram
IOL	Intraocular lens
ISCEV	International Society for Clinical Electrophysiology of Vision
L-C-D	Liquid-crystal-diffuser
LCD	Liquid crystal display
LOCS	Lens Opacities Classification System
mfERG	Multifocal electroretinogram
MTF	Modulation transfer function
NC	Nuclear colour
ND filter	Neutral density filter
NMDA	N-methyl-D-aspartic acid
NO	Nuclear opalescence
OCCCGS	The Oxford Clinical Cataract Classification and Grading System
ONH	Optic nerve head
ONHC	Optic nerve head components
ONL	Outer nuclear layer
OPL	Outer plexiform layer
P	Posterior subcapsular opacities
PDA	cis-2,3 piperidine dicarboxylic acid
PERG	Pattern electroretinogram
PSC	Posterior subcapsular cataract
RC	Retinal components
RMS	Root mean square
RP	Retinitis pigmentosa
RPE	Retinal pigment epithelium
SD	Standard Deviation
SEM	Standard Error of Mean
SNR	Signal-to-noise ratio
TR	Transmission ratio
TTX	Tetrodotoxin
UV	Ultra-violet
VA	Visual Acuity
VEP	Visual evoked potential
WHO	World Health Organization

Part I – Introduction & Literature Review

Chapter 1 - Introduction

Cataract is defined as a change of lens transparency in the eye. It is well known that aged people are more susceptible to cataract. The occurrence of light absorption and light scattering in a cataractous lens can reduce the patient's visual acuity (VA) and contrast sensitivity (CS) (Elliott et al., 1989, Elliott & Situ, 1998). Up to this time, there is no effective way to prevent or slow down the development of cataract. The only solution is through cataract surgery to remove the opaque lens and replace it with an intraocular lens (IOL) to compensate for the loss of optical power (Nordlund et al., 2000).

In 1999, the World Health Organization (WHO) and the International Agency for Prevention of Blindness launched a global initiative called "Vision 2020: Right to Sight". The objective of this initiative is to eliminate avoidable blindness by 2020 (Foster, 2001). According to the WHO, there are an estimated 180 million people worldwide who are visually disabled. In addition, about 40 to 45 million people are blind from varying causes that include cataract, glaucoma, trachoma and onchocerciasis. The most common cause is cataract, which accounts for 50% of blindness in the world (Foster, 2001). In Taiwan, the prevalence of cataract in those aged 50 years or older was 51% (Cheng et al., 2000). Nuclear cataract was the most prevalent type followed by posterior subcapsular cataract and cortical cataract (Cheng et al., 2000). In a pilot study in Hong Kong, the prevalence of cataract in the population has been estimated to be 19% (Van Newkirk, 1997).

Eye care practitioners sometimes find themselves in a dilemma to decide whether or not cataract surgery can help their patients improve their visual acuity,

when the cataract is so severe as to obscure the clinician's view of the fundus. Since the aged retina is also susceptible to many different age-related retinal diseases such as age-related macular degeneration, glaucoma or diabetic retinopathy (Kanski, 1999), clinicians may be uncertain whether the reduced visual acuity is due to optical or neural factors. Because of this, numerous techniques, including hyperacuity measurement, the blue field entoptic test, interference fringe techniques, the potential acuity meter, noise charts, electroretinogram (ERG) and visual evoked potential (VEP) have been developed for assessing retinal function behind cataract (Alio et al., 1993, Hurst & Douthwaite, 1993, Patel et al., 2001). Most of these are subjective methods and only visual electrophysiological tests, such as ERG and VEP, can provide an objective measurement for predicting post-operative visual function in a cataract patient. The electroretinogram (ERG) is an objective way to assess retinal function without the need of a subjective response from the patient. A previous study found that the amplitudes of the a-wave and b-wave of full-field (flash) ERG were slightly reduced in cataractous eyes, but not to a statistically significant degree (Cruz & Adachi-Usami, 1989). Another study on the effect of cataract on the pattern electroretinogram (PERG) showed that mild media opacities can diminish the amplitude of the PERG but not its latency (Mauck et al., 1996). The above findings suggest that the flash ERG is more resistant to media opacity than the PERG. However, subtle damage to the retina may not be detected by the flash ERG as it evokes a global response, which can mask localized retinal abnormalities. The development of the multifocal electroretinogram (mfERG) allows quick simultaneous recordings from many retinal locations in a single recording session of

approximately 4 to 16 minutes (Sutter & Tran, 1992). This technique, based on pseudo-random binary m-sequences, is an effective way to detect local retinal damage (Hood et al., 1998a, Chan & Brown, 1999, Marmor et al., 1999, Greenstein et al., 2000b, Huang et al., 2000). However, the effect of cataract or media opacity on the mfERG has not been well studied. Only one small study has been done on two subjects to demonstrate the effect of light scattering on the mfERG (Arai et al., 1999). This study showed that the central retinal responses decreased slightly with increased scattering level but the peripheral retinal responses did not show any obvious reduction. Thus, abnormal responses measured from a cataractous patient may not be caused by a neural problem alone; optical factors should also be considered in interpreting the data.

In this study, we will firstly investigate the effect of light scattering on the mfERG to see if its topography is affected by light scattering. Secondly, the effect of different degrees of cataract on the mfERG will be studied. In addition, we will study the effect of cataract on the mfERG by comparing mfERG topography in subjects before and after cataract surgery. In the final experiment, we will study the effects of aging on mfERG topography by comparing young subjects and elderly subjects with intraocular lenses (IOL).

Chapter 2 - Fundamentals of Electroretinogram

2.1 The Discovery of Electroretinogram

In 1848, Du Bois Raymond was the first to discover the existence of the resting potential in the eye (Wallis, 1966). He found that the cornea had a positive potential with respect to the posterior pole of the eye and this potential difference was independent of illumination. He thought that this resting potential was maintained by a metabolic process as it declined when the eye was removed from the animal. Later, it was confirmed that this ocular resting potential fluctuates, when a flash of light is used to stimulate the eye, and this complex response is called electroretinogram (ERG) (Holmgren, 1870).

2.2 Full-field (Flash) Electroretinogram

Full-field electroretinogram (ERG) is usually recorded with ganzfeld stimuli. This measured response represents the summed activities from different groups of cells in different retinal layers (Wallis, 1966). Thus, the resultant potential is the algebraic sum of all these cellular responses. The general waveform of the ERG mainly contains three components (a-wave, b-wave, and c-wave) (Figure 2.1). The base line in Figure 2.1 represents the potential of the cornea with respect to the retina.

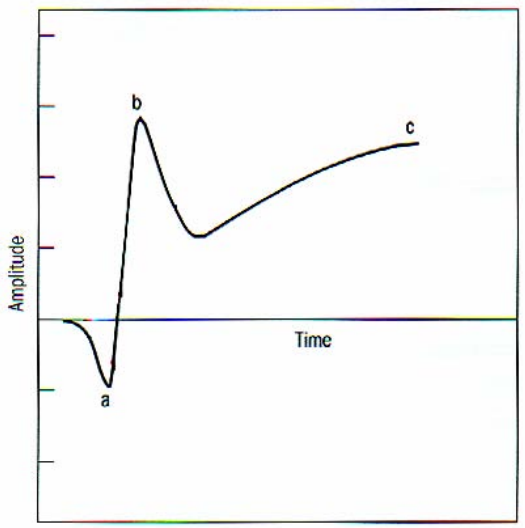

Figure 2.1 The waveform of full field ERG
(Adapted and modified from Fishman (2001)).

2.2.1 Origin of the a-wave

In a dark environment, a continuous flow of Na^+ ions into the outer segment of the photoreceptor and an outflow of K^+ ions from the inner segment form a loop of dark current, which is maintained by Na^+/K^+ pump. When light stimulates the outer segment, the influx of Na^+ ion will be reduced as the level of cyclic-GMP (cGMP) reduces (Molday, 1998). This process is called hyperpolarization, which is related to the origin of a-wave (Hood & Birch, 1990).

2.2.2 Origin of the b-wave

When the photoreceptor membrane is hyperpolarized, the amount of neurotransmitter released from photoreceptors is adjusted and this process causes a

depolarization of ON-bipolar cells or hyperpolarization of OFF-bipolar cells. Excited bipolar cells lead to depolarization of Müller cells. These changes of potential are responsible for the production of the positive b-wave (Newman & Odette, 1984). A study on the effect of retinal ischaemia due to occlusion of the central retinal artery clearly showed that the b-wave amplitude was reduced but the a-wave did not showed any changes, as photoreceptors get nutrients from the choriocapillaris and not from pre-retinal blood vessels (Barnett & Osborne, 1995, Block & Schwarz, 1998). These findings further strongly supported the view that the origin of the b-wave is different from that of a-wave.

2.2.3 Origin of the c-wave

Following the b-wave, there is a small positive wave called the c-wave. It is regarded as the hyperpolarization of the retinal pigment epithelium and the Müller cells (Celesia, 1988, Fishman, 2001). However, the high variation of c-wave amplitude and waveform limit its clinical application (Fishman, 2001).

2.3 Multifocal Electroretinogram (mfERG)

The development of the multifocal electroretinogram (mfERG) technique allows for multiple local retinal responses to be recorded within 4 to 16 minutes (Sutter & Tran, 1992). A matrix of hexagonal elements is displayed on a monitor and the stimulus matrixes can be in the form of 61, 103 or 241-hexagons (Figure 2.2). The size of the hexagons increases with eccentricity and inversely with the gradient of cone photoreceptor density in order to achieve equal magnitude of signals and similar signal-to-noise ratios at all retinal locations (Sutter & Tran, 1992). During the measurement, the subject needs to fixate at the central target of the stimulus pattern, and each hexagon has 50% probability of being black or white on each frame and changes in every 13.33ms (i.e. frame rate 75Hz), so that the overall luminance of the screen is stable during the recording period. The hexagons change their stage (black or white) according to a predetermined pseudo-random sequence called the binary m-sequence (Sutter, 1991). At different locations of the hexagons, the stimulus sequence is the same but it is lagged by different amounts. Thus, each retinal area is stimulated independently in a pseudo-random manner. With this technique, each of the stimulated retinal areas can produce a response with respect to the corresponding stimulus. The recorded potential is the response of the retina across the whole stimulated area and from this global response, the responses of localized retinal areas corresponding to a particular stimulus element can be extracted by calculations using a cross correlation method (Sutter, 2001).

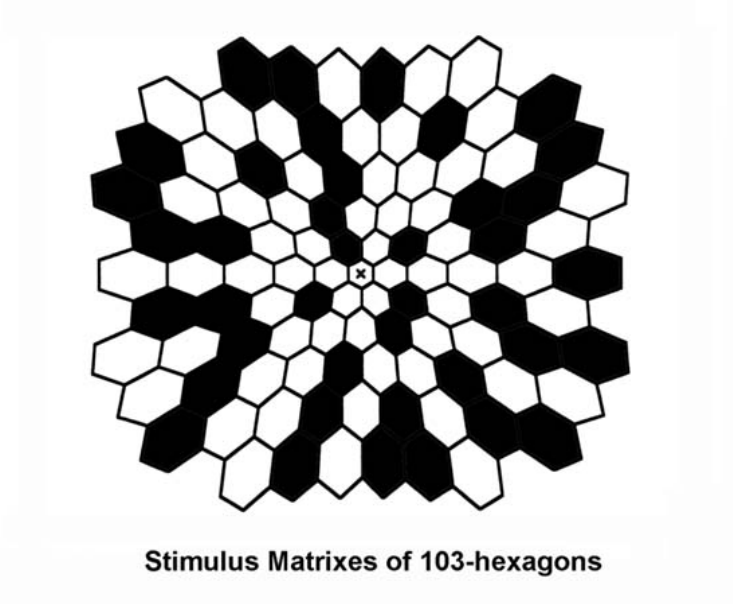

Figure 2.2. Stimulus matrixes of 103-hexagons with a central fixation cross.

The mechanism of signal derivation can be simplified as shown in Table 2.1 (Kondo et al., 1996). Suppose only seven retinal areas are being stimulated, the m-sequence is only 7 steps in length and the stimulus sequence is (1001110), where 1 is the presence of stimulus and 0 is the absence of stimulus. Each area of retina is stimulated with the same stimulus sequence but the other series are shifted one step from each other. With this stimulus sequence, responses due to area A can be calculated by adding the response at times 1,4,5 and 6 minus the response at times 2,3 and 7. The areas B to G result in no answer to the stimulus for area A, as each of the recorded single signal for area A is added and subtracted the same number of times, so cancellation occurs. On the other hand, responses due to area B can be calculated by adding the response at times 2,5,6 and 7 minus the response at 1,3 and 4. Similarly, areas A and C to G result in no answer to the stimulus for area B at this

time. This technique allows recording of different retinal area responses at the same time within a few minutes.

The signals of the mfERG are not, therefore, the “response” in the sense of direct electrical response from a local retinal area. The mfERG waveforms are mathematically derived signals, so they may be affected by adaptation effects and by the effects of scattered light on other retinal areas (Marmor et al., 2003).

Stimulus sequence									e.g. Response for Stimulus area A contributed by other individual stimulus areas							
Time	1	2	3	4	5	6	7		1	2	3	4	5	6	7	Resultant response with calculation
									∧	—	~	∧	∧	∧	—	
Area A	1	0	0	1	1	1	0		+	-	-	+	+	+	-	∧
Area B	0	1	0	0	1	1	1		-	+	-	-	+	+	+	= 0
Area C	1	0	1	0	0	1	1		+	-	+	-	-	+	+	= 0
Area D	1	1	0	1	0	0	1		+	+	-	+	-	-	+	= 0
Area E	1	1	1	0	1	0	0		+	+	+	-	+	-	-	= 0
Area F	0	1	1	1	0	1	0		-	+	+	+	-	+	-	= 0
Area G	0	0	1	1	1	0	1		-	-	+	+	+	-	+	= 0

Table 2.1. The simplified mechanism of signal derivation in mfERG measurement. (Please refer to the text in page 27 about the detailed explanation for this signal derivation).

Chapter 3 - Origin of Multifocal Electroretinogram (mfERG)

3.1 Comparison of full-field cone ERG to mfERG

The typical waveform of the mfERG is not exactly the same as that of the full-field cone ERG, but they are quite similar (Sutter & Tran, 1992). It is a biphasic wave with two negative and one positive components (Figure 3.1). The mfERG responses have a first negative component N1, followed by a large positive component P1 with latency about 37ms, and a small negative component N2 (Hood et al., 1997, Nagatomo et al., 1998).

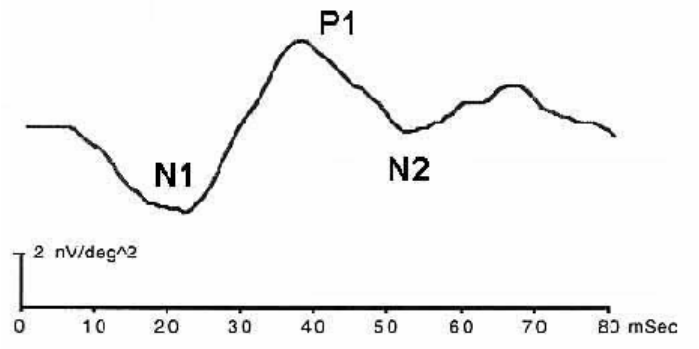

Figure 3.1. The first order kernel response waveform of the multifocal electroretinogram (mfERG).

As the waveform of the mfERG is similar to that of the full-field cone ERG, it is important to find out the relationship between the mfERG and the full-field cone ERG. Since the mfERG is usually measured under photopic condition, it is likely to relate to the cone response. If the components of the mfERG are related to the full-field cone ERG, the clinical application of mfERG will be greatly enhanced.

To compare the mfERG to the full-field cone ERG, the responses from mfERG under a slow frame interval condition (7F condition - the sequence of multifocal flashes was slowed down by interjecting 7 blank frames with the background intensity) were summed to produce a "summed mfERG" (Hood et al., 1997). It was found that the negative components of the mfERG and full-field cone ERG are similar, but the positive component shows significant differences. Firstly, the latency of the positive component of mfERG is shorter than that of the full-field cone ERG. Secondly, the waveform of the mfERG lacks multiple positive components when compared with the full-field cone ERG. The differences could be due to the paradigm of the mfERG technique being different from that of the full-field cone ERG. In the full-field cone ERG, the photopic background is present between flashes for at least one second, but there is no steady background present between the stimulations in the mfERG. Furthermore, the mfERG responses are mathematically derived through cross correlation (Sutter, 2001), but full-field cone ERG responses are obtained by direct recording and averaging. In order to minimize the paradigm difference between the mfERG and full-field cone ERG conditions, the sequence of multifocal flashes was slowed down by interjecting 7 blank frames with the background intensity (7F condition). The waveform of the mfERG under these specific conditions appeared to be the same as that of the full-field cone ERG. The first negative component of the mfERG did not show any significant change in the 7F condition, but the multiple positive components of the mfERG were observed (Figure 3.2).

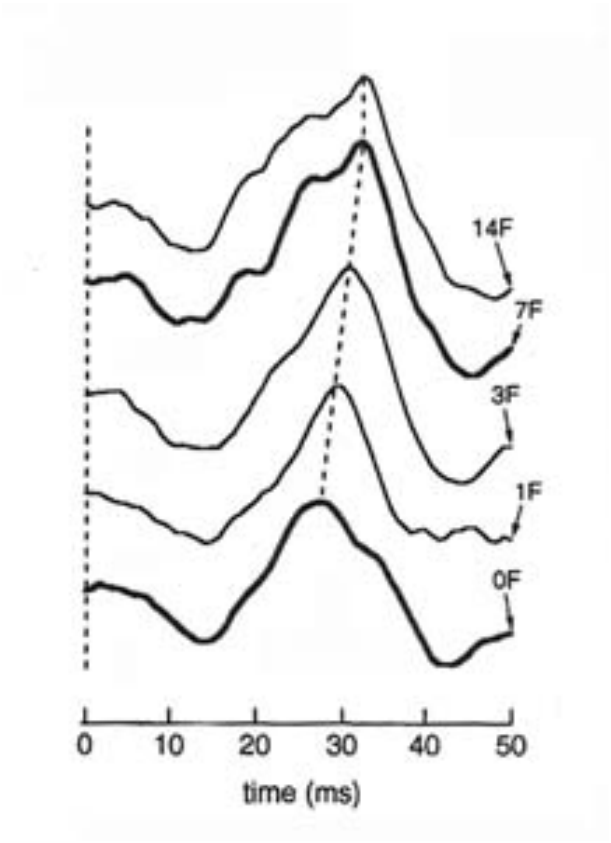

Figure 3.2. The effect of interjecting different numbers of background frames on the mfERG (Adapted from Hood et al. (1997)).

By comparing the effect of flash energy on the mfERG for the 7F condition and the full-field cone ERG, the implicit time of the third positive component and the implicit time of the first positive component in both cases decreased and increased, respectively, with increase in flash energy. In addition, increasing the background level decreased the implicit time of the third positive components and slightly increased the implicit time of the first positive components in both full-field cone ERG and mfERG (7F condition). This further indicated that the positive components in mfERG (7F condition) could be made up of the same components as the full-field cone ERG (Hood et al., 1997).

As the frame rate of stimulation did not affect the first negative component, it was suggested that the first negative component in both the fast condition and the 7F condition was made up of the same components as the a-wave in full-field cone ERG. Also, the change in the positive components of the mfERG with the 7F condition suggested that these positive components might contain similar positive components as in the full-field cone ERG (Hood et al., 1997).

Keating et al. (2002) tried to examine the construction of the mfERG responses by investigating different pulse trains embedded in the m-sequence. They also found that N1 and P1 components are generated by the same mechanisms of the standard full field ERG. In addition, N1 component is mainly contributed from the pulse trains where there is no change of state (i.e. 0-0 sequence and 1-1 sequence), so it includes a component from the interaction between two consecutive stimuli (Keating et al., 2002). Therefore the origin of the N1 and P1 components of the mfERG is similar to the full-field cone ERG (Hood et al., 1997, Keating et al., 2002).

3.2 Inner Retinal Contribution to the mfERG

Recording the mfERG from monkeys before and after the injection of Tetrodotoxin (TTX), which terminates the spiking activity of ganglion cells by blocking voltage-gated sodium channels, showed that there was a change of mfERG waveform and the responses became greater (Hood et al., 1999a). By comparing the pre-TTX response to the post-TTX response, there was a retinal naso-temporal topographical variation in the pre-TTX condition and this variation was not observed in the post-TTX condition. In addition, by grouping mfERG responses with increased distance from the optic nerve head (ONH) but with equal distance from the fovea, it was observed that the waveforms of the pre-TTX mfERG change with increase in distance from the ONH. The responses removed by TTX (the TTX component) also showed a prominent change of waveform with increase in distance from the ONH. Thus, part of the mfERG response was suggested to be measured from ONH. Hood et al. (1999a) also suggested that the mfERG responses from monkey contain ganglion cell activity, as a TTX component exists in the mfERG.

Another study showed that a ganglion cell component in humans was obvious in the mfERG, when the stimulus contrast was set at 50% (Hood et al., 1999b). They used a 100% contrast condition and showed that mfERG response waveforms recorded from monkeys changed from two positive peaks to a single peak after the injection of TTX and NMDA (N-methyl-D-aspartic acid) to block the ganglion cell responses. Secondly, when the stimulus contrast was 50%, the mfERG response waveforms measured from humans were similar to those from monkeys under 100% stimulus contrast without TTX injection and NMDA. Hood et al.

(1999a) next measured the mfERG at 50% contrast on glaucomatous patients, and the recorded mfERG waveforms were similar to those recorded in monkeys after injection of TTX and NMDA. By measuring the mfERG in patients with non-proliferative diabetic retinopathy at 50% stimulus contrast, only one positive peak was shown and this was similar to the result in monkeys after injection of TTX and NMDA (Figure 3.3). From these findings, it is deduced that the ganglion cell activity is measured by the mfERG in humans, but the findings do not correlate well with conventional clinical findings: for example, mfERG results do not match the result of visual field analysis very well (Fortune et al., 2001). Therefore, the use of the mfERG in the detection of ganglion cell activity needs to be further investigated.

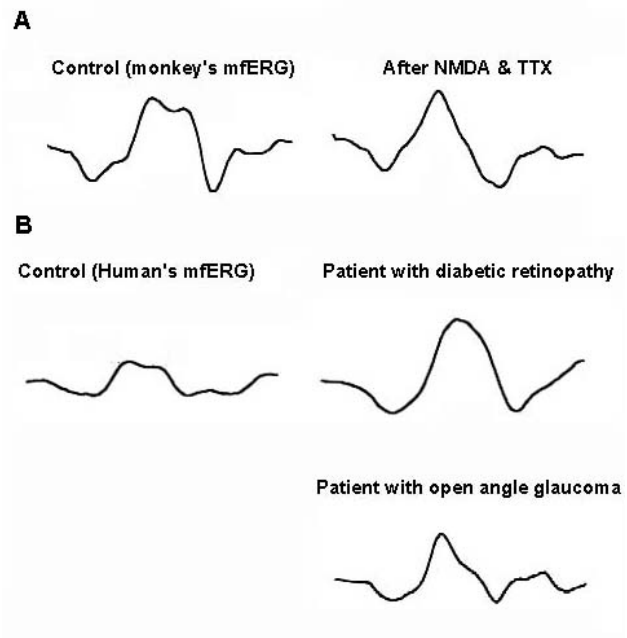

Figure 3.3. (A) The effect of TTX & NMDA on monkey mfERG with 100% stimulus contrast. **(B)** The waveform of human mfERG measured with 50% stimulus contrast in a normal subject, a patient with diabetic retinopathy and a patient with open angle glaucoma. (Modified from figures in Hood et al. (1999a)).

3.3 Two Components Hypothesis

3.3.1 The Optic Nerve Head Component of the Human mfERG

It was suggested recently that the mfERG measured in humans contains two components termed the “retinal component (RC)” and “optic nerve head component (OHNC)” (Sutter & Bearse, 1999). To investigate the properties of these two components, an algorithm was designed to separate them. They found that the latency of the RC was constant across the retinal area under stimulation. However, the latency of the OHNC increased with increasing distance from the optic nerve head. This latency delay was claimed to be due to the distance of the propagation of action potentials along unmyelinated axons, hence, the origin of this ONHC was thought to be from a source near the optic nerve head.

3.3.2 The Optic Nerve Head Component of the Monkey’s mfERG

The existence of the ONHC in the mfERG has been evaluated further in the monkey (Macaca mulatta) (Hood et al., 2001). By using special electrode configurations, a waveform, which is similar to the human OHNC, can be extracted. This component was measured by recording mfERG from the speculum of a Burian-Allen electrode, which was referenced to a DTL electrode (Dawson, Trick, and Litzkow electrode) on the unstimulated fellow eye, minus the mfERG response measured from the corneal electrode, which was referenced to the speculum on the stimulated eye. This extracted waveform contains two positive peaks at about 15ms and 36ms. The implicit time of the second positive peak increased from 34 to 39ms with increase in distance from the optic nerve head. By comparing the TTX

component and the ONHC in monkey mfERG, the waveform of the TTX component was similar to the ONHC (Hood et al., 2001). This suggested that TTX could remove a component which was similar to the ONHC, which may relate to ganglion cell activity.

3.4 Origin of the Primate mfERG and the Working Model of the Human mfERG

The mfERG waveforms recorded from monkeys were significantly different from those of the human mfERG (Frishman et al., 2000). However once the TTX or TTX+NMDA was injected, the mfERG waveforms of monkeys were similar to those from humans (Hood et al., 1999b, Frishman et al., 2000, Hood et al., 2001). After studying the effect of pharmacological agents on monkey mfERG, it was suggested that the origin of the mfERG is related to the ON-bipolar cells, OFF-bipolar cells, ganglion cells and cone photoreceptors (Hood et al., 2002). Once TTX and NMDA are injected into the monkey, only ON-bipolar cells, OFF-bipolar cells and cone receptors contribute to the mfERG. As APB (L-2-amino-4-phosphonobutyric acid) is a glutamate analogue that blocks the transmission between photoreceptors and ON-bipolar cells (Slaughter & Miller, 1983), the component removed by APB after injection of TTX + NMDA should be the contribution from the ON-bipolar cells. Furthermore, as PDA (cis-2,3 piperidine dicarboxylic acid) is a glutamate analogue that blocks cone input to OFF-bipolar cells and horizontal cells, it was assumed that the mfERG response recorded after TTX+NMDA+APB+PDA should only be the contribution from cone receptors. Since the contribution from ON-bipolar cells or cone receptors could be removed by

using pharmacological agents, the contribution from OFF-bipolar cells to the mfERG can be estimated by subtracting the responses. The contribution from both ON- and OFF-bipolar cells to the mfERG also decreased with increase in eccentricity, as the density of these cells also decreases with eccentricity. Therefore, the monkey mfERG was proposed to have a contribution from both outer and inner retinal cells (Hood et al., 2002). The outer retinal cells contributing to monkey mfERG are mainly ON-bipolar cells, OFF-bipolar cells and cone receptors. The inner retinal cells contributing to monkey mfERG may be ganglion cells and amacrine cells (Hood et al., 2001). From the above findings, a working model of the human mfERG was proposed (Figure 3.4) (Hood et al., 2002). This model suggests that cells contributing to the human mfERG are mainly from the outer retina. The leading edge of N1 is most probably related to the onset of the OFF-bipolar cells and is slightly contributed from photoreceptors. In addition, the leading edge of P1 is related to both the ON-bipolar cells and OFF-bipolar cells. The trailing edge of P1 is related to the depolarization of the ON-bipolar cells and is slightly contributed from the depolarization of the OFF-bipolar cells.

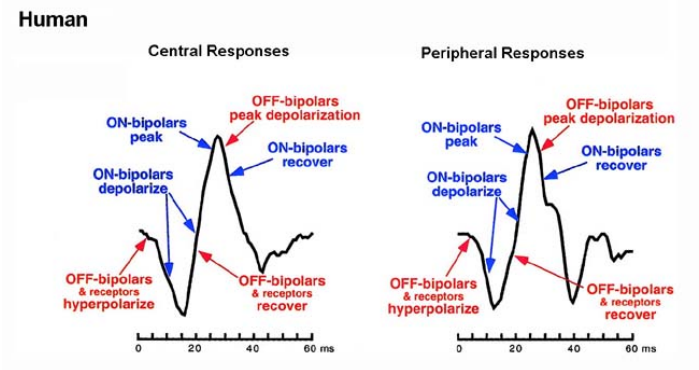

Figure 3.4. The working model of human mfERG based on the results from the monkey. (Modified from figures in Hood et al. (2002)).

In conclusion, the origin of the mfERG in humans is likely to be from the outer retinal layers such as from ON-bipolar cells, OFF-bipolar cells and photoreceptors (Hood et al., 2002). Responses from the inner retinal layers such as the ganglion cells can be measured by special measuring techniques (Hood et al., 1999b, Sutter & Bearse, 1999), which include using 50% stimulus contrast or using a special algorithm to extract it. However, using 50% stimulus contrast may not be sensitive enough to detect the inner retinal disease such as occurs in glaucoma in the clinical situation (Palmowski et al., 2000). Therefore, methods of using the mfERG for measuring ganglion cell activity are still under investigation. In addition, the proposed working model of the human mfERG is based only on results from monkeys. As the retinal structure of monkeys is somewhat different to that of humans, the origin of the mfERG measured from monkeys may not be exactly the same as in the human mfERG. Therefore, the origin of different components of human mfERG should be better understood by studying the effect of different retinal diseases on the mfERG (Hood, 2000).

Chapter 4 - Topography of the Multifocal Electroretinogram (mfERG)

The first study describing the topography of the mfERG in humans was by published by Sutter and Tran in 1992. They used a stimulus matrix with 241 hexagons, which covered the central visual field (about 23 degrees x 23 degrees) to measure the mfERG. They showed that response density decreased with increase in retinal eccentricity and the decrease was slightly lower in the nasal retina. These changes of response density with eccentricity were shown to be well-correlated with the cone density. It was suggested that the nasal-temporal variation was the result of higher cone density in the nasal retina (Sutter & Tran, 1992). In addition, the mfERG technique could identify the location of blind spot, which showed minimum response. Theoretically, there should have been no response recorded from the blind spot area, as there are no receptor cells. However, this could be explained in two ways (Sutter & Tran, 1992). Firstly, there may not be a single stimulus element that falls completely within the blind spot. Secondly, as the optic disc is highly reflective, light projected on it could be scattered or reflected onto other retinal areas to elicit a sizable residual response.

A study on 20 subjects with ages ranging from 21 to 76 years showed that both the amplitudes of N1 and P1 decrease with increasing eccentricity (Nagatomo et al., 1998). Nagatomo et al. (1998) suggested that this is due to the decrease in cone density with increase in eccentricity (Curcio et al., 1987) and not mainly due to the fact that luminance of the monitor decreases in the periphery. In addition, they found that the latencies of N1 and P1 decreased from the central retinal (about 5 degrees in diameter) to the parafovea (around 10 degrees in diameter) and then

increased again with increase in eccentricity. However, their study did not find any naso-temporal variation in terms of N1 latency, P1 latency, N1 amplitude and P1 amplitude. Moreover, they also found that there was a large variation of P1 amplitude among the subjects. We also agree the suggestion by Curcio et al. (1987) that the large variation of P1 amplitude is attributed to a large inter-subject variation in cone density (Curcio et al., 1987).

Verdon & Haegerstrom-Portony (1998) found that the variability of the mfERG response in the central retina was larger than in the peripheral response (Verdon & Haegerstrom-Portnoy, 1998). However, the variability of the central response was similar to the peripheral response in log units. There was no significant difference between (nasal versus temporal) response and between (superior versus inferior) response (Verdon & Haegerstrom-Portnoy, 1998, Li et al., 2001). In addition, latencies of N1 and P1 in the central retina tend to be slightly longer than in the peripheral regions (Verdon & Haegerstrom-Portnoy, 1998). However, studies using ring analysis did not show any change in N1 and P1 latencies with eccentricity (Parks et al., 1996, Li et al., 2001).

The latency variation of N1 and P1 with eccentricity is controversial (Parks et al., 1996, Nagatomo et al., 1998, Verdon & Haegerstrom-Portnoy, 1998). By plotting the latency into a topography map, the variation of latency at different retinal locations can be clearly seen (Seeliger et al., 1998). The latency of P1 was longer in the upper and lower borders of the stimulated field, macular and blind spot areas than the other regions. Longer P1 latency at the blind spot was suggested as a result of light reflection by the optic disc, so the reflected light would reach other

retinal areas with reduced intensity causing the latency to increase (Seeliger et al., 1998). Shorter latency of P1 was found in the parafoveal area and in the temporal retina (Seeliger et al., 1998). This further confirms that the latency of P1 tends to decrease from central to mid-peripheral retina and then increase to the peripheral retina (Nagatomo et al., 1998).

In summary, the amplitudes of N1 and P1 decrease with eccentricity (Nagatomo et al., 1998, Verdon & Haegerstrom-Portnoy, 1998, Li et al., 2001). Latency variation of N1 and P1 at different retinal locations can be revealed more clearly with a topography map (Seeliger et al., 1998). The reasons why some studies showed naso-temporal variation or superior-inferior variation, but others did not, still need to be further investigated (Sutter & Tran, 1992, Verdon & Haegerstrom-Portnoy, 1998, Li et al., 2001). Although the above studies used contact lens electrodes for measurement, it was reported that the type of electrode used for recording can affect the topography of the mfERG (Keating et al., 2000). With Burian-Allen contact lens electrode recordings, response density from upper retina was 36% higher than from lower retina, but the difference became only 16% when using a gold foil electrode (Keating et al., 2000). The contact lens electrode may more accurately reflect the topography of the mfERG, as it covers the whole cornea. As a gold foil electrode touches only part of the cornea, the potential derived from the cornea may be biased towards the signals generated by the superior retina (Keating et al., 2000).

The different results reported by different studies may be due to different methodologies (e.g. stimulus luminance and band-pass (Keating et al., 1996)). The

maximum stimulus luminance used by Seeliger et al. (1998) and Nagatomo et al. (1998) was 100cd/m^2, but the maximum stimulus luminance used by Verdon et al. (1998) was 200cd/m^2. Some studies used band-pass between 10Hz and 100Hz (Seeliger et al., 1998, Verdon & Haegerstrom-Portnoy, 1998) and the study by Nagatomo et al. (1998) used band-pass between 5 and 100Hz. The International Society for Clinical Electrophysiology of Vision suggested the use of a filter range of 3-300Hz or 10-300Hz (Marmor et al., 2003). Only the study by Li et al. (2001) followed this suggestion. As the filter setting could affect the mfERG waveforms (Marmor et al., 2003), the mfERG topography might also be affected by different filter settings (Han et al., 2004). In addition, the use of different age groups or races to study the mfERG topography may cause different findings (Parks et al., 1996, Nagatomo et al., 1998, Seeliger et al., 1998, Verdon & Haegerstrom-Portnoy, 1998, Li et al., 2001).

Chapter 5 – Factors Affecting the Multifocal Electroretinogram

5.1 Light Adaptation

The amplitude of the full-field cone ERG elicited by photopic stimuli increases during the first 10 to 20 minutes of light adaptation (Gouras & MacKay, 1989a). By measuring the mfERG every 2 minutes over a period of 16 minutes, the amplitude of N1 and P1 of the summed mfERG increased during light adaptation (Kondo et al., 1999). N1 amplitude increased 36% and P1 amplitude increased 47% during the 16 minutes of light adaptation. The latency of N1 and P1 also increased about 3% (0.5 msec) and 4% (0.8 msec), respectively during the 16 minutes of light adaptation. The increase of amplitude was larger in the peripheral than in the central retina, but the increase of latency was not related to retinal location. Similar results were found when using non-scaled hexagons as stimuli. In addition, the amplitude and latency of the second order kernel responses (see Appendices A3) showed similar variations during light adaptation. The exact mechanism of this phenomenon is not clear, but may relate to the activities of the rod system (Kondo et al., 1999). Therefore, Kondo and co-workers suggested that measuring the mfERG should be done on subjects in light adapted conditions to minimize inter-subject variation. However, a later study showed that both N1 and P1 amplitudes in central and peripheral retina were not affected by pre-adaptation conditions (Chappelow & Marmor, 2002). The difference in findings between these two studies may be due to the difference in recording times. The recording time in the latter research is much longer than the former, so the pre-adaptation condition may have a smaller effect on the mfERG when the recording time is more than 8 minutes.

As mentioned above, the full-field ERG amplitude increases during adaptation (Gouras & MacKay, 1989a). However, further increase of light level decreases photopic ERG amplitude, as greater amounts of photopigments are bleached (Gouras & MacKay, 1989b). Similar results also occur in mfERG recording (Kretschmann et al., 1998a). They found that the foveal responses were significantly decreased during foveal bleaching and there was a fast recovery of central responses after cessation of bleaching. In addition, latency of P1 increased from 32.1 to 34.2ms during foveal bleaching. However, responses from the extrafoveal macula did not change significantly during foveal bleaching or after foveal bleaching. It was interesting to note that the peripheral responses increased significantly, to about 113% of the pre-bleaching values during foveal bleaching (Kretschmann et al., 1998a). The exact reason for this finding is not known, but this study clearly shows that the peripheral retinal responses could be affected by the central foveal adaptation.

On the other hand, a recent study showed that lateral spread of adaptation could be observed in mfERG recording (Seiple et al., 2001). In the first part of the experiment, only the central hexagon was modulated and the surrounding hexagons were set at a constant luminance of either 0.45 cd/m^2, 172 cd/m^2, or 340 cd/m^2. P1 amplitude of the central response significantly decreased by 37.5 nV/deg^2 and N1 latency decreased by 4.2 ms when the surrounding luminance increased from 0.45cd/m^2 to 340cd/m^2. In order to investigate whether the above finding is related to the intra-ocular scattered light that reduces the contrast or increases the time-average mean luminance of the central hexagon, they further studied the effect of

contrast on the central response. A large reduction of stimulus contrast did not significantly affect the latency. In addition, the waveform of the central response changes from a single peak in the high contrast condition to double peaks with low contrast. This change was not observed in the first part of the experiment. Increasing the time-average mean luminance of the central hexagon did not affect the amplitude or latency of the central response. Furthermore, by placing a black annulus between the central hexagon and the surrounding hexagons, both the amplitude and latency of P1 were increased with increase in the size of the black annulus. Therefore, they concluded that lateral spread of adaptation could be observed by using the mfERG and it was not due to scattered light from the surrounding hexagons.

5.2 Ambient Room Lighting

Recently, room lighting was shown to affect mfERG responses (Chappelow & Marmor, 2002). Both central and peripheral N1 and P1 amplitudes of the mfERG decreased by about 25 to 30% as room luminance levels increased from –1.25 log cd/m^2 to 1.6 log cd/m^2. Latency of P1 also decreased as room luminance increased. In addition, the location of the blind spot in the mfERG topography could be more easily identified when the mfERG was measured in a light (1.56 log cd/m^2) rather than a darkened room. Therefore, the brightness of ambient room lighting during mfERG recording was clearly demonstrated to affect the mfERG responses. It was strongly recommended that the mfERG should be recorded in a fully lighted room, as amplitude and latency of mfERG responses can be affected by slight changes of room luminance (Chappelow & Marmor, 2002). Therefore, measuring the mfERG in

fully lighted rooms can achieve larger mfERG responses and the location of blind spot can be easily identified. However, we measured mfERG in a dim room in our experiment one, as we need to minimize unwanted light scattering when we place a liquid-crystal-diffuser in front of the patient's eye to produce light scattering effect.

5.3 Pupil Size

Pupil size controls the amount of light entering the eye. A weaker mfERG response is obtained at lower luminance levels (Brown & Yap, 1996), so a larger response could be obtained with a dilated than with a natural pupil. Chan and Brown (1998) demonstrated that mfERG responses increase significantly with the increase of pupil size. Changing the pupil size affected the macular response more than the peripheral response. A recent study by Gonzalez et al. (2004) also showed that both central and peripheral P1 amplitude increased significantly when the pupil size increased from 2 mm to 10mm in diameter. In addition, both central and peripheral P1 latency increased when pupil size decreased from 10mm to 2mm in diameter (Gonzalez et al., 2004). Although pupil dilation would vary the P1 amplitude, pupil dilation is still recommended as the signal-to-noise ratio (SNR) can be improved (Keating et al., 2000).

5.4 Refractive Blur

A previous study showed that refractive blur from –3D to +6D did not affect the latencies of the first order response (N1 and P1) and the second order response (N1P1) (Palmowski et al., 1999). These findings were observed in both central

(central 4 degrees) and peripheral responses (6-25 degrees). Similarly, the amplitudes of the first order response N1 and P1 from both central and peripheral regions were not affected by these levels of refractive blur. Amplitudes for the second order response N1P1 at central and peripheral regions were not affected by refractive blur. However, Chan & Siu (2003) found that N1 and P1 latencies were not affected by optical defocus, but the P1 amplitude decreased by about 10% with +1.00D defocus.

In conclusion, measuring mfERG with the optimal refractive correction is essential. Firstly, it may help observers to fixate the central target. This is important as eye movements may result in considerable noise (Kondo et al., 1995). Secondly, the sensitivity of mfERG to detect the small areas of retinal dysfunction may be affected as the defocused hexagons could stimulate larger retinal areas than expected (Palmowski et al., 1999). Hence, the contrast and luminance of the stimulus would be reduced by the refractive blur. As the reduction of contrast could reduce the mfERG responses (Brown & Yap, 1996), lower mfERG in subjects with refractive blur may be related to the change of stimulus contrast.

5.5 Degree of Myopia

Measuring the mfERG on myopic subjects has shown that the amplitudes of N1 and P1 were significantly lower and latencies of N1 and P1 were significantly longer in subjects with high myopia (Kawabata & Adachi-Usami, 1997). In addition, these changes of amplitude and latency were well correlated with the degree of myopia. Latencies of N1 and P1 showed a general increase with myopia at all

eccentricities. There was a general depression of N1 and P1 responses from central to peripheral regions (about 25 degrees form the macula) in all subjects with increase in myopia, but a tendency for greater reduction of responses in peripheral regions was noted in highly myopic subjects. It was claimed that this is due to axial elongation which mainly occurred in the peripheral retinal area (Kawabata & Adachi-Usami, 1997). In fact, a recent study has successfully shown that subjects with longer axial lengths have lower P1 amplitudes (the first-order kernel-K1) in the central and the paracentral regions (ring 3) (Chan & Mohidin, 2003). The central retinal region showed high rates of reduction in both N1 and P1 amplitudes. This further confirmed that weaker mfERG responses in highly myopic subjects may be related to the morphological changes associated with increased axial length.

5.6 Aging

Topography of the mfERG has been compared across different age groups (18-22, 33-37 and 48-52 years) and the averaged P1 response from the whole stimulated area did not show significant differences (Mohidin et al., 1999). In terms of retinal eccentricity, however, the P1 response at the central region decreased significantly with increasing age. In addition, there was no significant difference between the responses obtained at the periphery for all age groups. In this study, it was a pity that they did not show the effect of aging on the second order kernel responses and whether latency of P1 is affected in aged subjects, but they demonstrated that responses from male and female were the same in all age groups. They concluded that retinal function did not show significant changes between the

ages 18 to 37 years but tended to decline at about 50 years. A recent study showed similar findings (Nabeshima et al., 2002). P1 responses at all eccentricities significantly decreased at the age of 60 years, but latency of P1 did not change. Moreover, the N2P2 response in the second order kernel at all eccentricities tended to decrease with increasing age and there was a significant increase in response latency. It is important to point out that this study also included subjects with mild media opacities, so these findings could not indicate whether these age-related changes are due to optical, neural factors or both.

A similar study compared the mfERG topography in two age groups (19-30 and 60-74 years) (Jackson et al., 2002a). The first order kernel P1 scalar-product responses (see Appendices A3) in elderly subjects were significantly lower than those in young adults at all eccentricities and the reduction was maximal at the central region. In addition, P1 latency increased by about 1.31ms with increasing age. They claimed that the weaker response in elderly subjects was probably not due to optical factors, as retinal illuminance only reduced the mfERG response amplitude by 0.04 log unit. However, their study showed that the reduction of mfERG response was greater than 0.08 log unit in elderly subjects. Therefore, optical factors may not be the major contributor to the age-related decreased in the mfERG response. They suggested that neural factors such as slowed temporal adaptation in the aged retina would account in part for the reduced responses in aged subjects.

A later study suggested that the decline of mfERG responses with age was due to optical factors rather than neural factors (Fortune & Johnson, 2002). Firstly, they

showed that the first order kernel response density, the first order kernel P1 trough-to-peak amplitude, and the second-order kernel response density decreased at all eccentricities with increasing age. In addition, the decrease was the highest for the central retinal responses. They also found that latency of P1 increased with increasing age at all eccentricities, especially in the central retina. Since media opacities may affect the mfERG topography, they measured the crystalline lens optical density for each individual to calculate the effects of aging lens on retinal illuminance. They found that the retinal illuminance in young subject (age 21 years) is 0.12 log units greater than the oldest subjects (age 69 years). Since the mfERG was measured in subjects with natural pupil, the pupil size in the old subjects was smaller than the young subjects in about 1.7 mm diameters and the retinal illuminance in old subjects is 0.28 log units smaller than the young subjects. Therefore, the reduction in retinal illuminance due to age-related changes in lens density and pupillary miosis may have reduced the mfERG stimulus strength in about 0.4 log units. When the mfERG responses in each subjects was adjusted for reduced stimulus intensity, they found that only central responses for the first order kernel P1 trough-to-peak amplitude and the first order kernel P1 response density decreased significantly with increasing age. In addition, the latency of P1 did not change significantly with increasing age after the correction for stimulus intensity. However, the second-order kernel response density still showed significant reduction with increasing age in all eccentricities, especially in the central region. On the other hand, their study showed that the second-order responses decrease at all eccentricities especially in the central region with decrease in stimulus contrast.

These indicated that both contrast reduction and reduced stimulus intensity due to media opacities can reduce the mfERG responses. Since the adjustment of mfERG response for reduced stimulus intensity can compensate nearly all the aging effects, the remaining effects may be due to the effects of light scattering and reduced stimulus contrast. Therefore, two simulation experiments were done to prove that optical factors are largely responsible for the aging effects on mfERG topography (Fortune & Johnson, 2002). In the first simulation, the effects of age related lens changes were simulated by using a filter with a Wratten #96 neutral density filter, a yellow colored glass filter and a light scattering filter. In the second simulation, the mfERG were measured in young subjects with stimulus luminance and contrast reduced to a level predicted for an old subject (70 years old). The results of these two simulation experiments showed that the mfERG response amplitude and latency measured in these two conditions were very close to the value of the old subjects. Therefore, Fortune & Johnson (2002) suggested that effects of aging on mfERG responses are mainly from optical factors.

On the other hand, a recent study measuring mfERG from age 10 to 80 years (10 subjects on each decade) at two luminance levels (200 and 700 cd/m^2) showed that response density of P1 decreased and latency of P1 increased with increasing age under both conditions (Gerth et al., 2002). As mentioned before, stimulus luminance and contrast would be reduced in aged subjects as ocular media transmission reduces and light scattering occurs. Therefore, we could not simply conclude that the reduced response in aged people is due to neural factors without considering the optical factors. In their study, Gerth et al. (2002) assumed that there

was only 0.12 log units difference in luminance reaching the retina for young subjects (25 years old) and old subjects (75 years old). If stimulus luminance only reduces 0.12 log units, the old subjects (75 years old) should only have the response density of 0.0511 log units lower and latency of 0.00492 log units longer than the young subjects (25 years old). However, their study found that the old subjects (75 years old) have response density of 0.15 log units lower and latency of 0.0215 log units longer than the young subjects (25 years old). Therefore, the reduction of light transmission cannot fully account for the changes of mfERG responses. They then considered if it is due to intraocular light scattering. They assumed that light scattering could reduce stimulus contrast of 20% between young subjects and old subjects. According to their data, there were a reduction in log response density of 0.009 and a decrease in log latency of 0.024 if stimulus contrast reduces 20%. Therefore, the age-related reduction in retinal illuminance and the increase in intraocular light scattering cannot fully explain all of the age-related changes in mfERG responses (Gerth et al., 2002). Gerth et al. (2002) concluded that age-related changes in mfERG are due to both optical factors and neural factors.

5.7 Diurnal Variation

Previous studies have shown that diurnal variation exists for the scotopic and photopic ERG (Birch et al., 1984, Hankins et al., 1998). Therefore, diurnal variation may also exist for the mfERG. A recent study suggested that the influence of circadian rhythm on the mfERG is not significant (Heinemann-Vernaleken et al., 2000). Both the first order responses and the first slice of the second order responses (see Appendices A3) for the central and peripheral retina did not change significantly when the mfERG was measured at 10:30am, 1:30pm, and 4:30 pm. Therefore, the effect of circadian rhythm on mfERG can probably be ignored in clinical practice.

5.8 Light Scattering

For pattern ERG and pattern VEP, the responses are greatly affected by media opacities and conditions with light scattering (Tetsuka et al., 1992, Mauck et al., 1996). A recent study found that light scattering could reduce the central mfERG responses but the peripheral mfERG responses were increased under light scattering condition (Chan et al., 2002a). Another study measuring mfERG on subjects viewing through acrylic sheets which created light scattering to decrease VA from 20/20 to 20/70, showed that central responses decreased only slightly but peripheral responses were not affected (Arai et al., 1999). They claimed that the mfERG was not affected significantly by light scattering, and there is no need to consider the effect of scattering on patients with cataract. However, the sample size in this study was too small (n=2). Therefore, there is a need to study the effect of light scattering

with a larger sample to see if light scattering affects the mfERG topography. If light scattering affects the mfERG topography, it is necessary to study the effects of different degrees of cataract on the mfERG topography.

Chapter 6 - Clinical Application of Multifocal ERG

6.1 Detection of Localized Retinal Defects

The sensitivity and effectiveness of using the mfERG to extract focal retinal responses has been widely demonstrated (Bearse & Sutter, 1996, Yoshii et al., 1998, Arai et al., 1999, Marmor et al., 2002). When two of the stimulus hexagons were set not to modulate during mfERG recording, no responses were obtained from that position (Bearse & Sutter, 1996). A small retinal patch desensitized by partial bleaching of photopigments also reveals a localized reduction in response in the mfERG topography (Bearse & Sutter, 1996). An alternative approach to further support the view that the m-sequence technique can extract focal response was demonstrated by using mfERG stimulus hexagons to measure pupillary responses. In the study of Wilhelm et al. (2000), visual field sensitivity was deduced for 37 stimuli (covering the central 20 to 25 degree radius of visual field) which were flashed in a pseudorandom m-sequence at $1/9^{th}$ rate usually used for the mfERG measurements. The changing diameter of the pupil after stimulus presentation was analysed using the cross correlation technique of the VERIS system. The report by Wilhelm et al. (2000) indicated that pupillary responses to stimuli flashed at various locations across the visual field can be used to create a visual field plot when an adaptation of the VERIS system technique is used. It was also shown that when part of the stimulus screen was covered by cardboard, no pupillographic response was extracted in that area (Wilhelm et al., 2000).

A study placing different sizes of black circular paper ten degrees from the fixation point showed that reduced response density was only observed when the

size of the black paper was 5 degrees or more in visual angle (Yoshii et al., 1998). This study implied that it is difficult to use the mfERG to detect a visual field defect of less than 5 degrees diameter by using a 103 hexagon stimulus. Furthermore, the detection sensitivity for a small field defect would be lower if a small scotoma projected over two hexagonal stimulus elements (Yoshii et al., 1998). A later study also demonstrated that complete masking or half masking of stimulus hexagons with black paper or an ND filter could significantly reduce responses at that location (Marmor et al., 2002). Covering only 1/3 of a hexagon with black paper or ND filter also mildly reduced the response, but not by a statistically significant amount (Marmor et al., 2002). However, Brown and Yap (1995) showed that mfERG responses decreased significantly when a 0.4ND filter was placed over part of the screen. Marmor et al. (2002) showed that the sensitivity of using mfERG to detect stimulus masking would be higher if a higher resolution of stimulus pattern (e.g. 241 hexagons) is used. However, the drawbacks of using a high resolution stimulus pattern for recording mfERG are long recording time and poor signal-to-noise ratio. Therefore, the sensitivity of the mfERG in the detection of scotomata greatly depends on both the location and the size of the scotomata relative to the stimulus hexagons.

In addition, the mfERG responses could be recorded on an enlarged optic disc, as the optic disc reflects the stimulus light to other regions of the retina (Shimada & Horiguchi, 2003). As the stray light caused by media opacities (e.g. cataract) could also reflect the light to other regions of the retina, the sensitivity of mfERG to detect retinal defects could be affected by the stray light.

Clinical cases have demonstrated that the mfERG can be used to detect retinal defects or non-observable retinal dysfunction in patients with retinitis pigmentosa, branch retinal artery occlusion, pigmentary retinal dystrophy, age-related macular degeneration, and cytomegalovirus retinitis (Kondo et al., 1995, Bearse & Sutter, 1996).

6.2 Outer Retinal Disease

6.2.1 Retinitis Pigmentosa (RP)

An early study found that retinitis pigmentosa (RP) patients had a general reduction in both macular and peripheral responses in mfERG topography (Chan & Brown, 1998). In addition, latency was generally unchanged in the central retina but significantly delayed in the peripheral retina (Seeliger et al., 1998). A further study showed similar findings, but this study strongly indicated that amplitude reduction might not be sensitive enough to predict visual field defects (Hood et al., 1998a). Latency increase, however, appeared to be a good indicator for detecting early retinal changes in RP patients, as the local retinal areas with latency increases usually had a reduced sensitivity in Humphrey visual field analysis, and the retinal areas with normal latency usually had a normal sensitivity. However, the analysis of latency changes alone in mfERG topography could not give a complete picture of retinal dysfunction, as a later study clearly showed that retinal areas with reduced amplitude did not consistently correspond to the retinal area with a latency increase in patients with X-linked retinitis pigmentosa (Vajaranant et al., 2002). Retinal areas with latency delay or reduced amplitude might not show reduced sensitivity in the Humphrey visual field. On the other hand, retinal areas with reduced sensitivity might not show corresponding reduced amplitude or latency delay. Therefore, it has been alleged that the mechanisms for amplitude changes and latency changes in mfERG could be different (Vajaranant et al., 2002). Vajaranant et al. (2002) did indicate that the mfERG could provide an alternative method for detecting retinitis pigmentosa.

It is well known that mfERG was once regarded as cone-mediated responses (Sutter & Tran, 1992). Measuring mfERG in RP patients may not be sensitive enough to detect retinal functions, as the damage to the rod system is more predominant than to the cone system in RP patients (Kanski, 1999). For this reason, a method was devised to measure multifocal rod electroretinograms (Hood et al., 1998b). However, it has been shown that measuring multifocal rod electroretinograms on patients with RP provided no more information than measuring the cone-mediated mfERG, as there was a poor correlation between the multifocal rod electroretinograms and the rod system visual fields. It could not, therefore, act as an objective method for measuring rod system visual fields. Recently, it has been reported that mfERG for detecting early retinitis pigmentosa could be further improved by using wide field mfERG, which allows assessment of a 90 degree retinal field (Dolan et al., 2002).

6.2.2 Receptor cell dysfunction

A patient with enhanced S cone syndrome (ESCS) showed a significant reduction in P1 amplitude and a significant increase in P1 latency in the central retinal area. In the peripheral retina, the P1 amplitude also decreased almost to noise level and the N1 and P1 latency were extremely prolonged (Marmor et al., 1999). Patients with cone dystrophy showed greatly reduced or non-detectable responses in the entire test field (Kretschmann et al., 1998b). Another receptor cell dysfunction disease, complete type congenital stationary night blindness (cCSNB), can also be detected using the mfERG (Kondo et al., 2001). Nearly all local retinal areas had

delayed N1 and P1 latencies in patients with cCSNB. Only one of the subjects showed reduced response density. These findings imply that cCSNB mainly affects the latency rather than the response density. An additional finding of this research was that the second positive peak (latency at about 60msec) in the first order response was diminished in patients with cCSNB. The first slice of the second order responses in some of these patients were also severely reduced or even absent. Therefore, it appears that latency increase without amplitude reduction in the first order response is one of the characteristics of congenital stationary night blindness.

6.3 Inner Retinal Diseases

6.3.1 Ocular Hypertension & Glaucoma

An early study indicated that subjects with ocular hypertension can be detected by the mfERG (Chan & Brown, 2000). This study showed that both the first order responses (N1 and P1) and the second order responses were significantly reduced compared to normal subjects in both the central and peripheral retinal areas, but that the reduction was greater in the central than in the peripheral responses. Although this study did not show whether ocular hypertension would affect the latency of mfERG responses, the results of this study implied that mfERG could be a useful tool for the early detection of glaucoma. To further evaluate the effect of ocular hypertension on the mfERG, a later study examined the effect of chronic ocular hypertension on cynomolgous monkeys (Hare et al., 2001). Although this study showed that N1 and P1 amplitudes in first order responses did not show smaller responses than control eyes, the P2 amplitude in the first order responses and the first slice of second order responses were significantly reduced in eyes with ocular hypertension and the reduction was highly correlated with the density of the surviving ganglion cells. Therefore, these results further supported the view that mfERG responses could contain a significant contribution from the ganglion cells.

Furthermore, previous studies have shown that inner retinal activity could be suppressed by intravitreal injection of TTX and NMDA into a monkey's eye (Hood et al., 1999a, Frishman et al., 2000). Hood et al. (1999a) showed that the naso-temporal variation and oscillatory potentials in the monkey's mfERG could be removed by TTX and NMDA. A later study on monkeys showed that the effect of

experimental glaucoma on the first order responses and the first slice of second order responses in mfERG were similar to the effect of TTX and NMDA (Frishman et al., 2000). In addition, the naso-temporal variation and oscillatory potentials also disappeared in the experimental glaucoma eyes. Therefore, this previous study implied that the mfERG could detect glaucomatous damage. A human study showed that a glaucoma defect could be revealed in both the first order responses and the first slice of second order responses (Chan & Brown, 1999). N1 and P1 amplitudes in the first order responses were significantly lower than in normal control subjects and the reductions were more prominent in the central retina than peripheral retina. A later study indicated that glaucoma patients had similar N1 and P1 amplitudes when compared with normal subjects (Hasegawa et al., 2000). Only the latencies of N1, P1, and N2 were significantly increased in glaucoma patients. In addition, this study showed significant negative correlations between the latency (N1, P1 and N2) and the mean sensitivity value (dB) of static perimetry, but no correlation was found between the response density and mean sensitivity value (dB) of static perimetry. Hasegawa et al. (2000) suggested that latency changes were more sensitive than amplitude changes to glaucomatous visual field defects, but they emphasized that mfERG still cannot provide a more sensitive way to detect visual field defects in glaucoma patients than static perimetry, as the loss of sensitivity occurs before the mfERG latency becomes abnormal. Another study, however, has shown that abnormal mfERG responses do not spatially correspond to local sensitivity losses (Fortune et al., 2001).

Using lower contrast stimuli in the mfERG might be a better method to detect retinal damage caused by glaucoma, as larger inner retinal responses are elicited under 50% stimulus contrast (Hood et al., 1999b). When the stimulus contrast was set at 50%, the human mfERG waveform becomes a double peak similar to the waveform of the monkey's mfERG for 100% stimulus contrast. This study showed that the waveforms of glaucoma patients and patients with non-proliferative diabetic retinopathy were similar to monkeys' after TTX and NMDA. Although this method seemed to be a better way to detect inner retinal conditions, using 50% contrast stimulus is still not sensitive enough to detect glaucomatous visual field defects (Hood et al., 2000). In fact, the sensitivities of using either high or low contrast stimuli to detect glaucomatous retinal damage were similar (Palmowski et al., 2000). In conclusion, using mfERG as an objective method to detect glaucomatous visual field defects still needs further investigation.

6.3.2 Diabetic Retinopathy

An early study indicated that the topography of the mfERG in diabetic patients without retinopathy was similar to that in normal subjects in the first order responses (Palmowski et al., 1997). Only diabetic patients with non-proliferative diabetic retinopathy (NPDR) showed a significant increase in N1 and P1 latencies and significant reduction in P1 amplitude. They also demonstrated that the first slice of the second order responses could have a higher sensitivity than the first order responses for detection of subtle retinal change in diabetic patients, as diabetic patients without retinopathy had a significant reduction in amplitude in the second

order responses, and some patients with no retinopathy did not show any detectable second order responses.

That the first slice of the second order responses had higher sensitivity than the first order responses may be due to the fact that they are constructed from different manner. The first order kernel response is the difference between the mean responses to all white stimuli in the sequence and the mean responses to all black stimuli, while the second order kernel response represents the temporal interaction between two white stimuli separated by an integral number of stimulus base intervals (Hood et al., 1997, Keating et al., 2002, Sutter, 2002). Both first and second order responses are constructed from the same set of waveforms, but they are added and subtracted in a different manner (Keating et al., 2002). Hood et al. (2003) believed that the weaker second order responses indicated an abnormality in the retinal circuits and connections involved in adaptation.

As the second order responses, which reflect the interaction between the two consecutive focal flashes, are very sensitive for the detection of retinal function in the diabetic eyes, a later study examined a new stimulation protocol to detect retinal function (Shimada et al., 2001). In this protocol, each m-sequence step consists of four video frames. The first frame is a focal flash; the display is dark in the second frame; the third frame is a global flash; the display is dark again in fourth frame. With this stimulation protocol, the first order responses contain two components. The first component (direct response) is generated by focal flash. This component is expected to be reduced if the recovery from the preceding global flash is impaired. The second component (induced component) is generated by the effect of the focal

flash on the following global flash response 26.7 msec later. They found that “direct responses” were significantly reduced in diabetic patients without retinopathy, but “induced component” amplitude was not significantly reduced in diabetic patients without retinopathy. Therefore, these results showed that diabetic eyes without retinopathy had impaired rates of recovery from the preceding global flash, and this stimulation protocol could detect early retinal changes in the diabetic eye. Another study demonstrated that the mfERG (in standard condition) could reveal local retinal damage in diabetic patients without retinopathy (Fortune et al., 1999). Diabetic eyes with no retinopathy generally showed normal P1 amplitude. Retinal areas with significant defects, such as retinal oedema and haemorrhage, showed a corresponding increase in P1 latency. These findings suggest that the mfERG could be an early indicator of local retinal defects in diabetic eyes and that analysis of mfERG latency variation is necessary as it is sensitive to local retinal damages. In addition, this study clearly showed that the second positive peak (P2) (from 40 msec to 60 msec) in the first order responses was absent or reduced in ophthalmoscopically abnormal areas. A recent study by Schneck et al. (2004) further found that P1 latency showed the greatest sensitivity and association with local retinopathy than N1 latency and N2 latency. Therefore, latency change is a good indicator of local retinopathic changes. In summary, both the latency change and the first slice of second order responses are very sensitive for detecting early retinal changes in diabetic eyes (Palmowski et al., 1997, Fortune et al., 1999).

Chapter 7 - Grading and Classification of Cataract

Any type of opacification or discoloration of the crystalline lens, that reduces visual function below age-matched normal levels, is generally defined as cataract (Hockwin, 1994). The opacity may be localized in certain parts of the lens or diffused through the whole lens. With different locations and sizes of opacity, different degrees of image degradation will result. There are currently no objective guidelines to classify cataract for clinical and research uses, and this has stimulated the need for a good cataract classification system that allows clinicians and researchers to compare and quantify the degree of cataract. Numerous subjective cataract classification systems have been published and developed. Each of these systems has its own advantages and disadvantages, so different researchers have used different classification systems of their epidemiologic studies (Fujisawa et al., 1991, Thompson et al., 1997, Hall et al., 2001). Two famous subjective classification systems and objective grading devices will be introduced.

7.1 Subjective Classification System

7.1.1 The Oxford Clinical Cataract Classification and Grading System (OCCCGS)

The Oxford Clinical Cataract Classification and Grading System is a precise and comprehensive system. It provides a tool for detailed recording of different types of lens opacity. It uses standard diagrams and Munsell colour samples for grading of cortical cataract, posterior subcapsular cataract, and nuclear cataract (Sparrow et al., 1986).

In human lens, it can be roughly divided into 3 zones – lens capsule, cortex and nuclear. This classification system divides the lens structure into a number of discrete concentric shells by lens scattering properties. Therefore, it divides the cortex into C1 to C4. Numerous features can be graded in this system. For *cortical features*, such as anterior sub-capsular opacity, posterior sub-capsular opacity, cortical spoke opacity, waterclefts, vacuoles, retro-dots, focal dots, and anterior clear zone thickness, they can be graded. For *nuclear features*, such as nuclear brunscence and white nuclear scatter, they can also be graded. Moreover, any other features observed can be recorded in the recording chart. For each of the features, special techniques of slit lamp must be used to fully recognize them. Moreover, patient's pupil must be dilated to at least 8mm for grading the degree of cataract.

Recently, this system was modified and decimalised (Sparrow et al., 2000). This modified system simply divides each grade into 10 steps so each step is 0.1. This improvement makes the system more sensitive to the small changes of lens features and more convenient for examiners to perform comparisons between research studies.

This system is very comprehensive and is a good system for *in vivo* study of cataract, as it has good inter-observer and intra-observer repeatability (Sparrow et al., 1988, Sparrow et al., 2000). It is a good system for epidemiological or clinical studies such as clinical trials of anti-cataract drugs. However, the disadvantage of this system is that the time taken to grade a cataract is too long (Sparrow et al., 1986). It is quite complicated and it needs time to train an examiner to be familiar with this system.

7.1.2 Lens Opacities Classification System (LOCS)

The Lens Opacities Classification System was introduced in 1988 (Chylack et al., 1988). It was further improved into LOCS II in 1989 (Chylack et al., 1989). This improved system uses a series of coloured slit lamp and retroillumination standard photographs for examiners to grade the different degrees of nuclear, cortical and subcapsular cataract (Figure 9.1). It has been demonstrated to have good inter-observer and intra-observer reproducibility (Chylack et al., 1989).

As it is so simple to learn and use, LOCSII is widely used in different research areas, such as in the evaluation of visual function (Lasa et al., 1992, Chylack et al., 1993b, Lasa et al., 1995) and ocular physiology (Moss et al., 1995, Rouhiainen et al., 1996). Although LOCS II is applicable in clinical research, it still may be improved. The scaling intervals on all scales are unequal and the scale for nuclear opalescence and nuclear colour is small and coarse (Chylack et al., 1993a). Thus, it has been further improved into LOCS III (Chylack et al., 1993a) which provides more standard photographs for grading the severity of cataract. In addition, the standard photographs have been chosen using objective tests, such as chromaticity, nuclear density, and opacity area measurement; and a decimal scale has been used to improve sensitivity and reduce 95% limits. The most important factor with this system is its excellent inter-observer agreement. However, there are only four lens features being graded. They are 1) nuclear colour (NC), 2) nuclear opalescence (NO), 3) cortical opacities (C), and 4) posterior subcapsular opacities

(P). As with the “Oxford Clinical Classification and Grading System”, different slit lamp techniques should be used in evaluating each feature.

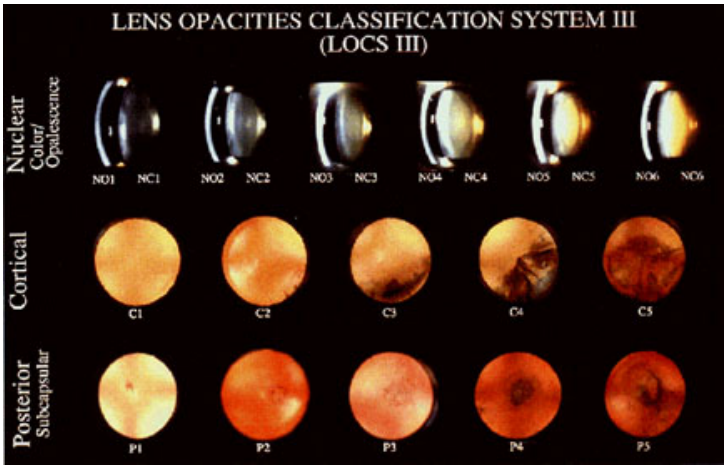

Figure 7.1. Standard photos for LOCS III (Adapted from Chylack et al. (1993)).

7.1.3 Comparison between Different Cataract Classification Systems

A recent study tried to compare the LOCS III and the OCCCGS (Hall et al., 1997). Although both systems use different techniques for assessing cataract features, there was a linear relationship in grading nuclear lens opacities between OCCGS and LOCS III. In addition, a linear relationship was found in grading posterior subcapsular cataract between these two systems. Although, there was no linear relationship in the cortical cataract grading scales between OCCCGS and LOCS III, there was a relationship between OCCCGS scale and the square of LOSCIII in cortical cataract grading. Overall, both systems had a good inter-observer repeatability (Sparrow et al., 1988, Chylack et al., 1993a, Hall et al., 1997).

7.2 Objective Instruments for Grading Cataract

Objective assessment of cataract is essential to the epidemiological or therapeutic study of cataract. Photography is an accurate and permanent method for recording the lens status and for detecting morphological changes of the crystalline lens in vivo (Sasaki et al., 1990). Photographs can be quantified by computer image analysis systems and can be used as a baseline for evaluating changes in the cataract over time. Two commonly used techniques for photographing are Scheimpflug photography and retro-illumination photography.

Scheimpflug Photography

A Scheimpflug slit image of the anterior eye segment allows a cross-sectional view of the lens. This provides information about the dimensions and optical densities of the lens. Lens opacities appear as brighter areas where light scattering from the lens back towards the camera occurs, so it can be used to assess the backward light scatter. As it is a cross-sectional image, it can only detect lens opacities in that plane of section. Opacities in other planes can be missed (Brown et al., 1987), so Scheimpflug photographs should be taken in different meridians to give a more comprehensive assessment of the lens. Therefore, nuclear cataract can be well demonstrated using Scheimpflug images.

Retro-illumination Photography

The retro-illumination image is taken using simultaneous axial illumination and photography of the lens (Brown et al., 1987). As an image taken by this method

can induce a specular reflection from the corneal surface, the reflected light can mislead the observer to regard it as a lens opacity. Therefore, a method was devised to solve this problem by using crossed polarized filters and an orange filter to eliminate the corneal reflection. This method can provide high resolution and high contrast of the image (Kawara & Obazawa, 1980) and shows lens opacities in the form of shadows on a bright background. Therefore, cortical cataract and posterior subcapsular cataract can be detected effectively by retro-illumination images.

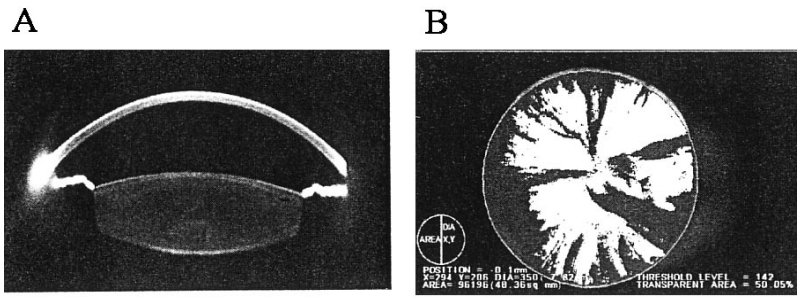

Figure 7.2 (A) Scheimpflug Photography. **(B)** Retro-illumination Photography.

7.2.1 NIDEK EAS-1000

The EAS-1000 is designed to evaluate the anterior segment of the eye. It consists of two units: a camera for image recording and a computer for image storage, system operation and image analysis (Wegener et al., 1992). This instrument can be used to assess radius of corneal curvature, corneal thickness, anterior chamber depth, angle of the anterior chamber, lens thickness, and back scattering light intensity by taking Scheimpflug and retroillumination images (Baez et al., 1992, Sakamoto et al., 1992).

In Scheimpflug images, the lens opacity is analyzed by measuring the intensity of scattered light, which is regarded as equal to the opacification density. The opacity density value is expressed as a computer-compatible tape (CCT) which quantifies the light scattering intensity level from 0 (min) to 255 (max) (Hayashi et al., 1998). In the analysis of the retro-illumination image, the brightness of different points on the digital images is graded in 256 steps from 0 to 255 brightness units (BUs). The higher BU values are more transparent (Wang & Woung, 2000). The Nidek EAS-1000 software defines threshold for cataract automatically at 12% below the brightest point of the histogram of density distribution (Gershenzon & Robman, 1999). The percentage transparency is then calculated with the selected lens area.

In conclusion, nuclear cataract can be demonstrated effectively using Scheimpflug images, while cortical cataract and posterior subcapsular cataract can be well detected by retro-illumination images. For this reason, Scheimpflug image and retro-illumination image should be used in conjunction to assess the state of the lens with cataract (Brown et al., 1987).

7.2.2 Topcon SL-45

This instrument is similar to the EAS-1000, which uses the Scheimpflug slit image for analysis of the anterior segment. The repeatability of using this instrument with the Perkin Elmer microdensitometer for measuring the optical density of lens nucleus and lens cortex was good (Datiles et al., 1987). A study for testing the ability in using Topcon SL-45 for grading the severity of nuclear opacities showed that there was a good relationship between the densitometer readings and subjective gradings of Scheimpflug photography (West et al., 1988). West et al. (1988) suggested that this instrument could effectively record and measure nuclear opacities.

7.3 Comparison of objective test and subjective test

Objective measurement can reduce inter-observer and intra-observer variability. The objective instruments, such as EAS-1000, have good repeatability and reliability (Lam et al. 2002). It can provide a less noisy continuous scale for monitoring lens changes, which is not possible with subjective classification. In addition, EAS-1000 is likely better than Topcon SL-45 as it can also be used to measure lens thickness and lens curvature. Objective measures are sensitive to small changes in lens occurring over short periods of time (Khu & Kashiwagi, 1990, Chylack et al., 1993c). In addition, the recording time for using objective tests are generally less than subjective tests (Chylack et al., 1993c). Time consumption is less for training in use of these machines as compared to other classification systems. However, most of the hardwares and softwares required for objective analysis are

expensive. On the other hand, subjective measurement systems are inexpensive, available and repeatable. However, we do not found any report about the repeatability and reliability of the LOCS III and OCCCGS. The decimalised scales of LOCSIII and the recent versions of OCCCGS are able to provide essentially continuous scales. However, subjective systems such as the Oxford Clinical Classification and Grading System and LOCS III require adequate training for the examiners before assessment. Comparing LOCS III to OCCCGS, LOCS III is much simple than OCCCGS, but OCCCGS allows us to record more lens features than LOCS III (e.g. cortical spoke opacity, waterclefts, vacuoles, retro-dots, focal dots). Thus, the ideal method for recording cataract is using objective methods when possible.

Chapter 8 - Effect of Cataract on Retinal Image

In human eyes, the crystalline lens contains highly ordered molecular and cellular components to maintain its transparency. Any microscopic disturbance of this complex structure can cause the lens to lose the transparency. Disturbances can be in the form of physical damage or of physiological changes to the lens. The most common cause of lens transparency loss is age-related cataract. When the lens transparency is reduced, the quality of retinal image will be affected. Therefore, our spatial vision and contrast sensitivity function (CSF) deteriorate with age. In fact, the modulation transfer function (MTF) in older subjects is lower than in young subjects (Artal et al., 1993). The decline of MTF with age was observed with every pupil diameter but it was more prominent with small pupils (Guirao et al., 1999). Guirao et al. (1999) believed that the decline of MTF was caused by the increase in ocular aberrations and light scattering which affect the retinal image quality. However, the studies by Artal et al. (1993) and Guirao et al. (1999) did not factor in age-related pupillary miosis. The study by Calver et al. (1999) found that the CSF and MTF of an old subject are worse than the MTF of a young subject with the same pupil size. However, old subjects had similar MTF to young subjects at their natural pupil diameters because old subjects had smaller wave-front aberration under natural pupil diameter condition than young subjects. Calver et al. (1999) suggested that the loss of CSF in older subjects is due to light scatter, light absorption, and/or neural changes rather than monochromatic aberration. In the cataractous eye, the quality of the retinal image is mainly influenced by light absorption and light scattering.

8.1 Anatomy and Physiology of the Lens

The adult lens comprises the capsule, epithelium, cortex, and nucleus. In longitudinal section, the lens has an onion-like structure. The cells in the lens are long. They wrap around in their layers from anterior to posterior. The central portion of the lens is the nucleus, which is the oldest part of the lens. The outer part is the cortex. The anterior surface of the lens is a single layer of epithelium cells, which is enclosed by a collagenous capsule (Vavvas et al. 2002).

The human lens has a high protein concentration. Eighty percent of the proteins are water-soluble and consist of crystallins which mainly divid into 3 types: α-, β-, γ-, crystallins (Slingsby & Clout, 1999). The α-crystallins are large macromolecular aggregates (600-4000kDa). The β-crystallins and γ-crystallins are about 20kDa and 18-20kDa in size respectively. The crystallins are not only the dominant structural elements in the lens, but also act as an active signal player in lens development. As the lens ages, there is an increase in the water insoluble proteins which would scatter light.

A number of factors are for the lens to maintain its transparency (Vavvas et al. 2002). Firstly, there are no blood vessels, lymph vessels, and nerves. Secondly, the orderly packaging of lens fibres can minimize the inter-cellular connective tissue. Thirdly, there are no cell nuclei besides the paraxial equatorial region. Fourthly, the cytoplasm in the lens is evenly dense in distribution within lens cells. Fifthly, the crystallins are uniformly packed within lens cells. These factors result in the maintenance of cytoplasmic refractive index and lens transparency. However,

alterations in the density of packing of lens proteins (e.g. protein aggregation caused by UV light) can cause opacities in the lens (Truscott, 2003).

With increasing age, the amount of the water insoluble proteins increases that leads to reduce the transparency of the lens and to cause light scattering. A recent study by Harmmond et al. (2000) clearly found that there is an increase in the optical density of the crystalline lens with age. They also showed that optical density in subjects with dark iris color is higher than subjects with light iris but the difference was only significant in subject with the age over 45 years. The relationships between age and lens optical density for dark iris subjects is “Lens Optical Density (410nm) =1.08 +0.0135 (age (years)), r = 0.68”. The relationship between age and lens optical density for light iris subjects was “Lens Optical Density (410nm) =1.15 + 0.010 (age (years)), r = 0.58”. In addition, the lens optical density had relationship with visual acuity. The relationship was the strongest in subjects with nuclear cataract, very weak in subjects with cortical cataract, and intermediate in subjects with posterior subcapsular cataract (de Waard et al. 1992).

8.2 Light Absorption

Light absorbed by the lens increases with an increasing degree of lens opacity and lens colouring. Measuring the light transmission ratio (TR) at wavelengths 450nm-650nm can give an objective indication of lens opacity and lens colouring (Seland et al., 1992). There is an increase in light absorption at wavelength 320nm and an increase in visible light absorption from wavelengths 400nm to 550nm, when comparing a young lens to an aged lens (Gaillard et al., 2000).

8.3 Light scattering

Light scattering can be physically divided into two types: a) Rayleigh scatter and b) Mie scatter (Figure 8.1). Rayleigh scattering occurs only when the particle size is very small compared to the wavelength of incident light rays. One feature of Rayleigh scattering is its symmetry in forward and backward directions. Mie scattering occurs when particle size is relatively larger than the wavelength of incident light rays. When the particle size increases, there is an increase of forward scattering (Heavens & Ditchburn, 1991).

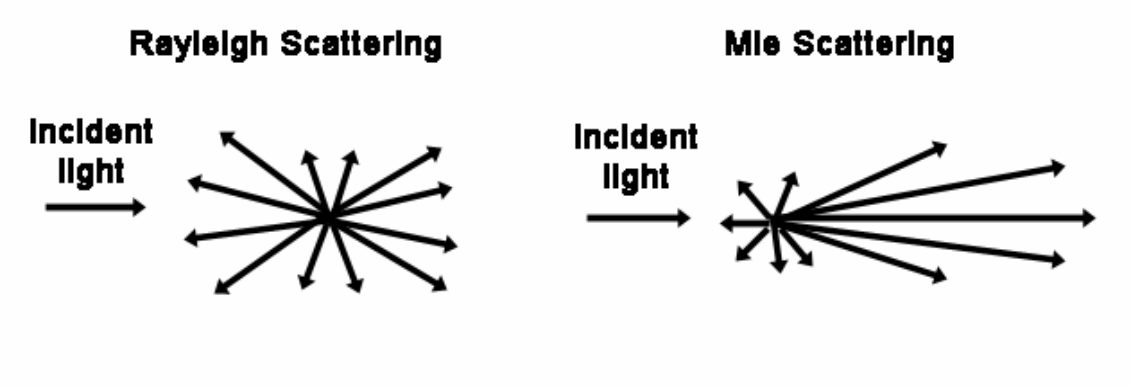

Figure 8.1. Rayleigh scattering and Mie scattering.

Light scattering in a cataract lens is mainly Mie scattering. With increasing age, the amount of insoluble protein increases especially in a cataractous lens (Kamei et al., 1987). The increase in insoluble protein is due to the increased aggregation of crystallins by high energy ultra-violet (UV) radiation. When high energy UV radiation is incident on the lens, the energy must be dissipated by breaking the chemical bonds of the lens proteins, which causes a change in molecular structure and leads to protein aggregation. Once these proteins are aggregated into appropriate size, the criteria for Mie scattering are met.

The method to measure the forward light scattering was first introduced by van den Berg (1986) called flickering glare source method. The design criteria for this apparatus were further developed by Beckman et al. (1991). This method uses a flickering ring-shaped glare source with a circular central test target in the centre. These two targets are shown alternately. Therefore, a flickering appearance will be perceived in the central area. If the subject's ocular media has opacities, the glare source adds stray light to the central test target. The subject can minimize the flickering luminance within central target by adjusting the luminance of the central circular target. Then the amount of forward light scattering can be estimated by knowing the adjusted luminance of the central circular target.

In fact, study had shown that different types of cataract could cause different effect on contrast sensitivity (Elliott & Gilchrist. 1989). Elliot & Gilchrist (1989) found that subject with cortical or nuclear cataract has reduced contrast sensitivity at high spatial frequencies. If the degree of nuclear cataract or cortical cataract is dense,

the medium spatial frequencies become increasingly affected. For subjects with posterior subcapsular cataract, contrast sensitivity at low spatial frequencies also reduces and the loss of contrast sensitivity is not related to visual acuity. Therefore, light scattering could be caused by very different anatomical changes in the three main morphological types of cataract, and those are nuclear, cortical, and posterior subcapsular cataract.

To measure the amount of backward light scattering, we can quantify the slit-lamp observation (e.g. LOCSIII and OCCCGS) or using objective instrument (e.g. Topcon SL-45 & NIDEK EAS-1000) to analyse the Scheimpflug and Retro-illumination images. By using the computerized analysis of Scheimpflug images, there is a positive correlation between the amount of light scattering in the lens and the age of subjects (Smith et al., 1992). The increase was most obvious after the age of 45 years.

Chapter 9 – Visual Assessment of Cataract

In the past, whether cataract surgery was performed or not was mainly based on a patient's visual acuity (VA). The Agency for Health Care Policy and Research (AHCPR) suggested the following guidelines for cataract surgery (O'Day, 1993).

1) Visual acuity (VA) is 6/15 or worse and the reduction is mainly due to cataract.
2) The patient finds difficulties in his/ her daily life, related to poor vision.
3) The expected visual improvement outweighs the potential risk, cost and inconvenience of surgery.

However, patients with VA better than 6/15 may also complain of significant visual problems (Holladay et al., 1987, Koch, 1989, Elliott & Hurst, 1990), suggesting that visual acuity cannot fully represent the performance of the visual system. In fact, AHCPR also recognizes the existence of these aspects of visual function. Therefore, they suggested that practitioners should carefully record the patient's symptoms. Practically, many additional tests can be used in conjunction with VA measurement to assess the visual performance of cataract patients. The most common clinical tests are contrast sensitivity and glare sensitivity tests.

9.1 Visual acuity

Measurement of visual acuity is a standard procedure for estimating visual disability of cataract patients. A high correlation has been found between visual acuity and the degree of nuclear cataract (Drews-Bankiewicz et al., 1992). There is a correspondence between the visual acuity and the degree of cataract by using Lens Opacities Classification System II (LOCS II) for cortical cataract ($r^2 = 0.65$), nuclear

(r^2 = 0.8) and posterior subcapsular cataracts (PSC) (r^2 = 0.36). The relationship of VA to the degree of cataract is the highest for nuclear cataract and the lowest for cortical cataract (Maraini et al., 1994).

However, the clinical visual acuity measurement may not completely show how poor the cataract patient's visual performance is, as visual acuity is normally measured at a high contrast level. In normal situation, the contrast levels of the objects around us are varied, so the measurement may overestimate the patient's visual performance (Brown, 1993).

9.2 Contrast Sensitivity (CS)

In 1978, Hess and Woo found that cataract affected contrast sensitivity at high spatial frequencies more than at low spatial frequencies (Hess & Woo, 1978). Their findings implied that cataract mainly scatters light at small angles. However, few subjects in this study showed contrast sensitivity loss at both high and low spatial frequencies, and the contrast sensitivity loss at low spatial frequencies was not related to VA. They suggested that light could also be scattered at wide angles to affect contrast sensitivity at low spatial frequencies. In addition, they suggested that CS measurement can provide information additional to VA test. By measuring CS in diabetic patients with cataract, CS test was shown to provide more information about cataract-related vision loss than VA (Chylack et al., 1993b).

Elliott and co-workers (1989) investigated the differences between VA and CS among cataract patients with different types of cataract. In all three types of cataract (nuclear, cortical, and posterior subcapsular cataract), there was a significant

decrease in CS at medium and high spatial frequencies (2 c/deg to 10 c/deg). The loss of CS also depended on the severity of cataract. Patients with posterior subcapsular cataract showed significant reduction in CS at low spatial frequency (1 c/deg) with increase in the severity of cataract, but it did not appear in patients with either nuclear or cortical cataract. They also showed that there is a significant correlation between LogMAR VA and CS at high spatial frequencies in all three types of cataract, but poor correlation was found at low spatial frequencies. This indicated that CS measured at low spatial frequencies would give additional information over LogMAR VA in assessing the visual function of cataract patients especially in cases of posterior subcapsular cataract. They also suggested that LogMAR VA would be a good indicator of visual function for cortical and nuclear cataracts, if LogMAR VA is worse than 0.5, but this was not for the case of posterior subcapsular cataract.

9.3 Glare

Cataract patients sometimes complain of poor vision in outdoor activities but have no problems in indoor activities. Neumann and co-workers found that 69.8% of cataract patients had outdoor VA at least 2 lines worse than the indoor VA and 21.7% of patients had outdoor VA at least 5 lines worse than indoor VA (Neumann et al., 1988). The VA measurements for these patients in indoor and outdoor showed dramatic differences, as outdoor environments are normally brighter than indoor environments. The bright light in outdoor environments can produce a veiling luminance on the retina caused by light scattering. This veiling luminance is

superimposed on the retinal image to reduce its contrast and decrease visual ability. Any bright light reducing visibility and/or causing discomfort is called glare. According to the American Academy of Ophthalmology (1990), glare can be traditionally divided into discomfort glare and disability glare (Muscat et al., 2001). *Discomfort glare* is the discomfort caused by the glare light without any measurable effect on the visual function. A common example of discomfort glare is when you walk out from a dark room to a bright environment. *Disability glare* refers to the reduced visibility of an object due to the presence of an extra light source in the visual field which causes light scattering in the ocular media. Reduced visibility of roadway markers in the presence of oncoming headlights is one of the common examples of disability glare. Cornea, lens and fundus are regarded as the main sources of disability glare (Vos, 2003). In addition, the degree of disability glare is depended on glare angle, age and ocular pigmentation. However, it is independent of wavelength. According to the *Age-adjusted Stiles Holladay equation* (Vos, 2003), the disability glare increases rapidly beyond the age of 60 years. It doubles at the age of 70 years and triples at the age of 83 years. Therefore, measurement of VA and CS still cannot fully describe the cataract patients' visual function. A number of authors have suggested that glare tests should be included in the evaluation of cataractous patients (Abrahamsson & Sjostrand, 1986, Elliott & Hurst, 1990, Regan et al., 1993).

Chapter 10 – The Aging of the Retina

10.1 Structural and Morphological Changes

10.1.1 Photoreceptors

In 1981, Gartner and Henkind investigated the effects of aging on human macula by studying 104 necropsy eyes from patients aged 3 to 96. They found that some nuclei of the photoreceptors in the outer nuclear layer (ONL) displaced into the outer plexiform layer (OPL) or into the layer of photoreceptors (rods and cones). At the macula, the displacement of the nuclei from ONL into the OPL is most prominent after age 50. The nuclei displaced into the photoreceptor layer were often enlarged and oval in shape with their long axes parallel to the rods and cones. The nuclei displaced into the OPL were also elongated with their long axes parallel to the oblique nerve fibres of Henle's layer. Gartner and Henkind believed that the nuclear displacement could be due to the traction exerted by other attached cells (e.g. bipolar cells). The reduced number of nuclei in the ONL was not only caused by the displacement of the cells into other layers but also by the reduced number of photoreceptors. Since the number of cells in the ONL was decreased and the number of axons in the OPL was decreased, the OPL was significantly thinner in the aged retina. The most important finding of this study is that the progressive degeneration of cells in the ONL and their photoreceptors can occur without significant changes in adjacent layers (Gartner & Henkind, 1981).

However, a later study showed that the foveal cone density did not decrease significantly with increasing age even in subjects as old as 95 years (Gao & Hollyfield, 1992). Cone density only decreased linearly with increasing age in the

peripheral retina. The average rate of cone loss was 16 cones/mm^2/year. In other words, about 6.7% and 23% of cones were lost at the fourth decade and ninth decade respectively. Similarly, rod density in the peripheral retina decreased with increasing age but not at a uniform rate. The decrease was most prominent between the second and fourth decades but was less prominent after the fourth decade. From their data, about 15 % of rods were lost between the second and fourth decades and about 32% were lost between the second and ninth decades. They, therefore, believed that rods are more vulnerable to loss during aging than cones.

Curcio and colleagues (1993) found that the photoreceptor mosaic of the aged retina is quite similar to the young retina, but the aged retina has two special cytological features. Firstly, there are many highly refractive intracellular inclusions near the ellipsoid myoid junction of the cones. These refractive particles are lipofuscin granules. Secondly, the nuclei of photoreceptors were displaced from the outer nuclear layer into adjacent layers. The mosaic of rod cells did not change significantly in the aged retina. However, the inner segment diameter of rods in aged retina was 13.5% larger than the rods in the young retina, so that the retinal area covered by rods remained constant throughout life. The inner segment diameter of cones does not change with increasing age. The cone density in the fovea (rod-free zone) and in para-fovea did not change significantly with increasing age. In the far peripheral retina, the cone density decreased about 22% from 20 years to 90 years. However, rod density in the inferior retina started to decrease in eyes of 44 years to 58 years. Then the decrease was widely spread across the central retina in eyes of 61 years to 75 years. When comparing retinas of about 37 years to 82 years, rod density

was greatly reduced in an annulus from 0.5 mm to 3mm eccentricity (corresponding to 1.8° to 10° of visual angle in the visual field) and only about 69% of rods remained in retina aged 82 years. In the far peripheral retina (beyond the equator), Curcio et al. (1993) did not find any significant changes of rod density in the aged retina.

10.1.2 Bipolar Cells

There is little information about the effects of aging on the anatomy of bipolar cells (Spear, 1993). Electrophysiological studies may provide information. The scotopic b-wave is generated from the depolarization of the on-bipolar cells (Shiells & Falk, 1999). An earlier full-field flash electrophysiological study showed that the scotopic b-wave amplitude decreased with increasing age (Weleber, 1981). This finding may imply that there are age-related changes in the function of on-bipolar cells. However, a study found that the middle-wavelength sensitive cone ERG b-wave did not decrease with increasing age (Suzuki et al., 1998). As middle-wavelength sensitive cone ERG b-wave is generated by the interaction of the on-bipolar and off-bipolar cells, this may imply that there is a balance between the age related changes of the on-bipolar and off-bipolar cells.

10.1.2 Ganglion Cells

Gao and Hollyfield (1992) showed that cell density in the ganglion cell layer (GCL) was decreased with increasing age in the peripheral retina. The loss was most prominent from the second and fourth decades. About 18% and 40% of cells in ganglion cell layer were lost in the fourth and ninth decades, respectively. In the

fovea, only 16% of cells in the ganglion cell layer were lost from the second to sixth decades. Another study found that the ganglion cell density around the macula (11º of visual field) and in the nasal retina are reduced by one-fourth in the aged retina (Curcio & Drucker, 1993). However, this study did not show that the aged retina has a lower ganglion cell density than the young retina in the periphery (Fig 10.1). The difference between these two studies may be due to the differences in morphometric methods and cell identification criteria.

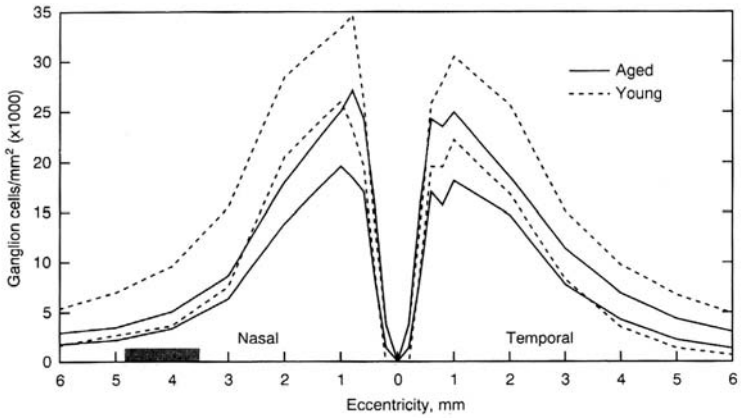

Figure 10.1. Density of ganglion cells in aged and young retina
(One standard deviation above and below the mean for each group is denoted by a pair of either solid or dashed lines.)
(Adapted from Curcio & Drucker (1993))

10.1.3 Retinal Pigment Epithelium (RPE)

Gao and Hollyfield (1992) demonstrated that the retinal pigment epithelium density at the fovea is stable from the second to the ninth decade, but the RPE density in the peripheral retina decreased linearly with increasing age. A recent study

found that apoptotic RPE cells increase significantly with age and are mainly found in the macula (Del Priore et al., 2002). Since the density of RPE cells at the macula does not decrease with age (Gao & Hollyfield, 1992), it is suggested that peripheral RPE cells might migrate to the macula to compensate for the loss of RPE cells. Another feature of RPE cells in the aged retina is that the amount of lipofuscin in RPE cells increases with age (Delori et al., 2001). Since lipofuscin can generate superoxide ions, hydrogen peroxide and lipid peroxide when it is exposed to light (Wassell et al., 1999), this may damage RPE cells and affects the RPE function. These age-related changes are regarded as part of the pathogenesis of age-related macular degeneration (ARM) (Boulton & Dayhaw-Barker, 2001).

10.1.4 Bruch's Membrane

In aged retina, it has been observed that the five-layered structure of Bruch's membrane becomes less ordered (Pauleikhoff et al., 1990). In addition, the amount of debris in or on Bruch's membrane is increased with increasing age (Bird, 1992). These debris are composed of neural fats, neural lipids and phospholipids (Pauleikhoff et al., 1990). These abnormal materials are widely believed to be derived from the degradation products of photoreceptor outer segment material within the RPE cells. During life, the degradation products accumulate progressively within RPE cells and ultimately pass to Bruch's membrane. With increasing age, those degradation products cannot effectively pass through Bruch's membrane to the choroidal capillaries, so more and more debris is accumulated at Bruch's membrane (Farkas et al., 1971, Feeney-Burns & Ellersieck, 1985). Since Bruch's membrane is

between the RPE cells and the choroid, the age-related change of Bruch's membrane would affect the diffusion of nutrient substances passing from the choroid to the RPE cells and finally affects retinal metabolism and its function.

10.2. Functional Changes - Dark Adaptation

One of the common visual problems suggested by the elderly is difficulty with night vision (Jackson et al., 1998). The loss of rod sensitivity or scotopic sensitivity can be caused by optical factors such as decreased pupil size and lens opacities (Daikoku et al., 1982, Pulos, 1989), but can also be due to the loss of rods and ganglion cells (Gao & Hollyfield, 1992, Curcio & Drucker, 1993, Curcio et al., 1993). Pulos (1989) suggested that lower scotopic sensitivity in the elderly is due to optical factors only, as he found that there was no significant difference in scotopic sensitivity between elderly and young subjects, after the correction of the optical factors. Since the oldest subject in that study was only 61 years old, the effect of aging beyond this point was not examined. Therefore, a later study was done on the same topic but it included subjects of 84 years old (Sturr et al., 1997). After correction for optical factors, Sturr et al. (1997) found that rod-mediated sensitivity for the older observers was 0.39 log units lower than that of the younger observers. A more comprehensive study was done to investigate the same topic at different retinal regions (4°, 7°, 32° and 38° both nasal and temporal) (Jackson et al., 1998). Their results further supported the finding of a lower scotopic sensitivity in the elderly, the reduction being due to both optical and neural factors. In addition, they further demonstrated that the sensitivity loss was not eccentricity dependent. This implied that anatomical changes of the retina with age could not fully explain these

findings, as other data have indicated that rod density in the far peripheral retina does not change with increasing age (Curcio et al., 1993).

Another problem frequently complained of by the elderly is substantial delays in adapting to darkness (Jackson et al., 1999). A recent study found that subjects 70 years old had a dramatic slowing in rod-mediated dark adaptation after the correction of optical factors (Jackson et al., 1999). The rod-cone break was significantly delayed (by almost 2 minutes) in 70 years old subjects when compared to 20 years old. The prolongated dark adaptation in elderly subjects implied that the visual cycle, the biochemical pathway responsible for rhodopsin regeneration, was slower in elderly subjects. In the normal situation, the rhodopsin regeneration requires a sufficient quantity of 11-cis-retinal, which is derived from vitamin A (Saari et al., 1998). In the aged retina, Bruch's membrane increases its thickness and decreases its hydraulic conductivity (Pauleikhoff et al., 1990, Moore et al., 1995). This may act as a barrier to affect the supply of vitamin A to rod outer segments through the retinal pigment epithelium, thus delaying the visual cycle in elderly subjects. Although evidence showed that the rhodopsin regeneration rate in elderly was slower than young subjects, a previous study indicated that there was no change or only a mild increase in rod photopigment density as a function of age (Liem et al., 1991). In addition, two studies have shown that foveal sensitivity and cone photopigment density decrease with age especially after 50 years (van Norren & van Meel, 1985, Kilbride et al., 1986). In addition, foveal cone photopigment regeneration was slowed with age (Coile & Baker, 1992). Therefore, both rods and cones exhibit an age-related change in functional status.

Part II - Experiments

Experiment I

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The effects of forward light scattering on the multifocal electroretinogram

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Chapter 11. Experiment 1 - Effect of forward light scattering on multifocal electroretinogram

Abstract

Purpose

To study the effects of forward light scattering on the multifocal electroretinogram (mfERG).

Methods

Thirty young normal subjects were recruited for this study. The mfERG was measured under five conditions: (1) no light scattering (stimulus contrast 93%), (2) mild light scattering (stimulus contrast 80%), (3) moderate light scattering (stimulus contrast 50%), (4) no light scattering (stimulus contrast 80%), and (5) no light scattering (stimulus contrast 50%).

Results

The amplitudes of N1 at all retinal eccentricities did not change significantly, but the amplitudes of P1 in the mid peripheral retina increased with the increase of forward light scattering. By comparing conditions 1, 4 and 5, it was shown that the amplitudes of N1 and P1 decreased significantly in the central retina when stimulus contrast reduced from 93% to 50%.

Conclusions

This study demonstrates that the topography and waveform of the mfERG could be affected by forward light scattering.

Introduction

One of the most common eye diseases in the elderly is cataract, which reduces vision by light scattering and light absorption (Hess & Woo, 1978, Elliott & Hurst, 1990, de Waard et al., 1992, Elliott, 1993, Cook et al., 1994, Gaillard et al., 2000, Foster, 2001). Cataracts are mainly divided into three types: a) nuclear cataract, b) cortical cataract, and c) posterior subcapsular cataract. Different types of cataract could cause different effect on contrast sensitivity (Elliott & Gilchrist, 1989). Elliot & Gilchrist (1989) found that subject with cortical or nuclear cataract could reduce contrast sensitive at high spatial frequencies. If the degree of nuclear cataract or cortical cataract is dense, the medium spatial frequencies become increasingly affected. For subjects with posterior subcapsular cataract, contrast sensitivity at low spatial frequencies also reduced and the loss of contrast sensitivity is not related to visual acuity. Therefore, light scattering could be caused by very different anatomical changes in the three main morphological types of cataract (i.e. nuclear, cortical, and posterior subcapsular cataract).

Light scattering or stray light is regarded as the reason for disability glare (Beckman et al., 1991). Stray light reduces the quality of vision because it reduces the image contrast (IJspeert et al., 1990). Stray light can be quantified in terms of point spread function, as the light scattering causes the shape of "Airy disk" to widen (IJspeert et al., 1990). To measure the glare, a direct measurement technique was introduced by Beckman et al. (1991). Stray light meter is a simple instrument in which a round central target is surrounded by a flickering circular light source acted as a glare source in front of a subject. The luminance of the central target can be

adjusted by the subject and it is initially at the minimum luminance level. Moreover, the central target is flickering in counter-phase to the circular light source. As forward light scatters within the eye, a visible flicker is seen on the central target. The subject only needs to adjust the luminance of central target until no flickering is observed (IJspeert et al., 1990, Beckman et al., 1991). IJspeert et al. (1990) reported that the stray light increased with age and the amount of stray light for a subject of 70 years old was the double of a subject of 20 years old.

Since the cataract is the main problem to cause light scattering, the most effective method of restoring good vision is by removing the cataract, but it is sometimes difficult to know whether reduced vision in cataract patients is due to media opacity or retinal degeneration. In fact, elderly patients with cataract often have other retinal diseases such as age-related macular degeneration (Shuttleworth et al., 1998). Therefore, it would be useful to know whether the loss of vision in a patient with cataract is mainly due to the media opacity or retinal problems before cataract surgery. Numerous methods have been developed for assessing the integrity and function of the retina behind the cataract (Halliday & Ross, 1983, Spurny et al., 1986, Elliott, 1993, Hurst & Douthwaite, 1993, Hurst et al., 1995, Fine & Rubin, 1999, Elliott et al., 2001). The full-field flash ERG is claimed to be a good method to assess the retinal function behind the cataract (Hurst & Douthwaite, 1993). However, this method may not be able to detect subtle retinal changes, as the full-field flash evokes a global response, largely from the peripheral retina.

The development of the multifocal electroretinogram (mfERG) allows quick simultaneous recording of many local retinal locations in a single recording session

of approximately 4 to 8 minutes (Sutter & Tran, 1992). This technique, based on pseudo-random binary m-sequences, has been shown to be an effective way to detect local retinal damage (Bearse & Sutter, 1996, Brown & Yap, 1996, Chan & Brown, 1998, Hood et al., 1998a, Chan & Brown, 1999, Marmor et al., 1999). The mfERG could potentially be a useful objective method for evaluating retinal function behind a cataractous lens, provided that the amplitude and latency of mfERG are not affected by media opacities.

Recent studies have shown that central retinal responses are significantly reduced under light scattering conditions (Arai et al., 1999, Chan et al., 2002a). In these studies, both luminance and contrast of the stimulus decreased with an increase of light scattering (Chan et al., 2002a). Backward light scattering reduces stimulus luminance. Forward light scattering produces a veiling luminance, which reduces stimulus contrast (Elliott, 1993).

It is not known whether the reduced responses in the central retina and the increased response from peripheral retina by light scattering are related to contrast reduction or luminance reduction. In this study, we investigated the effects of forward light scattering on mfERG by controlling both stimulus luminance and contrast.

Method

Subjects

Thirty young subjects (16 males and 14 females) aged 22 to 25 years were recruited from the Optometry Clinic at The Hong Kong Polytechnic University. All subjects had refractive errors less than ±3.00D and less than 1.00D astigmatism, and corrected visual acuity of 6/6 or better. To ensure that all subjects were free of retinal disease or abnormal ocular media in the tested eye, they received an eye examination including visual acuity assessment, biomicroscopy, tonometry and ophthalmoscopy. Research procedures in this study followed the tenets of the Declaration of Helsinki. All procedures were approved by the ethics committee of The Hong Kong Polytechnic University. Informed consent was obtained from all participating subjects after they were given an explanation of the study.

Stimulus Conditions

The VERIS Science 4.1 system (Electro Diagnostic Imaging Inc., San Mateo, CA, USA) was used to record the mfERG. The stimulus matrix consisted of 103 scaled hexagonal elements presented on a high resolution RGB 19" monitor (Sony, GDM-500P3, Japan) with frame rate of 75 Hz which was controlled by a video card (from Electro Diagnostic Imaging Inc.) in a Macintosh G3 computer. A size of 0.8 deg (pen diameter 1%) red fixation cross was used. This monitor subtended a viewing angle of 41° vertically and 53° horizontally at a viewing distance of 40cm. The diameters of different stimulus rings were: Ring 1 (central hexagon): about 2.3°; Ring 2: about 2.3° to 7.8°; Ring 3: about 7.8° to 14°; Ring 4: about 14° to 22.4°; Ring 5: about 22.4° to 31°; Ring 6: about 31° to 40°.

Recording Conditions

Pupils were dilated with 1% tropicamide (Mydriacyl, Alcon, Belgium) to a pupil size at least 6mm. A Dawson-Trick-Litzkow (DTL) electrode was used as an active electrode. The reference and ground electrodes (Ag-AgCl electrode) were attached to the ipsilateral outer canthus and forehead respectively. Only the right eye of each subject was tested and the left eye was patched during recording. The testing distance was 40cm. Refractive errors were fully corrected at that viewing distance. The signals were amplified by 100,000 with band-pass from 3-300Hz (Grass Instrument Co., Quincy, MA, USA) No line filter was used. A binary m-sequence of 2^{14} was used for recording the mfERG. Total recording time was 3 min 38 sec for each complete recording. While the m-sequence of 2^{14} may result in a slightly higher variation of responses than using an m-sequence of 2^{15}, we repeated the mfERG measurements five times for each subject, giving a total recording time of about 45 minutes. The total recording time for five measurements using the 2^{15} m-sequence is about 1hr15min for each subject.

We had tried the longer sequence (2^{15}) but most subjects felt tired and could not maintain good fixation with such a long recording time. Since good fixation is very important in the mfERG measurements, we compromised in choosing the shorter m-sequence (2^{14}) in this study. Each recording was collected in 16 segments of approximately 14 sec. Subjects rested for a few seconds between segments. Any segment with breaks of fixation, eye movements, or blinks was discarded and recorded again.

Measurement

The mfERG was measured on each subject in five conditions (Table 11.1). In all conditions, the average stimulus luminance and background luminance were maintained at 40cd/m^2. In condition 1, the black and white hexagons were 77 and 3 cd/m^2, respectively, with contrast about 93%. In conditions 2 and 3, a liquid-crystal-diffuser (L-C-D) (Edmund Scientific, Industrial Optics Division, NY, USA) was mounted in front of the subject. The transparency of this L-C-D filter can be varied by alteration of the voltage across it. Higher voltage provides higher transparency of the L-C-D filter. When the L-C-D filter was placed in front of the eye, both luminance and contrast of stimulus were reduced. As we were interested in investigating the effects of forward light scattering on the mfERG, the average luminance of the stimulus was kept constant by adjusting the luminance of the white hexagons using the VERIS Science 4.1 system. Since the L-C-D filter causes forward light scattering, the contrast of the stimulus is reduced by the light scattering.

Thus, different degrees of forward light scattering can be produced by different voltages across the L-C-D panel. In condition 2, a mild light scattering condition was created. In this condition, the best VA of all subjects was still about 6/6. The black and white hexagons behind the L-C-D filter were changed to 8 and 72 cd/m^2, respectively, with contrast about 80%. In condition 3, a moderate light scattering condition was created, and the best VA of all subjects was decreased to 6/9. The black and white hexagons behind the L-C-D filter were changed to 20 and 60 cd/m^2, respectively, with contrast 50%. In all conditions, the mean luminance was maintained at 40 cd/m^2 but the forward light scattering caused the stimulus contrast

to be reduced. By comparing the mfERG responses under these three conditions, we can examine the effects of forward light scattering on the mfERG.

Since the stimulus contrast is reduced when the amount of forward light scattering increases, mfERG responses would be expected to be affected by contrast reduction alone. Therefore, the effect of contrast on mfERG responses without the L-C-D filter was studied as a control experiment. The mfERG was measured under two conditions with stimulus contrast at 80% (condition 4) and 50% (condition 5). In condition 4, the black and white hexagons were set at 8 and 72 cd/m^2, respectively, with contrast 80%. In condition 5, the black and white hexagons were set at 20 and 60 cd/m^2, respectively, with contrast 50%. In these two conditions, the mean luminance was the same as in the first part of experiment, but there was no light scattering. The stimulus contrast was adjusted by the VERIS Science 4.1 program. All stimulus luminances were measured using a Minolta LS-110 photometer (Osaka, Japan), which was placed at the position of the eye, and behind the L-C-D filter for conditions 1, 2 and 3. The luminance of black and white hexagons was measured at the rings 3. The luminance of black and white hexagons was measured three times and an average was taken.

In addition, when we use the photometer to measure the luminance of black and white hexagons under different light scattering conditions, we found that the stimulus contrast induced by the scattering medium was very similar at different eccentricities from fixation target across the screen. The variation is less than 10% from the center to periphery. It agreed with the findings of Keating et al. (2000).

In this experiment, the mean stimulus luminance was lower than the standard conditions suggested by the International Society for Clinical Electrophysiology of Vision (Marmor et al., 2003). It is because the maximum luminance of the stimulus monitor was only 165 cd/m^2. Under light scattering conditions, part of the light will be scattered backward and it reduces stimulus luminance. As we need to maintain the mean luminance at constant level in all conditions in this experiment, the maximum luminance of the white hexagons was adjusted to a lower level (77cd/m^2) even in no light scattering condition. Therefore, the mean screen luminance of this experiment was lower than the standard conditions.

All mfERG was measured in a dim room in this experiment, as this would avoid unwanted light scattering caused by the room light incidences on the back surface (i.e. the side face towards the subject's eye) of the L-C-D filter. Since room lighting could affect mfERG responses, all conditions were measured in same room lighting condition. Therefore, we can directly compare the mfERG responses in different conditions.

Table 11.1. Summary of Stimulus Conditions.

Conditions	Luminance of White Hexagons (cd/m^2)	Luminance of Black Hexagons (cd/m^2)	Michelson Contrast of Stimulus (%)	Mean Luminance (cd/m^2)
1 (no light scattering)	77	3	93	40
2 (mild light scattering)	72	8	80	40
3 (moderate light scattering)	60	20	50	40
4 (no light scattering with stimulus contrast 80%)	72	8	80	40
5 (no light scattering with stimulus contrast 50%)	60	20	50	40

In order to demonstrate that the L-C-D filter can cause light scattering similar to that of cataract, the effect of light scattering induced by the L-C-D filter on the contrast sensitivity function (CSF) was measured using the software package 'Psycho for Windows' (Cambridge Research System) (Chan et al., 2002b). If the L-C-D filter has the similar effect of cataract, it would influence the CSF in a similar way of the cataract. Figure 11.1 shows the effect of different degrees of light scattering on the contrast sensitivity function for 8 healthy young subjects. The contrast sensitivity at high spatial frequencies (10 c/deg) was reduced significantly but contrast sensitivity at low spatial frequencies (0.5 c/deg) was only marginally reduced. These data illustrate that the L-C-D filter can produce light scattering effects similar to the nuclear and cortical cataract in real patients (Hess & Woo, 1978; Elliott et al., 1989).

In addition, the light scattering produced by this L-C-D filter is mainly Mie scattering (i.e. less light is scattered to greater angles away from the direction of light travel.), because the effect of light scattering produced by this L-C-D filter mainly affects the contrast sensitivity at high spatial frequencies. If the L-C-D filter produces a wide angled light scattering (e.g. Rayleigh scattering), the contrast sensitivity at low spatial frequencies will be affected significantly (Elliott, 1993).

Pelli-Robson letter chart was not used in this experiment, as it has relative poor sensitivity to mild light scattering and it only measure contrast sensitivity at one spatial frequency. Therefore, it may not provide a more sensitivity measure of light scattering than VA (Elliott, 1993).

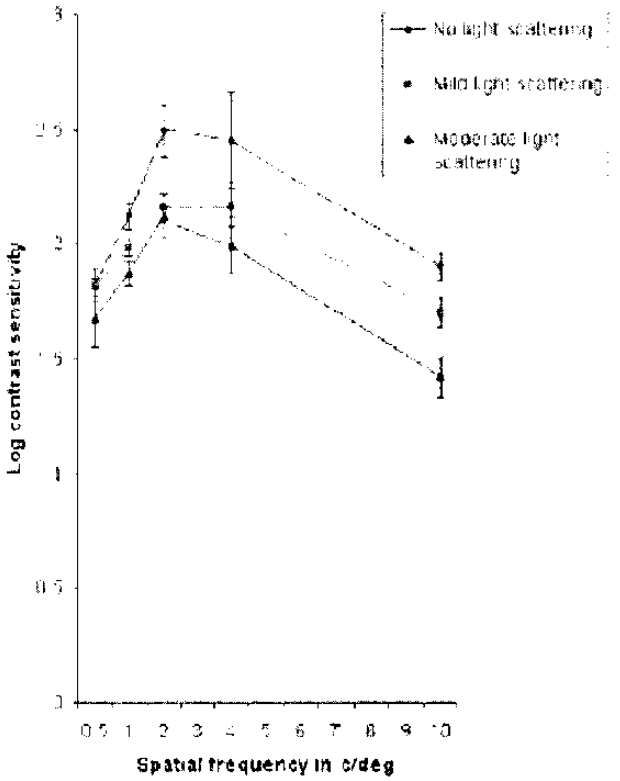

Figure 11.1 The effects of different degrees of light scattering produced by the liquid-crystal-diffuser (L-C-D) on contrast sensitivity function. Error bars are ± 1 standard deviation of the mean of 8 subjects.

Analysis

In data analysis, mfERG responses were grouped in two ways: **(a) Summed response**: 103 responses were summed together (Figure 11.2A), **(b) Six concentric rings**: responses with similar eccentricities from the central foveal response were grouped (Figure 11.2B). In this study, the first-order kernel responses were analyzed. In addition, only amplitudes and latencies of N1 and P1 were evaluated. We defined the first negative and positive deflections of the mfERG as N1 and P1 respectively. The amplitude of N1 was measured from the baseline to the first negative peak. Amplitude of P1 was measured from the first negative peak to the first positive peak. Latencies of N1 and P1 were defined as the time periods from the stimulus onset to the peak of N1 and P1, respectively. The effects of forward light scattering and contrast reduction on summed mfERG responses were evaluated by using one-way repeated measures ANOVA (RM ANOVA) and the Tukey multiple comparisons test was used as a post-hoc test. The effects of forward light scattering and contrast reduction on mfERG responses from the six concentric rings were evaluated by using two-way ANOVA and the Tukey multiple comparisons test was used as a post-hoc test. We use Tukey's post-hoc analysis instead of Scheffe post-hoc test, as Tukey's post-hoc test is suitable for comparing groups of equal sizes. On the other hand, Scheffe is more suitable for comparing groups of unequal sizes. When a significant interaction was found, multiple pairwise comparisons with the Tukey's test were used to compare each group. P-values less than 0.05 were considered statistically significant.

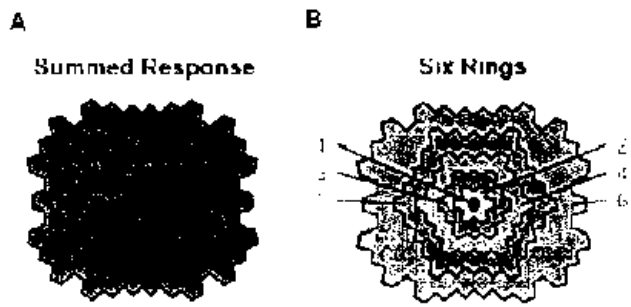

Figure 11.2. Responses from 103 stimulus hexagons are grouped into two ways. (**A**) Summed response. (**B**) Six concentric rings.

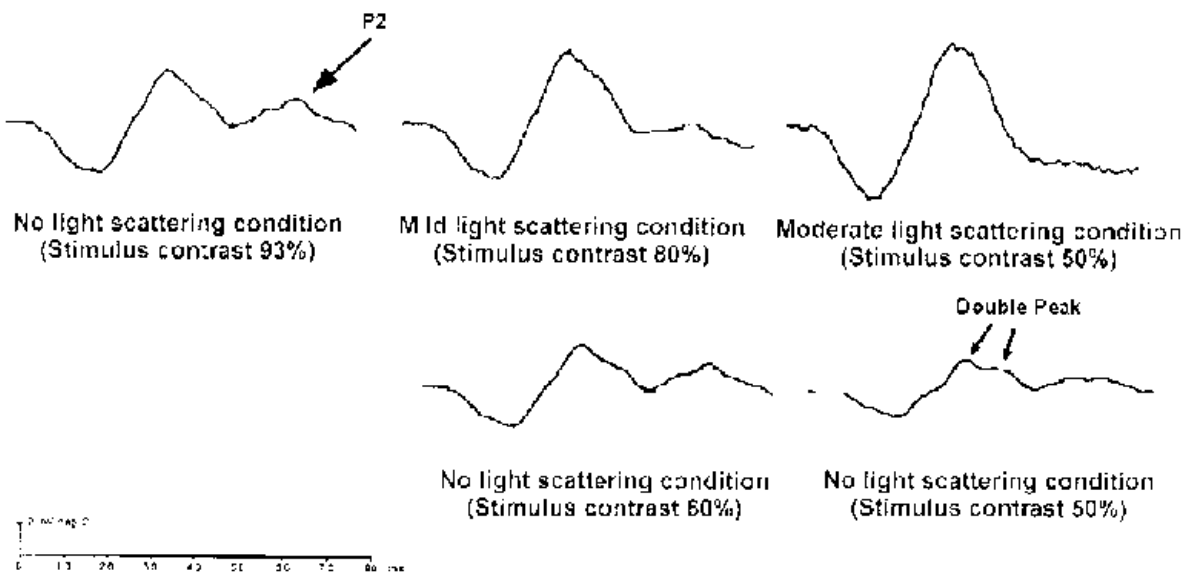

Figure 11.3. Summed response waveforms (first order kernel response). N1 and P1 amplitudes increased when light scattering levels increased. Under no light scattering condition, both N1 and P1 amplitudes decreased when stimulus contrast decreased.

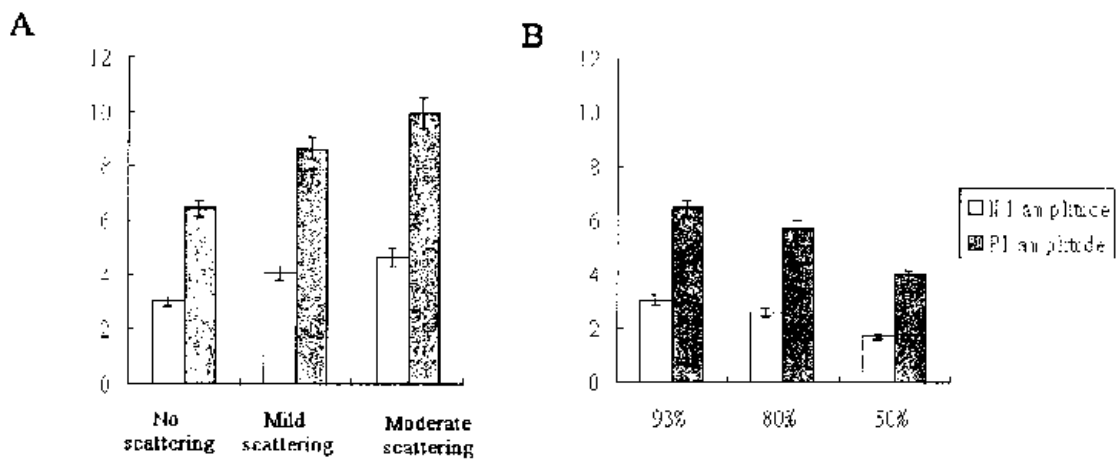

Figure 11.4. First order kernel response. (**A**) Mean N1 and P1 amplitudes of summed responses at three conditions: no light scattering, mild light scattering, and moderate light scattering. (**B**) Mean N1 and P1 amplitudes of summed responses at three different stimulus contrast conditions: 93%, 80%, and 50%. Error bars are ± 1 standard error of the mean.

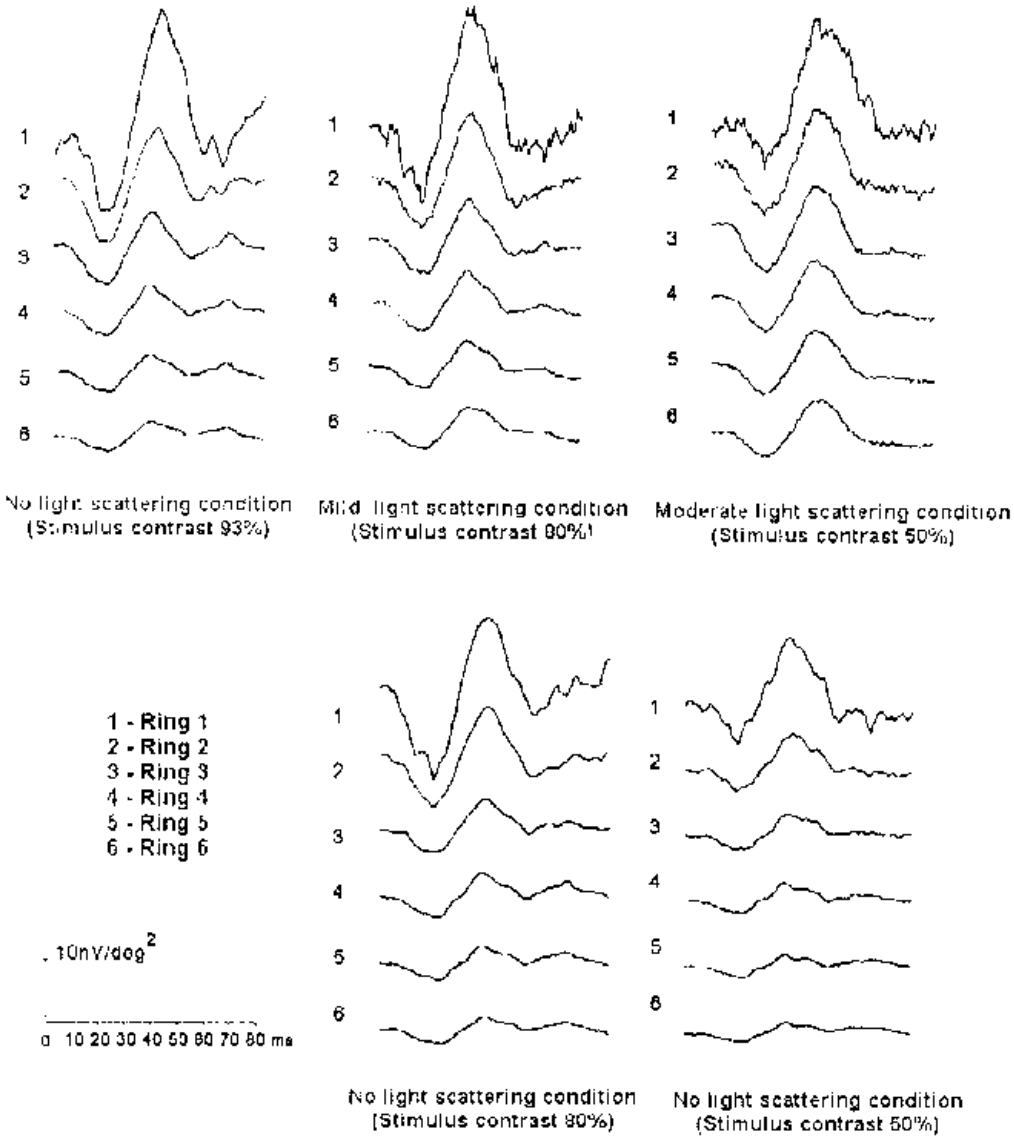

Figure 11.5. Response waveforms (first order kernel response) from six concentric rings. N1 and P1 amplitudes increased when light scattering levels increased. Under no light scattering condition, both N1 and P1 amplitudes decreased when stimulus contrast decreased.

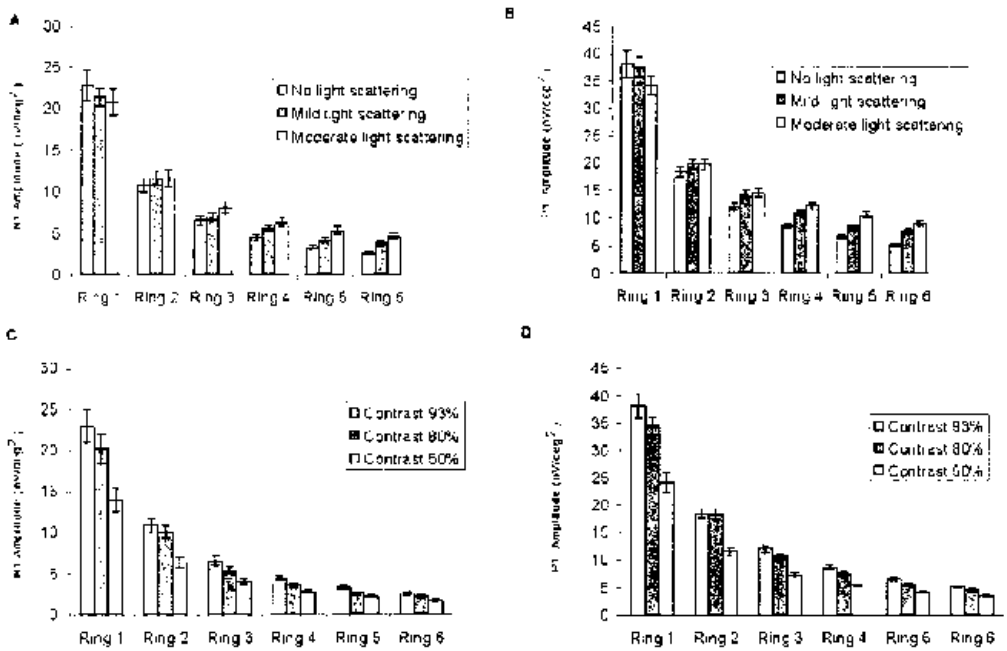

Figure 11.6. First order kernel response (**A**) Mean N1 amplitudes and (**B**) Mean P1 amplitudes of six concentric rings at three conditions: no scattering, mild scattering, and moderate scattering. (**C**) Mean N1 amplitudes and (**D**) Mean P1 amplitudes of six concentric rings at three stimulus contrast conditions: 93%, 80%, and 50%. Error bars are ± 1 standard error of the mean.

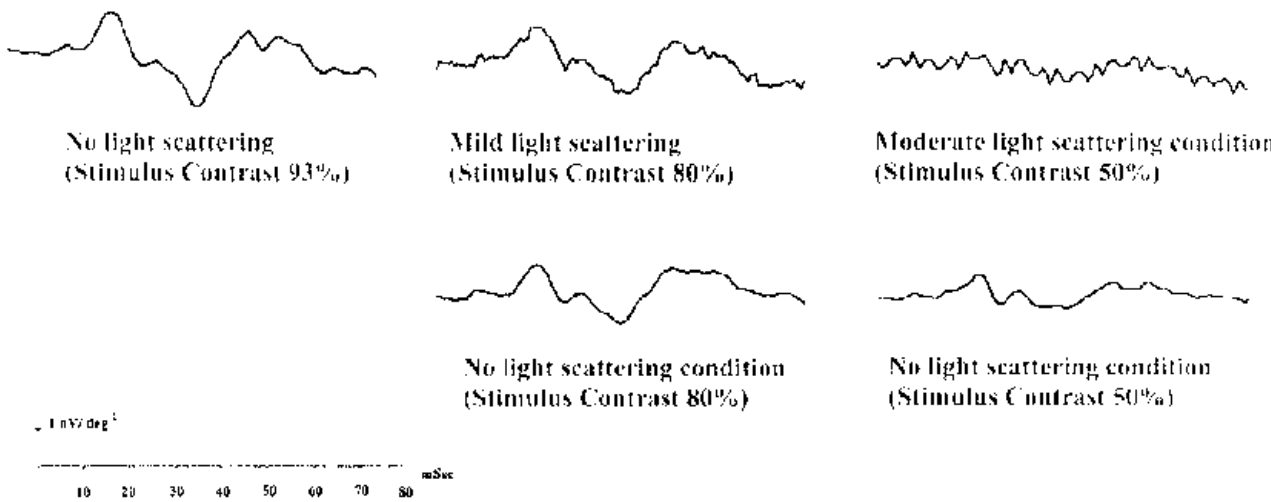

Figure 11.7. Summed response waveforms (First slice of the second order kernel response).
First slice of the second order kernel response was undetectable in moderate light scattering condition.

Table 11.2. Effect of forward light scattering on mfERG responses (first order kernel response) parameters and statistical findings: Summed response.

	No light scattering condition with stimulus contrast 93%	Mild light scattering condition with stimulus contrast 80%	Moderate light scattering condition with stimulus contrast 50%	RM ANOVA (df=2)	Tukey multiple comparison test
N1 amplitude (nV/deg^2)	3.04 ± 0.18	4.09 ± 0.25	4.68 ± 0.32	p< 0.000 * F=30.883	* ◆ ❖
P1 amplitude (nV/deg^2)	6.45 ± 0.27	8.66 ± 0.38	9.95 ± 0.53	p< 0.000 * F=67.250	* ◆ ❖
N1 latency (ms)	21.03 ± 0.29	20.72 ± 0.30	20.83 ± 0.32	p= 0.607 F=0.504	
P1 latency (ms)	37.31 ± 0.29	37.51 ± 0.27	38.62 ± 1.07	p= 0.273 F=1.328	

Data are presented as mean ± 1 SEM
* Significant difference (p< 0.05) among three conditions
* Results differ significantly between no light scattering condition and mild light scattering condition (p<0.05)
◆ Results differ significantly between no light scattering condition and moderate light scattering condition (p<0.05)
❖ Results differ significantly between mild light scattering condition and moderate light scattering condition (p<0.05)

Table 11.3. Effect of contrast on mfERG responses (first order kernel response) parameters and statistical findings: Summed response.

	No light scattering condition with stimulus contrast 93%	No scattering condition with stimulus contrast 80%	No scattering condition with stimulus contrast 50%	RM ANOVA (df=2)	Tukey multiple comparison test
N1 amplitude (nV/deg^2)	3.04 ± 0.18	2.60 ± 0.15	1.73 ± 0.12	p< 0.000 * F=38.64	* ◆ ❖
P1 amplitude (nV/deg^2)	6.45 ± 0.27	5.73 ± 0.25	4.01 ± 0.18	p< 0.000 * F=78.84	* ◆ ❖
N1 latency (ms)	21.03 ± 0.29	20.84 ± 0.34	20.75 ± 0.26	p= 0.647 F=0.439	
P1 latency (ms)	37.31 ± 0.29	37.19 ± 0.29	36.48 ± 0.37	p= 0.866 F=0.144	

Data are presented as mean ± 1SEM
* Significant difference (p< 0.05) among three conditions
* Results differ significantly between stimulus contrast 93% and stimulus contrast 80% (p<0.05)
◆ Results differ significantly between stimulus contrast 93% and stimulus contrast 50% (p<0.05)
❖ Results differ significantly between stimulus contrast 80% and stimulus contrast 50% (p<0.05)

Table 11.4. Effect of forward light scattering on mfERG responses (first order kernel response) parameters and statistical findings: Responses from six concentric rings.

		No light scattering condition with stimulus contrast 93%	Mild light scattering condition with stimulus contrast 80%	Moderate light scattering condition with stimulus contrast 50%	Two-Way ANOVA	Multiple Pairwise Comparisons with Tukey's Test
N1 amplitude (nV/deg²)	Ring 1	22.83 ± 1.92	21.42 ± 1.05	20.79 ± 1.53	**Scattering level** p=0.080 F[2,522]=2.539 **Ring** p=0.000* F[5,522]=216.323 **Interaction** p=0.412 F[10,522]=1.035	
	Ring 2	10.82 ± 0.79	11.66 ± 0.83	11.62 ± 1.09		
	Ring 3	6.56 ± 0.52	6.99 ± 0.40	8.11 ± 0.72		
	Ring 4	4.47 ± 0.30	5.62 ± 0.32	6.34 ± 0.52		
	Ring 5	3.31 ± 0.24	4.20 ± 0.23	5.34 ± 0.48		
	Ring 6	2.57 ± 0.17	3.86 ± 0.23	4.63 ± 0.39		
P1 amplitude (nV/deg²)	Ring 1	38.04 ± 2.40	37.42 ± 1.73	34.18 ± 1.71	**Scattering level** p=0.001* F[2,522]=6.949 **Ring** p=0.000* F[5,522]=368.987 **Interaction** p=0.008* F[10,522]=2.427	◆❖
	Ring 2	18.39 ± 0.90	19.88 ± 0.82	19.71 ± 1.09		
	Ring 3	12.00 ± 0.64	14.44 ± 0.64	14.65 ± 0.77		◆
	Ring 4	8.62 ± 0.41	10.99 ± 0.47	12.27 ± 0.61		◆
	Ring 5	6.60 ± 0.29	8.54 ± 0.38	10.60 ± 0.54		◆
	Ring 6	5.18 ± 0.26	7.81 ± 0.38	9.09 ±0.46		
N1 latency (ms)	Ring 1	20.79 ± 0.55	20.14 ± 0.51	20.88 ± 0.48	**Scattering level** p=0.326 F[2,522]=1.124 **Ring** p=0.066 F[5,522]=2.082 **Interaction** p=0.846 F[10,522]=0.561	
	Ring 2	19.95 ± 0.42	20.14 ± 0.48	20.33 ± 0.44		
	Ring 3	19.57 ± 0.60	20.64 ± 0.41	20.14 ± 0.36		
	Ring 4	20.02 ± 0.41	20.56 ± 0.39	20.67 ± 0.22		
	Ring 5	20.53 ± 0.45	21.03 ± 0.29	20.89 ± 0.32		
	Ring 6	20.94 ± 0.44	21.26 ± 0.27	20.81 ± 0.29		
P1 latency (ms)	Ring 1	39.19 ± 0.62	38.48 ± 0.53	38.85 ± 0.66	**Scattering level** p=0.068 F[2,522]=2.708 **Ring** p=0.023* F[5,522]=2.637 **Interaction** p=0.485 F[10,522]=0.952	
	Ring 2	38.29 ± 0.44	38.27 ±0.45	38.34 ± 0.49		
	Ring 3	37.53 ± 0.36	37.67 ± 0.30	37.61 ± 0.37		
	Ring 4	37.22 ± 0.32	37.15 ± 0.32	39.11 ± 1.04		
	Ring 5	37.31 ± 0.30	37.69 ±0.32	38.39 ± 0.47		
	Ring 6	38.03 ± 0.30	38.24 ±0.4.	38.63 ± 0.44		

Data are presented as mean ± SEM
* Significant difference (p< 0.05) among three conditions
✱ Results differ significantly between no light scattering condition and mild light scattering condition (p<0.05)
◆ Results differ significantly between no light scattering condition and moderate light scattering condition (p<0.05)
❖ Results differ significantly between mild light scattering condition and moderate light scattering condition (p<0.05)

Table 11.5. Effect of contrast on mfERG responses (first order kernel response) parameters and statistical findings: Responses from six concentric rings.

		No light scattering condition with stimulus contrast 93%	No light scattering condition with stimulus contrast 80%	No light scattering condition with stimulus contrast 50%	Two-way ANOVA	Multiple Pairwise Comparisons with Tukey's Test
N1 amplitude (nV/deg^2)	Ring 1	22.83 ± 1.92	20.15 ± 1.67	13.87 ± 1.43	**Contrast level**	★◆❖
	Ring 2	10.82 ± 0.79	10.04 ± 0.72	6.31 ±0.61	p=0.000* F(2,522)=27.684	★◆❖
	Ring 3	6.56 ± 0.52	5.38 ± 0.41	4.02 ± 0.30	**Ring**	◆
	Ring 4	4.47 ± 0.30	3.66 ± 0.25	2.76 ± 0.22	p=0.000* F(5,522)=200.692	
	Ring 5	3.31 ±0.24	2.64 ± 0.16	2.20 ± 0.15	**Interaction**	
	Ring 6	2.57 ±0.17	2.23 ± 0.13	1.71 ± 0.13	p=0.000* F(10,522)=4.296	
P1 amplitude (nV/deg^2)	Ring 1	38.04 ± 2.40	34.29 ± 1.62	24.03 ± 1.72	**Contrast level**	★◆❖
	Ring 2	18.39 ± 0.90	18.29 ± 1.00	11.55 ± 0.67	p=0.000* F(2,522)=59.20	◆❖
	Ring 3	12.00 ± 0.64	10.59 ± 0.59	7.20 ± 0.39	**Ring**	◆❖
	Ring 4	8.62 ± 0.41	7.42 ± .0.32	5.34 ± 0.23	p=0.000* F(5,522)=391.415	◆
	Ring 5	6.60 ± 0.29	5.55 ± 0.23	4.17 ± 0.18	**Interaction**	
	Ring 6	5.18 ± 0.26	4.59 ± 0.18	3.38 ± 0.16	p=0.000* F(10,522)=6.965	
N1 latency (ms)	Ring 1	20.79 ± 0.55	20.07 ± 0.41	20.09 ± 0.57	**Contrast level**	
	Ring 2	19.95 ± 0.41	20.03 ± 0.38	20.00 ± 0.47	p=0.098 F(2,522) 2.330	
	Ring 3	19.57 ± 0.80	20.48 ± 0.41	20.07 ± 0.49	**Ring**	
	Ring 4	20.02 ± 0.41	21.03 ± 0.65	20.25 ± 0.43	p=0.007* F(5,522)=3.245	
	Ring 5	20.53 ± 0.82	21.39 ± 0.65	20.94 ± 0.44	**Interaction**	
	Ring 6	20.94 ± 0.44	22.33 ± 0.85	20.80 ± 0.34	p=0.729 F(10,522)=0.696	
P1 latency (ms)	Ring 1	39.19 ± 0.62	37.17 ± 0.50	38.59 ± 0.59	**Contrast level**	
	Ring 2	38.29 ± 0.44	38.04 ± 0.46	38.00 ± 0.49	p=0.104 F(2,522)=2.274	
	Ring 3	37.53 ± 0.36	38.03 ± 0.41	37.30 ± 0.54	**Ring**	
	Ring 4	37.22 ± 0.33	37.03 ± 0.29	36.21 ± 1.27	p=0.003* F(5,522)=3.637	
	Ring 5	37.31 ± 0.30	37.02 ± 0.35	36.95 ± 0.49	**Interaction**	
	Ring 6	38.03 ± 0.30	37.42 ± 0.38	36.87 ± 0.45	p=0.493 F(10,522)=0.943	

Data are presented as mean ± 1 SEM

* Significant difference ($p < 0.05$) among three conditions

★ Results differ significantly between stimulus contrast 93% and stimulus contrast 80% ($p<0.05$)

◆ Results differ significantly between stimulus contrast 93% and stimulus contrast 50% ($p<0.05$)

❖ Results differ significantly between stimulus contrast 80% and stimulus contrast 50% ($p<0.05$)

Summed Response

The waveforms of mfERG for five different conditions were shown in Figure 11.3. N1 and P1 latencies did not change significantly when the amount of forward light scattering level increased. N1 and P1 amplitudes increased significantly in both mild light scattering condition (stimulus contrast 80%) and moderate light scattering condition (stimulus contrast 50%) (Figure 11.4A & Table 11.2).

Under conditions of no light scattering, N1 and P1 latencies did not change significantly in any reduced contrast conditions, but N1 and P1 amplitudes reduced significantly when stimulus contrast reduced from 93% to 80% and 50% (Figure 11.4B & Table 11.3).

Responses from six concentric rings

Effects of light scattering and contrast reduction on mfERG waveforms were shown in Figure 11.5, Table 11.4 and Table 11.5. There was no significant interaction of light scattering level and ring grouping for N1 latency, P1 latency, and N1 amplitude. There were no effects of light scattering on N1 latency, P1 latency, and N1 amplitude. However, there were statistically significant effects of ring grouping on P1 latency and N1 amplitude for each light scattering level. Tukey multiple comparisons test showed that P1 latency from ring 1 was significantly longer than ring 3 and N1 amplitude decreases significantly with increasing eccentricity as expected (Figure 11.6A).

There was a significant interaction between light scattering level and ring grouping for P1 amplitude. There were statistically significant effects of light

scattering on P1 amplitude and of ring grouping on P1 amplitude for each light scattering level. Multiple pairwise comparisons with Tukey's test showed that P1 amplitude from ring 1 decreased significantly in the moderate light scattering condition, but P1 amplitude from ring 4 to ring 6 increased significantly in the moderate light scattering condition (Fig. 11.6B).

Under the no light scattering condition, there were no statistically significant interaction effects for N1 latency and P1 latency. There were no effects of contrast reduction on N1 latency and P1 latency (Table 11.5), but there were statistically significant effects of ring grouping on these parameters for each contrast level. Tukey multiple comparisons test showed that N1 latencies from ring 2 and ring 3 were significantly shorter than ring 6, and P1 latencies from ring 1 and ring 2 were significantly longer than ring 4.

There were significant interactions between contrast level and ring grouping for N1 amplitude and P1 amplitude. There was a statistically significant effect of contrast reduction on N1 amplitude and P1 amplitude. There were statistically significant effects of ring grouping on N1 amplitude and P1 amplitude for each contrast level. In addition, multiple pairwise comparisons with Tukey's test showed that N1 amplitude from ring 1 to ring 3 decreased significantly with stimulus contrast 50% (Figure 11.6C). N1 amplitude from ring 1 and ring 2 also decreased significantly when stimulus contrast was 80%. P1 amplitude from ring 1 to ring 4 decreased significantly when stimulus contrast was 50%. P1 amplitude from ring 1 decreased significantly when stimulus contrast was 80% (Figure 11.6D).

Discussion

Our results showed that forward light scattering affects the topography of the mfERG. From the summed response, mfERG response amplitudes increased when the amount of forward light scattering increased. N1 and P1 latencies were not affected by the amount of forward light scattering. P1 amplitude from ring 1 decreased significantly in the moderate light scattering condition but P1 amplitudes from ring 3 to ring 6 were increased when the amount of forward light scattering was increased. In this study, the stimulus contrast reduced with the increase of forward light scattering due to a veiling luminance that was superimposed on the retinal image to cause a contrast lowering effect (Elliott et al., 1989). It has been shown that the mfERG responses decrease with reduction of stimulus contrast (Brown & Yap, 1996). Our control experiment also showed that N1 and P1 amplitudes decreased significantly at the central retina with reduced stimulus contrast, but N1 and P1 latencies at all retinal eccentricities did not change when stimulus contrast was reduced.

Previous studies have shown that decrease of stimulus luminance or contrast decreases both central and peripheral responses (Brown & Yap, 1996, Yoshii et al., 2000b, Gerth et al., 2002). In our study, we also demonstrated that the mfERG responses from central and peripheral retina decreased with the decrease of stimulus contrast, but only the decrease from central retina was statistically significant. As forward light scattering reduces contrast, both central and paracentral responses would be expected to decrease under light scattering. However, previous studies have reported that central responses were slightly decreased and mid-peripheral

responses did not decrease prominently with the increase of light scattering (Arai et al., 1999, Chan et al., 2002a). These studies used an acrylic sheet or a liquid crystal diffuser (L-C-D) to produce the light scattering. Both the acrylic sheet and the L-C-D can reduce the mean luminance and contrast of the stimulus. This could explain why they showed a significant reduction in central responses. In our previous study, we did not compensate for the reduction of mean luminance by the L-C-D filter (Chan et al., 2002a). Therefore, we could not isolate the effect of light scattering from mean luminance reduction. In the present study, the experimental design compensates for the mean luminance reduction produced by the L-C-D filter. This may explain why a larger mid-peripheral response was obtained under the light scattering condition. In our study, the mean luminance was kept constant for all conditions and only contrast was reduced with the increase of light scattering. By eliminating the luminance factor, we found that the central retina is sensitive to the changes of contrast. In the presence of light scattering, the mid-peripheral retina seems to be more responsive to forward light scattering than the central retina, as central retinal responses still decreased significantly with the increase of forward light scattering, but peripheral retinal responses increase significantly. In addition, similar results were found when we repeated this experiment using a non-scaled stimulus, in which all hexagons are the same size. Therefore, the larger size of the peripheral stimulus hexagons is not the reason to obtain larger responses in mid-peripheral retina under the light scattering condition.

Larger than normal scotopic flash ERG responses can be recorded in subjects with medium opacities, as the "Ganzfeld effect" produced by media opacities can

direct light to other parts of the retina (Galloway, 1988). Under these conditions a larger area of retina will be stimulated under the light scattering condition, so a considerably larger than normal response can be obtained in cataract cases. We speculate that similar phenomenon may occur in the mfERG. However, why this phenomenon was only observed at the peripheral retina and not at the central retina is still not clear. This may be due to the amplitude variation in the central retinal region which is relatively larger than in the peripheral retinal region (Verdon & Haegerstrom-Portnoy, 1998), so the change cannot be easily observed in the central retinal region. In our study, we also found that the amplitude variation in the central retinal region is larger than in the peripheral region. For example, the coefficient of variation for P1 amplitude in ring 1 under no scattering condition is 35% and the coefficient of variation in ring 6 is 28%. The central retinal responses decreased with the increase of forward light scattering may be related to the Stiles-Crawford effect. The Stiles-Crawford effect, light which is incident directly along the cone axis will be a more effective stimulus, may occur under forward light scattering condition. As part of the scattered light is likely to be incident at oblique angles, the probability of photon absorption should be reduced. On the other hand, the L-C-D filter reduces the contrast of the stimulus, as forward light scattering produces a veiling luminance, which is superimposed on the retinal image (Elliott, 1993); mfERG responses from the central retina could be decreased when the contrast of the stimulus is decreased (Brown & Yap, 1996, Chan & Brown, 1998, Yoshii et al., 2000b, Raz et al , 2002) because the macular response is more sensitive than the peripheral response to the contrast reduction of stimulus.

The effects of aging on the mfERG have been widely studied (Mohidin et al., 1999, Fortune & Johnson, 2002, Gerth et al., 2002, Jackson et al., 2002a, Nabeshima et al., 2002, Gerth et al., 2003, Seiple et al., 2003, Tzekov et al., 2004). It has been found that the central retinal responses decrease more rapidly than peripheral retinal responses with increasing age. Fortune et al. (2002) believed that the effect of aging is mainly due to optical factors, but Seiple et al. (2003) believed that it is due to neural factors. Gerth et al. (2002) demonstrated that it is related to both optical and neural factors. Gerth et al. (2003) further found that the isolated flash response decreased with increasing age and they believed that it is also related to both optical and neural factors. A recent study by Tzekov et al. (2004) found that the mfERG responses declined with age. It is well-known that the light scattering of the crystalline lens increases with increasing age especially in subjects above 45 years old (Siik et al., 1992, Hennelly et al., 1998). Our study clearly demonstrated that moderate light scattering would affect mfERG topography. Our results, therefore, also suggest an important role for optical factors in the effects of aging on the mfERG.

In this study, we noted that the mfERG waveform exhibited double peaks when the stimulus contrast was 50% as shown in Figure 11.3. However, the double peaks were not observed under the moderate light scattering condition with a stimulus contrast of 50%. What do these additional peaks imply? Previous studies suggested that measuring the mfERG in humans using a stimulus contrast level of 50% might elicit a ganglion cell component (Hood et al., 1999b, Hood et al., 2000, Palmowski et al., 2000), as the optic nerve head component saturates at 60% contrast and

mfERG responses could be dominated by other retinal components when it is measured at high stimulus contrast (>75%) (Sutter & Bearse, 1995). The waveform of the human mfERG changes from a single peak to double peaks when it is measured at 50% contrast. This double-peak mfERG waveform measured in humans was similar to the monkey mfERG measured at 100% contrast (Hood et al., 1999a). The waveform of the mfERG measured on patients with glaucoma or non-proliferative diabetic retinopathy using 50% stimulus contrast was reported to be similar to the effect of TTX (Tetrodotoxin) + NMDA (N-methyl-D-aspartic acid) on the monkey's mfERG measured using 100% stimulus contrast (Hood et al., 1999b). A recent study also demonstrated that this second positive peak was significantly diminished or reduced in glaucomatous eyes of cynomolgus monkeys (Raz et al., 2002). This finding implied that this second positive peak may represent the activities of inner retinal layers as well as ganglion cells. Our results indicate that the mild to moderate light scattering condition will diminish the second positive peak of mfERG responses and the second order kernel mfERG responses. This implies that the forward light scattering might affect inner retinal activity. Since forward light scattering can also affect the appearance of the double-peak, we therefore tentatively conclude that manipulation of stimulus contrast in diagnosis of ganglion cell malfunction may not be suitable for patients with cataract or with light scattering problems.

With moderate light scattering, a positive peak with latency at about 60ms disappeared (Figure 11.3; P2). In our previous study, we also reported this phenomenon (Chan et al., 2002a). This positive wavelet (P2) was still observable

even for stimulus contrast of 50%, but it was severely attenuated to noise level when the mfERG was measured under moderate light scattering conditions. A previous study has shown that this small positive wavelet (P2) was absent in patients with central retinal vein occlusion, but was present in normal subjects and in patients with maculopathies or with autosomal dominant optic atrophy (Kretschmann et al., 1998b). It has been proposed that this positive wavelet (P2) was generated from inner retinal layers and beyond the ganglion cells (Kretschmann et al., 1998b). This wavelet was also diminished or absent in patients with moderate non-proliferative diabetic retinopathy and in patients with the complete type of congenital stationary night blindness (Fortune et al., 1999, Kondo et al., 2001). In fact, this latter portion of the first-order kernel response is likely contributed from the first slice of the second-order kernel response (Hood, 2000, Sutter, 2000), that reflects the nonlinear mechanism of retina. Figure 11.7 shows that the first slice of the second order kernel response was undetectable in the moderate light scattering condition. This indicated that the P2 may be highly correlated with the first slice of the second-order kernel response. Previous studies suggest that the first slice of the second-order kernel response might show early inner retinal damage as diabetic patients without any retinopathy had reduced second-order responses (Palmowski et al., 1997) and patients with ocular hypertension had a relatively greater reduction of the first slice of the second-order response than the first-order responses (Chan & Brown, 2000). This wavelet (P2) was reported to be less prominent in glaucomatous eyes than in normal eyes of cynomolgus monkeys (Raz et al., 2002). The amplitude of this P2

wavelet is also smaller in hypertensive eyes and its amplitude is strongly correlated with the number of surviving ganglion cells (Hare et al., 2001).

Our study, therefore, illustrates that forward light scattering not only affects the amplitude of mfERG responses, but also reduces the higher order kernel response and P2 even without any retinal dysfunction. This may be because the veiling luminance caused by forward light scattering abolishes the antagonistic characteristics at inner retinal layers. Therefore, previous studies might misattribute the stray light effects to inner retinal dysfunction. The exact mechanisms of such findings are not fully understood and further studies on the origin of the human mfERG response components may help to explain why the double peaks and the late component (P2) were diminished when there is light scattering.

Figure 11.5 showed that as scattering level was increased, the mfERG waveforms acquired a noticeable higher frequency "oscillatory" component superimposed on the fundamental waveform of the first order kernel. This was not seen in the reduced contrast condition. We believed that this "oscillatory" component might be the noise during recording. The noise may be due to the addition of undesigned mfERG responses to the VERIS cross-correlation arising from straylight falling onto the elements of stimulus that are assumed to be dark. Moreover, the limitation of this experiment is that the total recording time is about 4 minutes in each mfERG recording. This is shorter than the standard condition (Marmor et al., 2001), which is about 8 minutes. Therefore the signal-to-noise ratio is relatively low. We used binary m-sequence of 2^{14} instead of 2^{15}, as we hope to reduce the recording time. If the recording time is too long, most subjects feel tired

and could not maintain good fixation. Under light scattering conditions, a very mild instability of fixation may occur (Fortune & Johnson, 2002), as the clarity of the central fixation mark may be affected. Also, the glare light caused by the I-C-D filter makes the subjects feel uncomfortable. Therefore, the "oscillatory" component may also be the cause of very mild instability of fixation.

In summary, the results in this study demonstrate that the topography of the mfERG is affected by forward light scattering. The paracentral retinal response is increased with increasing amounts of forward light scattering, but the P1 amplitude from the central retina decreased at moderate scattering condition. Therefore, clinicians using the mfERG on cataract patients or patients with light scattering problem but no cataract, should be aware of the confounding effect of forward light scattering on the mfERG. Since each laboratory may use different recording equipment (Marmor et al., 2003), we suggest that each laboratory should establish its normative values for different degrees of cataract or light scattering, if the clinicians have to use the mfERG to assess retinal function in the elderly or in patients with cataract.

Experiment II

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CLINICAL STUDY

Effects of different degrees of cataract on the multifocal electroretinogram

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Chapter 12. Experiment II - Effects of different degrees of cataract on the multifocal electroretinogram

Abstract

Purpose:

To study the effect of different degrees of nuclear cataract on the multifocal electroretinogram (mfERG).

Methods:

Multifocal electroretinograms were recorded from thirty elderly subjects with very mild, mild, or moderate nuclear cataract using a VERIS System (Version 4.1). The subjects were divided into three groups (10 in each group) according to their degree of nuclear cataract as classified according to the Lens Opacities Classification System III (LOCSIII). No subject had any significant eye disease or degenerative changes except for cataract. The mfERG responses were grouped into six concentric rings for analysis. Both the N1 and P1 amplitudes and the latencies of N1 and P1 of the first order responses were used for analysis.

Results:

Amplitudes of N1 and P1 from the central retina (14 degrees) were significantly reduced in patients with mild or moderate cataract when compared with subjects with very mild cataract. However, there was no significant reduction of N1 and P1 amplitudes in the para-central retina (14-40 degrees). Latencies of N1 and P1 were significantly longer in patients with moderate cataract.

Conclusions:

The mfERG responses from the central retina (central 14 degrees) were affected by the severity of cataract, but responses from the para-central retina (14 40 degrees) were not affected. This suggests that in interpreting the mfERG in subjects with mild or moderate cataract, some care should be taken as reduced amplitudes (N1 and P1) or increased latency (N1 and P1) will be expected from the central retina.

Introduction

The multifocal electroretinogram (mfERG) technique (Sutter & Tran, 1992) allows simultaneous recording of many local retinal responses within a short time. It has been shown that numerous retinal eye diseases can be detected by the mfERG (e.g. diabetic retinopathy, glaucoma, retinitis pigmentosa) (Chan & Brown, 1998, Chan & Brown, 1999, Fortune et al., 1999, Chan & Brown, 2000, Hood, 2000, Palmowski et al., 2000). In these studies, they reported that subjects had clear media. However, many patients with retinal eye disease (e.g. age-related macular degeneration) are elderly and some degree of lenticular change such as nuclear sclerosis is inevitable. For diagnostic purposes, it is important to know how changes in the ocular media affect the mfERG topography.

It has been reported previously that media opacities such as cataract can reduce the amplitudes of the a-wave and b-wave of scotopic flash ERG (Hurst & Douthwaite, 1993, Fishman, 2001). However, a larger than normal scotopic flash ERG response has also been recorded in patients with cataract (Galloway, 1988). It was suggested that this might be due to the light scattering effect (Ganzfeld effect) of the cataract (Galloway, 1988). A recent study showed that a subject with mild cortical cataract had lower than normal mfERG responses (Yoshii et al., 2000b).

By using acrylic sheets or liquid-crystal-diffusers, the light scattering effects of cataract have been simulated in mfERG studies (Arai et al., 1999, Chan et al., 2002a). In a study on two subjects, Arai et al. (1999) showed that central mfERG responses decreased slightly with increased scattering level, but that the peripheral responses did not show a corresponding reduction. Our own study on a larger group

of young subjects showed that the central mfERG responses decreased significantly with increasing light scattering but paradoxically, the peripheral responses increased with increasing light scattering (Chan et al., 2002a).

The aim of this study was to find out how nuclear cataract affects the mfERG. No subjects were chosen with other forms of cataract.

Method

Subjects

Thirty elderly subjects aged 50 to 75 years (mean age: 64 years) were recruited from the Optometry Clinic at The Hong Kong Polytechnic University. All subjects had mild to moderate nuclear cataract. The nuclear opalescence (NO) was classified and graded according to the Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993a) and their visual acuities (VA) were measured. Pelli-Robson letter chart was not used in this experiment, as it has poor sensitivity to mild light scattering and it only measures contrast sensitivity at one spatial frequency. Therefore, it may not provide more sensitivity measure of light scattering than VA (Elliott, 1993). Subjects with cortical or with posterior subcapsular cataract (more than grade 1 of LOCS III) were excluded. A previous study has shown that there is a positive correlation between VA and LOCS nuclear cataract grade [(y = -0.23 +0.0093x) (r^2 = 0.47) (y = logMAR VA and x = LOCS nuclear cataract grade)] (Elliott & Situ, 1998).

Subjects were divided into three groups with ten subjects in each group according to the degree of cataract (see Table 12.1). All subjects had refractive

errors of less than ±3.00D and less than 1.00D of astigmatism. The mfERG topography in subjects with very mild nuclear cataract acted as the normative values in this study, since subjects aged over 50 years old usually have very mild nuclear cataract, which causes light scattering (Siik et al., 1992, Smith et al., 1992).

To ensure that all subjects were free of retinal disease in the tested eye, all received an eye examination which included measurements of visual acuity and intraocular pressure. Ocular health was assessed using a slit lamp and indirect ophthalmoscopy. Research procedures used in this study followed the tenets of the Declaration of Helsinki. All procedures were approved by the ethics committee of The Hong Kong Polytechnic University. Informed consent was obtained from all participating subjects after they were given an explanation of the study.

Group	**Range of the best corrected visual acuity**	**LOCS III grading ± SD**	**Mean age (years) ± SD**
A (Very Mild Cataract)	$6/5 - 6/6^{-2}$	2 ± 0.2	61 ± 6.0
B (Mild Cataract)	$6/9$ to $6/9^{-2}$	4 ± 0.3	65 ± 6.7
C (Moderate Cataract)	$6/12$ to $6/12^{-2}$	5 + 0.3	66 + 4.85

Table 12.1. Characteristics of subject groups.

Stimulus Conditions

The VERIS Science 4.1 system (Electro Diagnostic Imaging Inc., San Mateo, CA) was used to record the mfERG. The stimulus matrix consisted of 103 scaled hexagonal elements presented on a Sony high resolution RGB 19" monitor (Sony, GPM-500P3, Japan) which had a frame rate of 75 Hz. The stimulus was controlled by a video card (from Electro Diagnostic Imaging Inc.) in a Macintosh G3 computer. The stimulus hexagons were individually modulated between white (165 cd/m^2) and black (3 cd/m^2) according to a pseudorandom m-sequence (Sutter & Tran, 1992). The luminance of the surround was set at 84 cd/m^2. A 0.8 deg red fixation cross was used. This CRT monitor subtended a viewing angle of 41° vertical and 53° horizontal at a viewing distance of 40cm. The diameter of the different stimulus rings were: Ring 1: about 2.3°, Ring 2: about 2.3° to 7.8°, Ring 3: about 7.8° to 14°, Ring 4: about 14° to 22.4°, Ring 5: about 22.4° to 31°, Ring 6: about 31° to 40°.

Recording Conditions

The pupils were dilated with 1% tropicamide (Mydriacyl, Alcon, Belgium) and all pupils were at least 6mm before recording commenced. The Dawson-Trick-Litzkow (DTL) electrode was used as the active electrode. The reference and ground electrodes (Ag-AgCl electrodes) were attached to the ipsilateral outer canthus and forehead respectively. The untested eye was occluded during recording. The testing distance was 40cm. Refractive errors were fully corrected for the viewing distance. The signals were amplified 100,000 times with band-pass set at 3-300Hz (Grass Instrument Co., Quincy, MA). All mfERG responses were spatially smoothed once by averaging each local trace with 17% of each of its six nearest neighbors. A binary

m-sequence of 2^{15} was used for recording the mfERG. The total recording time was 7 min 17 sec, divided into 32 segments (recording periods). Subjects rested for a few seconds between segments. Any segment with breaks of fixation, eye movements, or blinks was discarded and recorded again. The recording conditions were performed according to the guidelines of International Society for Clinical Electrophysiology of Vision (ISCEV) (Marmor et al , 2003).

Analysis

For data analysis, the mfERG responses were grouped in six concentric rings: responses with similar eccentricities from the central foveal response were grouped (Figure 12.1). In this study, the first order kernel responses were analyzed and only the amplitudes and latencies of N1 and P1 were evaluated. We defined the first negative and positive deflections of the mfERG waveform as N1 and P1 respectively. The amplitude of N1 was measured from the baseline to the first negative peak. The amplitude of P1 was measured from the first negative peak to the first positive peak. The latencies of N1 and P1 were defined as the time periods from the stimulus onset to the peak of N1 and P1 responses, respectively. The effects of different degrees of cataract on mfERG responses were evaluated by using two-way ANOVA. When a significant interaction was found, multiple pairwise comparisons with the Tukey's test were used to compare each group. P-values less than 0.05 were considered statistically significant.

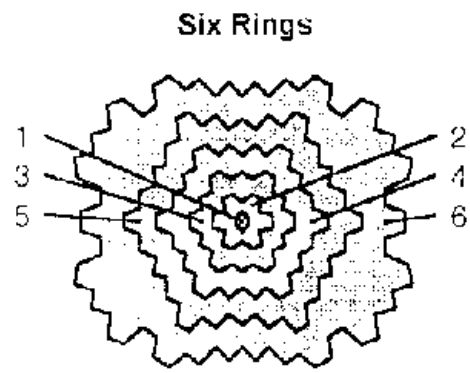

Figure 12.1. Responses were grouped into six rings for analysis

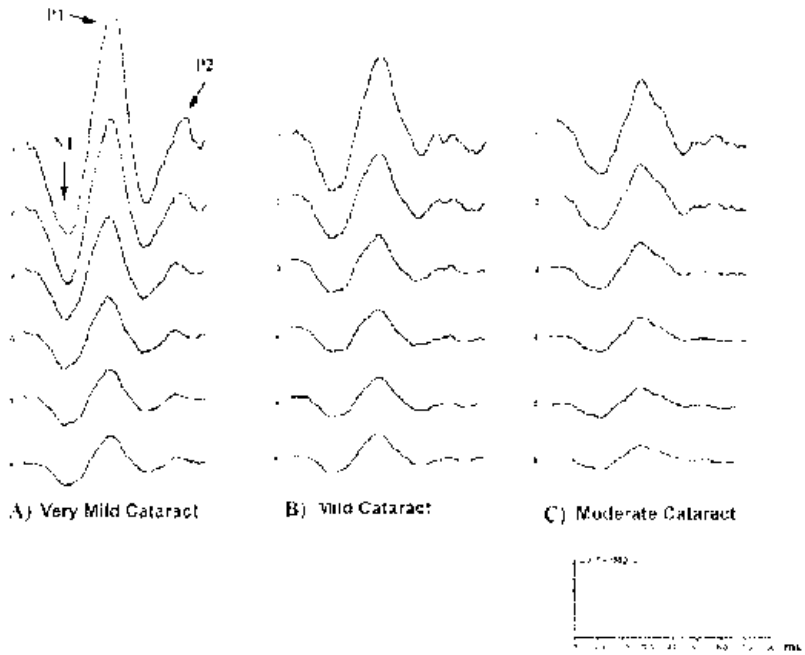

Figure 12.2. The mfERG (first order kernel response) from three subjects for the six concentric rings with spatial averaging. (A) Subject with very mild cataract. **(B)** Subject with mild cataract. **(C)** Subject with moderate cataract. N1 amplitudes from the central three rings (i.e. 1-3) were significantly reduced with increasing degrees of cataract. P1 amplitude showed a similar trend.

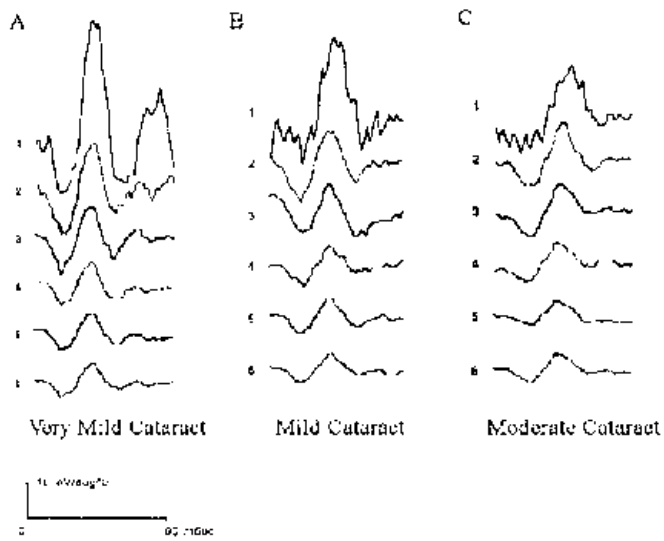

Figure 12.3. The mfERG (first order kernel response) from three subjects for the six concentric rings without spatial averaging. (**A**) Subject with very mild cataract. (**B**) Subject with mild cataract. (**C**) Subject with moderate cataract. N1 amplitudes from the central three rings (i.e. 1–3) were significantly reduced with increasing degrees of cataract. P1 amplitude showed a similar trend.

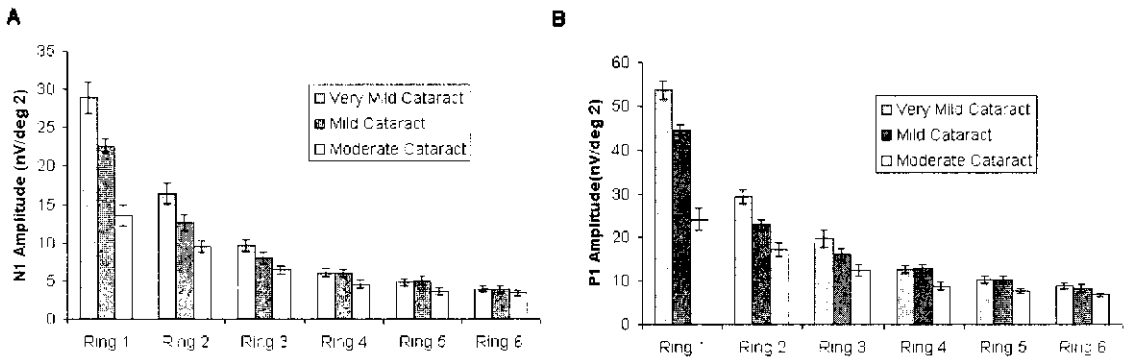

Figure 12.4. First order kernel response (**A**) Mean N1 amplitudes of the six concentric rings for three groups of subjects with cataract. (**B**) Mean P1 amplitudes of the six concentric rings for the three groups of subjects with cataract. Error bars are ± 1 standard error of the mean.

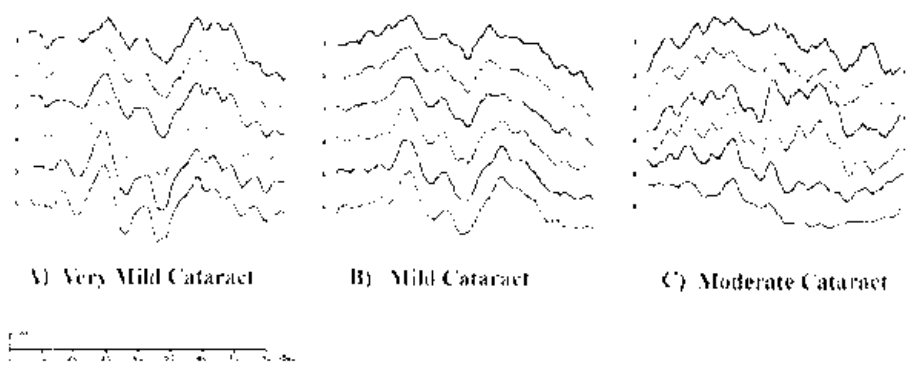

Figure 12.5. First slice of the second order kernel response waveforms for the six concentric rings from three subjects. **(A)** Subject with very mild cataract. **(B)** Subject with mild cataract. **(C)** Subject with moderate cataract. The first slice of second order kernel responses is not obvious in subject with moderate cataract.

Table 12.2. Effect of different degrees of cataract on mfERG responses (first order kernel response) parameters and statistical findings: Responses from six concentric rings.

		Very Mild Cataract	Mild Cataract	Moderate Cataract	Two way ANOVA	Multiple Pairwise Comparisons with Tukey's Test
N1 Amplitude (nV/deg2)	Ring 1	28.90 ± 2.06	22.69 ± 0.86	13.63 ± 1.34	Cataract level	✱◆❖
	Ring 2	16.44 ± 1.32	12.69 ± 1.08	9.51 ± 0.76	p=0.000* F[2,162]=45.53	✱◆❖
	Ring 3	9.64 ± 0.72	7.97 ± 0.73	6.45 ± 0.49	Ring	◆
	Ring 4	6.08 ± 0.54	6.07 ± 0.48	4.56 ± 0.54	p=0.000* F[5,162]=187.501	
	Ring 5	4.78 ± 0.48	5.04 ± 0.56	3.66 ± 0.44	Interaction	
	Ring 6	3.95 ± 0.36	3.94 ± 0.49	3.40 ± 0.40	p=0.000* F[10,162]=10.676	
P1 Amplitude (nV/deg2)	Ring 1	53.94 ± 2.09	44.55 ± 1.29	25.40 ± 2.54	Cataract level	✱◆❖
	Ring 2	28.10 ± 1.63	22.75 ± 1.18	17.46 ± 1.55	p=0.000* F[2,162]=62.183	✱◆❖
	Ring 3	19.43 ± 2.05	15.81 ± 1.39	12.87 ± 1.24	Ring	◆
	Ring 4	12.47 ± 0.72	12.52 ± 0.83	9.39 ± 0.97	p=0.000* F[5,162]=266.973	
	Ring 5	9.42 ± 0.81	10.08 ± 0.74	8.42 ± 0.6	Interaction	
	Ring 6	8.37 ± 0.66	8.04 ± 0.95	7.48 ± 0.51	p=0.000* F[10,162]=16.326	
N1 latency (ms)	Ring 1	20.74 ± 0.91	19.42 ± 0.66	21.74 ± 0.74	Cataract level	
	Ring 2	19.58 ± 0.59	19.67 ± 0.66	21.25 ± 0.78	p=0.000* F[2,162]=11.689	
	Ring 3	18.66 ± 0.63	20.14 ± 0.87	21.33 ± 0.79	Ring	
	Ring 4	18.91 ± 0.54	19.72 ± 0.68	20.75 ± 0.63	p=0.761 F[5,162]=0.520	
	Ring 5	19.32 ± 0.59	20.74 ± 0.51	20.93 ± 0.52	Interaction	
	Ring 6	19.17 ± 0.68	20.17 ± 0.64	21.59 ± 0.75	p=0.724 F[10,162]=0.700	
P1 Latency (ms)	Ring 1	37.73 ± 0.79	37.39 ± 0.66	38.56 ± 0.61	Cataract level	
	Ring 2	37.32 ± 0.55	36.49 ± 0.64	38.40 ± 0.65	p=0.000* F[2,162]=11.753	
	Ring 3	37.32 ± 0.21	36.91 ± 0.79	38.73 ± 0.62	Ring	
	Ring 4	36.64 ± 0.28	36.98 ± 0.45	38.24 ± 0.68	p=0.842 F[5,162]=0.410	
	Ring 5	37.64 ± 0.60	36.91 ± 0.31	38.40 ± 0.61	Interaction	
	Ring 6	37.72 ± 0.43	36.65 ± 0.41	38.49 ± 0.67	p=0.992 F[10,162]=0238	

Data are presented as mean ± 1 SEM
* Significant difference (P< 0.05) among three conditions
✱ Results differ significantly between very mild cataract and mild cataract (P<0.05)
◆ Results differ significantly between very mild and moderate cataract (P<0.05)
❖ Results differ significantly between mild cataract and moderate cataract (P<0.05)

Results

The effects of different degrees of cataract on mfERG waveforms were shown in Figure 12.2. Table 12.2 showed the mfERG waveform parameters and statistical findings (N1 latency, P1 latency, N1 amplitude, and P1 amplitude) when responses were grouped in six concentric rings. There were statistically significant effects of cataract on the N1 amplitude and P1 amplitude and there were statistically significant effects of ring grouping on N1 amplitude and P1 amplitude for each cataract level. In addition, there was a significant interaction between cataract level and ring grouping for both N1 amplitude and P1 amplitude. Tukey multiple comparisons test showed that N1 amplitude and P1 amplitude from ring 1 to ring 3 decreased significantly in the subjects with moderate cataract, but N1 amplitude and P1 amplitude from ring 4 to ring 6 did not change significantly in subjects with moderate cataract (Figure. 12.4A and Figure 12.4B).

There were statistically significant effects of cataract level on the N1 latency and P1 latency but there were no statistically significant effects of ring grouping on N1 latency and P1 latency for each cataract level. In addition, there was no significant interaction between cataract level and ring grouping for either N1 latency or P1 latency. Tukey multiple comparisons test showed that subjects with moderate cataract had significantly longer N1 latency and P1 latency than the subjects with very mild or mild cataract.

Discussion

A recent study on a single subject with cataract showed that mfERG responses (first order kernel response) were lower than in normal subjects of a similar age, but it was unclear whether the reduction was in the central retinal responses or peripheral retinal responses (Yoshii et al., 2000b). Our study confirms and extends this finding: the reductions in mfERGs were related to the severity of nuclear cataract but only over central retinal areas. In addition, our study found that the P2 amplitude in the first order kernel responses with a latency of about 60msec was reduced in subjects with moderate cataract (Figure 12.2). Recent studies have pointed out that this P2 response is related to the first slice of the second-order kernel response (Hood, 2000, Sutter, 2000, Shimada & Horiguchi, 2003). In Figure 12.4, these second-order responses demonstrate the effects of different degrees of cataract; the first slice of the second-order kernel response was undetectable in subjects with moderate cataract. This is likely to be the results of the forward light scattering produced by the cataract which abolishes the activities related to antagonistic characteristics at inner retinal layer.

It is well-known that the amount of light scattering (both forward light scattering and backward light scattering) increases with increasing age (Hemenger, 1990, Siik et al., 1992, Whitaker et al., 1993). Light scattering occurs due to the presence of insoluble proteins in the lens (Kamei et al., 1987). With increasing age, there is an increase in the amount of insoluble lens protein, so the amount of light scattering will increase as the criteria for Mie scattering are met (Heavens & Ditchburn, 1991). In the cataractous lens, forward light scattering reduces the

contrast of the retinal image. Backward light scattering reduces the amount of light reaching the retina, as light is scattered back from the eye toward the light source (Elliott, 1993).

Previous studies have shown that mfERG responses (P1 amplitude) from the central and para-central retina decreased linearly when stimulus luminance is decreased (Brown & Yap, 1996, Chan & Brown, 1998, Yoshii et al., 2000b, Fortune & Johnson, 2002). Yoshii et al. (2000b) also found that P1 latency increased linearly with decreased mean luminance. Brown and Yap (1996) showed that the mfERG responses decreased linearly at all retinal eccentricities when the stimulus contrast was decreased. On the basis of these reports, cataract should reduce central and peripheral mfERG responses and may increase P1 latency. In our study, we also found that subjects with moderate cataract had longer N1 and P1 latency than the subjects with very mild or mild cataract. However, we found that central retinal responses were decreased but peripheral retinal responses were not. This finding is slightly different from our previous studies (Chan et al., 2002a, Tam et al., 2004), which found that central mfERG responses were reduced, but peripheral retinal responses were increased under light scattering conditions. This may be due to the difference of luminance reduction between the real cataract cases and the simulated cases. Although the luminance reduction plays a role to influence the mfERG responses, we can conclude generally that light scattering caused by media opacities can affect the amplitude of mfERG responses (Galloway, 1988, Chan et al., 2002a).

There have been several studies on the effect of aging on mfERG topography (Mohidin et al., 1999, Fortune & Johnson, 2002, Gerth et al., 2002, Jackson et al.,

2002a, Nabeshima et al., 2002, Gerth et al., 2003, Tzekov et al., 2004). Mohidin et al. (1999) found that the decrease in response density in aged eyes was mainly within the central 10 degrees diameter of the retina, with no significant reduction in peripheral responses. However, the oldest subject in their study was only 52 years of age. Nabeshima et al. (2002) reported although the reduction of response density was the greatest in the central retina, the peripheral retinal responses also decreased with increasing age. Since the study from Nabeshima et al. included subjects with slight nuclear opacity, it is not clear whether the age-related effects on the mfERG were due to neural factors or optical factors. Fortune and Johnson (2002) adjusted their mfERG data for the effect of reduced lens transmission and pupil diameter in aged subjects to rule out the effect of optical factors and they found that the decline of mfERG responses with age could be attributed to optical factors. They concluded that neural factors only played a small role which was mainly restricted to the central (5 degrees) of the retinal responses (Fortune & Johnson, 2002). Gerth et al. (2002) calculated the effect of media opacities (decrease stimulus luminance and contrast) on mfERG topography and indicated that the effect of aging on mfERG is due to both optical and neural factors (Gerth et al., 2002). Gerth et al. (2003) further showed that the age-related decrease of the isolated flash response was due to both optical and neural factors. However, two recent studies strongly claimed that smaller mfERG responses in the elderly were due to neural factors rather than optical factors (Jackson et al., 2002a, Seiple et al., 2003). The most recent study by Tzekov et al. (2004) found that the age-related decrease of mfERG response was significantly higher for the superior than for the inferior retina. The results of these studies,

therefore, suggest that each laboratory should establish normative values for older adults (Mohidin et al., 1999, Fortune & Johnson, 2002, Gerth et al., 2002, Jackson et al., 2002a, Nabeshima et al., 2002, Seiple et al., 2003).

The results of our study suggest that the presence of cataract should be taken into consideration in the clinical application of the mfERG. According to the guidelines from the International Society for Clinical Electrophysiology of Vision, each laboratory should develop normative data as there may be variations in recording equipment and techniques (Marmor et al., 2003). We, therefore, also suggest that each laboratory should also establish its normative values for different degrees of cataract, if the clinician wishes to use the mfERG to assess retinal function behind a cataractous lens.

Experiment III

Comparing the multifocal electroretinogram before and after cataract surgery. ***Curr Eye Res,*** **30 (7), 593-599**

Chapter 13. Experiment III - Comparing the multifocal electroretinogram topography before and after cataract surgery

Abstract

Purpose

To determine how the topography of the multifocal electroretinogram (mfERG) is affected by nuclear cataract.

Methods

Multifocal electroretinograms were recorded from ten elderly subjects (10 eyes) with nuclear cataract of grade five (LOCS III) before and after cataract surgery (phacoemulsification). Their visual acuities before the cataract surgery were between 6/12 and 6/18. The postoperative period was from 2 to 3 months. None of the subjects had any significant eye disease apart from cataract. The mfERG responses were grouped into six concentric rings for analysis. Both the amplitudes and the latencies of N1 and P1 of the first order responses were used for analysis.

Results

N1 amplitude from ring 1, and P1 amplitude from ring 1 and ring 2 increased significantly after cataract surgery. N1 and P1 amplitude from ring 3 to ring 6 did not change significantly after cataract surgery. Latencies from ring 1 to ring 6 were not changed significantly.

Conclusions

Nuclear cataract affects the topography of the mfERG, reducing the amplitudes of central responses, so clinicians should be aware of these changes when interpreting mfERG responses in cataract patients.

Introduction

The conventional electroretinogram (Full-field flash) is a valuable tool for examination of retinal function. By choosing appropriate test parameters, rod and cone responses can be tested separately (Marmor & Zrenner, 1998). Since the full-field flash ERG can only represent the whole retinal summated response, it is unlikely to reveal subtle, local retinal damage. Although the pattern ERG and focal ERG can be used to assess macular function (Miyake, 1998, Holder et al., 2003), these responses still cannot provide topographical information to describe retinal function. The newly developed multifocal electroretinogram (mfERG) by Sutter and Tran (1992) has proved able to detect local retinal damage in retinal diseases such as age-related macular degeneration, diabetic retinopathy, and retinitis pigmentosa (Sutter & Tran, 1992, Chan & Brown, 1998, Ruether et al., 1998, Chan & Brown, 1999, Kretschmann et al., 1999, Marmor & Tan, 1999, Chan & Brown, 2000, Chappelow & Marmor, 2000, Hood, 2000, Kondo et al., 2001, Marmor et al., 2002, Hood et al., 2003).

In most clinical studies, the recruited subjects have been nominally without media opacities (Fortune et al., 1999, Kondo et al., 2001, Palmowski et al., 2002), as the confounding effects of media opacities (e.g. cataract) on mfERG topography should be ruled out. However, most patients with common retinal diseases such as age-related macular degeneration are elderly. In addition, patients with diabetic retinopathy are likely to develop cataract (Rotini et al., 2003). It is, therefore, important to understand the effects of cataract on mfERG topography, when we want to use mfERG in many common retinal conditions.

Previous studies have found that weaker central retinal responses can be recorded in simulated cataract conditions (Yoshii et al., 2000b, Chan et al., 2002a). A study comparing the mfERG topography before and after cataract surgery also showed that only the mfERG responses from the central retina change significantly after cataract surgery (Wordehoff et al., 2004). However, this study included subjects with various kinds of cataract (e.g. nuclear cataract, cortical cataract, and posterior subcapsular cataract). Previous studies have shown that different kinds of cataract have different effects on visual function (Elliott et al., 1989, Mataini et al., 1994). For example, nuclear cataract and cortical cataract mainly affect the contrast sensitivity at high spatial frequency, but posterior subcapsular cataract affects the contrast sensitivity at both high and low spatial frequencies (Elliott et al., 1989). Therefore, different kinds of cataract may have different effects on mfERG topography. As nuclear cataract is most common (Mitchell et al., 1997, Sasaki et al., 2002), we have investigated the effects of nuclear cataract on mfERG topography by comparing mfERG responses in patients before and after cataract surgery.

Method

Subjects

Ten elderly subjects aged 65 to 75 years (average age 69 years) were measured using the mfERG 2 weeks before and 2 months after cataract surgery (Phacoemulsification). All surgery was performed by the same surgeon; all subjects had intraocular lens (IOL) implantation. Six patients were female and four were male. All these subjects had nuclear cataract. The nuclear opalescence (NO) of all these subjects were grade 5 according to the Lens Opacities Classification System III (LOCS III) (Chylack et al., 1993a) and their preoperative best corrected visual acuities (VA) ranged from 6/12 to 6/18. After cataract surgery, all these subjects had the best corrected VA of 6/6.

To ensure that all subjects were free of retinal disease in the tested eye, they received an eye examination including visual acuity assessment, biomicroscopy, tonometry and indirect ophthalmoscopy. All of the subjects were in good general health. Research procedures in this study followed the tenets of the Declaration of Helsinki. All procedures were approved by the Ethics Committee of The Hong Kong Polytechnic University. Informed consent was obtained from all participating subjects after they were given an explanation of the study.

Stimulus Conditions

The VERIS Science 4.1 system (Electro Diagnostic Imaging Inc., San Mateo, CA, USA) was used to record the mfERG. The stimulus matrix consisted of 103 scaled hexagonal elements presented on a high resolution RGB 19" monitor (Sony,

GDM-500P3, Japan) with frame rate of 75 Hz, which was controlled by a video card (from Electro Diagnostic Imaging Inc.) in a Machintosh G3 computer. The stimulus hexagons were individually modulated between white (165 cd/m^2) and black (3 cd/m^2) according to a pseudorandom m-sequence (Sutter & Tran, 1992). The luminance of the surround was set at 84 cd/m^2. A 0.8 deg (pen diameter 1%) red fixation cross was used. This monitor subtended a viewing angle of 41° vertically and 53° horizontally at a viewing distance of 40cm. The diameters of different stimulus rings were: Ring 1 (central hexagon): about 2.3°; Ring 2: about 2.3° to 7.8°; Ring 3: about 7.8° to 14°; Ring 4: about 14° to 22.4°; Ring 5: about 22.4° to 31°; Ring 6: about 31° to 40°.

Recording Conditions

Pupils were dilated with 1% tropicamide (Mydriacyl, Alcon, Belgium) with pupil size at least 6mm. A Dawson-Trick-Litzkow (DTL) electrode was used as the active electrode. The reference and ground electrodes (Ag-AgCl electrode) were attached to the ipsilateral outer canthus and forehead, respectively. Only one eye of each subject was tested and the untested eye was patched during recording. The testing distance was 40cm. Refractive errors were fully corrected at that viewing distance. The signals were amplified by 100,000 with band-pass from 3-300Hz (Grass Instrument Co., Quincy, MA, USA). No line filter was used. A binary m-sequence of 2^{17} was used for recording mfERG. Total recording time was 7 min 17 sec in each complete recording. Each recording was collected in 32 segments of approximately 14 sec in each segment. Subjects rested for a few seconds between

segments. Any segment with breaks of fixation, eye movements, or blinks was discarded and recorded again.

Analysis

For the purpose of data analysis, the mfERG responses were grouped in six concentric rings; responses with similar eccentricities from the central foveal responses were grouped together (Figure 13.1). In this study, only the first order kernel responses were analyzed. In addition, amplitudes and latencies of N1 and P1 were evaluated. We defined the first negative and positive deflections of the mfERG as N1 and P1 respectively. The amplitude of N1 was measured from the baseline to the first negative peak. The amplitude of P1 was measured from the first negative peak to the first positive peak. The latencies of N1 and P1 were defined as the time periods from the stimulus onset to the peak of N1 and P1 responses, respectively. Differences between the results of pre- and post-cataract surgery were evaluated statistically using Two-way repeated measure ANOVA [using two conditions (pre-cataract and post-cataract) x 6 rings as the main factors]. When a significant interaction was found, multiple pairwise comparisons with Tukey's test were used to compare each group and p-values less than 0.05 were considered statistically significant.

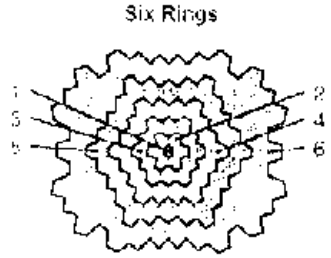

Figure 13.1. The mfERG responses were grouped into six rings for analysis.

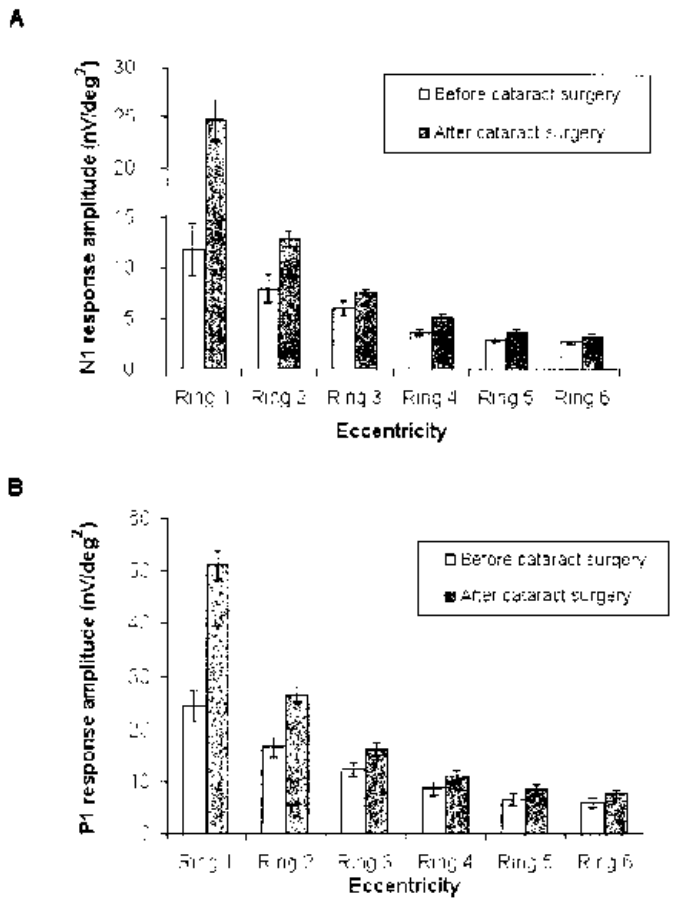

Figure 13.2. First order kernel response. **(A)** Multifocal electroretinogram N1 amplitude before and after cataract surgery. **(B)** Multifocal electroretinogram P1 amplitude before and after cataract surgery. Error bars are ± 1 standard error of the mean.

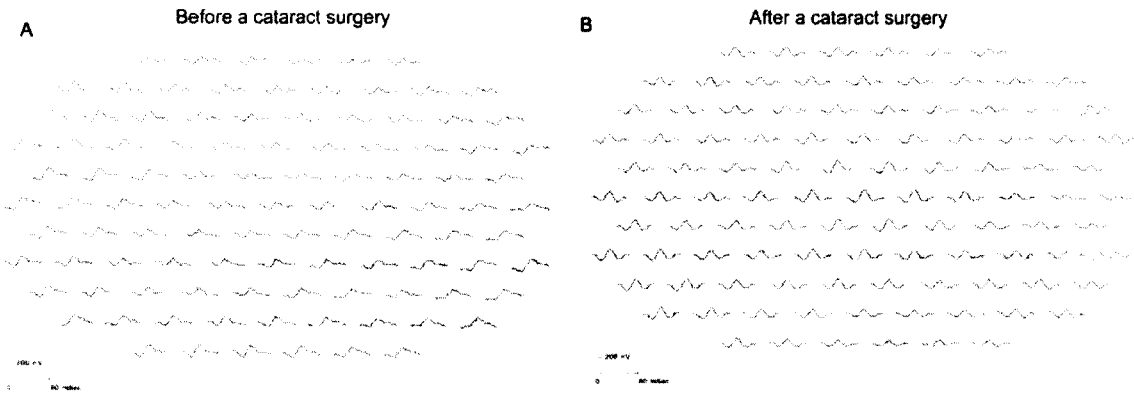

Figure 13.3. The mfERG topography (trace arrays) of a typical subject. **(A)** Before a cataract surgery. **(B)** After a cataract surgery.

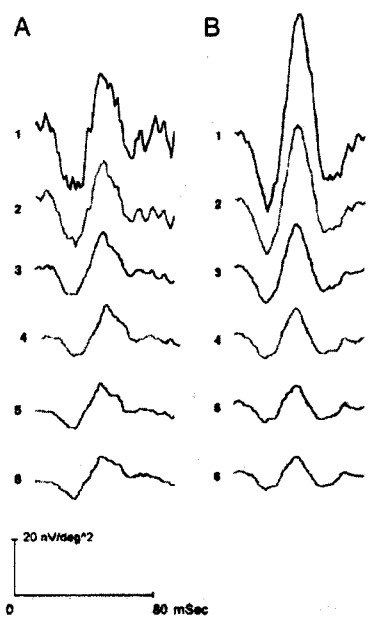

Figure 13.4. A typical subject's responses waveforms from six concentric rings. **(A)** Before a cataract surgery. **(B)** After a cataract surgery.

Table 13.1. Two-way repeated measure ANOVA was used to compare the results before and after cataract surgery.

	Ring	Before Surgery	After Surgery	Two-way repeated measure ANOVA	Multiple Pairwise Comparisons With Tukey's Test (Pre and Post)	Percentage increases post-surgery compared to pre-surgery
N1 amplitude (nV/deg^2)	1	11.80 ± 2.04	24.74 ± 2.61	**Condition** p= 0.000* F[1, 108] =34.31 **Ring** p= 0.000* F[1, 108] =56.98 **Interaction** p= 0.000* F[5, 108] =9.45	0.000*	110
	2	7.95 ± 0.73	12.92 ± 1.42		0.076	63
	3	5.98 ± 0.24	7.71 ± 0.70		0.994	29
	4	3.60 ± 0.37	5.05 ± 0.31		0.999	40
	5	2.89 ± 0.37	3.63 ± 0.17		1.000	26
	6	2.75 ± 0.20	3.28 ± 0.30		1.000	19
P1 amplitude (nV/deg^2)	1	24.40 ± 2.77	51.11 ± 2.98	**Condition** p= 0.000* F[1, 108] =69.44 **Ring** p= 0.000* F[1, 108] =108.13 **Interaction** p= 0.000* F[5, 108] =18.48	0.000*	109
	2	16.57 ± 1.42	26.46 ± 1.93		0.002*	60
	3	12.24 ± 1.17	16.12 ± 1.35		0.863	32
	4	8.69 ± 1.02	11.10 ± 1.26		0.996	28
	5	6.53 ± 0.86	8.49 ± 0.99		0.999	30
	6	5.98 ± 0.71	7.53 ± 0.96		1.000	26
N1 latency (ms)	1	20.26 ± 1.06	19.52 ± 0.85	**Condition** p= 0.06 F[1, 108] =3.61 **Ring** p= 0.313 F[1, 108] =1.202 **Interaction** p= 0.993 F[5, 108] =0.95		
	2	20.76 ± 1.18	19.85 ± 0.88			
	3	20.86 ± 0.60	19.78 ± 0.91			
	4	20.69 ± 0.67	20.02 ± 0.90			
	5	22.01 ± 1.00	21.19 ± 0.73			
	6	22.35 ± 0.94	20.60 ± 0.71			
P1 latency (ms)	1	35.24 ± 1.73	37.27 ± 1.73	**Condition** p= 0.674 F[1, 108] =0.178 **Ring** p= 0.931 F[1, 108] =0.279 **Interaction** p= 0.816 F[5, 108] =0.445		
	2	37.82 ± 1.49	37.19 ± 1.75			
	3	38.42 ± 0.65	36.96 ± 1.33			
	4	37.84 ± 0.83	37.51 ± 1.06			
	5	36.68 ± 1.96	38.09 ± 1.34			
	6	36.81 ± 2.06	37.89 ± 1.20			

All values are given as mean ± 1 standard error of the mean
*p<0.05 was considered statistically significant

Results

In Figure 13.2A and 13.2B, the N1 and P1 amplitudes are shown graphically to facilitate the comparison of mfERG responses before and after cataract surgery. In Figure 13.3A and 13.3B, the trace arrays of a typical subject before and after cataract surgery are shown. In Figure 13.4A and 13.4B, the waveforms from six concentric rings of a typical subject before and after cataract surgery are shown. Table 13.1 shows the mfERG waveform parameters (N1 amplitude, P1 amplitude, N1 latency and P1 latency) at different eccentricities before and after cataract surgery. N1 latency and P1 latency did not change significantly after cataract surgery. However, N1 and P1 amplitudes changed significantly after cataract surgery. As there was a significant interaction between conditions and rings for N1 and P1 amplitudes, it means that the effect of cataract surgery on N1 and P1 amplitudes depended on the rings. Therefore, we further used multiple pairwise comparisons with Tukey's test to find which rings show significant difference after cataract surgery. We found that the N1 amplitude in ring 1 increased significantly after cataract surgery. The P1 amplitude from ring 1 and ring 2 also increased significantly after cataract surgery. In addition, Table 13.1 shows that the percentage of reduction of N1 and P1 amplitudes due to cataract was considerably higher at ring 1 and ring 2 than from ring 3 to ring 6, but the percentage of reduction of N1 and P1 amplitudes due to cataract was similar from ring 3 to ring 6.

Discussion

A previous study reported that the amplitudes of the a-wave and b-wave of full-field (flash) ERG are not significantly reduced in cataractous eyes (Cruz & Adachi-Usami, 1989). Other studies reported that cataract can reduce the amplitudes of the a-wave and b-wave of scotopic flash ERG (Hurst & Douthwaite, 1993, Fishman, 2001). However, a larger than normal scotopic flash ERG response has also been recorded in patients with cataract (Galloway, 1988). Galloway (1988) suggested that this might be due to the light scattering effect (Ganzfeld effect) of the cataract since the response in the flash ERG is mainly generated by peripheral rods. These differing results may also be, in part, due to different types and different degrees of cataract in the subjects used.

In this study, we restricted our investigation to the effects of nuclear cataract (LOCSIII Grade 5) on mfERG topography. We found that both the N1 and P1 amplitudes from the central retina increased prominently after cataract surgery, but the peripheral responses did not change significantly.

Our results differ from those of Arai et al. (1999) who used various layers of acrylic sheets to simulate different degrees of cataract. They showed that with 10 acrylic sheets, which decreased visual acuity to 20/70 (6/21), the responses from ring 1 and ring 2 decreased only slightly and responses from the other rings did not decrease greatly. They, therefore, concluded that the mfERG is not sensitive to the effects of light scattering and that there is no need to consider the effects of cataract on mfERG recording in elderly patients (Arai et al., 1999). However, our study found that P1 amplitudes from ring 1 and ring 2 increased significantly after cataract

surgery. This agrees with the results of a case study presented by Yoshii et al. (2000b), which showed that a patient with cortical cataract had a weaker central mfERG response than normal elderly subjects. They advocated that optical factors should not be ignored when analyzing the mfERG topography in patients with cataract.

Those patients with cataract have weaker responses from the central retina and this can be explained as follows. Firstly, scattered light may be less effective in eliciting the cone response. According to the Stiles-Crawford effect, light which is incident directly along the cone axis will be a more effective stimulus. As part of the scattered light is likely to be incident at a range of angles, the probability of photon absorption should be reduced. Secondly, the stimulus luminance is reduced, as the cataract absorbs light of wavelengths from 450nm to 650nm (Seland et al., 1992) and the backward light scattering reflects light out of the eye (Elliott, 1993). Thirdly, the cataractous lens reduces the contrast of the stimulus, as forward light scatter produces a veiling luminance, which is superimposed on the retinal image (Elliott, 1993); mfERG responses from the central and peripheral retina decrease when the contrast or luminance of the stimulus is decreased (Brown & Yap, 1996, Chan & Brown, 1998, Yoshii et al., 2000b, Raz et al., 2002). Consequently, we might expect that a weaker mfERG response from the central and peripheral retina would be recorded from patients with cataract. However, in our study, the central retinal responses decreased significantly but the peripheral retinal responses did not change significantly. This suggests that the effects of a cataract are not only to decrease the

contrast and luminance of the retinal image, but also to act as a diffuser to cause light scattering and to produce the "Ganzfeld effect" (Burian & Burns, 1966).

In addition, the central responses in subjects with moderate degree of cataract appear noisier (Figure 13.4), as subjects with moderate degree of cataract may find difficult to fixate steadily at the central mark. Therefore, the very mild instability of fixation may occur in these subjects (Fortune & Johnson, 2002). Chisholm et al. (2001) suggested that poor fixation mainly affects the central responses, so only central responses appear noisier than peripheral responses.

In our previous studies, we found that forward light scattering could increase the peripheral mfERG responses and reduce the central mfERG responses (Chan et al., 2002a, Tam et al., 2004). In this study, the mean stimulus luminance was decreased by the cataract. Therefore, the fact that the peripheral mfERG responses are not significantly changed under media opacities may be due to the effects of forward light scattering, which counteracts the effects of luminance and contrast reduction. In addition, our previous studies found that the second positive peak (P2) was diminished under light scattering conditions (Chan et al., 2002a, Tam et al., 2004). In the present study, we found that the P2 was not obvious before cataract surgery (Figure 13.4A), but it was more prominent after cataract surgery (Figure 13.4B). This finding is similar to the study by Shimada and Horiguchi (2003), who also found that P2 was not obvious when responses were elicited by stray light (Shimada & Horiguchi, 2003).

In this experiment, we studied the influence of cataract on the mfERG topography by comparing mfERG responses before and after cataract surgery with

IOL implantation. As the surface and the edge of the IOL may cause light to scatter, it is possible that the optics of the IOL and residual lens capsule may affect the mfERG results especially under mydriasis. Recent studies showed that there may be internal reflections at the IOL surface and the IOL edge (Erie et al., 2001, Erie & Bandhauer, 2003). The IOL surface could cause internal reflections as light reflected from the fundus can be directed to the anterior surface of the IOL and then be reflected back to the retina to form a glare image (Erie & Bandhauer, 2003). Although the internal reflectivity of the IOL is higher than a clear human lens, the intensity of the internally reflected glare is very low (Erie & Bandhauer, 2003). In addition, the IOL edge would produce a glare image when light is directed to the eye at about 35 degrees to the optical axis (Holladay et al., 1999). As our stimulus is about 20 to 25 degrees to the optical axis, we would not expect substantial glare to be produced by the edge of the IOL. Therefore, the internally reflected light from an IOL edge and IOL surface would not significantly reduce the quality of vision in normal conditions (Tester et al., 2000, Erie & Bandhauer, 2003). In fact, a previous study found that the contrast sensitivity in subjects with IOLs is better than that of an age-matched phakic population (Tester et al., 2000). We, therefore, believe that the scattered light produced by the IOL and the residual lens capsule would not significantly affect the mfERG topography in this study.

A previous study investigated the effect of cataract on visual thresholds using Octopus automated perimetry before and after cataract surgery (Guthauser & Flammer, 1988). They found that the effects of cataract on the visual field sensitivity were slightly greater in the central than in the peripheral region, although this result

has not been confirmed in a study using the Humphrey Visual Field Analyzer (Lam et al., 1991). When we compared the percentage of response changes in our data (Table 13.1), we were able to demonstrate that cataract reduced mfERG topography mainly in the central retinal region, while having minimal effects in the para-central region. It also suggests that further investigation is needed to study why peripheral mfERG responses are not affected by media opacities but visual field thresholds are likely to be affected.

The results of this study indicate that nuclear cataract differentially reduces the central mfERG responses. Caution should be exercised when interpreting mfERG topography in patients with moderate to dense cataract. Previous studies found that the minimum size of scotoma that could be detected using the mfERG technique was about 5 degrees diameter in size (Yoshii et al., 1998, Marmor et al., 2002). As light can be re-directed to different parts of the retina in patients with cataract or other ocular media conditions resulting in light scattering (Beckman et al., 1992, de Waard et al., 1992, Elliott & Bullimore, 1993), the sensitivity of the mfERG to detect the area of retinal damage might be compromised. Further studies are required to investigate the sensitivity of the mfERG to detect scotomata in subjects with media opacities. In addition, the effects of cortical cataract and posterior subcapsular cataract on mfERG topography should also be studied.

Experiment IV

Eye (2004), 1–7

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Aging and mfERG topography

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CLINICAL

Tam, W.K., Chan. H., Brown, B., Leung, K.W., Woo, V., & Yap, M. (2005). Aging and mfERG topography. *Eye.* **In Press.**

Chapter 14. Experiment IV – Aging and mfERG topography

Abstract

Purpose

To study the effect of aging retina on the multifocal electroretinogram (mfERG).

Methods

Eighteen young subjects (aged 18-24 years) and thirty-six elderly subjects (aged 60--85 years) with intraocular lenses (IOLs) were recruited for this study. No subjects had eye diseases or media opacities. The mfERG was measured in standard conditions using the VERIS system (version 4.1). There were three groups of 18 subjects: 1) 18-25 years, 2) 60-70 years, and 3) 75-85 years. The mfERG responses were grouped into central, paracentral and peripheral regions for analysis. The N1 amplitude, P1 amplitude, N1 latency, and P1 latency of the first order kernel responses were analysed.

Results

Age had no effect on P1 latency, N1 amplitude, and P1 amplitude in all three regions; however, N1 latencies from central to peripheral regions were significantly longer for group 3 than for group 1.

Conclusions

This study suggests that measured age-related decreases in the mfERG responses are due to optical factors (decrease in retinal illuminance and light scattering) rather than retinal factors before the age of 70 years.

Introduction

Many aspects of human visual function change with age, including visual acuity, contrast sensitivity, colour vision, visual field, and dark adaptation (Johnson et al , 1989, Werner et al., 1990, Hurst & Douthwaite, 1993, Jackson et al., 1999). The decline of visual function with age could be due to optical and/or neural factors. Age-related optical changes include the light absorption, pupillary miosis, increased ocular aberrations, light scattering caused by the crystalline lens (Sample et al., 1988, Hennelly et al., 1998). Except pupillary miosis, all these changes have negative effects on our vision. Elliott et al. (1990) found that the contrast sensitivity of young subject with artificial senile miosis and neutral density filter is similar to the condition without senile miosis and neutral density filter. Therefore, Elliott et al. (1990) claimed that pupillary miosis, which reduces optical aberrations, has positive effects on our vision. Age-related neural changes include the loss of rods, cones, and ganglion cells (Gao & Hollyfield, 1992, Curcio & Drucker, 1993, Curcio et al., 1993). Psychophysical studies have demonstrated that rod-mediated sensitivity for older people is significantly lower than for younger people (Sturr et al., 1997, Jackson et al., 1998), and foveal cone sensitivity also decreases with increasing age especially after the age of 50 years (van Norren & van Meel, 1985, Kilbride et al., 1986). Earlier electrophysiological studies have shown age-related retinal changes using various ERG test protocols (Weleber, 1981, Birch & Anderson, 1992). Since the development of the multifocal electroretinogram (mfERG) (Sutter & Tran, 1992), age-related retinal changes can be studied topographically. The standard

mfERG allows us to examine the contribution of receptors and bipolar cells to the electrical response of the eye (Hood et al., 2003).

The effects of aging on mfERG topography have been studied extensively in recent years (Mohidin et al., 1999, Fortune & Johnson, 2002, Gerth et al., 2002, Jackson et al., 2002a, Nabeshima et al., 2002, Seiple et al., 2003). Most of these studies have found that both central and peripheral mfERG responses decrease with increasing age, but the decrease is more prominent in the central retina (Fortune & Johnson, 2002, Gerth et al., 2002, Jackson et al., 2002a, Nabeshima et al., 2002, Seiple et al., 2003). Fortune and Johnson (2002) concluded that the age-related changes are predominantly due to optical factors. However, Jackson et al. (2002a) and Gerth et al. (2002) argued that age-related changes in the mfERG topography are due to both optical and neural factors. In normal elderly subjects, the crystalline lens causes light scattering and would reduce the luminance and contrast of the retinal stimulus. As luminance, contrast, and light scattering are known to affect the mfERG topography (Brown & Yap, 1996, Chan et al., 2002a), lens changes will complicate the interpretation of the mfERG findings in the elderly. In addition, our experiment III and the study by Wordehoff et al. (2004) found that patients had larger mfERG responses after cataract surgery. One way to minimize the optical effects of media opacities on the mfERG of elderly subjects is to choose subjects who have had cataract surgery with the implant of an intraocular lens (IOL) (Owsley et al., 1985). Provided the posterior capsule is clear, the mfERG would then be expected to give a reasonable reflection of neural losses from the retina.

The aim of this study was to investigate the neural effects of aging on mfERG topography, by examining patients after IOL implant surgery; we compared mfERG topography in young subjects and two groups of older subjects with IOLs. Amplitudes and latencies of the first order kernel responses were analyzed in this study.

Method

Subjects

We examined 54 subjects whose ages ranged from 18 years to 85 years. Each of three groups contained eighteen subjects of similar age: 1) 18-25 years, 2) 60-70 years, 3) 75-85 years. All subjects in group 2 and group 3 had undergone cataract surgery (phacoemulsification) with IOLs implanted, without postoperative complications. The subjects in group 1 were students at The Hong Kong Polytechnic University and the subjects in group 2 and group 3 were recruited from a private eye clinic.

All of the subjects were in good general health. To ensure that all subjects were free of retinal disease in the tested eye, they received an eye examination including visual acuity assessment, biomicroscopy, tonometry, and indirect ophthalmoscopy. All subjects had the best corrected visual acuity of 6/6 or better and refractive errors less than + 3.00 D with less than 1.00 D astigmatism.

Research procedures in this study followed the tenets of the Declaration of Helsinki. All procedures were approved by the ethics committee of The Hong Kong Polytechnic University. Informed consent was obtained from all participating subjects after they were given an explanation of the study.

Stimulus Conditions

The VERIS Science 4.1 system (Electro Diagnostic Imaging Inc., San Mateo, CA, USA) was used to record the mfERG. The stimulus matrix consisted of 103 scaled hexagonal elements presented on a high resolution RGB 19" monitor (Sony, GDM-500P3, Japan) with frame rate of 75 Hz, which was controlled by a video card

(from Electro Diagnostic Imaging Inc.) in a Macintosh G3 computer. The stimulus hexagons were individually modulated between white (165 cd/m^2) and black (3 cd/m^2) according to a pseudorandom binary m-sequence (Sutter & Tran, 1992). The luminance of the surround was set at 84 cd/m^2. The monitor subtended a viewing angle of 45° vertically and 56.6° horizontally. A red central cross (0.8 deg; pen diameter 1%) was used to assist fixation. The diameters of different stimulus rings were: Ring 1: about 8.9°; Ring 2: about 8.9° to 25.2°; Ring 3: about 25.2° to 43.8°. These were modelled on those used by Seiple et al. (2003).

Recording Conditions

Pupils were dilated with 1% tropicamide (Mydriacyl, Alcon, Belgium) to pupil size at least 6mm. A Dawson-Trick-Litzkow (DTL) electrode was used as the active electrode. The reference and ground electrodes (Ag-AgCl electrode) were attached to the ipsilateral outer canthus and forehead, respectively. Only one eye of each subject was tested and the other eye was occluded during recording. Refractive errors were fully corrected for the 35 cm viewing distance. The signals were amplified by 100,000 with band-pass from 3-300 Hz (Grass Instrument Co., Quincy, MA, USA). No line filter was used. The binary m-sequence of 2^{15} was used for recording mfERG. Total recording time was 7 min 17 sec in each complete recording. Each recording was collected in 32 segments, each approximately 14 sec in length; subjects rested for a few seconds between segments. Any segment with breaks of fixation, eye movements, or blinks was discarded and recorded again. The recording conditions were performed according to the guidelines of International Society of Clinical Electrophysiology of Vision (ISCEV).

Analysis

For the purpose of data analysis, the mfERG responses were grouped into **three regions**: a) central, b) paracentral, and c) peripheral (Figure 14.1), as this will greatly improve the signal-to-noise ratio of the mfERG responses (Seiple et al., 2003).

In this study, the first-order kernel responses were analyzed. Amplitudes and latencies of N1 and P1 were evaluated. We defined the first negative and positive deflections of the mfERG waveform as N1 and P1, respectively. The amplitude of N1 was measured from the baseline to the first negative peak. The amplitude of P1 was measured from the first negative peak to the first positive peak. The latencies of N1 and P1 were defined as the time periods from the stimulus onset to the peak of N1 and P1 responses, respectively. The effects of aging on central, paracentral, and peripheral regions were evaluated by Two-way ANOVA (using three groups x 3 regions as the main factors). The Tukey HSD multiple comparisons test was used as *post-hoc* test. P-values less than 0.05 were considered statistically significant.

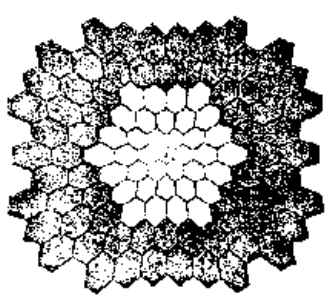


Figure 14.1. 103 local responses were grouped into three regions for analysis: central, paracentral, and peripheral.

Table 14.1. Effect of aging on mfERG responses (first order kernel response) parameters and statistical findings: Responses from three concentric rings.

		Group 1	Group 2	Group 3	Two-way ANOVA
N1 amplitude (nV/deg^2)	Region 1	15.77 ± 1.00	14.18 ± 0.82	13.57 ± 1.35	Group p= 0.257 F[2, 153] =1.372
	Region 2	6.49 ± 0.48	6.45 ± 0.54	5.69 ± 0.52	Region p= 0.000* F[2, 153] =176.6
	Region 3	3.74 ± 0.32	4.78 ± 0.33	3.99 ± 0.28	Interaction p= 0.379 F[4, 153] =1.059
P1 amplitude (nV/deg^2)	Region 1	32.98 ± 1.61	31.04 ± 1.54	31.25 ± 1.93	Group p= 0.551 F[2, 153] =0.599
	Region 2	13.84 ± 0.66	15.72 ± 0.98	14.38 ± 1.03	Region p= 0.000* F[2, 153] =290.4
	Region 3	8.03 ± 0.51	10.68 ± 0.88	8.88 ± 0.85	Interaction p= 0.376 F[4, 153] =1.065
N1 latency (ms)	Region 1	18.99 ± 0.35	19.09 ± 0.73	19.60 ± 0.55	Group p= 0.013* F[2, 153] =4.469
	Region 2	17.74 ± 0.22	19.15 ± 0.57	19.32 ± 0.59	Region p= 0.164 F[2, 153] =1.831
	Region 3	18.80 ± 0.36	19.47 ± 0.45	20.26 ± 0.49	Interaction p= 0.714 F[4, 153] =0.530
P1 latency (ms)	Region 1	33.63 ± 0.62	34.54 ± 0.73	34.69 ± 0.70	Group p= 0.183 F[2, 153] =1.717
	Region 2	34.41 ± 0.64	34.88 ± 0.73	35.43 ± 0.70	Region p= 0.376 F[2, 153] =0.983
	Region 3	34.58 ± 0.61	34.87 ± 0.78	35.63 ± 0.71	Interaction p= 0.991 F[4, 153] =0.069

Data are presented as mean ± 1 standard error of the mean
*** Significant difference (p< 0.05)**

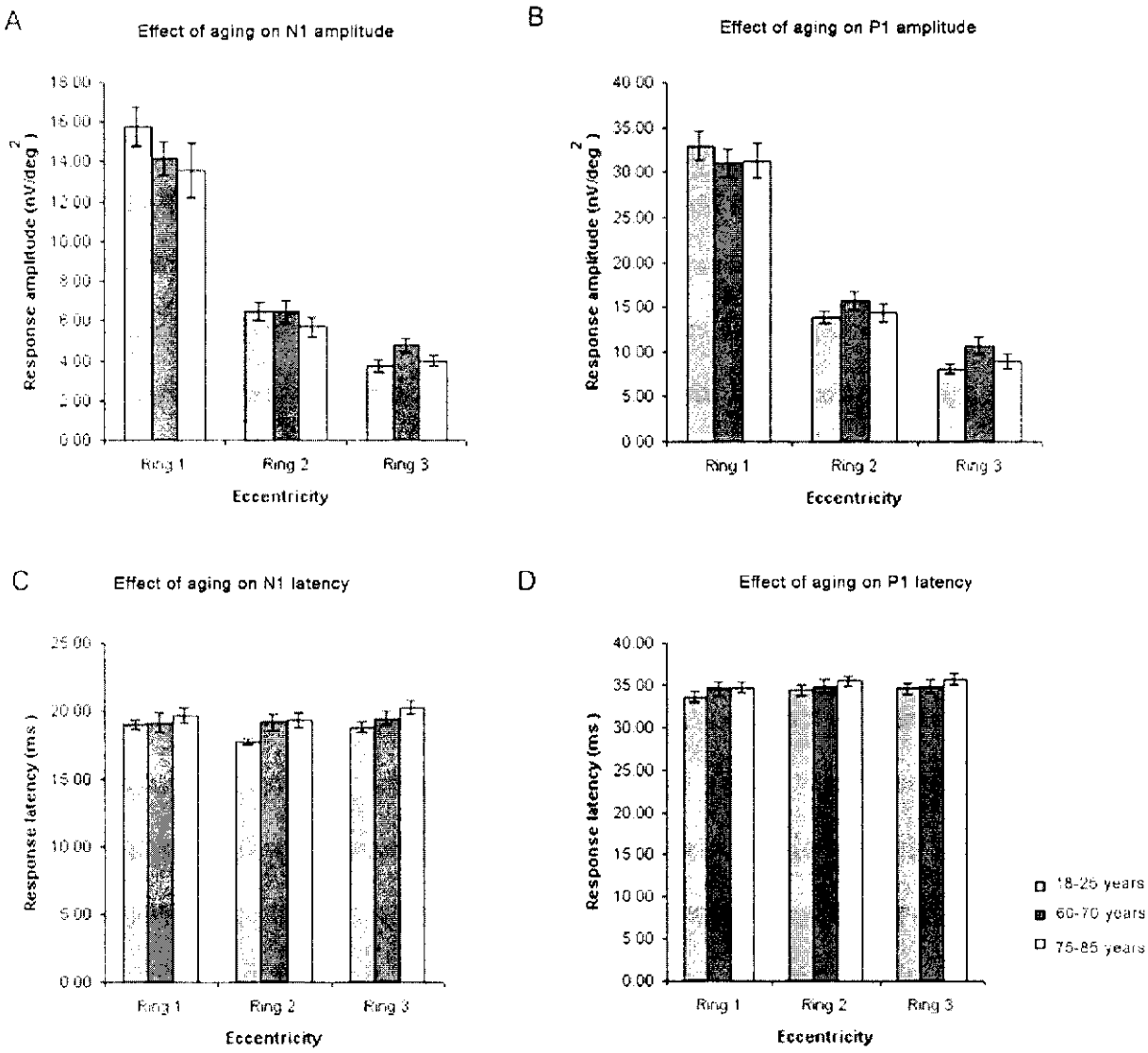


Figure 14.2. First order kernel response. **(A)** Mean N1 amplitude, **(B)** Mean P1 amplitude, **(C)** Mean N1 latency, and **(D)** Mean P1 latency of three regions for three groups of subjects of different ages. Error bars are ± 1 standard error of the mean.

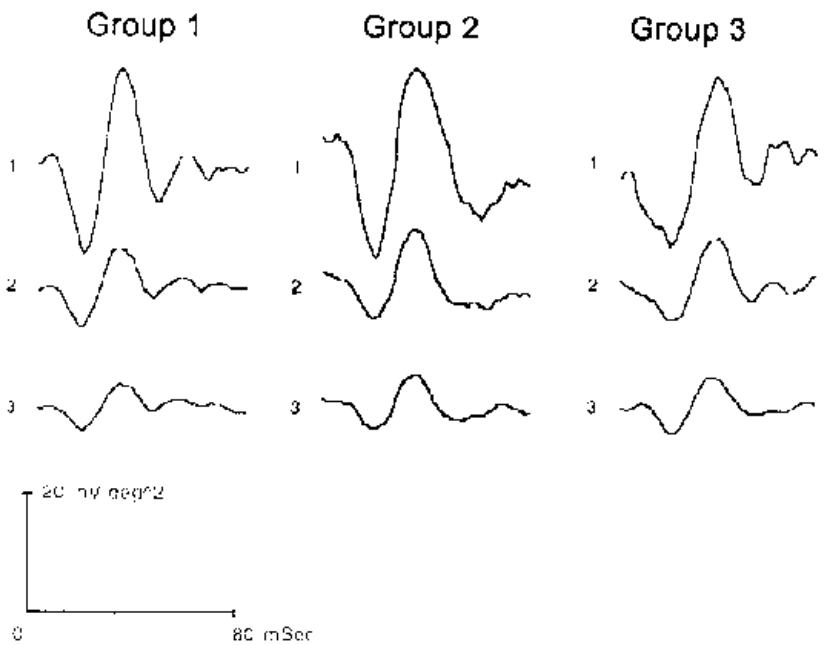


Figure 14.3. The mfERG (first order kernel response) from three of the subjects for the three concentric rings.

Results

There were no effects of age on N1 amplitude, but there were statistically significant effects of region grouping on this parameter for each age group (Table 14.1; Figure 1.42A). Tukey HSD multiple comparisons test showed that N1 amplitude among these three regions differed significantly. There was no significant interaction of age and region grouping for N1 amplitude. Findings were similar for P1 amplitude (Table 14.1; Figure 14.2B); P1 amplitude decreased significantly with increasing eccentricity as expected, but there were no statistically significant effects of age and no significant interaction effects.

There was a statistically significant effect on latency for the N1 component (Table 14.1; Figure 14.2C) with responses of the younger subject group (Group 1) faster than those of Group 2 by about 0.72 ms, and those of Group 2 faster than those of Group 3 by about 0.49 ms. However, Tukey HSD multiple comparisons test only showed that N1 latency from group 1 significantly differed from group 3 ($p=0.01$). There was no statistically significant retinal location effect and no significant interaction effect. For P1 latency, however, while the trends in timing of the responses were similar to those of the N1 response (Table 14.1; Figure 14.2D), there were no statistically significant effects for age group, regions and no interaction effect. The typical waveforms of the mfERG in these three groups of subjects were shown in Figure 14.3. The waveforms in these three groups of subjects did not show any significant differences.

Discussion

The number of cells in the human cerebral cortex decreases with increasing age (Henderson et al., 1980) and the function and anatomical structure of the retina also change in aging (Liem et al., 1991, Gao & Hollyfield, 1992, Curcio et al., 1993, Jackson et al., 2002b). It is well-known that visual functions such as visual acuity, contrast sensitivity, vernier acuity, and colour vision decrease with increasing age (Hurst & Douthwaite, 1993, Li et al., 2000). The decline of visual functions could be due to optical factors or neural factors. Therefore, when we study the effect of aging on visual function, the effect of cataract should not be ignored. Nevertheless, some of the visual functions are reported to be resistant to age-related changes. They include positional acuity, certain colour constancies, modulation-induced-desensitization, the Westheimer function, and the Stiles-Crawford effect of the first kind (Enoch et al., 1999), implying that not all kinds of neurons in the visual system are affected by age (Enoch et al., 1999).

In this study, we only found a main aging effect on mfERG N1 latency since only summed responses showed that N1 latency increased significantly when we compared young subjects and pseudophakic subjects over 75 years of age. Our results indicate that the age-related changes of the mfERG topography before age 70 years are caused by optical factors rather than neural factors.

An early study comparing the mfERG topography in different age groups (18-22 years, 33-37 years, and 48-52 years) showed that P1 amplitude from the central retina (10 deg in diameter) decreased significantly in the oldest group (Mohidin et al., 1999), but this study did not show changes in N1 amplitude, N1

latency, or P1 latency with increasing age. However, Tzekov et al. (2004) showed that P1 amplitude decreased and P1 latency increased with increasing age. They also found that the decrease of P1 amplitude from superior retina was faster than inferior retina, but the decrease was similar for both nasal and temporal retina. Nabeshima et al. (2001) also found that subjects over 40 years of age showed reduced P1 amplitudes from the central retinal region (6.4 degrees in diameter). Subjects over 50 years of age had lower P1 amplitudes in the central and peripheral retina (50 deg diameter) compared to 20 years old subjects. However, P1 latency did not change significantly with increasing age regardless of eccentricity.

Jackson et al. (2002a) examined the effect of pupil size and media opacities on mfERG topography. They demonstrated that pupil size and media opacities could reduce the mfERG responses. They concluded that both optical factors and neural factors (e.g. slowed temporal adaptation in the aged retina) caused the age-related changes in the mfERG topography. Without accounting for the effects of pupil size and media opacities, Jackson et al. (2002a) found that both central and peripheral retinal responses (N1 and P1) decreased with increasing age and the greatest reduction occurred in the central 10 degrees of the retina. They further showed that average N1 and P1 latencies in older subjects were longer than in young subjects. However, Dolan et al. (2003) showed that P1 amplitude and latency in the peripheral retina (60-90 degree of visual field) did not change significantly with increasing age even in subjects up to 75 years of age

Our results were similar to those of Fortune and Johnson (2002) which showed that P1 amplitude decreased and P1 latency increased at all eccentricities

with increasing age in normals: they attributed the decline of the mfERG responses with age to optical factors rather than to neural factors (Fortune & Johnson, 2002). After adjusting for the effects of pre-retinal optical factors, they demonstrated that P1 latency did not change significantly with age and only P1 amplitude from the central retina was slightly reduced with age. However, Gerth et al. (2002) showed that optical factors, which reduce retinal illuminance and increase intraocular scattering, could not fully account for the age-related decrease in mfERG response. They believed that the reduced responses in aged subjects might be attributable to age-related cell loss in the retina (Gerth et al., 2002). Their later study further demonstrated that the aging effect could also be observed in "isolated flash responses" as well as "adapted responses" (Gerth et al., 2003), implying a neural basis for the effect. Recently, Seiple et al. (2003) have claimed that the mfERG amplitude reduction with increasing age is mainly due to neural factors. They showed that central retinal responses decrease at a greater rate than the peripheral retinal responses. In addition, N1 latency and P1 latency tended to increase 0.02 ms and 0.03 ms per year, respectively; they examined subjects up to 81 years of age. All their subjects had VA of 20/25 and passed the Pelli-Robson contrast sensitivity test. However, the Pelli-Robson chart measures the contrast sensitivity (0.5-2 c/deg) just below the peak of contrast sensitivity function (2-6 c/deg). As media opacities (e.g. cataract) have a greater effect on high spatial frequencies (5-10 c/deg) than low spatial frequencies (1 c/deg) (Elliott et al., 1989), the Pelli-Robson chart does not provide a more sensitive measure of cataract than VA (Lempert et al., 1987, Elliott, 1993). Therefore, their criteria for subject recruitment cannot fully exclude the

subjects with ocular media problems and the confounding effects of pre-retinal optical factors on mfERG responses might still be present in their subjects. As a recent study by Wodehoff et al. (2004) has also found that cataract could reduce the mfREG responses, the pre-retinal optical factors should be noticed.

We believe that previous studies, which showed a significant decrease in mfERG response amplitude and an increase in mfERG response latency, may be due to the confounding effect of media opacities and the instability of fixation (Fortune & Johnson, 2002, Rudolph & Kalpadakis, 2002, Vrabec et al., 2004). The different results reported by different studies may be due to different methodologies (e.g. stimulus luminance and band-pass (Keating et al., 2000, Han et al., 2004)), assumptions (Fortune & Johnson, 2002, Jackson et al., 2002a), and different criteria for subject inclusion.

Since electroretinography is an objective method of assessing retinal function, the effects of age on the mfERG topography could be predicted theoretically by knowing the anatomical changes of aged retina and the origin of mfERG. An earlier well-known study by Gao and Hollyfield (1992) found that foveal cone density did not decrease significantly with increasing age, even in subjects up to the age of 95 years, but cone density at the equator decreases linearly with increasing age. Only 6.7% and 23% of cones at the retinal equator were lost at the fourth decade and ninth decade, respectively (Gao & Hollyfield, 1992). Their results were well supported by Curcio et al. (1993) who showed that cone density in the rod-free fovea and the extra-foveal region did not change significantly with increasing age even in a retina aged 90 years. Similarly, cone density in the

peripheral retina (beyond 43° diameter) decreased about 22% from 20 years to 90 years. In addition, cone inner segment diameter did not change significantly with increasing age (Curcio et al , 1993). In contrast to the loss of cones, rods appear to be more vulnerable to loss by aging. Both Gao and Hollyfield (1992) and Curcio et al. (1993) have found about 30% loss of rods to the ninth decade. The inner segment diameter of rods in the aged retina was 13.5% larger than that of the rods in the young retina so that the retinal area covered by rods remained constant throughout the life (Curcio et al., 1993).

Unfortunately, the effects of aging on the anatomy of bipolar cells in primates have not yet been reported (Spear, 1993). It has been reported that there are no age-related changes in amacrine cells within the central 5 mm of the retina (Curcio & Drucker, 1993). Anatomical studies have shown a progressive decrease in the number of ganglion cells with increasing age, with a more marked decrease in the peripheral retina (Gao & Hollyfield, 1992, Bonnel et al., 2003).

On the other hand, in the rest of visual system and neural system also have age related changes. Our brain uses one fifth of the total oxygen consumed by the body. It has relatively low levels of antioxidant defence, so it is easier to have oxidative damage (Batl & Birge, 2002). The age related neurodegenerative diseases, such as Parkinson's disease and Alzhiemer's disease, might be mediated by oxidative damage to neurons (Jackson & Owsley, 2003). In this study, we only provide the evidence about the contribution of optical and neural factors in aging within retinal level. Apart from the retina level, other parts of visual or neural system would also influence the performance of visual functions in aging.

The main contributions to the human mfERG are from the cells of outer retina. The leading edge of N1 is most probably related to the onset of off-bipolar cells with a small contribution from the cones. The leading edge of P1 is related to on-bipolar cells and off-bipolar cells (Hood et al., 2002). Under photopic conditions, it is believed that the rod system does not provide any contribution to the mfERG responses, as it is suppressed by the high frequency and high luminance stimulus (Keating et al., 2000, Hood et al., 2002). In addition, damage to retinal ganglion cells or amacrine cells does not affect the mfERG amplitude significantly (Hood et al., 2000, Fortune et al., 2001) and such damage has only mild effects on the mfERG waveform (Hood et al., 2002). The contribution of ganglion cells to the mfERG response would be revealed only under a specific condition and a specific setup (Sutter & Bearse, 1999). Since the mfERG may be predominantly generated by bipolar cells and such a contribution is driven by functional photoreceptors, only the damage of cone cells or bipolar cells greatly decreases the mfERG amplitude (Hood et al., 2003).

Our results have shown that central, paracentral or peripheral mfERG responses up to 44 degrees of the central retina do not change significantly with increasing age; this implies that the functional abilities of cone and bipolar cells do not decline significantly before 70 years of age. In addition, only the N1 latency of the mfERG responses in our eldest group was significantly longer than that of our youngest group. This suggests that age-related retinal changes occur late in life (i.e. after 70 years of age). Only N1 latency was increased in the eldest group, but P1 latency did not change significantly. This may imply that the cone cells or off-

bipolar cells might be more vulnerable than on-bipolar cells, anatomical studies may be useful to confirm these findings.

In this study, we assumed that cataract surgery has no detrimental effect on the retina. However, there is a possibility that cataract surgery has some effect on the retina. Some studies suggested that patients may develop cystoid macular edema, uveitis, retinal detachment, and elevated intraocular pressure after cataract surgery (Apple & Werner, 2001, McKellar et al., 2001). In this study, we used indirect ophthalmoscopy to examine the retina of the subjects to ensure they did not have any complications. However, very mild retinal complication may not be detected by this method. Therefore, this is one of the issues which may influence our results. In addition, although the new design of IOLs is free of aberration (Altmann et al., 2005), the old design of IOLs are known to increase optical aberrations. Since there is no study about the effect of aberrations on mfERG, we are not sure how this factor could affect the mfERG topography. Further study is required to study the effect of aberration on mfERG topography. In this study, the posterior capsular remnants were assessed after the cataract surgery. All subjects did not have significant post-surgical posterior capsular remnants before the mfERG measurement. so the effect of the opacities in the posterior capsule can be ignored.

In addition, all pupils in our subjects were fully dilated. The results of this study suggest that when we measure mfERG in patients (before 70 years of age) with IOLs, we should expect amplitude and latency values similar to those of young subjects. Increased latency and decreased amplitude of mfERG in patients (before 70 years of age) with IOLs is likely to imply abnormal retinal function.

Chapter 15. Conclusions and Suggestions for Future Research

The multifocal electroretinogram developed by Sutter and Tran (1992) has proved able to detect local retinal damage in retinal diseases such as age related macular degeneration, diabetic retinopathy, and retinitis pigmentosa. Most patients with these common retinal diseases are elderly. Since these patients are likely to have some degree of cataract which causes light scattering, it is important to study the effects of light scattering on the mfERG topography, before we use the mfERG to examine retinal function behind the cataractous lens.

In experiment 1, we found that forward light scattering decreased the central retinal responses and increased the mid-peripheral retinal response amplitude. It should be remembered that in this experiment the mean stimulus luminance was kept constant with the increase of forward light scattering, as forward light scattering does not reduce stimulus luminance and only reduces stimulus contrast. Only backward light scattering reduces the stimulus luminance. The results of this experiment showed that forward light scattering had effects on mfERG topography. Therefore, cataract is likely to affect mfERG topography. In experiment 2, we studied the effects of different degrees of cataract on mfERG topography. We found that the mfERG response amplitude was significantly reduced in patients with mild or moderate cataract. In addition, we found that cataract mainly affected the central retinal responses but the mid-peripheral retinal response amplitude did not change significantly. In experiment 3, we compared the mfERG topography in patients before and after cataract (with IOL implant) surgery. We found that cataract mainly reduced the central retinal responses not the peripheral responses. The results of

experiments 2 and 3 differed from those of experiment 1, as both forward light scatting and backward light scattering occurred in experiments 2 and 3. Therefore, the decrease of central retinal responses was due to the light scattering and the reduction of stimulus luminance. That the peripheral retinal responses were not affected by the cataract could be explained by the effects of forward light scattering, which counteract the effects of luminance reduction (Figure 15.1). The results of experiments 2 and 3 remind us that the confounding effect of cataract should not be ignored when interpreting mfERG topography in patients with cataract. The results of experiment 2 showed that different degrees of cataract affected the mfERG topography to different degrees. The International Society of Clinical Electrophysiology of Vision suggests that each laboratory should develop normative data for different age groups, as each laboratory may have variations in recording equipment. Therefore, we also suggest that each laboratory should establish its normative values for different degrees of cataract, if they want to use the mfERG to assess the retinal function behind a cataractous lens. Although experiment 2 showed that the subjects with moderate cataract had longer latency than the subjects with very mild cataract, it only increases N1 latency and P1 latency in about 1ms. This increase is within the normal variability of measurement and of no clinical significance. Our experiment 3 did not show statistically significant changes in N1 latency and P1 latency produced by cataract. In conclusion, the cataract has significant effect on the mfERG amplitudes in different retinal regions.

The effects of aging on mfERG topography have been studied extensively in recent years (Mohidin et al., 1999, Fortune & Johnson, 2002, Gerth et al., 2002,

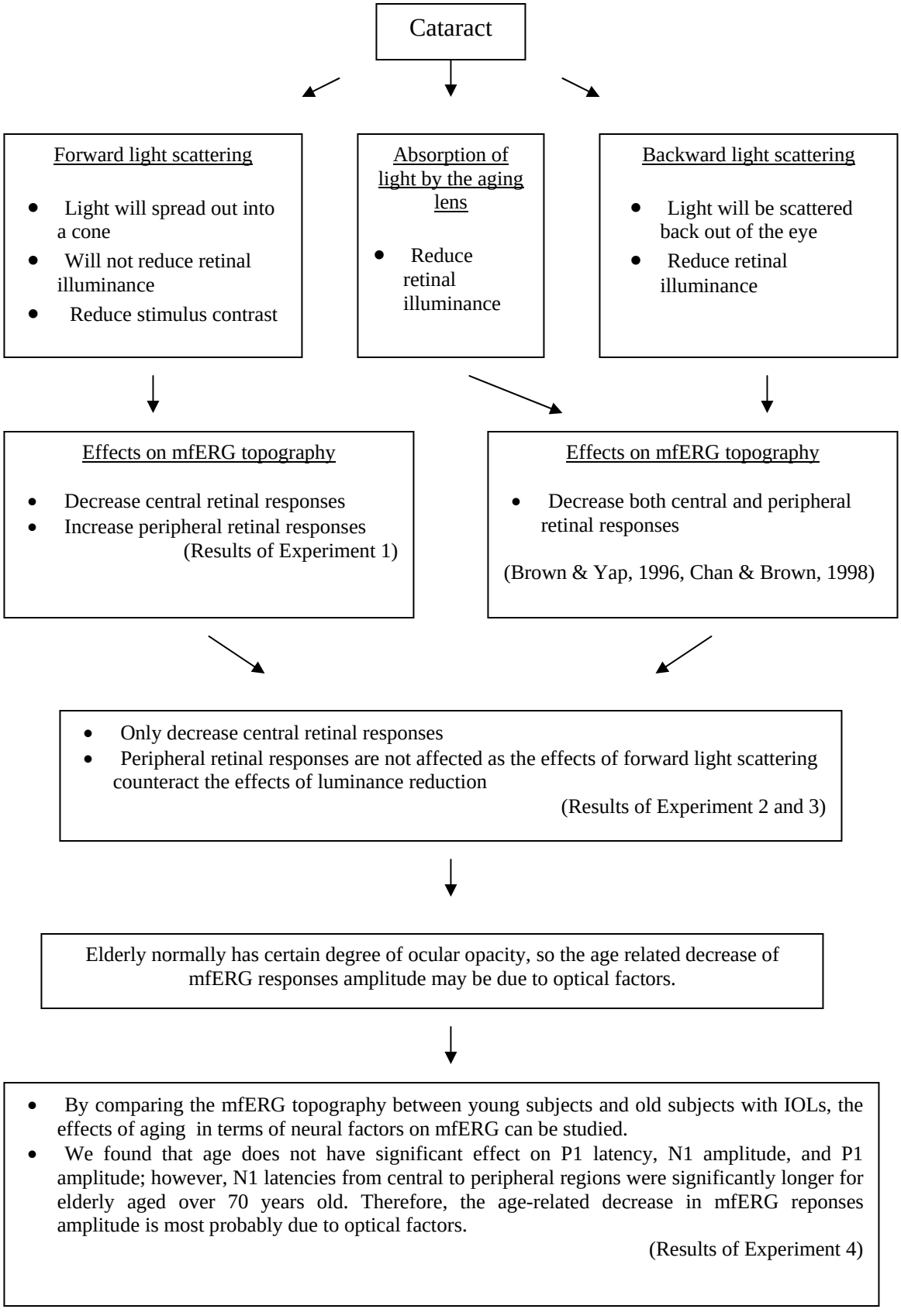
Jackson et al., 2002a, Nabeshima et al., 2002, Seiple et al., 2003). Most of these studies have found that both central and peripheral mfERG responses decrease with increasing age, but the decrease is more prominent in the central retina (Fortune & Johnson, 2002, Gerth et al., 2002, Jackson et al., 2002a, Nabeshima et al., 2002, Seiple et al., 2003). However, the age-related decrease of mfERG responses could be due to optical factors (e.g. lens opacities) and neural factors. Therefore, comparing the mfERG topography between young subjects and elderly subjects with IOLs is an excellent model for study of neural deficits in retina.

In experiment 4, we compared the mfERG topography between young subjects and elderly subjects with IOLs. We found that the mfERG topography did not change significantly before 70 years of age. Only the N1 latency from central to peripheral retina increased significantly after 70 years of age. This implies that the age-related decrease of the mfERG responses amplitude is due to optical rather than neural factors before the age of 70 years. When we measure the mfERG in patients with IOLs, aged less than 70 years, we should expect similar amplitude and latency values to those of young subjects. Increased latency and decreased amplitude in patients aged less than 70 years who have IOLs or clear ocular media is most likely to imply abnormal retinal function.

Since different types of cataract have different effects on CSF (Elliott et al., 1989), different types of cataract may have different effects on mfERG topography. Further studies about the effect of cortical cataract and posterior subcapsular cataract on mfERG are worth to be conducted. In addition, since media opacities can influence the mfERG responses, it is not known how much media opacities could

affect other multifocal responses, for example, multifocal oscillatory potentials and multifocal visual evoked potentials. Previous studies found that the multifocal oscillatory potentials were contributed by the rod-cone interaction and may be related to the bipolar-amacrine synapses (Wu & Sutter, 1995, Kurthnbach et al., 2000). It was also found that the multifocal oscillatory potentials could detect inner retinal diseases such as Type 1 diabetes without retinopathy (Kurtenbach et al., 2000). However, the effect of media opacities on multifocal oscillatory potentials has not yet been studied. Further studies can be done to investigate the effects of media opacities on these measures. Clinicians and scientists should know if cautions should be taken when measuring the multifocal oscillatory potentials and the multifocal visual evoked potential in patients with media opacities. A recent study by Kurtenbach et al. (2002) showed that there is a linear decrease in the amplitude and a linear increase in latency of the multifocal oscillatory potentials with age. In addition, the change of amplitude and latency are similar for both central and peripheral retina. Therefore, they suggested that there is an age-related impairment at the inner retina. Since this study only compared the subjects aged between 13 and 58 years, further studies can be done to investigate the effects of aging on multifocal oscillatory potentials by comparing young subjects and old subjects without optical influencing factors (eg. Subjects with IOL). This would provide further understanding of the age-related decreases in the multifocal oscillatory potentials and the multifocal visual evoked potential and their relations to optical factors or neural factors.

Figure. 15.1. Effects of cataract on the mfERG topography



Appendices

A. Technical aspects of multifocal electroretinogram

Numerous factors can affect the quality of the responses derived by the multifocal technique. Understanding these factors is important to help us in the comparison of the results of different studies as they may use different setups. Moreover, it can help to improve signal to noise ratio in the recording process and it can help us to design a better protocol for our experiments.

A1 Stimulus Setting

A1.1 Stimulus Luminance and Contrast

Early research showed that P1 amplitude decreases logarithmically with decreasing stimulus contrast from 96% to 32% (Brown & Yap, 1996). Moreover, the amount of amplitude reduction with decreasing stimulus contrast was similar for both central and peripheral responses. Therefore, central and peripheral retina might have a similar sensitivity to stimulus contrast.

With reducing the stimulus intensity by using a neutral density (ND) filter on part of the screen, it was found that amplitude of P1 at that localized area tends to decrease when a 0.2 ND filter is placed over the screen and a more prominent response reduction could be observed when a 0.4 ND filter was used (Brown & Yap, 1996). In addition, the amplitude of P1 decreased linearly with increased density of the ND filter from 0.1 to 1.0. A similar study worked on the effect of global luminance variation on mfERG also found that P1 amplitude also decreased when the mean luminance decreased (Yoshii et al., 2000b). At the same time, latency of

P1 increased linearly with decrease of mean luminance. Chan and Brown (1998) showed that the rate of P1 amplitude increase in the macula was greater than that in the peripheral retina when stimulus luminance increased (Chan & Brown, 1998). The topography of mfERG in a patient with a mild cortical cataract showed a pattern similar to normal subjects with an ND filter between –0.30 log to –0.52 log in front of the eye (Yoshii et al., 2000b). This reminds us that it is necessary to pay attention to the effect of media opacities on mfERG in aged subjects.

A1.2 Display Unit

There are two kinds of display unit for delivering the multifocal stimulus. They are cathode-ray tube (CRT) device and liquid crystal display (LCD) projection system (Keating et al., 2000). The most commonly used is the CRT device. An electron beam is used to show the stimulus and its refresh rate is high (up to 100Hz) so it enables a large number of signals to be averaged within a short time for improving the signal-to-noise ratio. For the LCD projection system, an electrical current passing through the liquid crystal is used to control the stimulus presentation; the refresh rate is lower than that in the CRT device.

CRT devices can provide a more uniform luminance across the field with only 10% luminance reduction from centre to periphery. However, LCD devices give a variation of about 30% (Keating et al., 2000). The most important difference between these two systems is that the pixel in LCD systems remains at a constant luminance until the next raster returns to that point. For CRT systems, the pixel will be updated as the raster passes (Figure A1.1). This means that pixel luminance will decay to zero in a short time before the next raster passes. Therefore, special cross

correlation software should be used when using LCD devices for delivering stimuli (Keating et al., 2001). Comparison of the mfERG responses measured from both display instruments with similar settings showed that the first order response obtained from the CRT device was larger than from the LCD device by 35%, but the second order responses obtained from the LCD device were larger than those from the CRT device by 24% (Keating et al., 2001). The difference was partly due to the fact that the LCD system will not add responses to the cross correlation where there are consecutive stimuli, but the CRT will add responses to the cross correlation even though the consecutive stimuli elicit a smaller response (Keating et al., 2001).

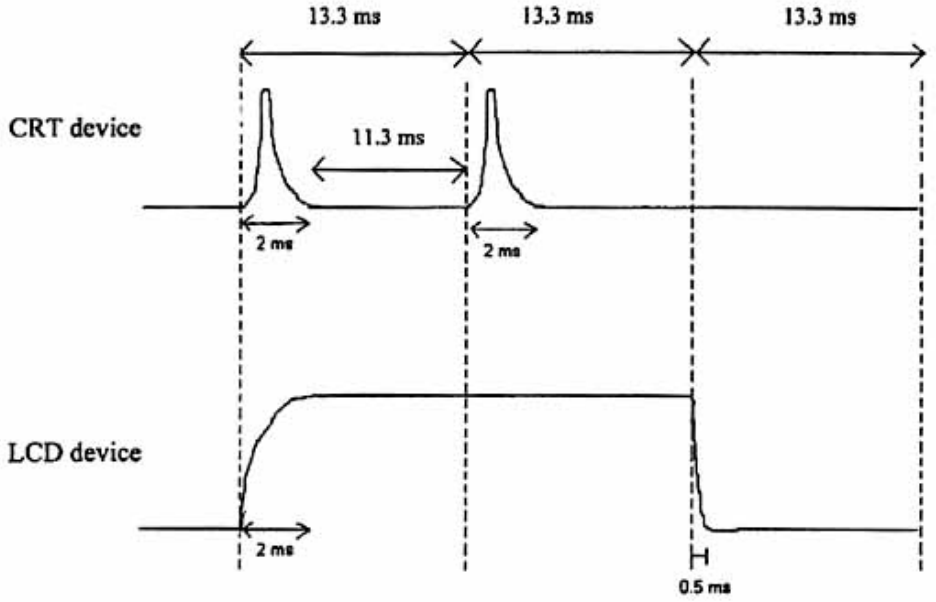


Figure A1.1 The luminance output graph for a black-white sequence for both cathode-ray tube (CRT) and liquid crystal display (LCD) systems. (Adapted from Keating et al. (2001)).

A2 Instrument Setup

A2.1 Electrodes

There are many types of electrodes for ERG measurement. The commonly used electrodes are Jet electrode, DTL electrode, Burian-Allen contact lens electrode, gold foil electrode, and C-glide (Arai et al., 1998). Studies comparing their relative effectiveness showed that the Burian-Allen contact lens electrode was the best for measuring both scotopic ERG and photopic ERG as it gives the highest responses. The relative amplitude measured with other electrodes were as follows: Burian-Allen contact lens electrode (100%) > Jet electrode (89%) > C-glide (77%) > Gold foil (56%) > DTL (46%) > Skin (14%). The commonly used electrodes for measuring mfERG are Burian-Allen contact lens electrode and DTL electrode (Hood et al., 1998b, Seeliger et al., 1998, Verdon & Haegerstrom-Portnoy, 1998).

A2.1.1 Burian-Allen Contact Lens Electrode

The advantage of using the Burian-Allen contact lens electrode is that it provides excellent signal to noise ratio. It covers the whole cornea during recording, so it can keep the cornea moist and reduce the blink rate, so that blink artifacts can be almost eliminated (Bearse & Sutter, 1996). In our clinical experience, however, some patients complain of dry eye when using this electrode, as no blinking is permitted. This uncomfortable sensation may cause the patient difficulty in maintaining steady fixation when measuring the mfERG. Moreover, it is not easy to insert for subjects with narrow palpebral fissures (Esakowitz et al., 1993). In

addition, the mfERG responses can be affected if small bubbles are trapped between the electrode and the cornea (Yoshii et al., 2000b).

A2.1.2 DTL Silver-impregnated Nylon Thread Electrode

This electrode is made up of filaments of spun nylon impregnated with silver. As this electrode is very thin, it can float on the tear film to measure ERG responses. It is claimed to have several advantages (Thompson & Drasdo, 1987). Firstly, it is a disposable fibre so that it maintains good hygiene. Secondly, the flexible nature of the DTL electrode allows it to be stretched and returned to its original position without any damage to the cornea when the patient blinks and it is easy to be put on to the patient's eye. Thirdly, the patient feels comfortable even in a long recording period without using any local anaesthesia.

A2.1.3 Comparison of Using Different Types of Electrodes for Recording mfERG

Mohidin et al. (1997) compared the repeatability and variability of four different types of electrode for measuring mfERG. Summed mfERG responses obtained from Jet contact lens electrodes, gold foil electrodes, DTL electrodes, and C-glide electrodes were shown to be repeatable when they were measured on different days. In addition, the responses obtained from C-glide electrodes had higher variability than Jet contact lens electrodes and gold foil electrodes. However, the variability of the gold foil electrodes was not significantly different to DTL electrodes. It is a pity that this study did not examine whether the topography of

mfERG would vary in using different types of electrode. Therefore, each type of electrode has its own advantages and disadvantages. There is no perfect electrode and the choice of electrode depends on situation (Barber, 1994).

A2.2 Filter Bandwidth

In recording full-field ERG, a wide bandwidth (0.3 to 300Hz) is generally used and recommended (Celesia et al., 1993). The function of bandpass filter is to avoid amplifier saturation (Keating et al., 2002). In recording mfERG, some studies have used a bandwidth from 10 to 300Hz (Yoshii et al., 1998, Fortune et al., 1999, Greenstein et al., 2000a, Hood & Zhang, 2000, Li et al., 2001) and some studies have used a bandwidth from 3 to 300 Hz, with high amplification for recording mfERG responses (Jackson et al., 2002a, Marmor et al., 2002). A recent study showed that the waveform of the full-field ERG was not greatly affected by high-pass filter, when it was set from 1Hz to 10Hz (Keating et al., 1996) (Figure A2.1). There is only a small reduction in b-wave amplitude and a fast return to baseline after the b-wave. However, an artificial positive component could be created in a negative ERG waveform when the high-pass filter is at 5Hz or 10Hz. In measuring the mfERG on a patient with upper branch retinal vein occlusion with these two settings (a bandwidth from 1-300Hz and a bandwidth from 10-300Hz) (Keating et al., 1996), a positive artifact component could be created by using a bandwidth from 10-300Hz and this artifact was not observed with a bandwidth from 1-300Hz. Thus, a wide bandwidth (with high-pass filter at least 3Hz) was strongly recommended in mfERG recording (Keating et al., 2000). The setting of the low-pass filter is also

important in mfERG recording as a low pass filter at 100Hz could suppress oscillatory potentials (Kretschmann et al., 2000).

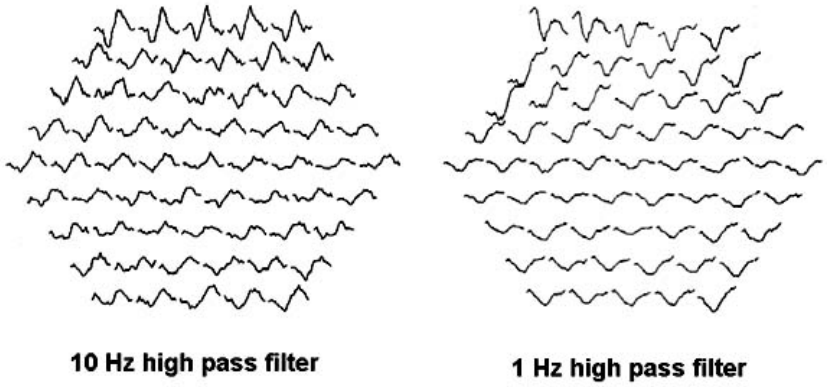


Figure A2.1. Multifocal ERG waveforms in a patient with upper branch retinal vein occlusion at two different bandpass settings. (Modified from figures in Keating et al. (1997)).

However, a recent study compared two bandwidth settings (10-100Hz and 10-300Hz) to measure mfERG (Han et al., 2004). The 10-100Hz bandwidth setting has higher signal-to-noise ratio and lower intersubject variability than 10-300Hz. In addition, they suggested that using a bandwidth from 10-100Hz was more sensitive for detection of retinal disease. In the past, there are no guidelines for using bandpass filter, so different studies had different settings. Now, the ISCEV has guidelines for us, and suggests to use filter range of 3-300Hz or 10-300Hz. After the consideration of all the setup from the previous studies, we chose 3-300Hz as the bandwidth in our study and this range is the optimal one in the mfERG measurement.

A2.3 Artifact Removal Procedure

In the VERIS system, there is a function called "Artifact Removal Procedure". This function can be maximally repeated 3 times (called 1st iteration, 2nd iteration and 3rd iteration). The VERIS manual recommends using this function for all analyses, as it can eliminate artifact like the noise caused by blinking and eye movement. Therefore, many studies on mfERG have used this function before analyzing their results (Palmowski et al., 1997, Kondo et al., 1999, Sutter & Bearse, 1999, Chappelow & Marmor, 2000). Under some circumstances, the latter part of response waveforms become noisier than the original waveform with increase in the number of iterations (Yoshii et al., 2000a). There was a tendency for the first order kernel response to be reduced after the 2nd iterations of "Artifact Removal Procedure", and the second order kernel response tended to reduce after the 1st iteration of the "Artifact Removal Procedure". In addition, "Artifact Removal Procedure" was demonstrated not only to affect the local response itself but also to affect the neighboring responses around it. Therefore, the "Artifact Removal Procedure" may distort the mfERG waveform, but it is mainly used for analysis of the second order kernel response, as it can reduce noise in the waveforms (Palmowski et al., 1997, Yoshii et al., 2000a). In our study, we normally did not use this function before analyzing the results.

A2.4 Notch filter (Line filter)

When we measure the mfERG, there may have environmental noise such as 50Hz mains frequency, which is emitted by surrounding environment (e.g. stimulus

monitor, etc.) (Bock et al., 2000). Therefore, a notch filter is recommended to use to eliminate the noise in different mfERG recording protocols. A recent study indicated that the mfERG responses could be greatly affected by notch filtering (Bock et al., 2000). By comparing the mfERG responses with and without using the notch filter, the first order kernel response amplitude of N1, P1, and N2 decreased when the notch filter was used. Latency of P1 and N2 increased by 5 ms and 8 ms, respectively with notch filter. Fourier analysis showed that when notch filter was not used, the first order kernel responses are mainly composed of waveforms with frequencies below 65 Hz and those main spectral components are between 19 and 47 Hz. When the notch filter was used, these main spectral components were clearly attenuated (Figure A2.2). Therefore, using a notch filter in measuring the mfERG should be avoided and we did not use the notch filter in our study.

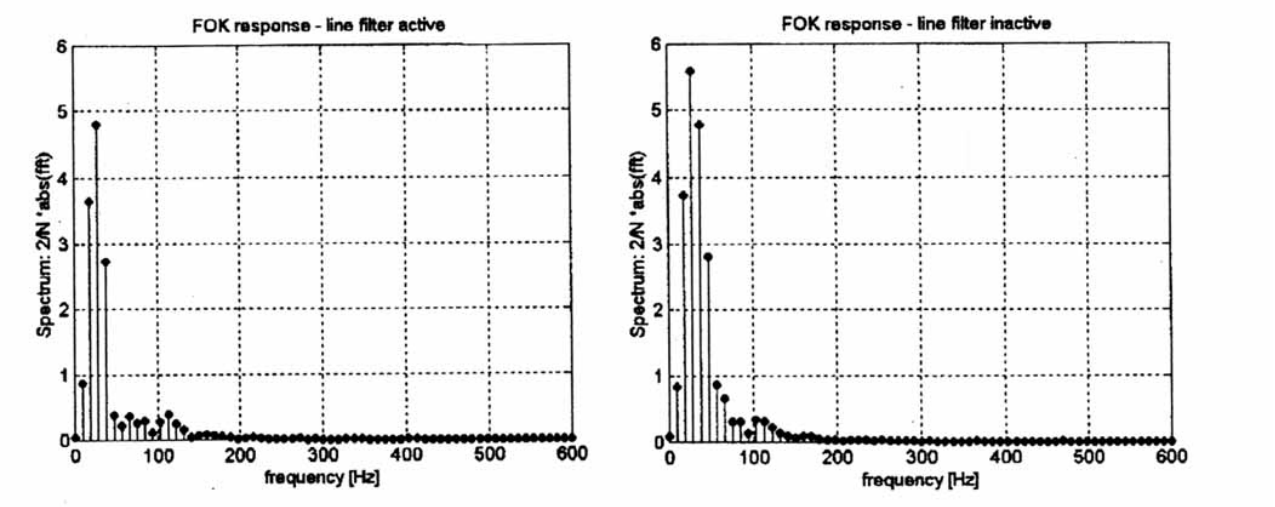


Figure A2.2 Spectral plots of the first order kernel responses of the same subject for two different filter setups (notch filter active and notch filter inactive). (Modified from figures in Bock et al. (2000)).

A3 Data Analysis

A3.1 First and Second Order Kernel

As previously mentioned, the technique of binary m-sequence with cross-correlation allows multiple local retinal responses to be extracted. Moreover, this technique makes it possible to derive the first and higher order kernel responses (Sutter & Tran, 1992, Sutter, 2000). Therefore, both the first order and the second order kernel responses are recorded at the same time to study the effect of different retinal diseases on mfERG (Palmowski et al., 1997, Chan & Brown, 2000, Palmowski et al., 2000).

The first order kernel response is the difference between the mean responses to all white stimuli and the mean response to all black stimuli in the sequence, while the second order kernel response represents the temporal interaction between two white stimuli separated by an integral number of stimulus base intervals. In another words, the second order kernel responses can indicate how the mfERG is influenced by the previous stimulus. The first slice of the second order kernel response represents the interaction between two consecutive white stimuli, so it is the measurement of the effect of an immediately preceding flash (Sutter, 2000). The second slice of the second order kernel response represents the interaction between two white stimuli with two intervening base intervals, so it is the measurement of the effect of the flashes two frames apart (Hood, 2000, Sutter, 2000). The second order kernel response of the mfERG is suggested to be an actual response generated in the inner retina (Hood, 2000). It is also estimated that 80% of the first slice of the second order responses is contributed by the inner retinal cells (e.g. ganglion cells)

(Raz et al., 2003). The presence of the second order response indicates the existence of short-term adaptation. The diminished second order response indicates an abnormality in the circuits and connections involved in adaptation rather than a missing component or cellular response (Sutter, 2001, Hood, 2003).

A3.2 Peak to Peak Amplitude, Root Mean Square, Scalar Product, and Curve Fitting Technique

The mfERG responses can be analyzed by four different methods. They are 1) Peak to peak amplitude, 2) Root mean square (RMS), 3) Scalar product, and 4) Curve Fitting Technique. Each method has its unique characteristics.

Peak to Peak Amplitude

This is a direct method for the calculation of mfERG response density using the peak-to-peak amplitude of response. In the VERIS system, a response amplitude is measured by selecting the point at the positive peak and the negative peak. The difference between these two points indicates the response amplitude. However, selecting the appropriate peaks would be difficult when the signal is highly noise contaminated (Fortune & Johnson, 2002).

Root Mean Square Amplitude (RMS)

Peak-to-peak amplitude is commonly used to measure the response amplitude in the traditional ERG. As the response from the mfERG is susceptible to noise contamination, root mean square (RMS) may also be used for analysis (Sutter

& Tran, 1992). This method does not take account of the waveform, and so both signal and noise can affect the result. Therefore, it can overestimate signal amplitude (Sutter & Tran, 1992).

Scalar Product

The scalar product is recommended for use when the signal is relatively weak and noisy. It can give an indication of how the mfERG response deviates from the normal value, as differences in response timing or shape also affect this value (Keating et al., 2000, Fortune & Johnson, 2002). The scalar product is formed by multiplying corresponding points in a template waveform by a recorded waveform. Then each multiplication is added to give this value (Keating et al., 2000). Thus, this method relies on comparison with a normal or standard waveform template (Verdon & Haegerstrom-Portnoy, 1998). By dividing scalar product amplitude by the area of a stimulus element, a 3D topography of mfERG responses can be formed (Figure A3.1). However, previous studies have reported that the scalar product is not sensitive in detecting latency changes; less than 5 msec of latency shift will not be detected (Keating et al., 2000). Therefore, sensitivity of mfERG in detection of retinal disease may be affected by using scalar product only. On the other hand, direct measurement of time to peak latency may provide additional information.

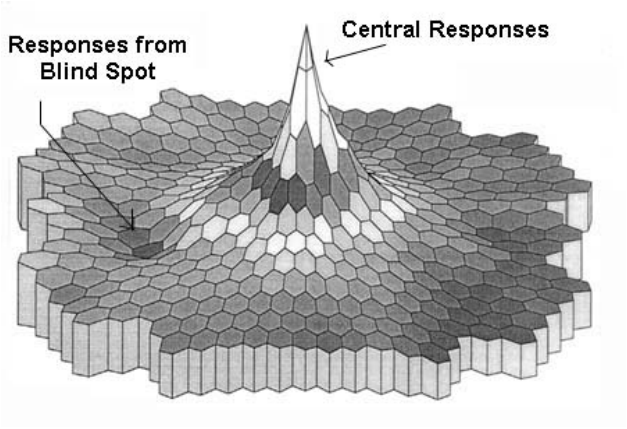


Figure A3.1 The 3D topography of mfERG responses.

Curve Fitting Technique

This "Curve Fitting Technique" was developed by Hood and Li (1997). In this method, a single template is obtained from the control subjects. Then the template would be fitted to the records of the patients. The template can be scaled in both amplitude and time. A perfect fit to the template would produce a statfit of 0.0. A statfit of 1.0 means the fitting is poor. This method is suggested to be useful to analyze the mfERG responses with low signal-to-noise ratio (Hood & Li, 1997).

B. Measurement of Refractive Errors and Visual Acuity

Measurement of Refractive Errors

Subjective refraction was used to measure the refractive errors. To determine the astigmatism, a cross-cylinder with an astigmatic interval of 0.25D was used.

Measurement of Visual Acuity

To measure the visual acuity, a Snellen letter chart was used. Subject is seated comfortably in the examination chair and wears the best corrected lens. The untested eye is covered. A mirror was placed in front of the subject 3 meters away. The Snellen letter chart was placed above the subject's head. Therefore, the effective viewing distant is 6 meters. The background luminance of the Snellen letter chart was 150 candela per square meter. Snellen fraction (e.g. 6/6) was used for recording the visual acuity.

Measurement and Grading of cataract

We use LOCS III to grade the nuclear opalescence. For assessing nuclear opalescence (NO), it is graded by comparing the lens image viewed by optic section technique to standard photographs.

Consent Form

Research Study Information Sheet

Title of Project:

The effect of light scattering on the multifocal electroretinogram

Project Leader:
Dr. Henry Chan (Department of Optometry and Radiography)
Office: HJ 505 Tel: 2766-7937

Why is the study being performed?
Multi-focal electro-retinogram (ERG) is a useful objective clinical measurement that assess the functions of the retina. The aim of this study is to characterise the effects of light scattering (an important optical component) on ERG measurement.

What do volunteers for the study have to do?
If you volunteer for the study you will be asked:

1. *to sign an informed consent form that states you understand the information presented on this sheet.*
2. *give the information about the history of your ocular and general health, age, medication, allergic history*
3. *your pupil will be enlarged by putting eye drop; measuring sensor will be put on near the eye; you will be asked to look at a computer screen during recording which lasts about 15-20 minutes*
4. *you may experience mild superficial eye irritation for a few seconds after putting eye drops. The pupil enlargement will cause a transient loss of near focus and glare sensation. The disturbance will last 3-4 hours.*

Can a volunteer withdraw from the study?
Yes, you can stop participating in the study at any time with no penalty.

Can I get more information on the study?
Yes, contact **Dr. Henry Chan (2766-7937)** *and he will answer any questions you may have.*

This study was approved by the Ethics Committee of the Hong Kong Polytechnic University. However, if you think there are any procedures that seem to violate your welfare, you may complain in writing to the Ethics Committee of the Hong Kong Polytechnic University.

Consent Form

I agree to take part in the project entitled:

The effect of light scattering on the multifocal electroretinogram

Project Leader:

Dr. Henry Chan

* I have read and understood the information presented to me.
* I have had an opportunity to ask questions about the study, and these have been answered to my satisfaction.
* I realize I may not benefit personally from taking part in the study.
* I realize I can withdraw from the study at any time with no penalty.
* I realize that the results of this study may be published, but that my own results will be kept confidential, and that I will not be identified personally in any published work.

Name.....
Signature.....

Witness.....
Signature.....

Date.....

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