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Impairment of retinal adaptive circuitry in the myopic eye

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Abstract

Previous studies have proposed that the inner retina is affected in myopes. This study aimed to investigate the changes in adaptive circuitry of the inner retina in myopia, using the global flash multifocal electroretinogram (global flash mfERG) with different levels of contrast (luminance modulation). Fifty-four myopes had global flash mfERG recorded with different contrasts. The direct component (DC) and the induced component (IC) of the mfERG response were pooled into 6 regions for analysis. The response amplitudes and implicit times at different contrasts were also analysed. Results showed that myopes had significant reduction in the paracentral DC amplitude for the 29 and 49% contrasts and in the paracentral IC amplitude at all contrasts measured. The peripheral IC amplitude for the 49% contrast was also reduced. No significant change was found in implicit time for either DC or IC response. Refractive error explained about 14% of the variance in DC and 16% of the variance in IC amplitude respectively; axial length could not account for additional variance in either paracentral DC or IC amplitudes in the hierarchical regression models used. We concluded that the paracentral retinal region in myopes showed signs of impaired retinal adaptation, suggesting a functional loss at the inner retinal layer. In addition, functions attributed to the outer retinal layer showed only small changes due to myopia.

Introduction

Axial elongation is the primary anatomical change which differentiates myopia from emmetropia (Atchison, Jones, Schmid, Pritchard, Pope, Strugnell, & Riley, 2004; Lam, Edwards, Millodot, & Goh, 1999), leading to an alteration of the regular arrangement of retinal neurons (Beresford, Crewther, & Crewther, 1998; Crewther, 2000; Liang, Crewther, Crewther, & Barila, 1995). This alteration may, in turn, affect signal transmission among different retinal layers. A variety of visual functions of the myopic eye are reduced compared with emmetropes and this reduction in visual performance has been associated with retinal stretching (Aung, Foster, Seah, Chan, Lim, Wu, Lim, Lee, & Chew, 2001; Chen, Woung, & Yang, 2000; Chui, Yap, Chan, & Thibos, 2005; Jaworski, Gentle, Zele, Vingrys, & McBrien, 2006; Liou & Chiu, 2001; Mantyjarvi & Tuppurainen, 1995; Rudnicka & Edgar, 1995; Rudnicka & Edgar, 1996; Subbaram & Bullimore, 2002).

Multifocal electroretinography (mfERG), developed by Sutter & Tran (1992), can examine multiple retinal areas simultaneously to measure subtle changes in response topography. Both the first and second order kernels of mfERG responses are mathematically derived from retinal responses by using a cross-correlation method (Sutter, 2000). The first order kernel mfERG response, which is the average response to a focal flash by subtracting the response to the flash from the response to a dark frame

(Hood, 2000; Sutter, 2000), is dominated by the responses of photoreceptors, ON- and OFF-bipolar cells (Hood, 2000; Hood, Frishman, Saszik, & Viswanathan, 2002; Hood, Seiple, Holopigian, & Greenstein, 1997; Ng, Chan, Chu, Siu, To, Beale, Gilger, & Wong, 2008). On the other hand, the first slice of second order kernel response is the interaction of immediately preceding frames on a current frame, which is derived from the subtraction of the responses without change of stimulation in frames (i.e. white-to-white or black-to-black) from the responses with change of stimulation in frames (i.e. white-to-black or black-to-white). It represents the multiplicative temporal interaction of responses separated by a delay of 1 frame (Hood, 2000; Sutter, 2000). The second order response predominantly initiates from the amacrine cells and retinal ganglion cells with some contribution from ON- and OFF-bipolar cells (Ng et al., 2008).

There is ample evidence that mfERG components are affected by myopia development. The first order kernel response has been reported to be reduced and delayed with the increase of myopic refractive error (Chen, Brown, & Schmid, 2006a; Kawabata & Adachi-Usami, 1997; Luu, Lau, & Lee, 2006; Wolsley, Saunders, Silvestri, & Anderson, 2008) or axial length (Chan & Mohidin, 2003). Several studies have suggested that the attenuation of the mfERG response is due to axial elongation (Chan & Mohidin, 2003; Kawabata & Adachi-Usami, 1997; Westall, Dhaliwal, Panton, Sigesmun, Levin, Nischal, & Heon, 2001) and this functional loss was attributed to

outer retina (Chan & Mohidin, 2003; Kawabata & Adachi-Usami, 1997). Chen and her co-workers (2006a) found a significant response delay of 1.3 to 3.1 ms in myopia but showed that axial length and refractive error could only account for, respectively, 15% and 27% of the total variance of the mfERG delay. Since an increase in implicit time in ocular diseases may be related to a damage in the inner plexiform layer (Hood, 2000), Chen and her colleagues (2006a) proposed that the remaining variance of implicit time in myopia might be caused by altered synaptic connections at the inner plexiform layer. On the other hand, the first slice of second order kernel response has also been found to be reduced in amplitude by 5 to 10% for each millimetre of axial length elongation, indicating that inner retinal function is probably impaired in the myopic eye (Chan & Mohidin, 2003).

The conventional mfERG, which measures the interactive response to continuous flashes, presents flashes at 13.3 ms intervals (75Hz), so that before the response elicited by one focal flash has completed, a second flash may be presented; this results in superimposition of the waveforms of successive flashes (Hood, 2000). Although the higher order kernel response probably represents inner retinal activity, the use of higher order kernels is limited by a poor signal-to-noise ratio.

The mfERG with the global flash paradigm, which measures the dynamics of inner retinal processing by incorporating dark frames and a periodic full screen global flash

stimulus within the classic m-sequence stimulation (Chu, Chan, & Brown, 2006; Chu, Chan, Ng, Brown, Siu, Beale, Gilger, & Wong, 2008; Fortune, Bearse, Cioffi, & Johnson, 2002; Shimada, Bearse, & Sutter, 2005; Sutter, Shimada, Li, & Bearse, 1999), involves a direct response to focal flash, called direct component (DC) (Shimada et al., 2005), and a larger non-linear component originating from the interaction between focal flash and periodic global flash, called induced component (IC) (Bearse, Sutter, & Stamper, 2000; Sutter et al., 1999). These DC and IC responses have been shown to reflect predominantly the outer (Chu et al., 2008) and inner (Chu et al., 2008; Fortune et al., 2002; Shimada et al., 2005; Sutter et al., 1999) retinal activities, respectively. This stimulation paradigm has identified retinal defects in glaucoma patients (Chu et al., 2006; Fortune et al., 2002), which are presumed to originate in the inner retina.

Adaptive responses are thought to mainly take place in inner retina and we hypothesized that these adaptive functions are likely to be impaired in myopic eye. Several studies have suggested that the mfERG measurement with lower contrast stimulation can increase the relative contribution of inner retina cells to the mfERG response (Bearse & Sutter, 1998; Chan, 2005; Hood, Greenstein, Frishman, Holopigian, Viswanathan, Seiple, Ahmed, & Robson, 1999; Palmowski, Allgayer, & Heinemann-Vemaleken, 2000). This study aimed to investigate retinal function, especially the inner retina, in myopic eyes by using the global flash mfERG paradigm with different

contrasts, in an attempt to characterize aspects of adaptive functions of the myopic eye.

Methods

Subjects

Fifty-four subjects aged from 19 to 29 years (mean = 21.9 ± 1.9 years; median = 22.0) with refractive errors from plano to −8.13 D (spherical equivalent) (mean = -4.00 ± 2.16 D; median = -3.75 D) and astigmatism of equal to or less than 1.00 D were recruited from the Optometry Clinic of The Hong Kong Polytechnic University. All subjects received a thorough ophthalmic eye examination including subjective refraction and ocular health assessment by a registered optometrist. Subjective refraction was performed 30 minutes after the instillation of 1 drop of 0.4% Oxybuprocaine (Agepha Pharmaceuticals, Austria, Europe) and 2 drops of 1% Tropicamide (Alcon Laboratories, Inc., Fort Worth, TX, USA) at 5 minute intervals. The subjective refraction ended at reaching the best visual acuity with maximum plus optical correction. Ocular health assessment included slit lamp examination and ophthalmoscopy. Colour vision was also examined with the 24-plate version of Ishihara colour vision test. The inclusion criteria were corrected LogMAR visual acuity of 0.00 or better in both eyes, normal colour vision, cup-to-disc ratio of less than 0.50 with normal neuroretinal rim appearance, similar optic nerve head appearance in both eyes

and myopic crescent of less than 0.5 disc diameter. Subjects with ocular pathological changes, clinically significant fundus degeneration, systemic disease, a history of epilepsy or a family history of pathological myopia or retina disease were excluded from this study.

Subjects were informed of the nature and the risks of the experiment. Consent was obtained from each subject after the study had been explained and all enquiries had been answered. This study adhered to the tenets of the Declaration of Helsinki and was approved by the Human Ethics Committee of The Hong Kong Polytechnic University.

Multifocal ERG stimulation

The stimulus pattern, consisting of 103 hexagons scaled with eccentricity (stretch factor = 10.46), was presented on a 19 inch RGB computer monitor (Model no: GDM-500PS, Sony, Tokyo, Japan) using the Visual Evoked Response Imaging System (VERIS Science 4.1, EDI, San Mateo, CA, USA). The hexagonal pattern subtended 44° vertically and 52° horizontally at a working distance of 33 cm. To maintain constant retinal image size among all subjects (Rabbetts, 2007), the spectacle corrective lenses were placed at the anterior focal plane of the tested eyes during the mfERG recording.

The global flash paradigm, which contained four video frames, started with a multifocal flashes frame, followed by a dark frame (3 cd/m^2), a full screen global flash (162 cd/m^2) and a second dark frame in each slice of the pseudorandom binary m-

sequence (2^{13}) (Chu et al., 2006; Fortune et al., 2002; Shimada, Li, Bearse, Sutter, & Fung, 2001). As illustrated in Figure 1a, in the frames containing multifocal flashes, each hexagon was either a dark or bright stimulus according to the m-sequence with a stimulation rate of 75 video frames per second. To investigate the retinal adaptive changes at different contrasts, the luminance-difference of the multifocal flashes was set at 142, 89, 70 and 43 cd/m^2, corresponding to the stimulus contrasts of 96, 65, 49 and 29%, respectively. The mean luminance of the multifocal flashes and the background was 73 cd/m^2 for all contrast levels. The total recording time for each condition was approximately 7.5 minutes. Each subject was tested 4 times, once with each contrast and the order of presentation of the contrasts was randomised across subjects.

Multifocal ERG Recording

The pupil of the tested eye of each subject was dilated to at least 7 mm before mfERG recording. A Dawson-Trick-Litzkow (DTL) electrode was used as the active electrode. Gold-cup surface electrodes were placed about 10 mm lateral to the outer canthus of the tested eye as reference and at the central forehead as ground electrode. An amplifier (Model: P511K, Grass-Telefactor, West Warwick, RI, USA) was used to amplify and filter the signals (Gain: x100,000; Band pass: 10 to 300 Hz). The instantaneous compound ERG was monitored by the examiner using the VERIS

program. The recording process for each contrast was separated into 32 slightly overlapping segments and a short rest was provided between segments. If a segment was contaminated with artifacts such as blinks or small eye movement, the segment was discarded and re-recorded immediately.

Axial length measurement

The axial length of the tested eye was measured with an optical biometer (IOL master, V.4.08, Carl Zeiss Meditec, Inc., Dublin, CA, USA). Five readings were taken to obtain a mean value and the data were used if the signal-to-noise ratio for each reading was greater than 2.00 and the range of the five readings was less than 0.10 mm. The mean axial length of the subjects was 25.33 ± 1.14 mm (range 22.52 – 28.00 mm; median = 25.29 mm).

Analysis

Amplitudes and implicit times of the DC and IC responses were measured for each retinal region (Figure 1b). The amplitudes of DC (DC_{amp}) and IC (IC_{amp}) response were evaluated by using peak-to-peak measurement. The DC amplitude was measured from the first negative trough to the first positive peak while the IC amplitude was calculated from the second distinct peak to the subsequent trough. The implicit time of DC (DC_{IT}) response was measured from the onset of the stimulus to the peak of the DC response while the implicit time of IC (IC_{IT}) response was measured from presentation of the

global flash (i.e. 26.6 ms) to the IC response peak (Figure 1c).

Statistical Packages for the Social Sciences (SPSS 15.0, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Since both refractive error (Kawabata & Adachi-Usami, 1997; Luu et al., 2006) and axial length (Chan & Mohidin, 2003) were found to influence the mfERG response, hierarchical multiple regression was separately performed to investigate the contribution of axial length on the global mfERG responses at different regions, in addition to the effect of refractive error. This statistical method not only allows us to assess sets of independent variables at various levels with the control of each factor at preceding levels but also evaluates the contribution of each factor involved. Since refractive error has a greater effect on mfERG response than axial length (Chen et al., 2006a), refractive error was used in Step 1 of the hierarchical regression model and both refractive error and axial length were used in Step 2. Bonferroni correction with level of significance (α) set at 0.008 was used to correct for the multiple comparisons between different retinal regions.

Results

There was a strong correlation between refractive error and axial length indicating that the myopia was primarily axial in nature (Pearson's correlation: $r = -0.803$; $p < 0.0001$) (Figure 2).

Direct Component (DC) and Induced Component (IC) responses

Every subject had mfERG waveforms with distinct DC and IC responses at all the contrasts and regions. At 96% contrast, the mean DC response and mean IC response reached its peaks at, respectively, 29.8 ms (after the onset of the stimulus) and 30.1 ms (after the presentation of the periodic global flash.). Both the DC and IC response peaked slightly later at central region (i.e. Ring 1 and 2) (Figure 3). The waveform of the mfERG responses at other contrasts shared similar characteristics but each retinal region reached its peak slightly earlier under lower contrasts compared to high contrasts (data not shown).

Table 1 summarizes the results from the hierarchical regression analysis in determining the independent effects of refractive error and axial length on the DC and IC amplitudes. When refractive error was entered into the first step of this model, it explained 11% to 14% of the variance in DC response from ring 2 to ring 4 at 29% contrast. At these regions, the DC response decreased significantly as myopia increased at this contrast (only the scatter plot of ring 3 is shown) (Figure 4). In addition, about 19% of the variance in DC amplitude of ring 3 at 49% contrast was attributed to refractive error (Table 1) and the amplitude also decreased with increasing myopic refractive error (Figure 4). The DC amplitude was not affected by refractive error for the remaining contrasts or other retinal regions. When both refractive error and axial

length were included in the second step of this model, only the DC amplitude of ring 2 at 49% contrast made a further contribution to the model (adjusted R^2 change = 0.177, F change (1,51) = 12.077, p = 0.001). However, axial length did not account for any additional change of DC response in the remaining contrasts or other retinal regions (all $p > 0.05$). Both central (Ring 1) and peripheral DC (Ring 5 and 6) amplitude were unaffected by either refractive error or axial length (Table 1).

With regards to the IC amplitude, refractive error accounted for 13% to 23% of the variance in IC amplitude for ring 3 at all contrasts, i.e., it reduced significantly as the myopic refractive error increased (Table 2, Figure 4). In addition, refractive error explained 12% to 17% of the variance in IC amplitude for ring 4 at all contrasts measured but not 65% contrast. Similar findings were also observed at low to moderate contrasts (i.e. 29, 49 and 65 %) but not at high contrast for ring 5 and ring 6. However, only the IC amplitude of these two regions at 49% contrast reached the Bonferroni corrected significant level (Table 2). When axial length was added as a secondary explanatory variable in this model, it did not account for the extra variance in the IC amplitude for all the contrasts (all $p > 0.05$) (data not shown).

Neither DC nor IC implicit time showed significant association with refractive error at all the contrasts tested (data not shown). The implicit time virtually remained constant as refractive error increased. The addition of axial length as a secondary

variable in the model could not account for extra variance in either DC or IC implicit time (all $p > 0.05$).

Discussion

Our findings showed that the paracentral (Ring 3, eccentricity = 4.6° ~ 8.9°) DC response of myopes reduced significantly as a function of the magnitude of myopia at low (29%) and moderate (49%) contrast but not at high contrast. The direct component (DC) is the response to the focal flash and reflects the interactive response between focal flash and the periodic global flash in the preceding m-sequence stimulation. The DC thus reflects retinal adaptive changes (Chu et al., 2006; Chu et al., 2008; Shimada et al., 2005; Sutter et al., 1999). This component involves a larger contribution from the outer retinal activity (Chu et al., 2008) and a smaller contribution from the inner retina (Chu et al., 2008; Sutter et al., 1999). Decreasing the contrast increases the contribution of the inner retina to the mfERG response with conventional m-sequence stimulation (Bearse & Sutter, 1998; Chan, 2005; Hood et al., 1999; Palmowski et al., 2000). Recently, we have reported that there are some oscillatory-like wavelets originating from the inner retina superimposed on the DC waveform. One of these oscillatory wavelets contributes to the peak of DC response and saturates at moderate to high contrasts, while the activities of the outer retinal components including photoreceptors, ON- and OFF-bipolar cells increase linearly as contrast increases (Chu et al., 2008).

Thus, compared to higher contrasts, it is likely that the DC response at low contrasts involves a larger contribution from the inner retina. Reduction of the DC response amplitude has also been reported in eye diseases affecting the inner retina (Chu et al., 2006; Shimada et al., 2001). Taken together, we speculate that the attenuated DC response amplitude in myopes at low and moderate contrasts is probably a consequence of impaired adaptive function at the inner retinal level.

The paracentral (Rings 3 and 4, eccentricity = 4.6° ~ 13.5°) IC response of myopic eyes was reduced at all contrasts measured and a similar effect was observed in the peripheral (Rings 5 and 6, eccentricity = 13.5° ~ 25.4°) IC response at low and moderate contrasts. The induced component, which is an adaptive response produced by the global flash in the concurrent m-sequence stimulation, predominantly reflects the activity of the inner retina (Chu et al., 2008; Fortune et al., 2002; Shimada et al., 2005; Sutter et al., 1999). The IC response has been suggested to originate primarily from amacrine cells and retinal ganglion cells in porcine eyes (Chu et al., 2008). An attenuated IC response has also been identified in glaucoma patients whose inner retina was impaired (Chu et al., 2006; Fortune et al., 2002). So, an attenuated IC response in our study is further evidence of impaired adaptive function of inner retina in myopes.

In contrast to our findings, Chen and her colleagues (2006b), who also carried out mfERG measurements with the global flash paradigm at high contrast, found that the

response amplitude of both DC and IC increased with increasing myopic refractive error but did not reach statistical significance. In their study, the DC and IC amplitudes of subjects had been statistically adjusted to compensate for the change in response due to the variance of axial length among different myopic subjects. However, the adjustment may not be applicable to each retinal region as previous studies have demonstrated that the effect of myopia and axial length on retinal function is different with changing eccentricities (Chan & Mohidin, 2003; Chen et al., 2006a; Kawabata & Adachi-Usami, 1997). This statistical manipulation has presumed a uniform effect of myopia/axial length on retinal function and might not be an ideal method to study retinal function in myopic eyes. Since a substantial relationship exists between refractive error and axial length, using axial length as a co-variate may remove the shared variance with refractive error and cannot really reflect the influence of refractive error on mfERG response.

The conventional mfERG is a measure of the temporal interactive response to successive flashes (Hood, Holopigian, Greenstein, Seiple, Li, Sutter, & Carr, 1998). The focal flash presented before the response due to the preceding focal flash is fully developed. Thus, an adaptive response triggered by a sequential flash superimposes on the waveform of the previous flash (Hood, 2000). This response is an inverted second order kernel response, which was named the induced component by Sutter (2000), mainly overlaps the late portion of the first order waveform and leads to an early and

sharp P1 response (Hood, 2000). Chen and her colleagues (2006a) investigated the mfERG response in myopic eyes and found a delayed P1 response using the conventional mfERG without significant change in amplitude. Hood (2000) suggested that the delay in timing is likely to be caused by an attenuated "induced component" response, leading to a shift of the peak of the P1 response waveform and the attenuated "induced component" presumably from altered synaptic transmission at the inner plexiform layer. The global flash paradigm separates the higher order response by inserting a dark frame between two flashes. So, the reduced IC response without a significant change in the DC response as found in our results, especially at the higher contrasts, supports the above hypothesis that the delayed response may be caused by an altered synaptic connection in the inner plexiform layer.

The second order kernel response obtained with a conventional mfERG, which reflects the retinal adaptive changes and mainly represents the activity from amacrine cells and retinal ganglion cells with some contribution from ON- and OFF-bipolar cells (Hood, 2000; Hood et al., 2002; Ng et al., 2008), is reduced in myopes not only at retinal eccentricity of 5° to 13° but also at 18° to 25° (Chan & Mohidin, 2003), which is consistent with our findings. The pattern electroretinogram response, which mainly represents the activity from the inner retina, is also reduced with longer eyeballs (Hidajat, McLay, Burley, Elder, Morton, & Goode, 2003). Psychophysical

measurements of temporal vision including the critical fusion frequency and the temporal modulation sensitivity also show poorer performance in myopic eyes (Chen et al., 2000). These results indicate that the myopic eye takes longer to recover from temporal stimulation. All this evidence further supports our findings that the adaptive function of the myopic eye is impaired.

In the conventional full-field ERG, the entire retina is stimulated with a homogenous diffuse light. In myopic human eye, both the scotopic and photopic b-waves, as well as the oscillatory potentials, of the flash ERG response are reduced (Perlman, Meyer, Haim, & Zonis, 1984; Westall et al., 2001). They are also reduced in animals models of myopia (Fujikado, Kawasaki, Suzuki, Ohmi, & Tano, 1997). Recent studies using primates have shown that the photopic b-wave is partially affected by some third-order neurons such as amacrine cells and ganglions cells (Bui & Fortune, 2004; Mojumder, Sherry, & Frishman, 2008), in addition to the contribution from ON-bipolar cells, OFF-bipolar cells, horizontal cells and Müller cells (Sieving, Murayama, & Naarendorp, 1994). In addition, the oscillatory potentials probably originate from inner plexiform cells (Wachtmeister, 1998). Thus, the attenuated response in myopic eyes does not relate simply to the decline in cell density or physiological change in the outer plexiform cells but may also include cells of the inner plexiform layer.

We are surprised to find that retinal function in the paracentral region was more

affected in myopes, and that peripheral retina response was partially attenuated at low contrasts. We noted that central response was not affected. Visual sensitivity is generally depressed in myopes (Aung et al., 2001; Chihara & Sawada, 1990; Rudnicka & Edgar, 1995; Rudnicka & Edgar, 1996) and is predominantly affected at eccentricity from 15° to 20° (Chihara & Sawada, 1990). Orientation discrimination in the myopic eye is mildly changed at the fovea but is markedly reduced at an eccentricity of 15°, suggesting non-uniform stretching of the posterior part of the globe (Vera-Diaz, McGraw, Strang, & Whitaker, 2005). The retinal thickness in the paracentral region at eccentricity from 1.5 to 3 mm (i.e. ~ 5° to 10°) were found to be thinner in myopic eye (Lam, Leung, Mohamed, Chan, Palanivelu, Cheung, Li, Lai, & Leung, 2007; Luo, Gazzard, Fong, Aung, Hoh, Loon, Healey, Tan, Wong, & Saw, 2006; Wu, Chen, Chen, Chen, Shin, Yang, & Kuo, 2008). Beyond the central region, the dendrites of secondary and tertiary neurons like bipolar cells, amacrine cells and ganglion cells synapse horizontally with several presynaptic retinal neurons (Curcio & Allen, 1990; Kolb & Dekorver, 1991; Kolb, Linberg, & Fisher, 1992; Kolb & Marshak, 2003). It is likely that the dendrites of these neurons may be influenced as a result of retinal thinning, which in turn affects the physiological function of the retina. The results of the current study are in agreement with all of these previous studies that the paracentral retinal region is vulnerable and foveal function seems to be relatively preserved in myopic

eyes.

We found that myopic refractive error predominantly affected the retinal function at the paracentral region from 5° to 14° of eccentricity. In contrast, common central retinal diseases such as age-related maculopathy and glaucoma mainly affect the parafoveal (eccentricity from 2.5° to 4°) (Maguire & Vine, 1986; Sarks, Sarks, & Killingsworth, 1988) and mid-peripheral regions (beyond 20° of eccentricity) (Henson & Hobley, 1986), respectively, at the early stage of the disease. These results imply that more attention to potential functional deficits in myopic patients at the paracentral retina is needed.

An increase in spacing of photoreceptors (Beresford et al., 1998; Crewther, 2000) and inner retinal neurons (Teakle, Wildsoet, & Vaney, 1993) have been reported in animal models of myopia, and similar findings have been observed in myopic human eyes as a result of axial elongation (Chui, Song, & Burns, 2008; Chui et al., 2005; Kitaguchi, Bessho, Yamaguchi, Nakazawa, Mihashi, & Fujikado, 2007). Since the mfERG result presents as the magnitude of the response per unit area, the strength of the mfERG signal will also be affected by the neuron density (Sutter & Tran, 1992). However, Luu and co-worker (2006) only found weaker mfERG responses in axial myopic adults but not in myopic children with similar magnitude of refractive errors; this suggests that reduced mfERG response associated with a decline in cell density is

not a key factor in the mfERG response.

Our regression analysis indicates that refractive error only accounts for 14% and 16% of the variance in the DC and IC amplitudes, respectively. Reduced retinal illuminance and increased electrical resistance have already been excluded as confounding factors in accounting for reduction in mfERG response (Kawabata & Adachi-Usami, 1997; Luu et al., 2006). Dopamine level and dopamine metabolism are reduced in animal myopia models (Guo, Sivak, Callender, & Diehl-Jones, 1995; Morgan, 2003; Pendrak, Nguyen, Lin, Capehart, Zhu, & Stone, 1997; Stone, Lin, Laties, & Iuvone, 1989) and dopaminergic amacrine cells play a significant role in governing the general state of adaptation of the retina (Slaughter, 1990). Additionally, the lack of an adequate amount of dopamine in patients with Parkinson's disease gives a weaker retinal response to a light stimulus, suggesting the importance of dopamine in maintaining normal retinal function (Jaffe, Bruno, Campbell, Lavine, Karson, & Weinberger, 1987). Hence, it is expected that the adaptive function in the myopic eye should also be reduced. In addition, delayed mfERG responses have recently been found to be linked with reduced paracentral retinal thickness between outer plexiform and retinal nerve fibre layers in myopes (Wolsley et al., 2008). Moreover, the sensitivity of cone photoreceptors was reduced in a chicken model of form-deprivation myopia because of the changes in geometry of the photoreceptors (Westbrook, Crewther, &

Crewther, 1999). Thus, the remaining variance of the global flash mfERG response may be associated with functional changes such as alteration of the biochemical reactions in the retina, retinal thinning and subsequent synaptic alterations, and reduced sensitivity of the photoreceptors in response to myopic changes.

Previous studies on chicks have demonstrated that the process of eye growth is regulated locally by visual stimuli (Diether & Schaeffel, 1997; Gottlieb, Fugate-Wentzek, & Wallman, 1987; Troilo, Gottlieb, & Wallman, 1987; Wallman, Gottlieb, Rajaram, & Fugate-Wentzek, 1987) and the paracentral retina in higher primates is also involved in regulating eye growth (Smith, Kee, Ramamirtham, Qiao-Grider, & Hung, 2005; Smith, Ramamirtham, Qiao-Grider, Hung, Huang, Kee, Coats, & Paysse, 2007). In humans, the myopic eye usually has a relative hyperopic peripheral refraction (Mutti, Hayes, Mitchell, Jones, Moeschberger, Cotter, Kleinstein, Manny, Twelker, & Zadnik, 2007; Mutti, Sholtz, Friedman, & Zadnik, 2000). In addition, a longitudinal study of a group of pilots indicated that individuals with hyperopic refraction in the peripheral retina were more prone to develop axial myopia (Hoogerheide, Rempt, & Hoogenboom, 1971). This implies that the paracentral retina of human eye may have certain mechanism to detect defocus, even if it is not the site with the highest resolving power across the retina. We hypothesized that local hyperopic defocus in the peripheral retina would trigger retinal thinning, leading to reduced retinal function and inferior visual

performance in the paracentral retinal region.

Conclusions

In the myopic eye, the paracentral IC amplitude was significantly reduced at all contrasts measured and paracentral DC amplitude was significantly reduced at low and middle contrasts only, in which refractive error attributed to about 16% and 14% of the variance in IC and DC amplitude respectively. This study suggests that the adaptive function of inner retina was impaired in myopic eye and was predominantly affected at the paracentral retina.

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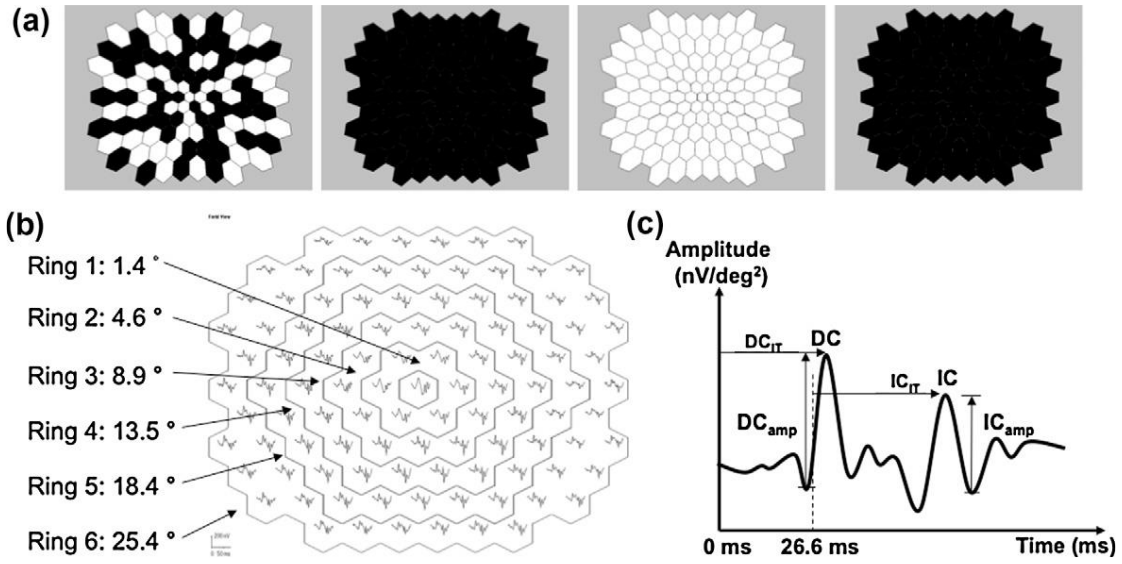

Figure 1. (a) Schematic diagram showing the video frame sequence of the global flash paradigm. The four frame sequence contained 1) a 103 stimulus array governed by m-sequence stimulation (multifocal flashes frame), followed by 2) a dark frame, 3) a (full screen) global flash and 4) a dark frame. (b) Each local response was pooled into 6 rings and was averaged to determine the effect of the magnitude of myopia on the retinal response at different regions. The eccentricity boundaries of each pooling region are labelled by the arrows. (c) A schematic diagram showing the first order kernel response waveform consisting of a direct component (DC) followed by an induced component (IC) (See text for details).

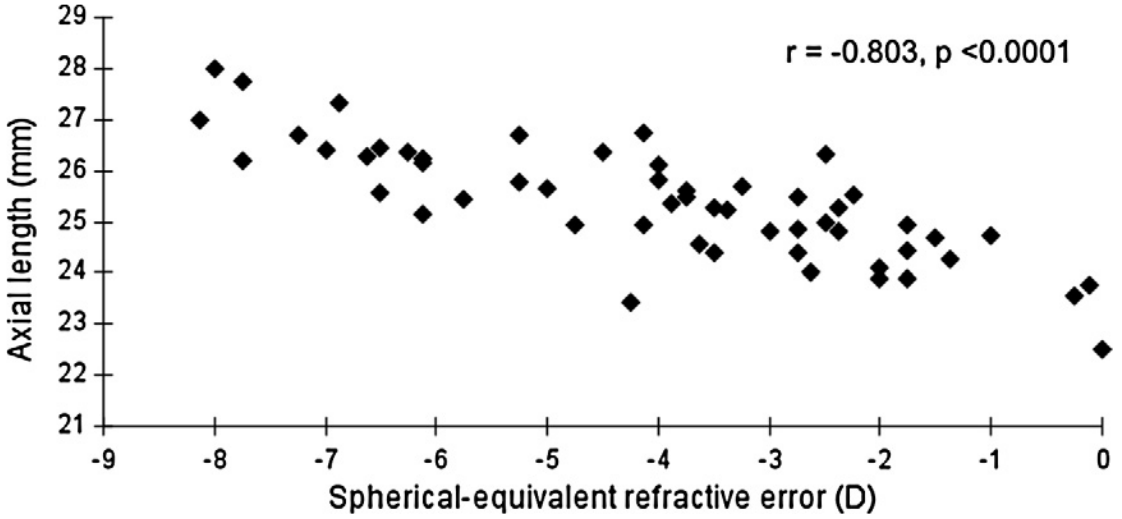

Figure 2. Correlations between refractive errors and axial length for our subjects (n = 54).

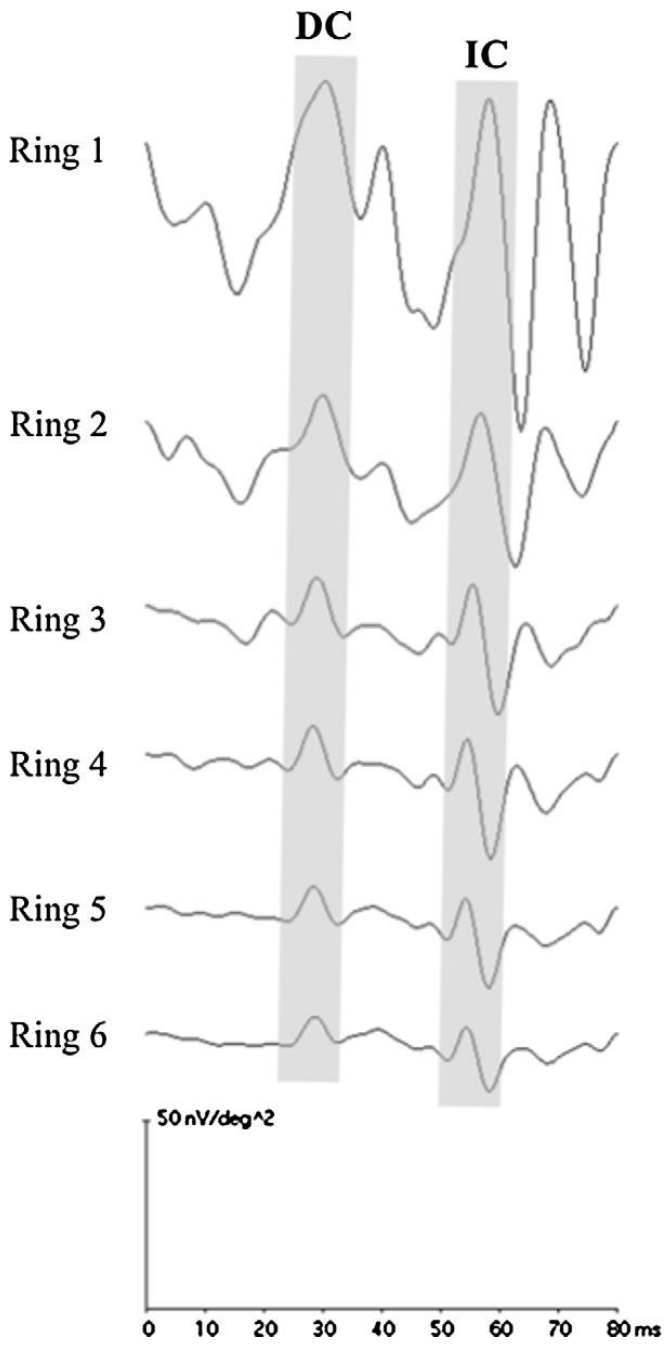

Figure 3. The waveforms of the ring-averaged responses from central (Ring 1) to peripheral (Ring 6) retina of a subject (SE = −1.38D) at 96% contrast. The waveform consisted of two distinct peaks corresponding to the DC and IC responses as highlighted in the figure.

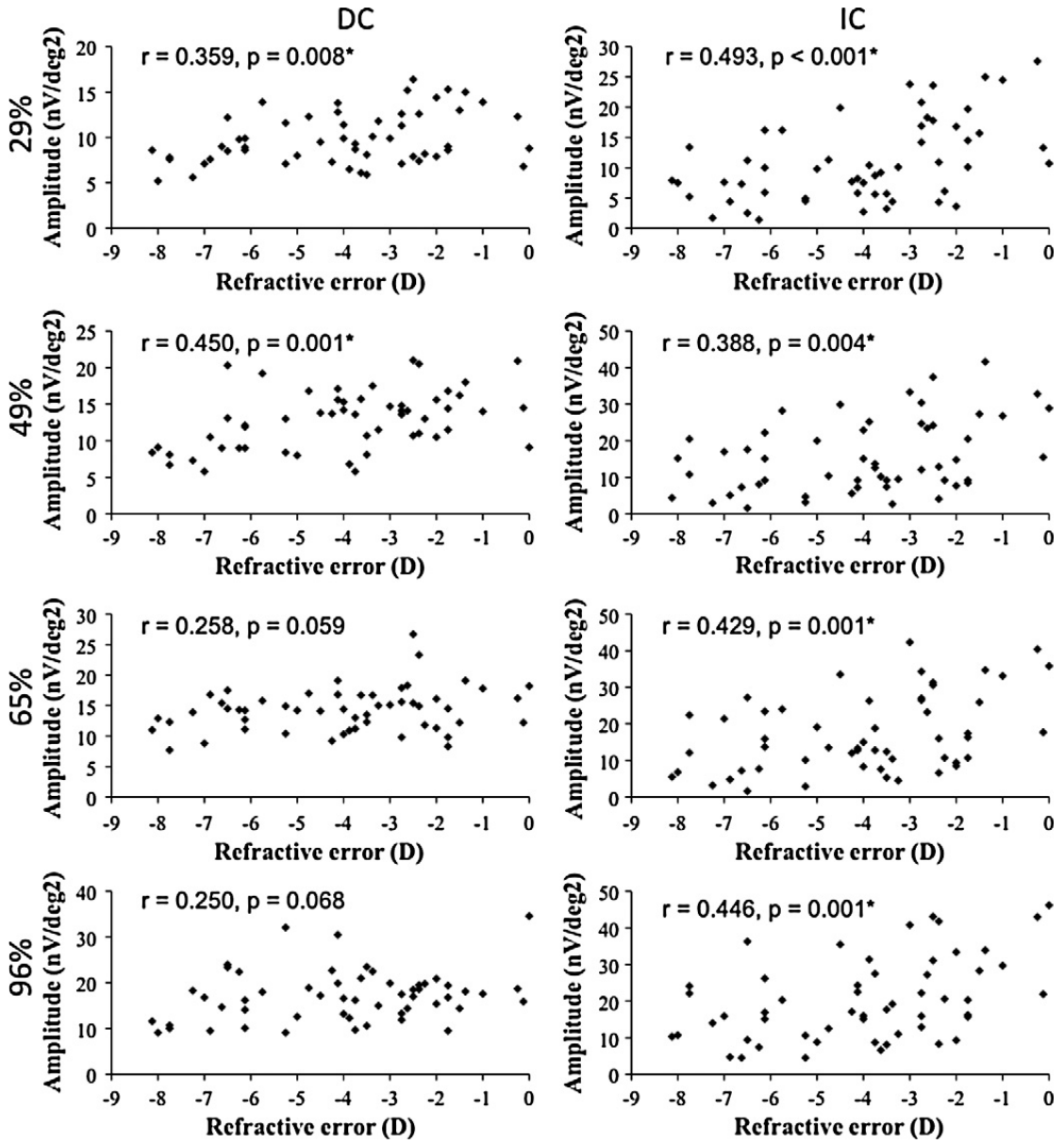

Figure 4. Scatter plots showing the relationship between global flash mfERG responses (Ring 3) and refractive errors at the four contrasts: 29% (top), 49% (second), 65% (third) and 96% (bottom). The DC response decreased significantly with increasing myopic refractive error at 29% and 49% contrasts (marked with '*') but not at 65% and 96% contrasts. In contrast, the IC response decreased significantly as a function of refractive error at all contrasts measured (marked with '*').

Table 1. A hierarchical regression analysis was conducted to study the effect of refractive error and axial length on DC amplitude. Refractive error (RE) was entered into Step 1, and refractive error and axial length (RE+AL) were entered into Step 2 (RE) of these models. The table shows the adjusted R square (adjusted R^2), F value (F) and p-value (p) for each step of the models.

		Contrast (%)											
		29%			49%			65%			96%		
Region		adjusted R^2	F	p	adjusted R^2	F	p	adjusted R^2	F	p	adjusted R^2	F	p
Direct Component (DC)													
Ring 1	RE	0.006	1.302	0.259	0.026	2.415	0.126	-0.008	0.576	0.451	0.015	1.780	0.188
	RE + AL	-0.014	0.643	0.530	0.098	3.863	0.027	-0.026	0.317	0.730	0.019	1.501	0.233
Ring 2	RE	**0.137**	**9.431**	**0.003**	0.055	4.098	0.048	-0.015	0.240	0.627	0.015	1.803	0.185
	RE + AL	0.137	5.200	0.009	0.221	8.524	0.001	-0.025	0.347	0.709	0.018	1.499	0.233
Ring 3	RE	**0.112**	**7.670**	**0.008**	**0.187**	**13.195**	**0.001**	0.049	3.722	0.059	0.045	3.474	0.068
	RE + AL	**0.098**	**3.876**	**0.027**	**0.174**	**6.572**	**0.003**	0.030	1.833	0.170	0.101	3.982	0.025
Ring 4	RE	**0.114**	**7.839**	**0.007**	0.026	2.430	0.125	-0.003	0.854	0.360	0.011	1.589	0.213
	RE + AL	0.110	0.742	0.393	0.032	1.318	0.256	0.001	1.019	0.368	0.023	1.624	0.207
Ring 5	RE	0.020	1.042	0.312	-0.019	<0.001	0.983	-0.014	0.273	0.603	<0.001	0.985	0.326
	RE + AL	0.046	1.225	0.302	-0.035	0.093	0.911	-0.034	<0.001	0.875	0.036	1.985	0.148
Ring 6	RE	0.039	3.175	0.081	-0.013	0.304	0.584	0.023	2.227	0.142	-0.008	0.602	0.441
	RE + AL	0.065	2.843	0.068	-0.028	0.279	0.758	0.004	0.006	0.939	0.034	1.936	0.155

Table 2. A hierarchical regression analysis was conducted to study the effect of refractive error and axial length on IC amplitude. Refractive error (RE) was entered into Step 1, and refractive error and axial length (RE+AL) were entered into Step 2 (RE) of these models. The table shows the adjusted R square (adjusted R^2), F value (F) and p-value (p) for each step of the models.

		Contrast (%)											
		29%			49%			65%			96%		
Region		adjusted R^2	F	p	adjusted R^2	F	p	adjusted R^2	F	p	adjusted R^2	F	p
Induced Component (IC)													
Ring 1	RE	-0.018	0.062	0.804	0.015	1.795	0.186	-0.017	0.092	0.763	0.020	2.063	0.157
	RE + AL	-0.036	0.091	0.913	0.001	1.022	0.367	-0.037	0.046	0.955	0.013	1.340	0.271
Ring 2	RE	0.057	4.211	0.045	0.012	1.648	0.205	0.060	4.368	0.042	0.074	5.218	0.026
	RE + AL	0.039	2.065	0.137	-0.005	0.856	0.431	0.046	2.291	0.111	0.092	3.690	0.032
Ring 3	RE	**0.229**	**16.706**	**<0.001**	**0.134**	**9.208**	**0.004**	**0.169**	**11.759**	**0.001**	**0.183**	**12.877**	**0.001**
	RE + AL	**0.220**	**8.488**	**0.001**	0.123	4.705	0.013	**0.153**	**5.781**	**0.005**	**0.170**	**6.425**	**0.003**
Ring 4	RE	**0.124**	**8.523**	**0.005**	**0.121**	**8.280**	**0.006**	0.094	6.513	0.014	**0.173**	**12.116**	**0.001**
	RE + AL	0.112	4.342	0.018	0.117	4.498	0.016	0.088	3.568	0.035	**0.158**	**5.961**	**0.005**
Ring 5	RE	0.077	5.447	0.023	**0.144**	**9.945**	**0.003**	0.083	5.794	0.020	0.051	3.835	0.056
	RE + AL	0.096	3.812	0.029	**0.151**	**5.727**	**0.006**	0.100	3.943	0.026	0.032	1.884	0.162
Ring 6	RE	0.080	5.616	0.022	**0.150**	**10.333**	**0.002**	0.100	6.866	0.011	0.058	4.269	0.044
	RE + AL	0.090	3.633	0.034	**0.145**	**5.489**	**0.007**	0.100	3.953	0.025	0.040	2.112	0.132