Bilateral Changes in Foveal Structure in Individuals with Amblyopia

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**Purpose:** To examine foveal structure in amblyopia using spectral-domain optical coherence tomography (SD-OCT).

**Design:** Prospective, cross-sectional study.

**Participants and Controls:** Two subject groups were recruited to the study: 85 amblyopes (34 adults, 51 children) and 110 visually normal controls (44 adults, 66 children).

**Methods:** A detailed eye examination, including an SD-OCT scan, was performed in all participants. A total of 390 eyes of 195 subjects were imaged using a 3-dimensional (3D) macula scan covering a nominal 6×6-mm area with a resolution of 256×256 (65 536 axial scans). Data from the B-scans bisecting the fovea both horizontally and vertically were fitted with a mathematical model of the fovea to determine a range of foveal parameters.

**Main Outcome Measures:** Foveal thickness, foveal pit depth, and foveal pit slope.

**Results:** Bilateral differences between the eyes of amblyopes compared with visually normal controls were found. The difference between foveal structure in amblyopic participants relative to structure in subjects with normal vision persisted even when variables such as age, ethnicity, axial length, and sex were taken into account. Amblyopes showed increased foveal thickness (8.31 μm; P = 0.006) and a reduction in pit depth in the horizontal meridian (10.06 μm; P = 0.005) but not in the vertical meridian (P = 0.082) when compared with subjects with normal vision. Foveal pit slopes were found to be approximately 1 degree flatter in the nasal (P = 0.033) and temporal (P = 0.014) meridians in amblyopes, but differences between amblyopes and controls in the superior (P = 0.061) and inferior (P = 0.087) meridians did not reach statistical significance. No statistically significant interocular differences were found in the foveal structure between amblyopic and fellow eyes.

**Conclusions:** Differences were found in the foveal structure in both eyes of amblyopes compared with subjects with normal vision. These differences consisted of increased foveal thickness, reduced pit depth when measured along the horizontal meridian, and flattening of the nasal and temporal sides of the foveal pit.

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Amblyopia is a developmental disorder of vision in which there is reduced visual acuity (typically unilateral) despite optimal refractive correction in an eye that is structurally normal and free from pathology.1 The remainder of the visual system is also presumed to be healthy, and the visual deficit is associated with the presence in early life of strabismus, anisometropia, or, less commonly, an obstruction (e.g., congenital cataract) along the visual axis.2,3 Since the pioneering studies of Hubel4 and Hubel and Wiesel5,6 in which animals were deprived of normal stimulation in their early lives by means of lid suture or experimentally induced strabismus, the site of the visual deficit in amblyopia has been considered to be cortical. The primary visual cortex represents the earliest point in the visual pathway where the inputs from the 2 eyes are mixed, and it was in the visual cortex that the experimental manipulations of Hubel and Wiesel5,6 were most apparent. Although the amblyopia in animal studies was artificially created and generated mostly by lid suture, the results from the animal literature were believed to be applicable to the human form of the condition in which monocular deprivation accounts for an extremely small proportion of cases of human amblyopia.3,7 Although there were claims of retinal8,9 or thalamic10,11 involvement in amblyopia, these claims were dismissed12 or the differences found were considered to be secondary to the cortical changes. Amblyopia in humans, like its animal counterpart, traditionally has been thought to represent visual loss that is cortical in origin.13

With the advent of new investigative techniques, fresh evidence is emerging from a range of different sources that the effects of amblyopia may be apparent in precortical areas of the human visual pathway. For example, neuroimaging studies of the lateral geniculate nucleus (LGN) suggest that it may be functionally14 different in human amblyopes. Structural abnormalities of the globe in both eyes of amblyopic individuals have been reported.15 Retinal in-
volvement in amblyopia has always been particularly controversial. The availability of optical coherence tomography (OCT) has enabled clinicians and researchers to examine the retinal structure in human amblyopes with the aim of finally answering the question of whether the retina is structurally normal in amblyopes. A large number of studies have appeared in the recent literature in which retinal nerve fiber thickness and macular volume and thickness have been assessed in amblyopes. This study is concerned with the possibility that there may be foveal involvement in amblyopia. Although the structure–function relationship is not well established for the human fovea (see “Discussion”), the study of foveal structure in amblyopes represents an important topic for research. If the foveal structure actually is different in amblyopia, it could pose a serious challenge to definitions for this common condition that refer, explicitly or implicitly, to eyes that are structurally sound.

Table 1 provides a summary of the articles about this topic that have recently appeared and that use OCT. It is clear that as yet no consensus exists about whether the amblyopic fovea is structurally abnormal. What possible reasons may account for the differing conclusions concerning foveal structure in amblyopes? First, whereas some studies have used the fellow eye as the eye against which to compare the amblyopic eye, others have compared the foveae of both eyes of amblyopes with those of visually normal controls (Table 1). This is crucially important because interocular comparison in amblyopes obviously cannot reveal whether both eyes of amblyopes differ from the eyes of subjects with normal vision. As indicated, precisely this claim has been made in relation to overall globe structure. Second, the age profile of participants in previous studies has varied considerably, with amblyopes and subjects with normal vision being compared in adult and child samples. It is conceivable, for example, that differences could exist in the foveal structure of amblyopic adults compared with adults with normal vision, but the same differences might not exist in children, or differences might exist between amblyopic children and visually normal children, but the same may not apply in adults. Third, differences in foveal structure may be associated with ethnic differences; this has been reported in recent studies of foveal structure. This study examines foveal structure in amblyopes in comparison with visually normal control subjects, taking into account factors of ethnicity, age, sex, and axial length. The hypothesis that we wanted to test is that the foveal structure of either eye of amblyopic individuals does not differ from that in subjects with normal vision. Finally, whereas all previous studies of amblyopic foveal structure have relied on the metrics that are the output by the instrument’s software, the accuracy of which will naturally depend on the robustness of the algorithm applied, this study assessed the detailed foveal structure by reconstructing the topography of the fovea using mathematical modeling and custom software.
Materials and Methods

Subjects

This was an exploratory study designed to compare multiple features of foveal structure in amblyopes and visually normal subjects, and for this reason, no formal calculation relating to sample size was conducted in advance. A total of 195 subjects were recruited (Table 2): 34 adult amblyopes and 51 amblyopic children, 45 visually normal adult controls, and 65 visually normal children. The participants were recruited from the staff and student populations at the University of Bradford (via the University’s Eye Clinic), via local optometry practices, via the ophthalmology and orthoptic clinics at local hospitals, and from the local community via a press release. The visually normal children were recruited from 3 local schools. Details of the mean and range of spherical equivalent refraction error for the 4 groups are provided in Table 2 along with visual acuity (mean and range) and presumed cause.

Participants received a full eye examination that included recording ocular history and subjective refraction, which, in the case of amblyopic children, was completed after cycloplegia. Cycloplegic refractions were not conducted in adults (adults or controls) or visually normal children. Visual acuity (logarithm of the minimum angle of resolution) was measured with best correction. Cover testing (at distance and near, with and without full refractive correction) also was conducted. For the purposes of this study, amblyopia was defined as a reduction in visual acuity in the amblyopic eye of \( > +0.2 \) logarithm of the minimum angle of resolution with at least 2 lines of difference between the amblyopic eye and the fellow eye.\(^1,2\)\(^7\)\(29,30\) and anisometropia was defined as 1-diopter difference in spherical equivalent refractive error.\(^28\) Ethics committee (National Health Service, Bradford, UK) approval was obtained before commencement of the study. All of the participants gave informed written consent, and the study was conducted according to the tenets of the Declaration of Helsinki.

Image Processing

Both eyes of participants were imaged using the 3-dimensional (3D) OCT-1000 (Topcon, Tokyo, Japan). The scan type for all scans in this study was a 3D macula scan covering a nominal 6×6-mm area, comprising a 256×256 grid of A-scans (65,536 axial scans). Because the area of the retina that was actually imaged depends on the axial length, it was necessary to take account of the axial length of individual eyes before the determination of foveal parameters for that eye. This was taken into account with a magnification factor based on recognized formulas\(^29,30\) and modified for the OCT by Leung et al.\(^31\) After acquisition and processing of the 3D OCT scan with the standard Topcon procedure, 2 individual B-scans, 1 that bisected the fovea horizontally and 1 that bisected in the vertical meridian, were identified. The steps involved in processing images to extract the foveal structural parameters are shown in Figure 1. The calibration tool of the 3D OCT-1000 was used to delineate horizontal and vertical scales on the individual B-scans (Fig II) that had retinal layers segmented by the Topcon OCT software. These images were exported into a shareware software package, DataThief III (B. Tummers, DataThief III, 2006; available at: http://datathief.org/, accessed July 1, 2009), in which the inner limiting membrane (ILM) and retinal pigment epithelium (RPE) layers were manually annotated to provide x and y coordinates for data export. This data exportation was undertaken by one of the authors (AB) who was aware of the participant grouping (amblyope or control) but was not aware which was the amblyopic eye and which was the fellow eye in the amblyopes. All images were annotated and had data exported before the modeling of foveal morphology process outlined next. The ILM-RPE data derived from DataThief III were then exported into the commercial program MATLAB (MathWorks, Inc., Natick, MA) and, by calculating the difference between the ILM height values and a polynomial fitted to the RPE data (Fig III), absolute retinal thicknesses were determined (Fig III). A customized
MATLAB program was designed emulating the procedure advocated by Dubis et al.\textsuperscript{32} to automatically identify key anatomic landmarks of the fovea. The modeling of foveal morphology was undertaken by the second author (IEP), who had no knowledge of the visual status of the scan being processed.

**Modeling Foveal Morphology**

The shape of the human fovea has been shown to fit well with the mathematical model of the Gaussian curve.\textsuperscript{33} More recently, however, Dubis et al.\textsuperscript{32} used a difference of Gaussian (DoG) function (Equation 1) to model the shape of the fovea. The DoG provides a good mathematical fit because it captures the contour of the foveal pit and the foveal rim, key areas to measure both the diameter and depth. The DoG is fitted to the retinal data thickness measurements using least-squares analysis, and the anatomic landmarks (Fig 1) were obtained on the basis of the zero-crossings of the first and second derivatives of the DoG function. The foveal thickness and the nasal and temporal retinal thicknesses were determined from the absolute retinal thickness values at the horizontal locations of the zero-crossings of the first derivative of Equation 1 (Fig IV). Likewise, the foveal slopes were the values of the first derivative corresponding to the zero-crossings of the second derivative of Equation 1.

\[
F(x) = a_1 x \left( \exp \left( \frac{- (x - \mu_1)^2}{-2\sigma_1^2} \right) \right) - a_2 x \left( \exp \left( \frac{- (x - \mu_2)^2}{-2\sigma_2^2} \right) \right) + \text{constant}
\]

Equation 1: Parameters of first and second Gaussians, respectively, \(\mu_1\) and \(\mu_2\) = means of the Gaussian curves, \(\sigma_1, \sigma_2\) = standard deviations of curve 1 and curve 2, \(a_1\) and \(a_2\) = heights of curve 1 and 2.

All participants in the study had a single 3D macular scan for each eye. From each 3D scan, 2 B-scans were selected (1 horizontal, 1 vertical) that bisected the center of the fovea. The B-scan that bisected the fovea was identified as the scan that showed the brightest foveal reflex or the greatest separation between the inner/outer photoreceptor segment boundary and the RPE layer. Of the 780 B-scans, 38 (5\%) were excluded (12 from adult amblyopes, 1 from a visually normal adult, 20 from visually normal children, and 5 from amblyopic children) because of an inability of the modeling process to identify all 5 zero-crossings in the first and second derivatives of Equation 1 within the extent of the B-scan. These poor modeling fits seemed to originate from lateral movement artefacts or accommodative fluctuations caused by unstable or eccentric fixation that particularly affected the vertical scans of the children and the amblyopes.

**Results**

**Interocular Differences in Foveal Structure: Subjects with Normal Vision and Amblyopes**

The interocular differences in the measured foveal parameters for the visually normal control subjects and, separately, for the amblyopic participants are shown in Table 3 (available at http://aaojournal.org). The results for adults and children are considered together for this analysis. In subjects with normal vision, a high degree of symmetry is evident, with none of the right eye versus left eye foveal parameters demonstrating a statistically significant difference. The same result was found in amblyopes: there was no difference between the amblyopic and fellow eyes, although the amblyopic eye versus fellow eye difference in inferior retinal thickness \((P = 0.054)\) and temporal retinal thickness \((P = 0.064)\) fell just short of reaching statistical significance (Table 3, available at http://aaojournal.org). Thus, our results indicate a high degree of interocular symmetry between the 2 eyes in both subjects with normal vision and amblyopes for all of the foveal parameters investigated (Table 3, available at http://aaojournal.org), indicating that the fovea of the amblyopic eye and its fellow eye are structurally similar. Although no statistically significant interocular differences were found in foveal structure between the 2 eyes of amblyopes or subjects with normal vision, the range of interocular differences was generally greater in amblyopes (Table 3, available at http://aaojournal.org). We compared the interocular differences in amblyopes and controls using the robust equality of variation test. This analysis revealed that the range of interocular differences is greater in amblyopes for the following 4 parameters: retinal thickness at the nasal margin \((P = 0.017)\) and the slopes on the superior \((P = 0.017)\), nasal \((P = 0.036)\), and temporal \((P = 0.003)\) sides of the foveal pit (Table 3, available at http://aaojournal.org).

**Influence of Age, Ethnicity, Sex, and Axial Length**

To compare amblyopic and visually normal control eyes, differences in age, ethnicity, sex, and axial length between the amblyope and control groups were taken into account.\textsuperscript{26,34–37} To do this, regression analyses were conducted that adjusted for discrete variables (ethnicity: South Asian vs. non-Asian; sex: male or female) and continuous variables (age, axial length). These results are presented in Table 3 (available at http://aaojournal.org).

Sex was not found to influence any of the foveal parameters. Ethnicity was found to be a significant determinant in all foveal parameters, with the exception of pit depth. Foveal thickness was reduced in the participants of South Asian origin (average difference, 11.75 \(\mu m\); \(P<0.001\)), and retinal thickness measurements

![Figure 1](https://example.com/figure1.png)
were lower in the nasal (P = 0.001), temporal (P<0.001), superior (P<0.001), and inferior (P = 0.001) meridians, confirming an overall thinner retina in individuals of South Asian ethnicity64,36,37 (Table 3, available at http://aoajournal.org).

Age was found to influence foveal thickness and pit depth, with the foveal thickness increasing significantly with age (P<0.001) and the pit depth becoming significantly shallower with age (vertical meridian, P<0.001; horizontal meridian, P = 0.001). In addition, retinal thickness was found to increase with age in the nasal meridian (P = 0.03), and the slopes of the foveal pit decreased with age (nasal, P < 0.001; temporal, P<0.001; superior, P = 0.009; inferior, P = 0.008) (Table 3, available at http://aoajournal.org).

Axial length was found to be a determinant of foveal thickness and pit depth, with foveal thickness increasing by 2.46 μm (P = 0.033) and pit depth (horizontal) decreasing by 2.65 μm (P = 0.049) per 1-mm increase in axial length (Table 3, available at http://aoajournal.org).

Foveal Structure in Amblyopic Eyes Compared with Visually Normal Eyes

After adjustment for ethnicity, age group, sex, and axial length, the presence of amblyopia was associated with an increased foveal thickness (+8.31 μm, P = 0.006) and a reduction in pit depth in the horizontal meridian (−10.06 μm, P = 0.005) but not in the vertical meridian (P = 0.082) when compared with controls. Foveal pit slopes were found to be approximately 1 degree flatter in the nasal (P = 0.033) and temporal meridians (P = 0.014) in amblyopes, but differences between amblyopes and controls in the superior (P = 0.061) and inferior meridians (P = 0.087) did not reach statistical significance (Table 3, available at http://aoajournal.org).

Strabismic versus Nonstrabismic Amblyopia

Only 2 of the adult amblyopes did not have strabismus, and for this reason, a comparison of strabismic versus nonstrabismic amblyopes was restricted to the amblyopic children, of whom 36 had strabismus (with or without anisometropia) and 15 had anisometropia only (no strabismus) (Table 2). The t tests of strabismic versus nonstrabismic eyes in amblyopic children showed no significant differences between amblyopic eyes with and without strabismus for any of the foveal parameters investigated (Table 4).

Discussion

A number of recent studies have examined foveal topography in amblyopia (Table 1). Many of these studies did not include visually normal control subjects and simply compared the amblyopic eye with its fellow eye. The majority of these studies found no difference in foveal structure between the 2 eyes of amblyopes (Table 1), as did the current study. Our results suggest that the lack of visually normal control subjects hides structural differences that exist between individuals with and without amblyopia, not between amblyopic and fellow eyes. A small number of studies have included control subjects; these studies have reported increased foveal thickness measures in amblyopes compared with the control group.21–23 Huynh et al21 reported an
increased foveal thickness (12 μm) in amblyopic children (aged 6 and 12 years) compared with eyes from control children. The variation in results among previously published studies may be due to the lack of control groups, variation in age of the sample, or differences in ethnicity. The results we present indicate that foveal structure does differ between amblyopic and visually normal control eyes but not between amblyopic and fellow eyes. The magnitude of the structural change is not dependent on the depth of amblyopia because both eyes of amblyopes are similarly affected.

There is evidence from monocular deprivation studies in animals that bilateral structural adaptation to deprivation can occur. This reported structural change, arising from the presence of monocular deprivation in chickens, resulted in elongation of the outer segment of the photoreceptors, both rods and cones. The use of mathematical modeling in this study has allowed detailed structural measurement of the fovea in amblyopia for the first time, producing an overall picture of the fovea including pit depth, a parameter not reported in previously studies. Our study indicates that the reduced pit depth is most likely caused by thickening of the fovea, probably as a result of changes at the level of the photoreceptors.

Ethnicity is a significant factor influencing foveal structure. South Asian ethnicity was found to be associated with decreased foveal thickness at the nasal, temporal, superior, and inferior rims, but with no difference in the depth of the foveal pit compared with non-Asians. The profile of the fovea in our participants of South Asian origin is, therefore, of a flatter overall structure. Huynh et al also reported a reduction of foveal thickness in children of East Asian ethnicity. In a study of visually normal children using OCT, El-Dairi et al found black children to have reduced foveal thickness (176 μm) in comparison with white children (198 μm). In visually normal adults, 2 studies of foveal thickness found measurements reduced in black compared with white adults. In the current study, age also was found to be a significant determinant of foveal shape. Adults demonstrated an increase in foveal thickness, a decrease in pit depth, and shallower pit slopes, indicating that structural change of the fovea continues into adulthood. For these reasons, we suggest that future studies should take the factors of age and ethnicity into account. Of note, the age-related changes in foveal structure we have observed closely match the changes associated with the presence of amblyopia.

Primary or Secondary Structural Changes?

The structural differences reported in this study could, in theory, have 3 possible origins. First, they could represent the primary cause of the deficit that has been diagnosed as amblyopia. Their subtle nature means that they could go undetected until detailed structural investigations are conducted. If this were true, strabismus or anisometropia would be secondary phenomena that might add to the visual deficit, but they would not represent its primary underlying cause. There are 2 lines of evidence that potentially support such an explanation. One is that strabismus and anisometropia are rarely present and persistent from birth in the numbers that would be required to account for the prevalence of amblyopia in the population. The other is that structural abnormalities of the eye are thought to be associated with conditions such as fetal alcohol syndrome. If these abnormalities are subtle, they could go undetected but still perhaps lead to development of strabismus or interfere with emmetropization and potentially cause anisometropia.

The second potential explanation for our finding that foveal structure in amblyopes differs from controls is that the structural differences arise as a consequence of the visual loss caused by the presence in early life of persistent anisometropia or strabismus. A third possible explanation is that the anisometropia/strabismus and the structural abnormalities could have been caused by a defect that is hidden in the visual system (e.g., a primary cortical deficit that leads to both the anisometropia/strabismus and the structural changes). Although this cross-sectional study cannot tell us whether the structural differences we observed came before or after the amblyopia, the evidence strongly suggests that they are a secondary phenomenon. Structural defects that represent the root of the visual deficit would be expected to be greater in the eye showing the poorer visual acuity and to correlate with the extent of the visual deficit. Neither of these is evident in our data. Bilateral structural defects of the retina have been observed in animal models of amblyopia; such effects are apparent not only after lid suture but also after defocus, even in the nondefocused eyes of animals that have been unilaterally blurred. Bilateral increases in foveal thickness also have been reported in ophthalmic conditions, such as retinopathy of prematurity, ocular albinism, and foveal hypoplasia. This evidence points to the observed structural differences being an effect rather than a cause of the visual loss. The bilateral structural change at the retinal level that we report is consistent with changes previously described in the parvocellular cells of the LGN in monkeys. In the study by Slopere, the parvocellular cells associated with both the deprived and undeprived eyes shrank almost equally by 25% to 30% after a period of monocular deprivation. These parvocellular LGN cells are the target cells for the retinal ganglion cells, and this may further support a retrograde effect on the retina.

An advantage of our study over previous studies is that previous studies of foveal structure in amblyopia have relied on the foveal measures that are output by the algorithm of the instrument, whereas in the present study the foveal structure was reconstructed using customized software. The advantage conferred is that poor or unsteady fixation is less likely to mean that a result cannot be obtained. A number of studies had significantly high exclusion rates (up to 50%) among the amblyopic cohorts. Huynh et al failed to achieve a scan result in 15% of children, whereas in the current study only 5% of scans were excluded even though our children were younger than those in the study by Huynh et al.

The significant number of participants recruited to this study in comparison with other studies (Table 1) (with the exception of 1 population-based study) is beneficial in allowing for the statistical analysis of correlated factors.
A drawback of our study is that amblyopic and nonamblyopic groups were not balanced in terms of ethnicity, particularly among the adults. Also, we had limited information about the treatment history of our amblyopic adults, and the age range of children who participated was narrow (Table 2). However, we were able to take the potentially confounding variables of age and ethnicity into account using regression analysis (Table 3, available at http://aaojournal.org). Another drawback is that the researcher who annotated the scans (AB) was aware whether individual scans were from amblyopes or controls, although not which was the amblyopic eye and which was the fellow eye. This introduced a potential source of bias. We do not believe that this affected our findings because the OCT software segmented the layers that the researcher then annotated and because modeling of the foveal morphology only took place once the scans for all participants had been segmented, annotated, and exported to the DataThief software. Thus, we had no knowledge about any emerging pattern of results before full completion of the analysis.

Our processing of the OCT scans to model foveal morphology measures the distance from the ILM to the RPE, producing the foveal thickness measurement that we have shown to be increased in amblyopes. However, we do not know exactly which layer or layers in the retina may be responsible for this increased thickness. Given the centrifugal displacement of the inner retinal cells toward the rim of the fovea pit, the increased foveal thickness is most likely to be formed as a result of changes at the level of the photoreceptors, but we cannot confirm if this is at the level of the inner or outer segments of the photoreceptors.

**Functional Significance of Structural Differences**

It is difficult to know whether the structural differences observed in both eyes of our amblyopic subjects carry any functional significance. Clearly they cannot in themselves be the cause of the visual acuity loss because the 2 eyes of amblyopes are structurally similar and interocular visual acuity differences are required to diagnose unilateral amblyopia. The structure–function relationship is not well established for the fovea. This was highlighted recently by Marmor et al. who described 4 subjects without a foveal pit, the increased foveal thickness is most likely to be formed as a result of changes at the level of the photoreceptors, but we cannot confirm if this is at the level of the inner or outer segments of the photoreceptors.

### References

Footnotes and Financial Disclosures

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