Comparison between anisometropic and strabismic amblyopia using functional magnetic resonance imaging

Mi Young Choi, Kyoung-Min Lee, Jeong-Min Hwang, Dong Gyu Choi, Dong Soo Lee, Ki Ho Park, Young Suk Yu

Abstract

Aims—To assess calcarine activation with functional magnetic resonance imaging (fMRI) in patients with anisometropic and strabismic amblyopia.

Methods—14 amblyopes (eight anisometropic and six strabismic) were studied with fMRI using stimuli of checkerboards of various checker sizes and temporal frequencies. While T2* weighted MRI were obtained every 3 seconds for 6 minutes, patients viewed the stimuli monocularly with either the amblyopic or sound eye.

Results—Amblyopic eyes showed reduced calcarine activation compared with contralateral sound eyes in fMRI in all subjects. The calcarine activation from amblyopic eyes in anisometropic amblyopes was more suppressed at higher spatial frequencies, while that from amblyopic eyes in strabismic amblyopes was more suppressed at lower spatial frequencies.

Conclusion—These results suggest that fMRI is a useful tool for the study of amblyopia in humans. The calcarine activation via amblyopic eyes because of anisometropia or strabismus has different temporospatial characteristics, which suggests differences in the neurophysiological mechanisms between two types of amblyopia.

Ambylopia is defined as abnormally low visual acuity, even with optical refractive correction and in the absence of any obvious retinal or central pathology. Clinical experience indicates that amblyopia is caused by form vision deprivation and/or abnormal binocular interaction in early childhood. Amblyopia can be induced during a critical period in experimental animals by unilateral eyelid suturing, optically induced anisometropia, and surgically induced strabismus. The animal studies showed that the number of visual cortical neurons which can be activated by visual stimulation of the amblyopic eye were reduced, and that shrinkage of cells in layers of the lateral geniculate nucleus connected to the amblyopic eye had occurred.

In an earlier study with human anisometropic and strabismic amblyopia, histological findings of the lateral geniculate nucleus were consistent with those in the animal studies. However, the normal finding of ocular dominance columns in the human cases of anisometropic amblyopia and accommodative esotropia was reported by Horton et al. These findings suggest that ocular dominance columns are probably no longer susceptible to shrinkage at the age after the critical period for amblyopia. Also, they speculated that milder forms of amblyopia may have a different cortical basis from amblyopia produced by early, severe form deprivation.

Recently, cortical activity in amblyopes has been studied with new techniques to understand the neurophysiological mechanism underlying amblyopia. Blood oxygenation level dependent functional magnetic resonance imaging (fMRI) utilizes the focal uncoupling of cerebral blood flow and metabolism to detect focal brain activation. In the previous fMRI studies with normal subjects, visual stimulation produced an increase in the intensity of MRI signals in the primary visual cortex. In the case of amblyopes, there were fewer activated fMRI voxels during amblyopic stimulation than during normal eye stimulation. However, the functional imaging studies in human amblyopes have suggested that striate cortex activity levels remain unchanged, while others draw the opposite conclusion.

The purpose of the present study was to examine ocular findings and calcarine activation in the case of amblyopes, and to evaluate differences in the response patterns between anisometropic and strabismic amblyopes.

Subjects and methods

This study was performed in accordance with our institution’s guidelines and the Declaration of Helsinki, and the protocols were approved by the institutional review board of the Seoul National University Hospital.

This study involved amblyopic subjects, having best corrected visual acuities in the sound eye of 6/6 or better, and 6/18 or less in the amblyopic eye, with no history of ophthalmological or systemic diseases. In the group of anisometropic amblyopias, patients with anisometropia, defined as a spherical difference between the two eyes of 3.0 dioptres or more, and with no heterotropia in the alternate cover test were included. Strabismic amblyopes had esotropia or exotropia of at least 15 prism dioptres in the alternate cover test and a spherical difference between the two eyes of less than 0.5 dioptre. All subjects gave written informed consent for the procedure after the purposes, risks, and benefits of this study were explained.

Subjects were positioned supine in the magnet, and silicone cups were placed over the ears.
Table 1 The ophthalmological findings of nine amblyopes

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Sex</th>
<th>Strabismus, amount (PD)</th>
<th>BCVA (Refractive error based on cycloplegic refraction)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>M</td>
<td>Eso, 50</td>
<td>6/6 +0.50Ds = +0.50Dc A90</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>M</td>
<td>Eso, 16</td>
<td>6/6 +0.25Ds = +0.25Dc A90 6/60 +0.75Ds = +0.50Dc A90</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>F</td>
<td>Eso, 35</td>
<td>6/6 +0.25Ds = +0.25Dc A90 6/60 +0.25Ds = +0.50Dc 90A</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>F</td>
<td>Eso, 25</td>
<td>6/6 +1.00Dm = +0.50Dc A180 6/45 +1.00Dm = +0.50Dc A180</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>F</td>
<td>Ortho</td>
<td>6/6 +0.50Ds = +0.25Dc A90 6/60 +0.25Ds = +0.75Dc A90</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>M</td>
<td>Ortho</td>
<td>6/6 +0.25Ds = +0.25Dc A90 6/30 +1.00Dc</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>M</td>
<td>Ortho</td>
<td>6/6 +0.25Ds = +0.25Dc A90 6/60 +0.25Ds = +0.75Dc A90</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>M</td>
<td>Ortho</td>
<td>6/6 +0.25Ds = +0.50Dc A90 6/18 +3.75Ds = +1.00Dc A90</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>F</td>
<td>Ortho</td>
<td>6/45 +1.50Ds = +0.50Dc</td>
</tr>
</tbody>
</table>

PD = prism dioptre, BCVA = best corrected visual acuity, Eso = esotropia, Eexo = exotropia, Ortho = orthotropia, Ds = spherical lens dioptre, Dc = cylinder lens dioptre

using a gradient EPI sequence (surface coil on the occiput, TR 3 seconds, TE 60 ms, FA 90, FOV 24 × 24 cm, matrix size of 128 × 128, 15 slices 3 mm thick). The total time for one fMRI experiment was 6.0 minutes. The images were reconstructed off-line, exported to volumes, and aligned for movement correction using an AIR3.0 package. Gaussian filtering was performed to enhance signal to noise ratio for a biologically appropriate signal, other than intrinsic filtering embedded during reconstruction of MRI form K-space data.

During each trial, fMRI scan was also performed on the sound eye during monocular viewing with a plus lens to produce optical defocus with the same level of visual acuity as the amblyopic eye at a 6 metre viewing distance. A checkerboard frequency of 8 Hz was used as a visual stimulus, because this rate had been previously found to maximise cerebral blood flow and minimise the scan time.

Sagittal slices from a conventional MRI were used to identify the plane of the calcarine fissure bilaterally. We derived the visually activated state after the region of interest was determined around the calcarine sulcus according to anatomical T1 weighted images obtained simultaneously with the functional data. Postprocessing was performed on a SPM96 package (Statistical Parametric Mapping version, Wellcome Department of Cognitive Neurology, University College London). Changes in calcarine activation induced by visual stimuli were identified and comparisons were performed between the basal and activated states.

The statistical significance of the differences was evaluated using a group t test for each voxel in the region of interest. We compared the results of fMRI for amblyopic and sound eyes of all amblyopes. Also, we evaluated the differences in the response patterns between anisometropic and strabismic amblyopes.

Results

Fourteen subjects ranged in age from 5 to 32 years. Eight had anisometropic amblyopia (anisohypermetropic amblyopia in seven, anisomypic amblyopia in one) and the range of the differences in spherical equivalents of two eyes was 3.5–8.3 dioptres. Six had strabismic amblyopia (esotropia in four, exotropia in two); the range of the deviation was 16–50 prism dioptres. No abnormalities in the ERG of amblyopic eyes were found, and no differences in the ERG responses of the amblyopic and sound eye were found in any subject.

We excluded five subjects who showed significant movement artefacts in the fMRI results, in spite of the attempt to correct for them. Therefore, four anisometropic and five strabismic amblyopes were analysed. Data collected during the study, including visual acuity and refractive status are summarised in Table 1.

The calcarine activation in amblyopic eyes, either due to anisometropia or strabismus, showed a reduced response compared with the sound fellow eyes (Fig 1). There was statistical significance at 0.25–1° in anisometropic amblyopes and at 0.5–2° in strabismic amblyopes.
In amblyopes, optical blur of the sound eyes reduced calcarine activation, but there was no statistically significant difference induced by optical blur (p > 0.05, Fig 2, Table 2). The trend towards a reduction was seen in the variable temporal frequency, especially in anisometropic amblyopia, and this finding was statistically significant at 4 and 8 Hz (p < 0.05, Fig 2, Table 2).

**Discussion**

Cortical activity in human amblyopia has recently been reported using new techniques. Demer *et al* employed positron emission tomography (PET) for amblyopic adults and control subjects. They found that activity in the primary visual cortex of amblyopes was 5–6% less during the stimulation of the amblyopic eye than during the stimulation of the sound eye. Kabasakal *et al* showed the visual cortex response of the amblyopic eye to light stimulation was severely reduced when compared with the sound eye, in an evaluation using brain single photon emission computed tomography. These results were verified by our study.

Our data demonstrate that the fMRI signal changes in the calcarine activation were, in general, smaller when viewed with amblyopic eyes than with sound eyes, in all amblyopes. These results probably reflect that amblyopia has an associated abnormality in or before the calcarine cortex. However, the lack of formed images on the retina of the amblyopic eye could lead to profound defects in calcarine activation. To exclude this possibility, we investigated the calcarine activation during viewing by an amblyopic eye that was simulated by

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**Table 2**  p Value of percentage of functional MRI signal between amblyopic eye or defocused eye and sound eye in amblyopes

<table>
<thead>
<tr>
<th></th>
<th>Spatial frequency (cycles/degree)</th>
<th>Temporal frequency (Hz)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Anisometropic amblyopia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amb and sound</td>
<td>0.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Defocused and sound</td>
<td>0.37</td>
<td>0.43</td>
</tr>
<tr>
<td>Strabismic amblyopia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amb and sound</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Defocused and sound</td>
<td>0.11</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Amb and sound = p value between amblyopic eyes and sound eyes. Defocused and sound = p value between sound eyes defocused with optical blur and sound eyes.

**Figure 2** The average percentage change of functional MRI signal in anisometropic and strabismic amblyopias. Solid circles represent the sound eyes, open circles show the defocused response in sound eyes, and rectangles represent amblyopic eyes. Asterisks mean that the difference between the two responses was statistically significant (p<0.05).
optically blurring the sound eye to reduce visual acuity to a comparable level. Monocular stimulation of the sound eye showed a reduced calcarine activation during viewing with optic blur; however, there was no statistical significance whether the condition was blurred or not. This finding is different from the previous report that optic blur reduced activity in primary visual cortex by about 8% in control subjects.\(^{14}\)

In the previous study using PET, the cortical response evoked by the amblyopic eye was significantly reduced in the ipsilateral Brodmann areas 18, 19 with no significant change in the Brodmann area 17 in the cases of strabismic amblyopia.\(^{22}\) These findings are different from those of the present study that anisometropic as well as strabismic amblyopes show a reduced fMRI response in calcarine cortex.

Reports of the effects of spatial frequency or temporal frequency on visual perception have been mixed. Cascairo et al reported that the contrast visual acuity in both anisometropic and strabismic amblyopes was reduced for the amblyopic eye at higher contrast levels.\(^{30}\) On the other hand, others have demonstrated that strabismic amblyopes had abnormalities only at the higher spatial frequency, whereas anisometropic amblyopes were found to have an abnormal function in both the low and high spatial frequency ranges.\(^{27–29}\) The present study evaluated calcarine activation according to variable frequency, showing statistical significance at 0.25–1° in anisometropic amblyopes and at 0.5–2° in strabismic amblyopes.

In terms of the temporal frequency, the regional cerebral blood flow response peaked at 7–8 Hz in normal subjects.\(^{10}\) We observed a significant difference at 4 and 8 Hz in anisometropic amblyopes, while there was no significant difference at any level of temporal frequency in the calcarine activation of strabismic amblyopes. The reason for the discrepancy between the two types of amblyopes is unclear and requires further investigation focusing on the change in properties of visual stimuli.

Anderson et al reported that the extent of the deficit was not correlated with either Snellen acuity or contrast sensitivity by magnetoencephalography.\(^{11}\) In contrast, functional activation of the calcarine cortex was reduced in amblyopic eyes compared with sound eyes to a degree more closely correlated with visual acuity than were visual evoked potential amplitude to the same stimulus.\(^{12}\) Unfortunately, the statistical analysis for these data could not be performed in this study because of the limited number of subjects.

In this study, five of 14 subjects were excluded because of movement artefacts. As could be expected, children could not cooperate as well as adults. This is the reason why a third of subjects could not be adequately studied in the study using functional MRI. Similarly, six of 17 subjects were excluded in another fMRI study.\(^{23}\)

Several limitations in the present study should be noted. One limitation of this study is the wide range in age of the recruited subjects. Although age related changes in calcarine activation have yet to be established by fMRI study, the previous imaging studies demonstrated age related differences in calcarine activation during visual perception.\(^{23–33}\) Therefore, the age related difference should be considered in evaluating calcarine activation. However, we did not have age matched control subjects, because inasubject comparison between normal eye and amblyopic eye was the main focus in the present study. In the future, a controlled comparison with age matched control subjects with normal visual acuity may be warranted. A second limitation is that this study involved a small patient population. Including more amblyopes in a further study would be extremely helpful in investigating the calcarine activation in amblyopia. Lastly, the scanned volume in this study was limited near the occipital lobe because of the use of surface coil to obtain maximum resolution at the pericalcarine areas. Therefore, extrastrate areas and the area anterior to them were poorly visualised for further analysis.

In conclusion, the decreased calcarine activation obtained from the amblyopic eyes relative to the sound eyes in amblyopic patients, and calcarine activation via amblyopic eyes due to anisometropia and strabismus show different temporo-spatial characteristics. These findings suggest that the calcarine activation is abnormal in both types of amblyopia and that the neurophysiological mechanisms, which underlie the development of these conditions are quite different.

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