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Assessment of neuroretinal function in a group of functional amblyopes with documented LGN deficits

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Abstract

Purpose: In this study we examine neuroretinal function in five amblyopes, who had been shown in previous functional MRI (fMRI) studies to have compromised function of the lateral geniculate nucleus (LGN), to determine if the fMRI deficit in amblyopia may have its origin at the retinal level.
Methods: We used slow flash multifocal ERG (mfERG) and compared averaged five ring responses of the amblyopic and fellow eyes across a 35 deg field. Central responses were also assessed over a field which was about 6.3 deg in diameter. We measured central retinal thickness using optical coherence tomography. Central fields were measured using the MP1-Microperimeter which also assesses ocular fixation during perimetry. MfERG data were compared with fMRI results from a previous study.
Results: Amblyopic eyes had reduced response density amplitudes (first major negative to first positive (N1-P1) responses) for the central and paracentral retina (up to 18 deg diameter) but not for the mid-periphery (from 18 to 35 deg). Retinal thickness was within normal limits for all eyes, and not different between amblyopic and fellow eyes. Fixation was maintained within the central 4° more than 80% of the time by four of the five participants; fixation assessed using bivariate contour ellipse areas (BCEA) gave rankings similar to those of the MP-1 system. There was no significant relationship between BCEA and mfERG response for either amblyopic or fellow eye. There was no significant relationship between the central mfERG eye response difference and the selective blood oxygen level dependent (BOLD) LGN eye response difference previously seen in these participants.
Conclusions: Retinal responses in amblyopes can be reduced within the central field without an obvious anatomical basis. Additionally, this retinal deficit may not be the reason why the LGN BOLD (blood oxygen level dependent) responses are reduced for amblyopic eye stimulation.

Introduction

Amblyopia is a developmental disorder of the visual system resulting in reduced visual acuity and reduced contrast sensitivity in one eye, as well as a range of accompanying conditions including fixation and eye movement disorders,[1] disorders of spatial coding,[2] contrast sensitivity,[3,4] position coding[5–7] and global sensitivity.[8–11] It is accompanied by disruption of binocular vision and stereopsis.

There is an extensive literature in primate and non-primate models that suggests that the major deficit in experimental amblyopia, whether surgically induced, optically induced or induced by deprivation, is at the cortical level. Much available evidence supports the notion that the

retina[12–16] and LGN[17–19] are not affected. The primary site of the deficit in amblyopia was thought to be cortical,[20,21] however the LGN has long been known to exhibit structural deficits specific to the deprived eye input in animals[22–24] and a number of studies have also questioned the structural integrity of the LGN in humans with amblyopia.[25–27]

In two related studies, Hess and colleagues have provided fMRI evidence of an LGN deficit to broadband stimuli (achromatic/chromatic; spatial/temporal)[28] as well as a selective deficit to red/green chromatic stimulation, implicating the parvocellular layers that receive input from the amblyopic eye.[29] These findings in humans receive support from some previous animal studies.[18,30–33]

In these fMRI studies by Hess and colleagues the broadband stimulus contained luminance, contrast and colour modulation and a broad spatial frequency spectrum, while the narrowband stimulation was a spatio-temporal sinusoidal stimulus containing just chromatic red/green, blue/yellow or achromatic contrast modulation. Since the fMRI responses reflect synaptic and inter-cellular activity within the LGN, any differences between the responses to fellow versus amblyopic eye stimulation could merely be a consequence of reduced input from the amblyopic eye due to a primary anomaly at the retinal level. To address this issue we assessed the structural and functional integrity of the inner retina of the amblyopic eye in the same participants who have been shown to have anomalous geniculate function. Retinal structure was assessed using optical coherence tomography (OCT). The functional assessment was obtained using microperimetry and the multifocal electroretinogram (mfERG).[34] In particular, we used a slowed stimulation version of this technique which is known to bias responses to cells in the inner retina.[35–37]

In this paper we examine OCT and mfERG data (response density amplitudes) to determine if there is a structural retinal deficit and additionally we compare mfERG data with previously obtained fMRI data[28,29] (average%BOLD response) to assess the possible functional retinal contribution to the reduced fMRI responses.

Methods

Participants

We tested five of the seven amblyopes who had participated in the previous fMRI studies conducted by Hess *et al.*[28,29] Three had strabismic amblyopia, one had anisometropic amblyopia, and one was amblyopic because of a combination of visual deprivation and strabismus. All were adults (aged 35–67 years) and had amblyopia of many years standing. The clinical characteristics of these participants are shown in *Table 1*.

The multifocal electroretinogram

The multifocal electroretinogram (mfERG) (VERIS 5.1, www.veris-edi.com) was recorded monocularly for each eye using DTL-electrodes with the unrecorded eye occluded. The reference electrode was at the outer canthus of the recorded eye and the ground electrode at the central forehead. Participants were corrected for the test distance using the EDI eye monitor/refraction unit. Pupils were dilated (Tropicamide 0.5%, www.alcon.com) and recordings were made under ambient room light conditions. The visual stimulus consisted of 103 scaled hexagons displayed on a high luminance 7 inch monitor and subtended approximately 35 deg × 30 deg in extent. A two degree fixation cross was provided at the centre of the display. Fixation was also aided by the stable outline of the whole mfERG target, and the fact that the participants were asked to fixate the centre of this array (*Figure 1*, left panel). They were further assisted in their fixation by the edges of the CRT screen. Fixation had only to be held steady for about 25 s at a time because of the nature of the mfERG task (see below).

Retinal signals were band pass filtered (10–300 Hz), sampled every 0.83 ms and amplified (50,000×, Grass P5 amplifier (www.grass-telefactor.com)). A camera allowed viewing of the eye under test during signal acquisition, and the ERG signal was monitored for fixation artifacts, which could have contaminated the data. Segments that were

Table 1. Subject characteristics (Modified from Table 1[28])

Subject/Type	Age	Eye	Refraction (D)	Acuity	Alignment	History
DL/deprivation	37	R	+8.25/−1.00 × 90		ET	Surgery for RET aet 9y (×2)
		L	+0.25	6/6		
CF/strabismic	43	R	−2.75	6/6		Surgery for LET in infancy and at aet 25y
		L	−3.00	6/240	LXT,LhypoT	
SH/anisometropic	35	R	+7.00/−3.00 × 150	6/30	Ortho	First Rx aet 19y
		L	+2.50/−1.25 × 80	6/4.5		
BB/strabismic	67	R	+0.50/−0.50 × 160	6/5		Surgery aet 7y, for large angle LET
		L	+1.00/−0.25 × 180	6/600	LET	
JL/strabismic	51	R	+0.75	6/5		Patching, aet 2 y Surgery aet 5y
		L	+0.75	6/48	LET	

CF, counts fingers; RET, right esotropia; LET, left esotropia; LXT, left exotropia; LhypoT, left hypotropia.

Figure 1. Sequence of four frames in the slow flash mfERG paradigm. Frames are presented at 75 Hz (13.3 ms intervals).

contaminated with blinks, small eye movements or artefacts were rejected and re-recorded.

All participants were tested with the slow flash mfERG; hexagons (ranging in size from about 3.2 to 5 deg in horizontal extent) flickered according to a pseudorandom binary m-sequence, and three blank frames were inserted between steps of the pseudorandom sequence (*Figure 1*). Each step in the binary m-sequence (2^{13}-1 steps in length) was four frames long. In the first frame, each hexagonal patch had a 50% probability of being white (200 cd m^{-2}) or black (3 cd m^{-2}) and the next three frames remained dark grey (mean luminance 26 cd m^{-2}). The slow flash response is generated predominantly by ON and OFF bipolar cells, but reflects less temporal nonlinear processing than the conventional fast flicker mfERG due to slowing of the stimulation sequence.[37]

Recordings were divided into 16 segments which overlapped slightly in time and the stimulus sequence in the overlapped section was repeated; the repeated sections of the record were excised by the VERIS software so that one continuous record was available for analysis. Participants were given breaks of 5–10 s between segments, resulting in a total recording time of about 7 min per eye. Whether amblyopic or fellow eye was tested first was chosen randomly. Participants used the inbuilt refraction unit of the VERIS system to perform their own refraction and adjust the focus of the target hexagons when presented as a static display (*Figure 1*, left panel). This task is easily accomplished, even by severe amblyopes, as the distinction between sharp and blurred high contrast images does not form part of the deficit in amblyopia. The inbuilt refractor has no scale for assessing refraction (see Discussion for further consideration of refraction and amblyopia).

Optical coherence tomography

Retinal thickness was assessed using the Cirrus Optical Coherence Tomography system (www.meditec.zeiss.com). The Macular cube 512 × 128-scan pattern was chosen. The caliper was moved across the retina to ensure that it was centred on the foveal pit when fixation was not central in the amblyopic eye.

Microperimetry and fixation assessment

Microperimetry and fixation assessment were performed using the MP-1 Microperimeter (www.nidek-intl.com/products/diagnosis/mp-1.html). In addition to a threshold assessment of sensitivity for 6 min of arc targets in the central retina, this system gives an assessment of fixation stability. The system gathers real-time fundus images at 25 Hz.

Retinal autotracking was performed by selecting a region of interest (for example a particular vessel or vessel junction), and the stimuli are referenced to this point in the image. Movements of this point are monitored with respect to the internal reference frame of the instrument to provide an index of fixation.

To assess central field sensitivity, we used the Humphrey 10-2 program of the MP-1 system with a grid of 68 stimuli and a Goldmann V stimulus size to cover the central 20 degrees (diameter) of the field. Thresholds were estimated with a 4-2 double staircase strategy.[38] The white background was 1.27 cd.m^{-2} (four asb) and the stimulus luminance ranged from its highest level (0 dB attenuation, 127 cd.m^{-2}) to lowest level (20 dB attenuation, 2.54 cd.m^{-2}). Stimulus presentation duration was 200 ms.

All participants in the study gave written informed consent; the tenets of the Declaration of Helsinki and the requirements of the University Human Research Ethics Committee of the Queensland University of Technology were followed. Participants were advised of their right to withdraw from any procedures at any time without prejudice.

Data analysis

Retinal thickness values were assessed according to the nine subfields defined by the Early Treatment Diabetic Retinopathy Study.[39] These are a central region 1 mm in diameter, and concentric circles, 3 mm and 6 mm in diameter. The inner and outer concentric circles contain four regions each, thus making nine subfields in all.

The Nidek Microperimeter gives a continuous assessment of eye position during the test, and provides statistics on the two-dimensional distribution of eye position. This is translated to 'time within a specific region', and these data

are presented; the device can also output a file of fixation positions at the time of detection of perimetry targets. These have been converted to 'bivariate contour ellipse areas'[40] (BCEA) describing fixation for four of five participants.

The mfERG data were averaged into five concentric rings and trough to peak N1-P1 response densities and P1 implicit times were measured. Data were compared by two way ANOVA with eye (fellow/amblyopic) and ring (eccentricity) as factors.

Pearson correlation coefficients were calculated between the BOLD fMRI responses and the mfERG responses for the same participants. All comparisons have been made in terms of right/left eye differences. We computed average BOLD activation (%BOLD change in the averaged haemodynamic response function) for each eye and mfERG response (response density amplitudes) averaged across the stimulus field for each eye. For the narrowband stimulus, we used the BOLD average responses to luminance, red/green, and blue/yellow stimuli for each eye and compared this to the central/peripheral mfERG difference (a measure of the foveal specificity of the deficit). This was derived by differencing the central and peripheral mfERG responses (i.e. central mfERG-peripheral mfERG for the 9.5° radius) and these measures were compared between eyes. Since the BOLD average response reflects blood flow changes as a result of increased metabolic activity it is biased towards field potential changes resulting from the summed intracellular potential changes within the LGN, which include synaptic activity; any differences between the responses to fixing versus amblyopic eye stimulation could be a consequence of reduced input from the amblyopic eye due to a primary anomaly at the retinal level. In such a case one would expect the reduced responsivity of LGN, as measured with fMRI to correlate with reduced responsivity of the retina, as measured with mfERGs across our participants.

Results

OCT findings

There were no consistent differences in central retinal thickness measures between participants; the average difference in central retinal thickness was 9μ, with the amblyopic eye having greater thickness but this was made up of values ranging from +14 to −30μ.

There was no significant difference in retinal thickness in the subfields between the two eyes of any of the participants (one-way ANOVA; $p > 0.05$). The near circumfoveal subregion (fields 2–5) was, on average, thicker than the more peripheral (fields 6–9) by about 40 μm for both amblyopic and fellow eyes (one-way ANOVA $p < 0.0001$) (*Figure 2*).

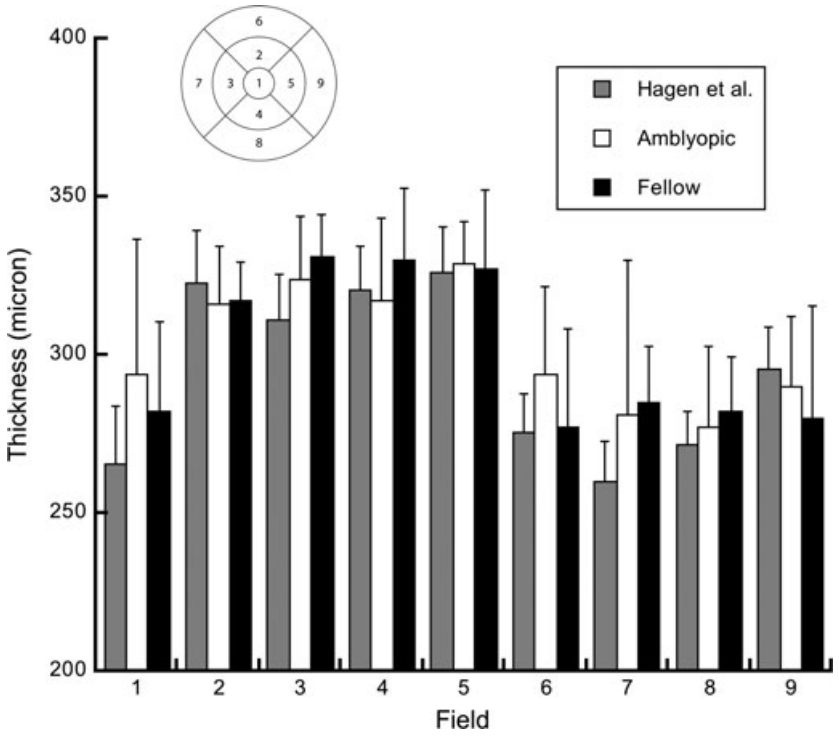

Figure 2. Retinal thickness for OCT measures for amblyopic and fellow eyes. They are compared with those of Hagen *et al.*[41] for the nine central fields as defined by the Early Treatment Diabetic Retinopathy Study Research Group39 (see inset at top left). Error bars show one S.D.

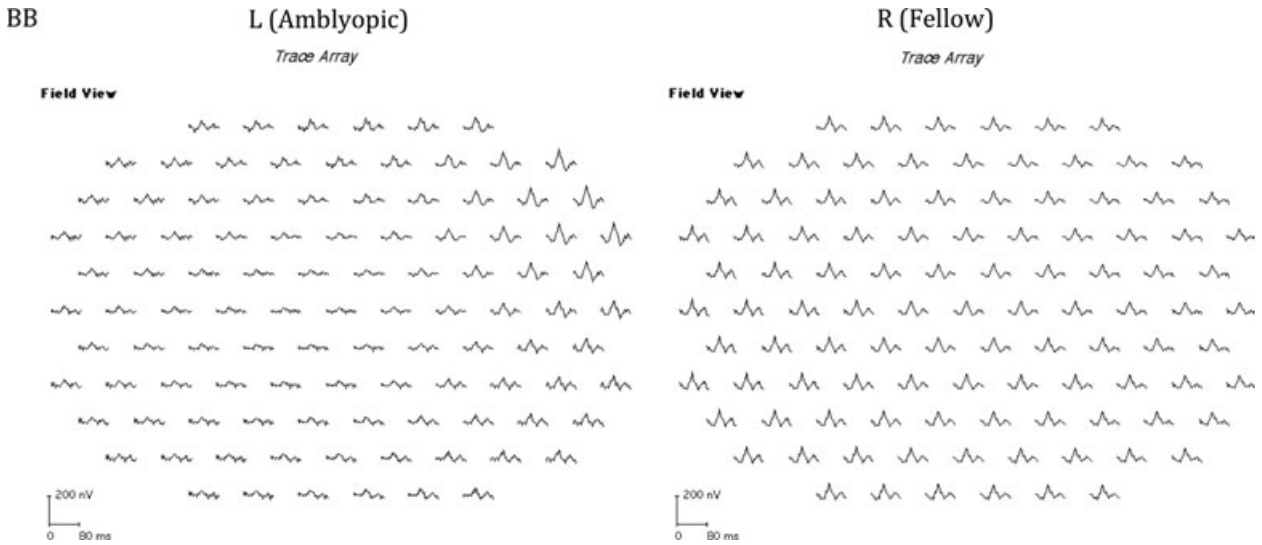

Figure 3. Individual hexagon responses for all 103 hexagons for R (fellow) and L (amblyopic) eye of subject BB.

The variation seen in *Figure 2* is within the variability of the Cirrus OCT system.[41] Central thicknesses (Field 1 in *Figure 2*) are higher than averages reported by Hagen *et al.*[41] but within their 95% confidence limits.

mfERG results

Central neuroretinal responses were depressed for the amblyopic eye of participant BB as shown in *Figure 3*; peripheral neuroretinal responses were comparatively large in the temporal periphery. Similar differences in central response and asymmetries in response were seen in the remaining participants. Amblyopic eyes in general showed reduced N1-P1 response densities in the central retina, although average peripheral responses were similar in amplitude to those of fellow eyes.

Concentric ring averages are illustrated for one participant in *Figure 4*. The ring averages (schematic shown centrally), for the amblyopic and fellow eye of participant BB are shown on the left, and the right, respectively. The N1-P1 response densities decrease with increasing eccentricity.

All of the participants showed reduced central mfERG N1-P1 response densities in the amblyopic eye, compared to the fellow eye; there were, on average, significant reductions in response densities for rings 1, 2 and 3 (one way ANOVA: $p < 0.001$; $p < 0.001$; $p = 0.06$) (*Figure 5*).

Response densities decreased with eccentricity but there was variability in the response profile and an interaction between eye and eccentricity (*Figure 5*) (two way ANOVA; $p < 0.001$), indicating differential effects of eccentricity on responses for the amblyopic and fellow eyes. Fellow eyes had greater central N1-P1 responses than amblyopic eyes, but in the periphery the averaged responses of the eyes were essentially the same. This can most clearly be seen in the responses of participants DL, CF and BB in *Figure 5*.

The implicit time data showed a similar pattern to that of the response density data, with differences at the fovea and no differences in the periphery, but the individual data were considerably more variable (see *Figure 6*). Central IT values (ring 1) are 1–2 ms longer than peripheral IT values (ring 2–4) on average (see upper right plot). Two-way ANOVA showed significant differences with eccentricity (rings) ($p < 0.05$), but no differences between eyes (i.e. no effect of amblyopic/fellow eye), and no interaction of eye with eccentricity.

Microperimetry

The mean sensitivity values for the Humphrey 10-2 visual field program were all within the normal range[42, 43] in four participants who could perform the task. These average values were 19.6 $\pm$ 0.4 dB and 19.4 $\pm$ 0.7 dB in fellow and amblyopic eyes, respectively; participant DL was unable to complete microperimetry due to her poor visual acuity.

Fixation was within 4 deg at least 67% of the time and largely central (except for participant JL); it was within 2 deg between 13% and 56% of the time (*Figure 7*, *Table 2*). The fixation patterns of the fellow eyes of the four participants who completed this test are shown in the lower panels of *Figure 7*, for direct comparison with the patterns of the amblyopic eyes. The only participant to show a striking difference between eyes is JL (see below). Fellow eyes show better fixation, with three participants having 80% of fixation within 2 deg and all four having 99%–100% within 4 deg (*Table 2*).

Table 2. Fixation data from Nidek P-1 Microperimeter; BCEA (Bivariate contour ellipse area) is calculated from fixation data output from the Microperimeter. (JL has multiple fixation loci.)

Participant	Amblyopic Within 2/4 deg (percent)	Fellow Within 2/4 deg (percent)	BCEA (Amblyopic) (log min arc sq)	BCEA (Fellow) (log min arc sq)
CF	36/84	80/99	4.41	3.37
SH	49/86	49/96	4.15	4.1
BB	56/91	97/100	4.13	3.38
JL	13/67	100/100	5.08	1.73

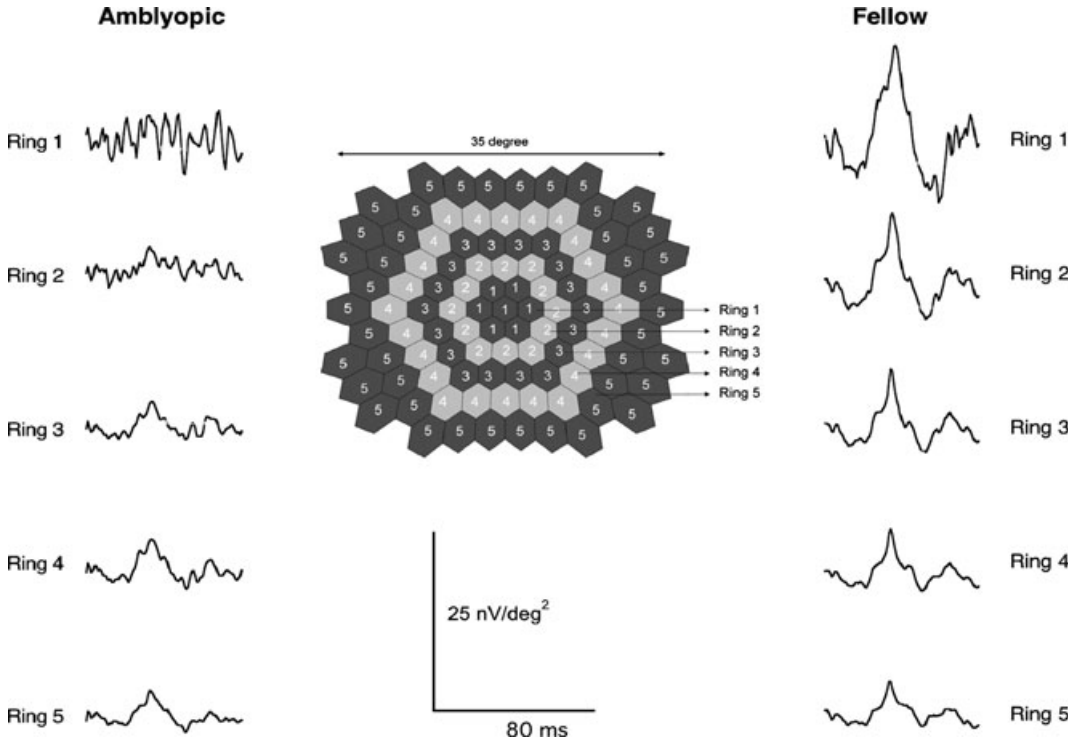

Figure 4. Ring responses for an individual subject (BB), showing reduction in response with increasing eccentricity (ring number), and reduced response in the amblyopic eye (see also Figures 3 and 5). Central N1-P1 response densities (ring 1) are shown at the top and more peripheral responses (rings 2–5) below.

Bivariate contour ellipse area (BCEA) was calculated from the fixation data output from the Nidek device for each perimetry session. *Table 2* shows BCEA values for the amblyopic eye, fellow eye, and the ratio of fixation areas in amblyopic eye/fellow eye. These are a factor of 10 or less, except for JL, who has multiple fixation loci in her amblyopic eye and extremely good fixation at the foveal locus of her fellow eye (*Figure 7*). There is no significant correlation between mfERG response and BCEA for either amblyopic eye ($r = 0.68$; $p > 0.05$ $n = 4$) or fellow eye ($r = 0.04$; $p > 0.05$ $n = 4$) in these participants, although these correlation analyses lack statistical power because of the small numbers of participants involved.

Correlation of mfERG results with fMRI results

Correlations were calculated between BOLD average response differences (differenced between right and left eye) and mfERG response differences (differenced between right and left eyes), either averaged across the entire stimulus field or targeting just the foveal selective mfERG deficit. The average BOLD measure represents the integrated hemodynamic response function[28,29]. As

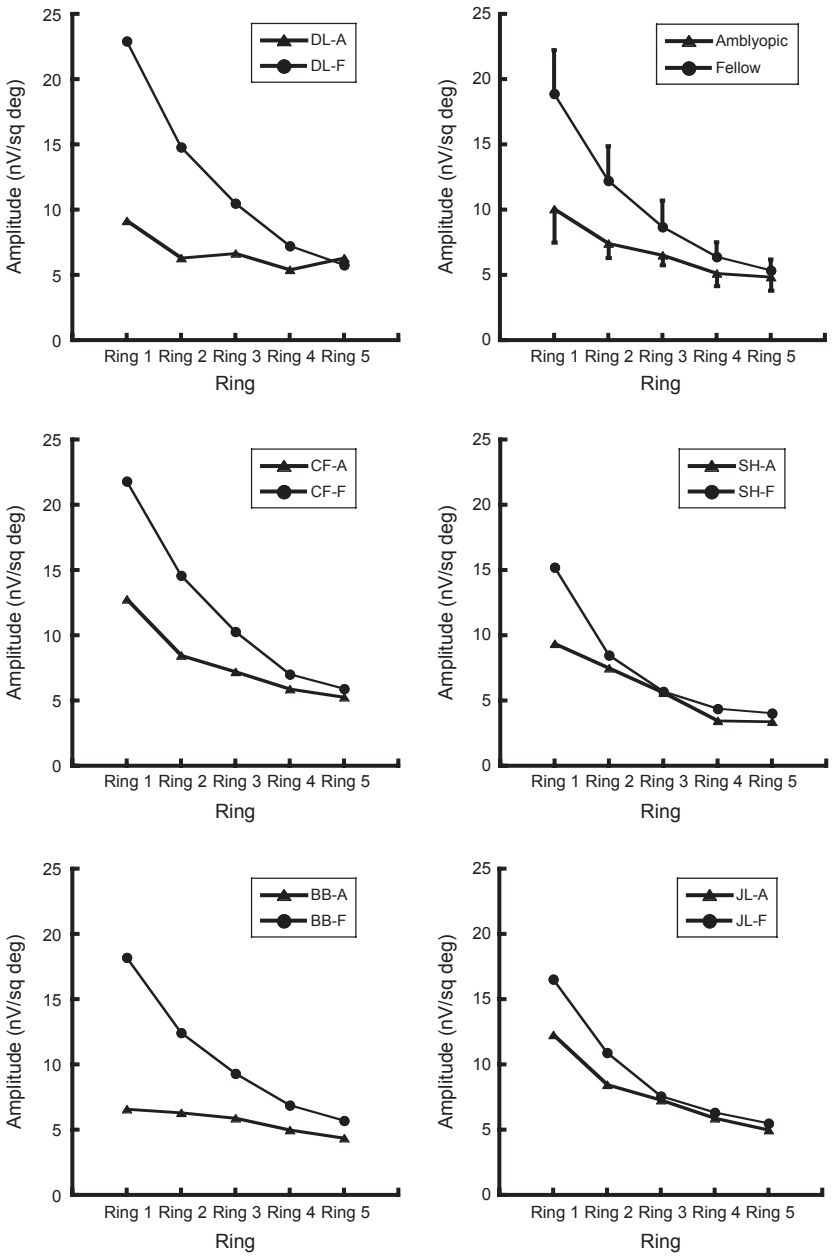

Figure 5. N1-P1 response densities as a function of eccentricity (Ring number) for five amblyopic subjects, showing data for amblyopic and fellow eyes. Data for all subjects combined, together with standard deviations are shown in the top right panel.

detailed in *Table 3* none of these were statistically significant. As noted above, these correlation analyses lack statistical power.

Discussion

First, all participants showed reduced mfERG response densities in the central retina (rings 1–3; about 19 deg) of the amblyopic eye compared to their fellow eye, and in general the responses normalised in the mid-periphery of the retina. We did not find any associated structural deficits. There are a number of possible explanations for reduced mfERG amplitudes in the central field other than reduced functional integrity of centrally-located retinal cells (e.g. reduced fixation stability, ametropia and the refractive correction) and these are discussed below. Second, since we found no correlation between the reduced mfERGs and reduced BOLD LGN deficits, we conclude that the previously reported LGN deficit may not have a retinal origin.

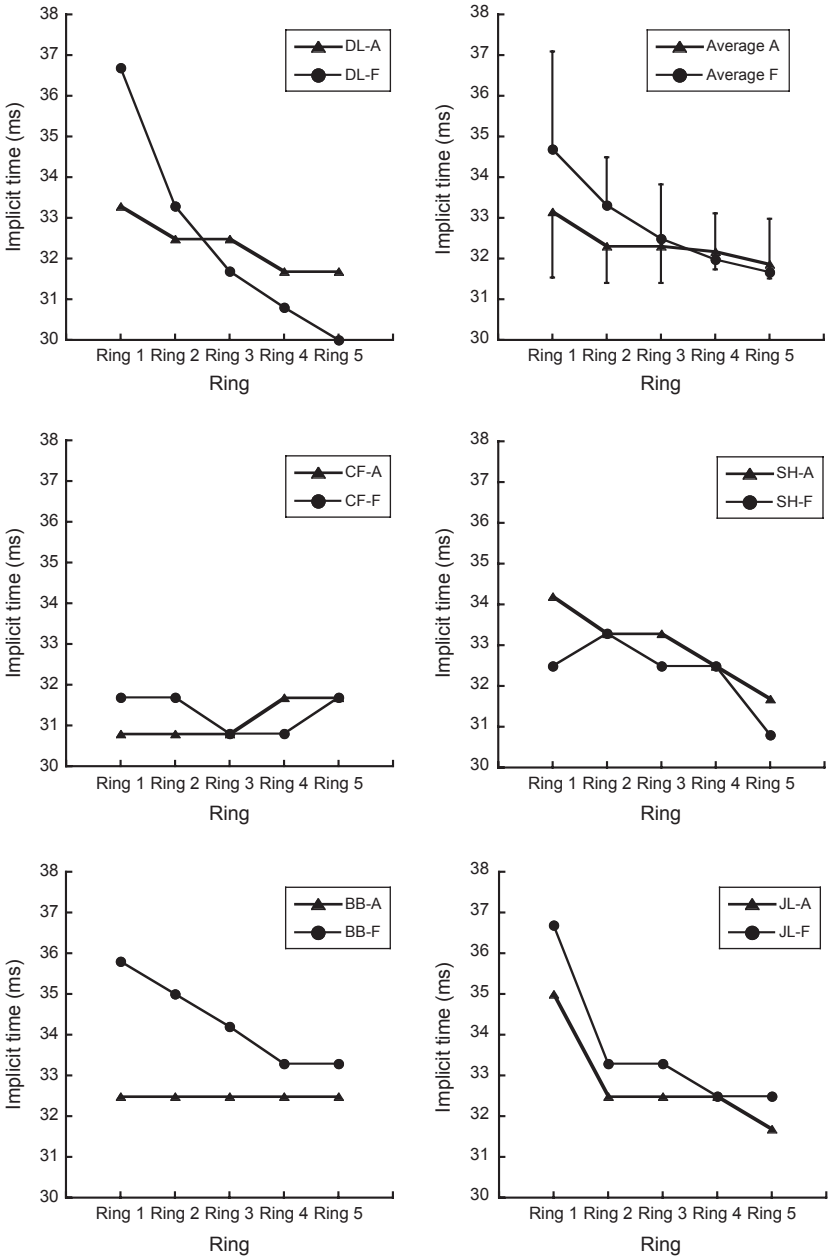

Figure 6. Implicit times as a function of eccentricity (Ring number) for five amblyopic subjects, showing data for amblyopic and fellow eyes. Data for all subjects combined, together with standard deviations are shown in the top right panel.

Fixation and mfERG

Here we must consider the region of the retina over which we are assessing 'central' mfERG function. The region of the 35 degree mfERG stimulus field which we used to assess central retinal function (for amblyopic and fellow eyes) included the central seven hexagons (the single central hexagon and the next ring which surrounds it). This field subtends about 6.3 deg (see central inset of *Figure 3*). The fixation records from the MP-1 indicated a reasonable degree of fixation stability in all participants whose MP-1 sensitivity could be assessed, and certainly their fixation could be maintained within 6.3 deg for the 25 s necessary to complete each segment of the mfERG task (see *Figure 7*, which shows fixation patterns over approximately 15 min). However, loss of foveal fixation on the central hexagon can also produce marked losses of mfERG response signal (see below).

Two participants (SH, BB) show fixation patterns which do not differ greatly between amblyopic and fellow eyes, show relatively small difference in BCEA and yet they show

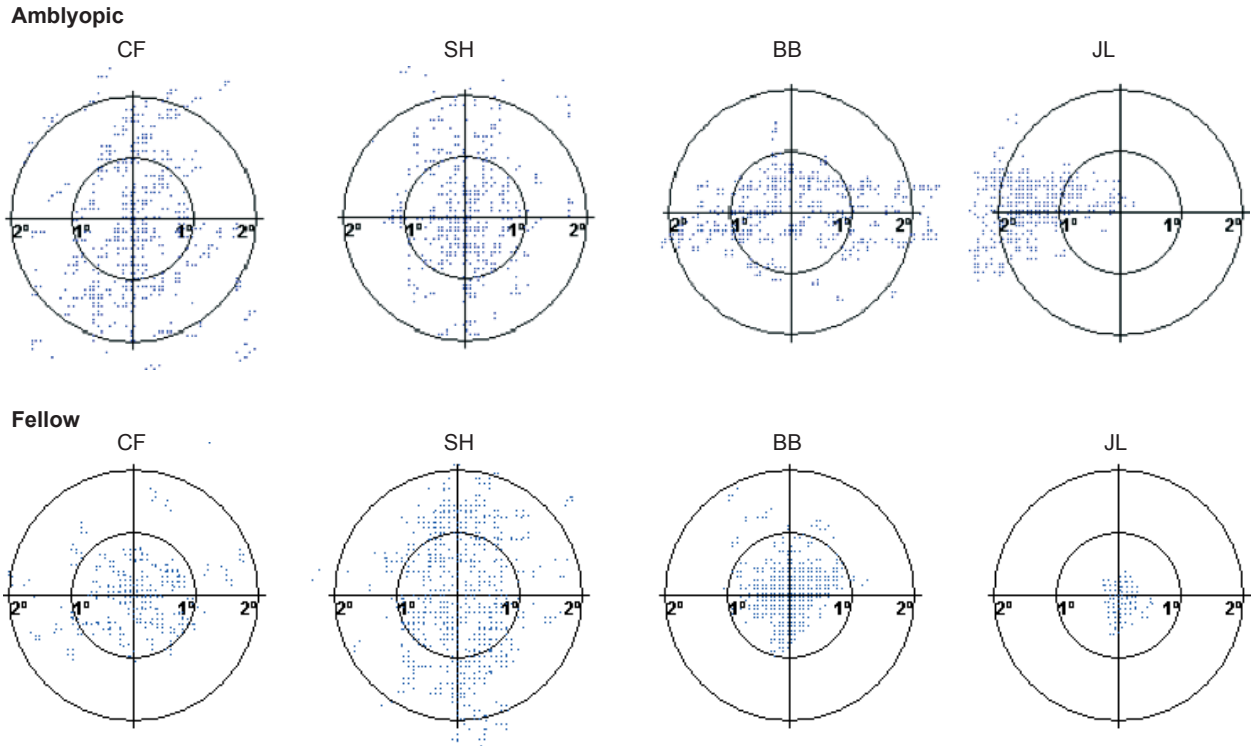

Figure 7. Fixation patterns as recorded by the Nidek MP-1 Microperimeter over a measurement period of approximately 15 min. Blue dots are recorded fixation points at the time of detection of perimetry presentations. JLK has two other fixation loci on the nasal (R) side of the pattern shown, which account for her larger bivariate contour ellipse value (see *Table* 2). Upper row shows patterns for amblyopic eyes, lower row shows patterns for fellow eyes.

markedly reduced central mfERG response in the amblyopic eye. As noted above, there was no significant correlation between BCEA and mfERG response in either amblyopic or fellow eye, although the power of this test is low because of the small number of subjects available.

Chu, Chan & Leat[44] examined the mfERG in 20 participants with normal vision who were asked to make controlled eye movements; fixation was moved regularly so that 51.2% of the time fixation was central and 12.2% of the time fixation was at each end of the fixation cross provided. Fixation crosses of 2°, 4° and 6° were used. For the 2° unsteady fixation, central (one hexagon, subtending only 2.4 deg) N1 and P1 amplitudes were unaffected; for 4° unsteady fixation, N1 amplitude was unaffected but P1 was reduced by some 30%. Implicit times of the mfERG N1-P1 responses were unaffected in any of the eye movement conditions which they used.

The idea that fixation instability has small effects on the mfERG does not accord with the conclusions of Zhang *et al.*[45] who reported mfERG data in a group of six amblyopes. They measured eye movements using a dual Purkinje image eye tracker prior to mfERG measurements, and found a selective central field mfERG deficit in amblyopic eyes that was tightly correlated with fixation instability. However the mfERG findings of Zhang *et al.* were assessed in terms of the P1-N2 amplitude instead of the more usual N1-P1 derivation that we used. In addition, their final analysis was biased toward amblyopia with gross nystagmus by the inclusion of two control participants with 2 Hz and 4 Hz nystagmus superimposed, and the data of the amblyopes were normalised to the 4 Hz data of the control participants before correlation and regression analysis. These issues make it difficult to compare their results with ours.

MP-1, fixation and perimetry

Shah and Chalam[46] used the MP-1 microperimeter in control participants and found that the total mean fixation stability values within 2° and 4° were 86% and 96%, respectively. They reported that older participants had worse fixation, with the 2° value declining by 3.4% per decade. Thus our amblyopic participants are worse (on average) than controls for the central (2°) fixation hexagon by a factor of about two, but considerably less than this for the 4° region. We would expect that fixation effects would be

Table 3. Pearson correlations for LGN (BOLD response) and retinal (mfERG). All differences refer to right eye minus left eye responses. Average BOLD responses are calculated for broadband checkerboards[28] or narrowband gratings[29] with achromatic, red/green and blue/yellow stimulation

Broadband fMRI stimulation (right eye-left eye):	
Averaged BOLD difference[28] vs mfERG difference averaged across eccentricity	$r = 0.258$ (ns)
Narrowband fMRI stimulation (right eye-left eye):	
Averaged BOLD difference[29] vs mfERG difference averaged across eccentricity	$r = 0.324$ (ns)
Average red/green BOLD difference[29] vs mfERG difference averaged across eccentricity	$r = 0.431$ (ns)
Average BOLD difference[29] vs foveal/peripheral mfERG difference (foveal erg-peripheral_9.5° ring)	$r = 0.662$ (ns)
Average red/green BOLD difference[29] vs foveal-peripheral mfERG eye difference (foveal erg-peripheral_9.5° ring)	$r = 0.468$ (ns)

minimal on ring 2 results, which encompass the region with a radius from 3.15° to 6.3°.

Bivariate contour ellipse area (BCEA) values (see *Table 3*) are within the range of values for those seen in age-related maculopathy patients[40]; we have been unable to find BCEA values for amblyopes in the literature for comparison with our own. The BCEA values for fellow eyes shown in *Table 2* (apart from that of participant JL) are of the expected value given the findings of Shah and Chalam[46] and the known disruption of fixation seen in fellow eyes of some amblyopes.[47]

The BCEA values we have measured reflect the ordering seen in the 2° and 4° scores from the MP-1, except for reversal of the positions of BB and SH in the 4° rankings.

MfERG and refractive error

It is known that high refractive errors in the absence of amblyopia can lead to reduced mfERGs. This has been shown for myopes[48, 49] and for this reason we rejected participant DG (−15.00 D/−2.25 D × 180) from our study even though she had been part of the original fMRI studies to which we compared our results.[28, 29] There is evidence that the mfERG response is reduced in cases of high myopia[48] and reduces as myopia increases.[49] A number of our participants had moderate to high levels of ametropia (DL + 8.0 D, SH + 7.0 D, CF −3.0 D) that could have impacted on the mfERG results. This is a possible confounding factor in our results.

Refractive correction

Refracting a severe amblyope can be quite difficult. If the visual loss is cortical then it is especially important to ensure optimal retinal focus for stimuli that are hard to see because the mfERG has a strong and monotonic dependence on stimulus contrast.[50] Any reduction in retinal contrast will reduce the mfERG amplitude. While even severe amblyopes can accurately detect image blur[51, 52] the accuracy of our subjective refraction may have resulted in a residual refractive error with a resultant loss of retinal contrast. This would produce a selective loss of mfERG amplitude for the central retina; Chan & Siu[53] have reported a 12% loss in P1 amplitude for a 3D error in refractive correction and no loss in central N1 amplitude, so that any loss for N1P1 amplitude should be less than 12%, assuming that our self-refractions are within 3D. It is possible that the amblyopes used the refractor to optimize contrast on the peripheral retina, while ignoring the less functional (or suppressed) central retina.

OCT and amblyopia

OCT data from three out of five of our amblyopic participants are consistent with those of previous studies,[54, 55] which found essentially no difference in macular thickness between amblyopic and fellow eyes. Values for participants SH (age 35) and BB (age 67) appear thicker by about 40μ in both central and circumfoveal regions of both eyes but this is within the expected normal range measured by Hagen *et al.*[41]

Relationship to LGN fMRI results

The unique aspect of this study is that this group of participants has been extensively studied with fMRI and a reduced LGN%BOLD response (both in terms of the averaged value and the peak value) has been identified[28] that is thought to be selective for the parvocellular layers.[29] Since we report reduced mfERG responses in this same group of participants, it is of special interest to know the relationship of the retinal and LGN deficits across the participants, to ascertain whether the reduced LGN response could be due to an attenuated input from the amblyopic eye.

If it were the case that the previously reported geniculate fMRI deficit[29] was solely the result of a reduced retinal input, we would have expected a significant correlation between the mfERG and geniculate fMRI deficits across our participants. There was no correlation between our measures of retinal and geniculate dysfunction for either broadband or narrowband stimuli (see *Table* 3). This result supports the view that the retinal deficit is not the sole explanation for the geniculate abnormality revealed by fMRI. This is true irrespective of whether the fMRI and mfERG deficits are computed as an average across the entire field or limited to the fovea where it is maximal. This conclusion however cannot be definitive because the exact relationship between these two measures (i.e. the mass electrical activity in the

inner retinal layers and the BOLD activity in the LGN) is not known for normal vision.

Conclusion

We have shown reduced central mfERG responses in a small group of amblyopes who have previously exhibited reduced BOLD responses (both in terms of the averaged response and the peak response) from the LGN in a manner that suggests a selective reduction of parvocellular function. There is no significant correlation between the retinal and geniculate deficits for similar stimulating conditions. We did not find any associated gross structural retinal deficits. We cannot conclude that the reduced LGN responses have a retinal basis because of the lack of a correlation between the retinal and geniculate losses.

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Disclosure

All authors report no conflicts of interest.

References

1. Higgins KE, Daugman JG & Mansfield RJ. Amblyopic contrast sensitivity: insensitivity to unsteady fixation. *Invest Ophthalmol Vis Sci* 1982; 23: 113–120.
2. Hess RF, Campbell FW & Greenhalgh T. On the nature of the neural abnormality in human amblyopia; neural aberrations and neural sensitivity loss. *Pflug Arch - Eur J Physiol* 1978; 377: 201–207.
3. Hess RF & Howell ER. The threshold contrast sensitivity function in strabismic amblyopia: evidence for a two type classification. *Vision Res* 1977; 17: 1049–1055.
4. Levi M & Harwerth RS. Spatio-temporal interactions in anisometropic and strabismic amblyopia. *Invest Ophthalmol Vis Sci* 1977; 16: 90–95.
5. Hess RF & Holliday IE. The spatial localization deficit in amblyopia. *Vision Res* 1992; 32: 1319–1339.
6. Lagreze WD & Sireteanu R. [Errors of monocular localization in strabismic amblyopia. Two-dimensional distortion]. *Klin Monatsbl Augenheilkd* 1992; 201: 92–96.
7. Levi DM & Klein SA. Spatial localization in normal and amblyopic vision. *Vision Res* 1983; 23: 1005–1017.
8. Aaen-Stockdale C & Hess RF. The amblyopic deficit for global motion is spatial scale invariant. *Vision Res* 2008; 48: 1965–1971.
9. Simmers AJ, Ledgeway T & Hess RF. The influences of visibility and anomalous integration processes on the perception of global spatial form versus motion in human amblyopia. *Vision Res* 2004; 45: 449–460.
10. Simmers AJ, Ledgeway T, Hess RF & McGraw PV. Deficits to global motion processing in human amblyopia. *Vision Res* 2003; 43: 729–738.
11. Simmers AJ, Ledgeway T, Mansouri B, Hutchinson CV & Hess RF. The extent of the dorsal extra-striate deficit in amblyopia. *Vision Res* 2006; 46: 2571–2580.
12. Cleland BG, Crewther DP, Crewther SG & Mitchell DEM. Normality of spatial resolution of retinal ganglion cells in cats with strabismic amblyopia. *J Physiol (Lond)* 1982; 326: 235–249.
13. Cleland BG, Mitchell DEM, Gillard-Crewther S & Crewther DP. Visual resolution of ganglion cells in monocularly-deprived cats. *Brain Res* 1980; 192: 261–266.
14. Crewther DP, Crewther SG & Cleland BG. Is the retina sensitive to the effects of prolonged blur? *Exp Brain Res* 1985; 58: 427–34.
15. Hess RF & Baker CL. Assessment of retinal function in severely amblyopic individuals. *Vision Res* 1984; 24: 1367–1376.
16. Hess RF, Baker CL, Verhoeve JN, Tulunay Keesey U & France TD. The pattern evoked electoretinogram: its variability in normals and its relationship to amblyopia. *Invest Ophthalmol Vis Sci* 1985; 26: 1610–1623.
17. Blakemore C & Vital-Durand F. Effects of visual deprivation on the development of the monkey's lateral geniculate nucleus. *J Physiol (Lond)* 1986; 380: 493–511.
18. Levitt JB, Schumer RA, Sherman SM, Spear PD & Movshon JA. Visual response properties of neurons in the LGN of normally reared and visually deprived macaque monkeys. *J Neurophysiol* 2001; 85: 2111–2129.
19. Zele AJ, Wood JM & Girgenti CC. Magnocellular and parvocellular pathway mediated luminance contrast discrimination in amblyopia. *Vision Res* 2010; 50: 969–976.
20. Barnes GR, Hess RF, Dumoulin SO, Achtman RL & Pike GB. The cortical deficit in humans with strabismic amblyopia. *J Physiol (Lond)* 2001; 533(Pt.1): 281–97.
21. Zele AJ, Pokorny J, Lee DY & Ireland G. Anisometropic amblyopia: spatial contrast sensitivity deficits in inferred magnocellular and parvocellular vision. *Invest Ophthalmol Vis Sci* 2007; 48: 3622–31.
22. Einon G, Ikeda H & Tremain KE. Perikaryal size of cells in the lateral geniculate nucleus and amblyopia in cats reared with convergent squint [proceedings]. *J Physiol (Lond)* 1978; 278: 50P.
23. Guillery RW. Binocular competition in the control of geniculate cell growth. *J Comp Neurol* 1972; 144: 117–129.
24. Tremain KE & Ikeda H. Relationship between amblyopia, LGN cell "shrinkage" and cortical ocular dominance in cats. *Exp Brain Res* 1982; 45: 243–252.

25. Barnes GR, Li X, Thompson B, Singh KD, Dumoulin SO & Hess RF. Decreased gray matter concentration in the lateral geniculate nuclei in human amblyopes. *Invest Ophthalmol Vis Sci* 2009; 51: 1432–1438.
26. von Noorden GK, Crawford ML & Levacy RA. The lateral geniculate nucleus in human anisometropic amblyopia. *Invest Ophthalmol Vis Sci* 1983; 24: 788–790.
27. von Noorden GK & Crawford ML. The lateral geniculate nucleus in human strabismic amblyopia. *Invest Ophthalmol Vis Sci* 1992; 33: 2729–2732.
28. Hess RF, Thompson B, Gole G & Mullen KT. Deficient responses from the lateral geniculate nucleus in humans with amblyopia. *Eur J Neurosci* 2009; 29: 1064–1070.
29. Hess RF, Thompson B, Gole GA & Mullen KT. The amblyopic deficit and its relationship to geniculo-cortical processing streams. *J Neurophysiol* 2010; 104: 475–483.
30. Chino YM, Smith EL (3rd), Yoshida K, Cheng H & Hamamoto J. Binocular interactions in striate cortical neurons of cats reared with discordant visual inputs. *J Neurosci* 1994; 14: 5050–5067.
31. Ikeda H, Tremain KE & Einon G. Loss of spatial resolution of lateral geniculate nucleus neurones in kittens raised with convergent squint produced at different stages in development. *Exp Brain Res* 1978; 31: 207–220.
32. Sherman SM, Wilson JR & Guillery RW. Evidence that binocular competition affects the postnatal development of Y-cells in the cat's lateral geniculate nucleus. *Brain Res* 1975; 100: 441–444.
33. Yin ZQ, Crewther SG, Wang C & Crewther DP. Pre- and post-critical period induced reduction of Cat-301 immunoreactivity in the lateral geniculate nucleus and visual cortex of cats Y-blocked as adults or made strabismic as kittens. *Mol Vis* 2006; 12: 858–866.
34. Sutter EE & Tran D. The field topography of ERG components in man–I. The photopic luminance response. *Vision Res* 1992; 32: 433–446.
35. Rangaswamy NV, Hood DC & Frishman LJ. Regional variations in local contributions to the primate photopic flash ERG: revealed using the slow-sequence mfERG. *Invest Ophthalmol Vis Sci* 2003; 44: 3233–3247.
36. Shimada Y, Bearse MA Jr & Sutter EE. Multifocal electroretinograms combined with periodic flashes: direct responses and induced components. *Graefes Arch Clin Exp Ophthalmol* 2005; 243: 132–141.
37. Bearse MA Jr, Han Y, Schneck ME & Adams AJ. Retinal function in normal and diabetic eyes mapped with the slow flash multifocal electroretinogram. *Invest Ophthalmol Vis Sci* 2004; 45: 296–304.
38. Cornsweet TN. The staircase method in psychophysics. *Am J Psychol* 1962; 75: 485–491.
39. Early Treatment of Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study Report No. 1. *Arch Ophthalmol* 1985;103:1796–1806.
40. Crossland MD, Dunbar HMP & Rubin GS. Fixation stability measurement using the MP1 microperimeter. *Retina* 2009; 29: 651–656.
41. Hagen S, Krebs I, Haas P *et al.* Reproducibility and comparison of retinal thickness and volume measurements in normal eyes determined with two different Cirrus OCT scanning protocols. *Retina* 2011; 31: 41–7.
42. Feigl B & Zele AJ. Macular function in tilted disc syndrome. *Doc Ophthalmol* 2010; 120: 201–203.
43. Springer C, Bultmann S, Volcker HE & Rohrschneider K. Fundus perimetry with the Micro Perimeter 1 in normal individuals: comparison with conventional threshold perimetry. *Ophthalmology* 2005; 112: 848–854.
44. Chu PH, Chan HH & Leat SJ. Effects of unsteady fixation on multifocal electroretinogram (mfERG). *Graefes Arch Clin Exp Ophthalmol* 2006; 244: 1273–1282.
45. Zhang B, Stevenson SS, Cheng H, *et al.* Effects of fixation instability on multifocal VEP (mfVEP) responses in amblyopes. *J Vis* 2008; 8: 16.1–14.
46. Shah VA & Chalam KV. Values for macular perimetry using the MP-1 microperimeter in normal participants. *Ophthalmic Res* 2009; 41: 9–13.
47. Bedell HE & Flom MC. Bilateral oculomotor abnormalities in strabismic amblyopes: evidence for a common central mechanism. *Doc Ophthalmol* 1985; 59: 309–321.
48. Chan HL & Mohidin N. Variation of multifocal electroretinogram with axial length. *Ophthalmic Physiol Opt* 2003; 23: 133–140.
49. Kawabata H & Adachi-Usami E. Multifocal electroretinogram in myopia. *Invest Ophthalmol Vis Sci* 1997; 38: 2844–2851.
50. Brown B & Yap MK. Contrast and luminance as parameters defining the output of the VERIS topographical ERG. *Ophthalmic Physiol Opt* 1996; 16: 42–48.
51. Hess RF, Pointer JS, Simmers A & Bex P. Border distinctness in amblyopia. *Vision Res* 2003; 43: 2255–2264.
52. Simmers AJ, Bex PJ & Hess RF. Perceived blur in amblyopia. *Invest Ophthalmol Vis Sci* 2003; 44: 1395–1400.
53. Chan HL & Siu AW. Effect of optical defocus on multifocal ERG responses. *Clin Exp Optom* 2003; 86: 317–322.
54. Repka MX, Kraker RT, Tamkins SM, Suh DW, Sala NA & Beck RW. Retinal nerve fiber layer thickness in amblyopic eyes. *Am J Ophthalmol* 2009; 148: 143–147.
55. Huynh SC, Samarawickrama C, Wang XY, *et al.* Macular and nerve fiber layer thickness in amblyopia: the Sydney Childhood Eye Study. *Ophthalmology* 2009; 116: 1604–1609.