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Sign-dependent changes in retinal electrical activity with positive and negative defocus in the human eye

Wing-cheung Ho, On-ying Wong, Yuen-chi Chan, Sze-wai Wong, Chea-su Kee, Henry Ho-lung Chan

Laboratory of Experimental Optometry (Neuroscience), School of Optometry, The Hong Kong Polytechnic University, Hong Kong SAR, China.

Corresponding Author

Dr. Henry HL Chan

Address: School of Optometry, The Hong Kong Polytechnic University.

Hung Hom, Kowloon, Hong Kong SAR, China

Telephone number: (852) 2766 7937

Fax number: (852) 2764 6051

E-mail address: henryhl.chan@polyu.edu.hk

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Abstract

The purpose of this study was to investigate the effect of optical defocus on changes of electrical response as a function of retinal region. Twenty-three subjects (aged 19-25y) with normal ocular health were recruited for global flash multifocal electroretinogram (mfERG) recordings under control (fully corrected) condition, and short-term positive defocus (+2 D and +4 D) and negative defocus (-2 D and -4 D) conditions. The amplitudes and implicit times of direct (DC) and induced (IC) components of mfERG responses were pooled into 6 concentric rings for analyses. The mfERG responses demonstrated more significant changes in amplitude in paracentral retinal regions than in the central regions under defocused conditions. The paracentral DC amplitude showed a significant reduction under negative defocus conditions. In contrast, the paracentral IC amplitude showed a significant increment under positive defocus conditions. Interestingly, the central IC response showed significant reduction in amplitude only to negative defocus, while increasing its amplitude to positive defocus. However, the DC and IC implicit times were virtually unaffected under defocused conditions. Our findings suggest that human retina is able to differentiate defocused signals and to identify positive and negative defocus. It shows that paracentral retina reacts more vigorously to optical defocus than does central retina.

Introduction

Most animal species including chicks (Wallman, Adams, & Trachtman, 1981), monkeys (Bradley, Fernandes, Lynn, Tigges, & Boothe, 1999; Smith, Hung, & Harwerth, 1994), tree shrews (Norton & McBrien, 1992), and guinea pigs (Zhou, Qu, Xie, Wang, Jiang, Zhao, Wen, & Lu, 2006) are born with hyperopia and undergo an emmetropization process, which is similar to that in humans (Edwards & Lam, 2004; Mayer, Hansen, Moore, Kim, & Fulton, 2001). Emmetropization matches the refractive power of the eye with its focal plane (retina) during postnatal development (Norton & Siegwart, 1995; Wallman & Winawer, 2004). Increasing evidence demonstrates that disruption of normal visual experience may lead to a myopic eye in a variety of animal species (Hodos & Kuenzel, 1984; Howlett & McFadden, 2006; Marsh-Tootle & Norton, 1989; Smith, Bradley, Fernandes, & Boothe, 1999; Wallman, Turkel, & Trachtman, 1978). Similarly, human infants born with congenital ptosis (Hoyt, Stone, Fromer, & Billson, 1981) or media opacities (Nathan, Kiely, Crewther, & Crewther, 1985) also develop myopia.

It is believed that eye growth is a visually-guided process, and that the eye can compensate for optically imposed positive defocus (focal point of the eye placed in front of the retina by using a positive lens) and negative defocus (focal point of the eye placed behind the retina by using a negative lens) (Howlett & McFadden, 2009; Hung,

Crawford, & Smith, 1995; Irving, Sivak, & Callender, 1992; Nathan et al., 1985; Norton & Siegwart, 1995; Smith & Hung, 1999). In the case of regionally imposed defocus, the posterior contour of the eyeball shows compensatory eye growth in the defocused region, indicating that eye growth is regulated by local visual signals (Diether & Schaeffel, 1997; Smith, Hung, Huang, Blasdel, Humbird, & Bockhorst, 2010). Despite optic nerve section (Choh, Lew, Nadel, & Wildsoet, 2006; Schmid & Wildsoet, 1996; Wildsoet, 2003) or ciliary nerve section (Schmid & Wildsoet, 1996), the eye compensates for induced defocus. These findings imply that the retina can detect the sign of defocus, despite the lack of signal processing from higher visual center(s).

The amacrine cell has been hypothesised to be one of the key retinal cells in detecting eye growth signals because of its sign-dependent changes in ZENK expression (Fischer, McGuire, Schaeffel, & Stell, 1999). It is still unknown whether other retinal cells are involved in detecting optical defocus. In addition, Liu and Wildsoet (2011) have recently found that imposing peripheral defocus in chicks (with clear central vision in regions of varying size) has profound effects on the refractive error development of the whole globe. Their study implies that different parts of the retina react differently to different optical defocused signals. However, the basis for the regional variations in discriminating the defocused signals is still unknown. The lack of appropriate tools to measure the regional retinal activity to defocus has been one of

the barriers in this research.

The flash (Ganzfeld) electroretinogram measures the summed electrical response of the whole retina but cannot provide topographical details of the response. The multifocal electroretinogram (mfERG) however can assess short-term electrical activity of multiple retinal loci in response to light stimuli (Sutter & Tran, 1992). The global flash mfERG, which incorporates a periodic global flash within conventional mfERG stimulation, enhances the activity from inner retinal neurons, for example, amacrine cells and retinal ganglion cells (Chu, Chan, Ng, Brown, Siu, Beale, Gilger, & Wong, 2008; Sutter, Shimada, Li, & Bearse, 1999). In addition, the global flash mfERG provides responses from outer retinal cells such as photoreceptors, ON and OFF-bipolar cells (Chu et al., 2008).

We were hopeful that we would obtain acute changes of retinal response to optical defocus in this study; Zhu and co-workers had chicks wear +10 D or -7 D or -8.6 D lenses for 10 minutes. There were increases or decreases in choroidal thickness in response to the positive or negative lenses respectively (Zhu, Park, Winawer, & Wallman, 2005). The aim of our study was to investigate the short-term effects of optical defocus on retinal activity in humans in different retinal regions using the global flash mfERG.

Methods

Subjects

Twenty-three young adults aged from 19 to 25 years (mean = 22.5 ± 1.3 years) were recruited. They received a comprehensive eye examination including cycloplegic subjective refraction and ocular health assessment. All had best corrected logMAR visual acuity of 0.00 (6/6) or better, astigmatism of 1.00 D or less, normal color vision and ocular health. Subjects with any ocular pathology, any known systemic disease, or history of epilepsy were excluded from this study. The refractive errors (spherical-equivalent) of the subjects ranged from +1.50 to -5.25 D (mean = -1.92 ± 0.42 D, median = -2.13 D) and astigmatism ranged from 0.00 to -1.00 D (mean = -0.47 ± 0.36 D, median = -0.50 D).

After detailed explanation of the study, all subjects gave informed consent. This study adhered to the tenets of the Declaration of Helsinki and was reviewed and approved by the Human Ethics Committee at The Hong Kong Polytechnic University.

Multifocal ERG stimulation

The stimulus array consisted of 103 non-scaled hexagons presented on a 22 inch color liquid crystal display (Model: 2232GW plus, SAMSUNG, Tianjin, China). The stimulus pattern subtended 29° horizontally and 24° vertically at a working distance of 67 cm. The Visual Evoked Response Imaging System (VERIS 6.0.6d19; Electro-Diagnostic Imaging Inc., San Mateo, CA, USA) was used to present the global flash

mfERG stimulation. The stimulation sequence consisted of a multifocal flash frame, a dark frame, a full screen global flash and a dark frame in each cycle (Shimada, Li, Bearse, Sutter, & Fung, 2001) (Figure 1). For the multifocal flash frame, each hexagonal stimulus was temporally modulated between bright and dark, according to a pseudo-random binary m-sequence stimulation. The frame rate was 75 Hz (see Figure 1).

Multifocal ERG recording

One eye from each subject was chosen at random for mfERG recording. Two drops of 1% Tropicamide (Alcon Laboratories Inc., Fort Worth, TX, USA) were instilled with a 5-minute interval before measurements commenced. A Dawson-Trick-Litzkow (DTL) electrode was placed behind the lower eyelid to contact with the cornea as the active electrode. Gold-cup reference and ground electrodes were placed 10 mm lateral to the outer canthus of the tested eye and at the central forehead, respectively. The fellow eye was occluded during recording.

The mfERG signal was filtered between 10 and 300 Hz and was amplified 100,000 times (Model: 15A54, Physiodata Amplifier system, Grass Technologies, Astro-Med, Inc., West Warwick, RI, U.S.A.). The total recording time for each condition was 7 minutes and 17 seconds with the 2^{12} binary m-sequence used; the record was divided

into 32 slightly overlapping segments. The signal was monitored by the examiner using the real-time response provided by the VERIS system and any segments contaminated with blinks or other artifacts were re-recorded immediately. The room luminance was about 240 lux.

The mfERG was measured after the pupil was dilated to at least 7 mm in diameter and the cycloplegic effect was steady (see below). Spherical ophthalmic lenses of 35 mm diameter were used to correct refractive errors as well as to impose different amounts of optical defocus including control (fully corrected), positive defocus (+2 D and +4 D) and negative defocus (-2 D and -4 D) conditions. The order of defocused conditions was randomized. The mfERG examination started immediately after the corrective lens was placed in front of the subjects. Most subjects took about 10 minutes to complete each set of mfERG recordings for a particular defocused condition.

Evaluation of cycloplegic effect

The cycloplegic effect was tested 20 minutes after the instillation of the eye drops and was also assessed before and after the mfERG examination under each defocused condition. This was done to ensure that the cycloplegic effect was constant throughout the experiment. The same examiner, who was masked to the defocused condition to be used, measured the residual accommodation of the tested eye of all subjects using the

push-up method. Subjects were corrected according to the subjective refraction with the near addition power of +2 D which resulted from this test. The end point used was the subject's report of blur in the line of letters at their best visual acuity when the target was slowly moved from a working distance of 50 cm toward them. The residual accommodation was the amplitude of accommodation measured minus 2 D (i.e. the near addition power given). Five readings were obtained to give an average result. The mfERG examination began if the difference in residual accommodation for 3 consecutive measurements measured at 5-minute interval was equal to or less than 0.25D. To impose a certain magnitude of negative defocus, the residual accommodation was compensated for to ensure constant levels of retinal defocus (e.g. if a subject was found to have 1D of residual accommodation, -3D was used to achieve -2D of defocus). Most subjects were found to have 1 to 2 D of residual accommodation. The data set was omitted if the difference in residual accommodation measured before and after mfERG examination was greater than 0.25 D.

Data Analysis

The mfERG responses were pooled into 6 concentric rings for analysis (Figure 2a). The amplitudes and implicit times of the direct (DC) and induced (IC) components in the first order kernel were analyzed (Figure 2b). The DC amplitude was measured from

the first negative trough to the first positive peak while the IC amplitude was measured from the second positive peak to the second negative trough. The implicit time of the DC was measured from the presentation of the multifocal flash while that of IC was measured from the presentation of the global flash (i.e. at 26.6 ms).

Statistical analysis and presentation

Statistical analysis of the data was carried out using the Statistical Packages for the Social Sciences (SPSS 15.0, SPSS Inc., Chicago, IL, USA). Repeated measures analysis of variance (ANOVA) was applied to investigate the effect of defocus on mfERG responses. Post-hoc tests with Bonferroni adjustment were done to correct the level of significance due to multiple comparisons of different retinal regions. The level of significance was set at 0.05.

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Results

Figure 3 shows the typical global flash mfERG waveform measured under control, +4 D defocus, and -4 D defocus conditions from one subject. Under optically corrected conditions, the waveform consisted of two distinct peaks in all six regions, with the first and second peaks corresponding to the DC and IC responses, respectively. The DC

amplitude was markedly reduced under negative defocus conditions but only mildly reduced under positive defocus conditions. In contrast, the IC amplitude was minimally changed under negative defocus conditions but moderately increased under positive defocus conditions. The two distinct peaks are still present under +2 D and -2 D defocus, and the amplitudes of DC and IC responses show similar changes for the same sign of defocus (data not shown).

Figure 4 illustrates the average DC and IC amplitudes (mean ± SEM) under different defocused conditions. Optical defocus had a significant effect on the DC amplitude ($F=15.62$, $p<0.001$); DC amplitude was significantly reduced under negative defocus only. Compared to the in-focus condition, the DC amplitude reduced significantly under -2 D defocus from rings 3 to 6 (all $p<0.003$) and under -4D defocus from rings 1 to 6 (all $p<0.007$). The highest reduction in response was at ring 4 under -2D defocus and ring 5 under -4D defocus, respectively, demonstrating about 28% and 26% decrement in amplitude.. However, the DC amplitude did not show any statistically significant change for either +2 D or +4 D of defocus for any of the six regions (all $p>0.05$).

On the other hand, optical defocus also had a significant effect on the IC amplitude ($F=11.35$, $p<0.001$). IC amplitude was markedly increased under positive defocus, but

only at rings 4 and 5 under +2 D defocus, and at rings 3 to 5 under + 4 D defocus were there statistically significant increment (all $p<0.001$). The highest increment in response was shown at ring 4, demonstrating 34% and 39% increment of amplitude, respectively, under +2 D and +4 D defocus. In contrast, IC amplitude was generally reduced under negative defocus. The reductions in amplitude were significant under -4 D defocus at rings 1 ($p=0.002$) and 3 ($p=0.006$), and under -2 D defocus at rings 1 ($p=0.007$) and 2 ($p=0.002$). Furthermore, IC amplitude showed no significant change for ring 6 under any of the defocused conditions.

Figure 5 shows the average DC and IC implicit times at various retinal regions for the five different optical defocused conditions. Optical defocus did not have significant effect on the DC implicit time ($F=1.61$, $p=0.18$). However, the implicit time at ring 2 demonstrated progressively shortened implicit time from in-focus to higher magnitude of positive defocus, though this was not statistically significant. In contrast, optical defocus had significant impact on the IC implicit time ($F=5.97$, $p=0.002$). The IC response from rings 2 to 6 also showed a systematic change in implicit time from negative to positive defocus, depending on the eccentricity. Specifically, compared to the in-focus condition, the IC implicit times at rings 2 and 3 showed gradually shortened implicit time with increasing magnitudes of positive defocus, in which only the change

of implicit time under +4 D defocus at rings 2 ($p=0.002$) and 3 ($p<0.001$) was statistically significant. The implicit time for these regions under negative defocus was almost the same as for the in-focus condition (all $p>0.05$). In contrast, the IC implicit time for rings 4 to 6 tended to lengthen under negative defocus and shorten under positive defocus. For both direction of defocus, the change in implicit time was even higher with increasing magnitudes of defocus in these regions (although not reaching a statistically significant level). The IC response for ring 1 did not show an obvious pattern-specific change in implicit time.

Discussion

Our key finding is that different signs of defocus can affect different components of the global flash mfERG response (Figure 4). The DC amplitude was significantly reduced under negative defocus, especially in the paracentral region (rings 4 and 5, eccentricity from 6.5 to 11.7°; but the amplitude was not significantly altered by positive defocus (Figure 4). In contrast, the IC response showed a remarkable increment in amplitude under positive defocus, especially in the paracentral regions (ring 4, eccentricity from 6.5 to 9.1°), but it changed only minimally, though significantly in certain regions, under conditions of negative defocus (Figure 4). The DC response represents the average response to a focal flash (of the multifocal flash stimulus) (Chu, Chan, & Brown, 2006; Chu et al., 2008; Sutter et al., 1999). It predominantly reflects the activity from outer retinal cells such as ON- and OFF- bipolar cells, with relatively small contribution from photoreceptors and other inner retinal cells (Chu et al., 2008). The IC response, which represents the adaptive responses to the global flash in the current m-sequence stimulation (Sutter et al., 1999), primarily reflects activity from inner retina including amacrine cells and retinal ganglion cells (Chu et al., 2008). Reduced IC response has also been found in retinal diseases affecting the inner retina such as glaucoma (Chu et al., 2006; Fortune, Bearse, Cioffi, & Johnson, 2002). Our results have shown that negative and positive defocus predominantly affect DC and IC

responses respectively, suggesting that the sign of defocus is probably decoded differentially in inner and outer retina.

In addition to the sign-preference of the mfERG response amplitude, the time domain of the mfERG response also demonstrated similar sign-dependence to the defocused signals but the change was less obvious than that in the amplitude domain. The DC implicit time was progressively shortened for ring 2, from negative defocus to positive defocus, but this was not found in other regions (Figure 5). In addition, there was systematic change in IC implicit time from negative defocus to positive defocus, even though the trend was different at various eccentricities (Figure 5). Specifically, the IC implicit time was almost unchanged for rings 2 and 3 under negative defocus but was progressively shortened under positive defocus in these regions, especially for +4 D defocus. However, the IC implicit time demonstrated different trends under different defocused conditions in the paracentral retina (i.e. rings 4 and 5). It tended to be lengthened and shortened, respectively, under negative and positive defocus. The sign-dependent change in the IC implicit time generally matches with the significant change of both DC and IC amplitudes in the paracentral region (i.e. rings 4 and 5). The underlying alteration in biochemical activity within the retina is still unknown. However, peripheral defocus is well known to affect the refractive error development of the eye (Mutti, Hayes, Mitchell, Jones, Moeschberger, Cotter, Kleinstein, Manny,

Twelker, & Zadnik, 2007; Smith, Hung, & Huang, 2009). According to our study, optical defocus also affects the time domain of the mfERG response. Additionally, changes in the time component of the mfERG response have been shown in progressing myopes by Chen and co-workers, although a different stimulation protocol was used (Chen, Brown, & Schmid, 2006). The underlying changes in the IC implicit time at paracentral region under optical defocus may represent retinal signals involved in eye growth. Further investigation is needed to explore the signal cascade between defocus and eye growth to clarify this issue.

In the chick eye, the blockage of ON and OFF pathways has been shown to inhibit compensatory response towards induced negative and positive defocus respectively (Crewther & Crewther, 2003), indicating that the detection of defocused signals is probably initiated at the retinal level and involves two different pathways. Moreover, in chicks, the level of ZENK expression in glucagon-containing amacrine cells (Fischer et al., 1999) and retinoic acid synthesis (Mertz, Howlett, McFadden, & Wallman, 1999) are dependent on the sign of defocused signals. In monkeys, on the other hand, the activities of ON-bipolar cells and GABAergic amacrine cells have been shown to be focus-sensitive. These cells have been shown to be more reactive for in-focus stimuli and those with positive defocus, compared to those with negative defocus, by using immunocytochemical markers (Zhong, Ge, Smith, & Stell, 2004). This indicates that

bipolar cells as well as amacrine cells are involved in detecting defocused signals. Taken together, this evidence suggests that retinal activity changes differently when the retina is presented with defocused signals of opposite signs. This evidence is consistent with the global flash mfERG responses we have seen over the range of defocus used in this study.

Paracentral vision may have a profound effect on the growth process of the whole eyeball. The foveal region is vital for spatial vision because of its high resolution. However, imposing peripheral negative defocus, leaving clear central vision, could cause myopia accompanied with axial elongation, in both chicks (Liu & Wildsoet, 2011) and monkeys (Smith et al., 2009). However, peripheral defocus has no effect on axial refraction development if the peripheral defocus is in the far retinal periphery (beyond 50°, at least for the chick's eye) (Liu & Wildsoet, 2011; Schippert & Schaeffel, 2006). In addition, Liu and Wildsoet (2011) recently used two-zone concentric bifocal lenses and found that peripheral defocus is more important than central defocus in refractive error development in the chick. It seems clear that the retinal region sensitive to defocus is not limited to the central visual area. In humans, a longitudinal study of refractive error development showed that children with hyperopic refraction in the paracentral retina went on to develop axial myopia (Mutti et al., 2007). Thus, the paracentral retina appears to have a profound influence on the refractive error development of the whole

globe, probably because the paracentral retina reacts more vigorously to optical defocus than does the central retina.

Our study focused on the central retina to about 15° eccentricity but does not include the region beyond 15°. It should be noted that previous studies have shown the magnitudes of peripheral refraction (in spherical-equivalent) and astigmatism were insignificant up to eccentricity of 20° (Millodot, 1981). While we used single-power spherical ophthalmic lenses to induce optical defocus in this study, the effect of peripheral refraction should be considered in the investigation of the retinal response to optical defocus in the peripheral retina (i.e. beyond the central 30°).

A recent study has reported that the correction of low to moderate myopes with single vision spherical lenses would induce negative defocus in the peripheral retina (Lin, Martinez, Chen, Li, Sankaridurg, Holden, & Ge, 2010). In view of the fact that the paracentral retina is more reactive to optical defocus and the eyeball actively grows towards the focal plane of the eye in the paracentral or peripheral regions, one of the key factors in myopia control should be related to the effectiveness of controlling the peripheral refraction. Modifying current myopia control lenses to suit individual needs is likely to be a future direction for effective control of myopia progression.

Conclusions

In conclusion, paracentral retina showed greater change than central retina in DC

and IC amplitudes under defocused conditions. Moreover, different components of the global flash mfERG response are differentially affected by negative and positive defocus. These results suggest that paracentral retina gives reduced DC responses to negative defocus and increased IC responses to positive defocus. This study provides evidence that human retina not only identifies optical defocus, but also differentiates the sign of optical defocus.

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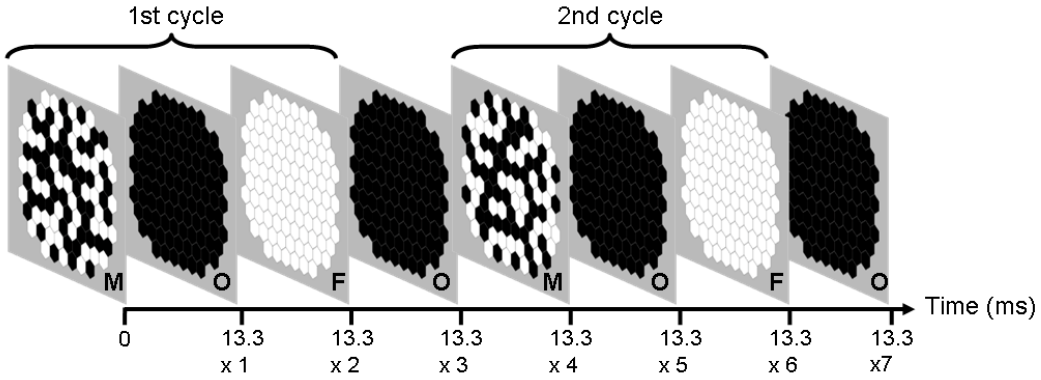

Figure 1. The stimulus array of the global flash mfERG consisted of 103 non-scaled hexagons and started with a multifocal flash frame (M), followed by a dark frame (O), a full screen global flash frame (F) and a dark frame (O) in each cycle. The video frame rate was 75 Hz and each frame interval was 13.3 ms. For the multifocal flash frame, the luminance of the bright and dark stimuli were 180 cd/m^2 and 1 cd/m^2 respectively. The luminance of the global flash frame was 180 cd/m^2 and that of the background was 90 cd/m^2.

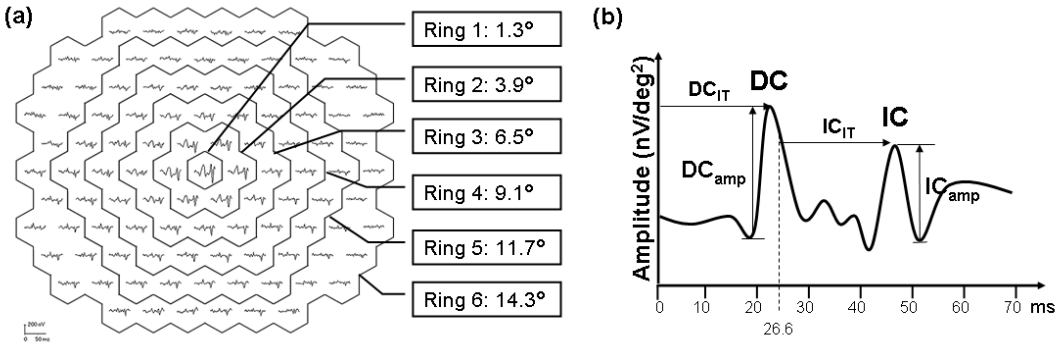

Figure 2. (a) The 103 local responses were grouped into 6 regions. The eccentricity boundary of each ring was indicated in the figure. (b) A schematic diagram showing the typical first order kernel global flash mfERG waveform. The waveform consisted of

direct (DC) and induced (IC) components. See text in details for the definitions of DC amplitude (DC_{amp}), IC amplitude (IC_{amp}), DC implicit time (DC_{IT}) and IC implicit time (IC_{IT}).

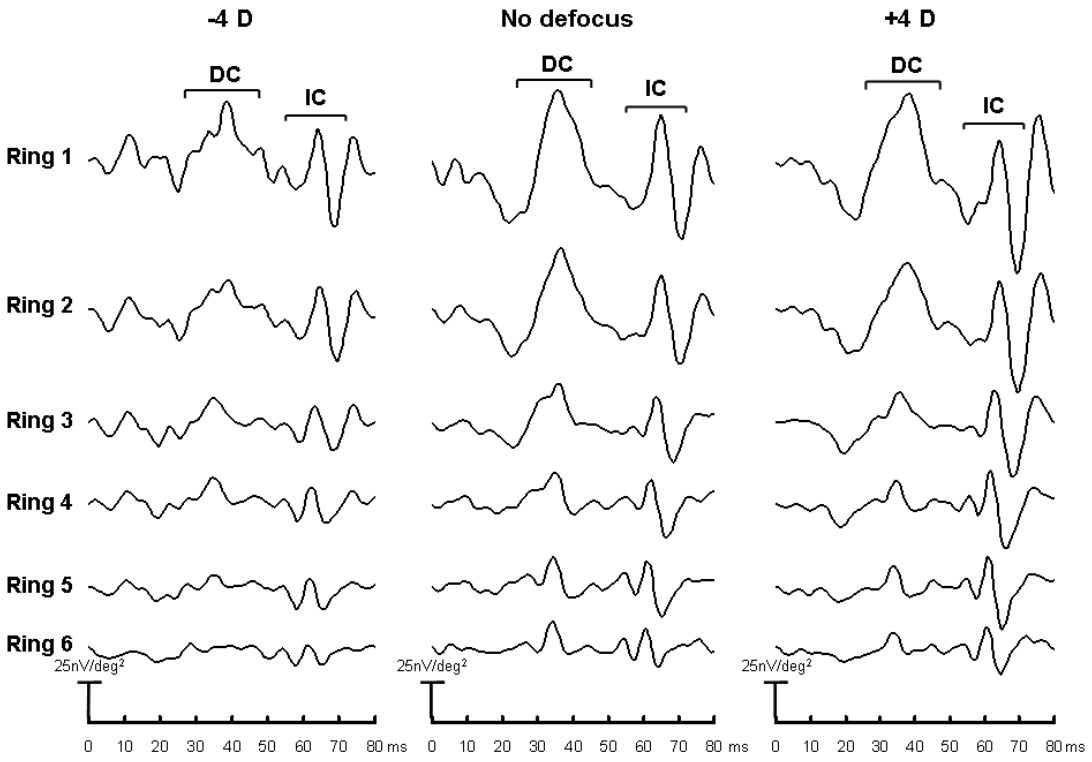

Figure 3. The typical global flash mfERG waveform measured from one subject for control (fully corrected; centre), -4 D defocus (left) and +4 D defocus (right) for six different retinal regions.

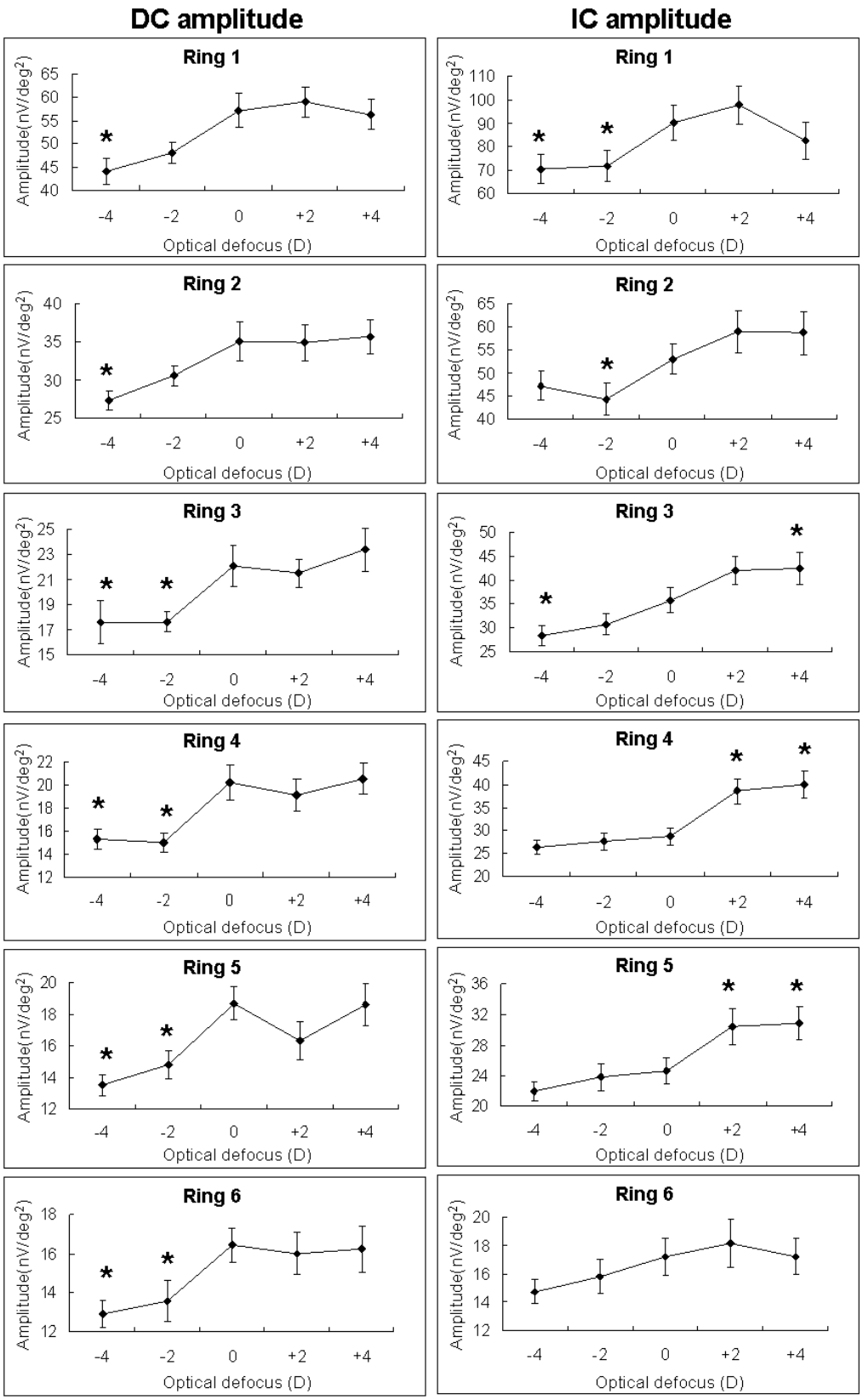

Figure 4. Average DC and IC amplitudes under various defocus conditions for different regions. Those marked with an asterisk "*" are statistically different from the in-focus

(0) condition. The error bars indicate the standard error of the mean (SEM).

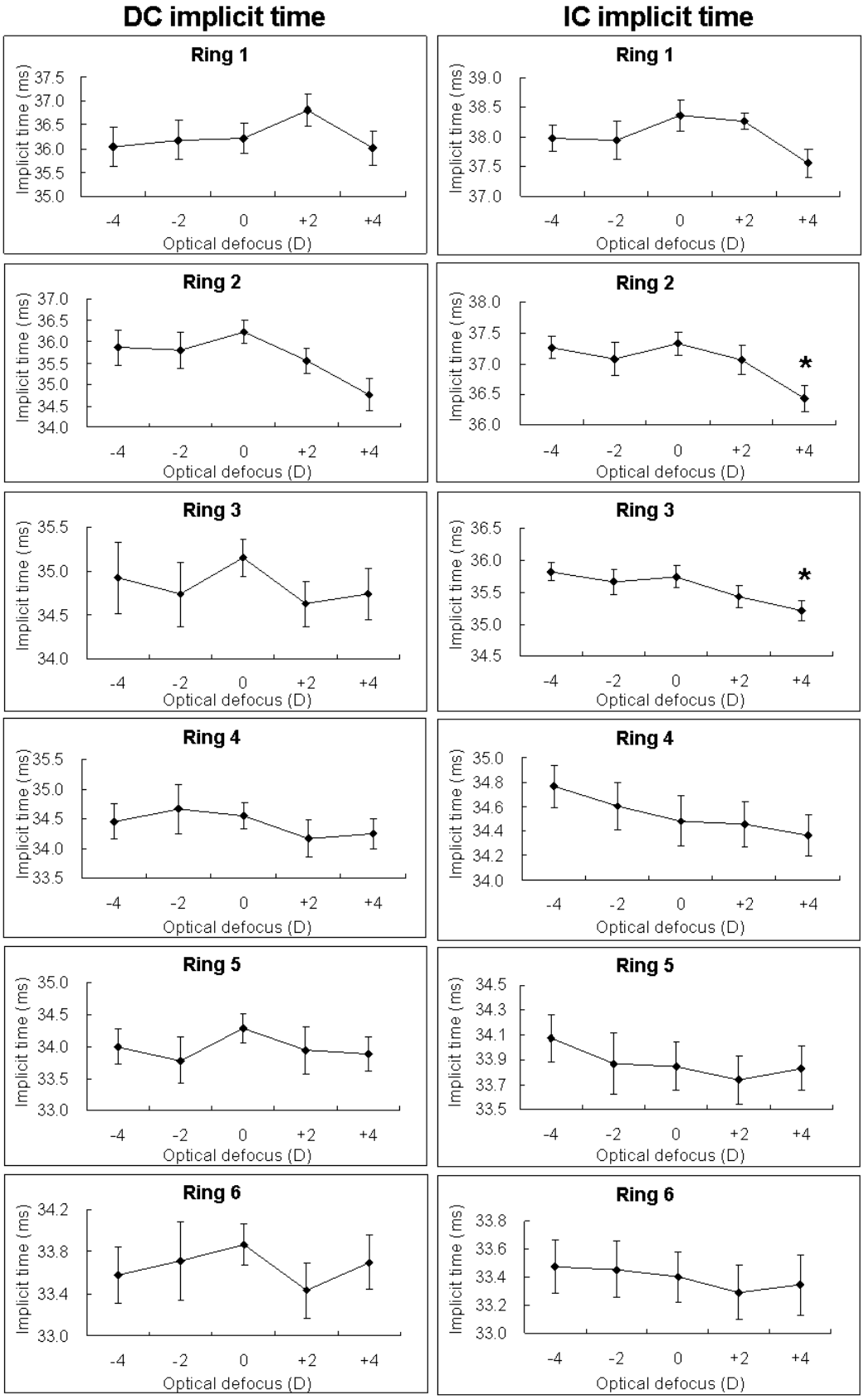

Figure 5. Average DC and IC implicit times under various defocus conditions for different regions. Those marked with an asterisk "*" are statistically different from the in-focus (0) condition. The error bars indicate the standard error of the mean (SEM).