

# 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.

(Br J Ophthalmol 2001;85:1052-1056)

Department of  
Ophthalmology,  
College of Medicine,  
Chungbuk National  
University, Korea  
M Y Choi

Department of  
Neurology  
K-M Lee

Department of  
Ophthalmology,  
College of Medicine,  
Seoul National  
University and Clinical  
Research Institute,  
Seoul National  
University Hospital,  
Korea  
J-M Hwang  
K H Park  
Y S Yu

Department of  
Ophthalmology,  
College of Medicine,  
Hallym University,  
Korea  
D G Choi

Department of  
Nuclear Medicine,  
College of Medicine,  
Seoul National  
University, Korea  
D S Lee

Correspondence to:  
Young Suk Yu, MD,  
Department of  
Ophthalmology, College of  
Medicine, Seoul National  
University, 28  
Yeongun-Dong,  
Chongro-Ku, Seoul 110-744,  
Korea  
ysyu@snu.ac.kr

Accepted for publication  
28 March 2001

esotropia was reported by Horton *et al.*<sup>9 10</sup> These findings suggest that ocular dominance columns are probably no longer susceptible to shrinkage at the age after the critical period for amblyopia.<sup>9</sup> Also, they speculated that milder forms of amblyopia may have a different cortical basis from amblyopia produced by early, severe form deprivation.<sup>9</sup>

Recently, cortical activity in amblyopes has been studied with new techniques to understand the neurophysiological mechanism underlying amblyopia.<sup>11-16</sup> Blood oxygenation level dependent functional magnetic resonance imaging (fMRI) utilises the focal uncoupling of cerebral blood flow and metabolism to detect focal brain activation.<sup>17-23</sup> In the previous fMRI studies with normal subjects, visual stimulation produced an increase in the intensity of MRI signals in the primary visual cortex.<sup>17-22</sup> In the case of amblyopes, there were fewer activated fMRI voxels during amblyopic stimulation than during normal eye stimulation.<sup>11</sup> However, the functional imaging studies in human amblyopes have suggested that striate cortex activity levels remain unchanged,<sup>12 17</sup> while others draw the opposite conclusion.<sup>13 24 25</sup>

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

Amblyopia is defined as abnormally low visual acuity, even with optical refractive correction and in the absence of any obvious retinal or central pathology.<sup>1</sup> 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.<sup>2-6</sup> 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.<sup>2-5</sup>

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.<sup>7 8</sup> However, the normal finding of ocular dominance columns in the human cases of anisometropic amblyopia and accommodative

Table 1 The ophthalmological findings of nine amblyopes

No	Age	Sex	Strabismus, amount (PD)	BCVA (Refractive error based on cycloplegic refraction)	
				Normal eyes	Amblyopic eyes
1	5	M	Eso, 50	6/6 +0.50Ds	6/120 +0.50Ds = +0.50Dc A90
2	8	M	Eso, 16	6/6 +0.25Ds = +0.25Dc A90	6/30 +0.75Ds = +0.50Dc A90
3	23	F	Exo, 35	6/4. (emmetropia)	6/60 +0.25Dc, 90A
4	23	F	Eso, 25	6/6 +1.00Ds = +0.50Dc A180	6/45 +1.00Ds = +0.50Dc A180
5	6	F	Ortho	6/6 +0.50Ds	6/60 +4.25Ds = +0.75Dc A90
6	8	M	Ortho	6/6 -0.25Ds	6/30 -10.00Ds
7	9	M	Ortho	6/6 +0.25Ds	6/45 +5.75Ds = +2.00Dc A180
8	9	M	Ortho	6/6 +0.25Ds = +0.50Dc A90	6/18 +3.75Ds = +1.00Dc A90
9	15	F	Ortho	6/45 +1.50Ds	6/30 +4.50Ds

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

to reduce the gradient noise. To minimise non-visual cerebral activity, subjects were asked to lie without any movement. During the study, the subject viewed stimuli through prism glasses on a screen positioned at the subject's feet. The visual stimuli were black and white checkerboard patterns at spatial frequencies of 0.25, 0.5, 1, and 2 cycles/degree of visual angle (that is, individual check sizes of 2, 1, 0.5, 0.25 degrees of visual angle, respectively). Each subject received alternating stimuli at temporal frequencies of 2, 4, 8, and 16 Hz. During monocularly applied visual stimuli, fMRI scans were set to use T2\* weighted images at 1.5T

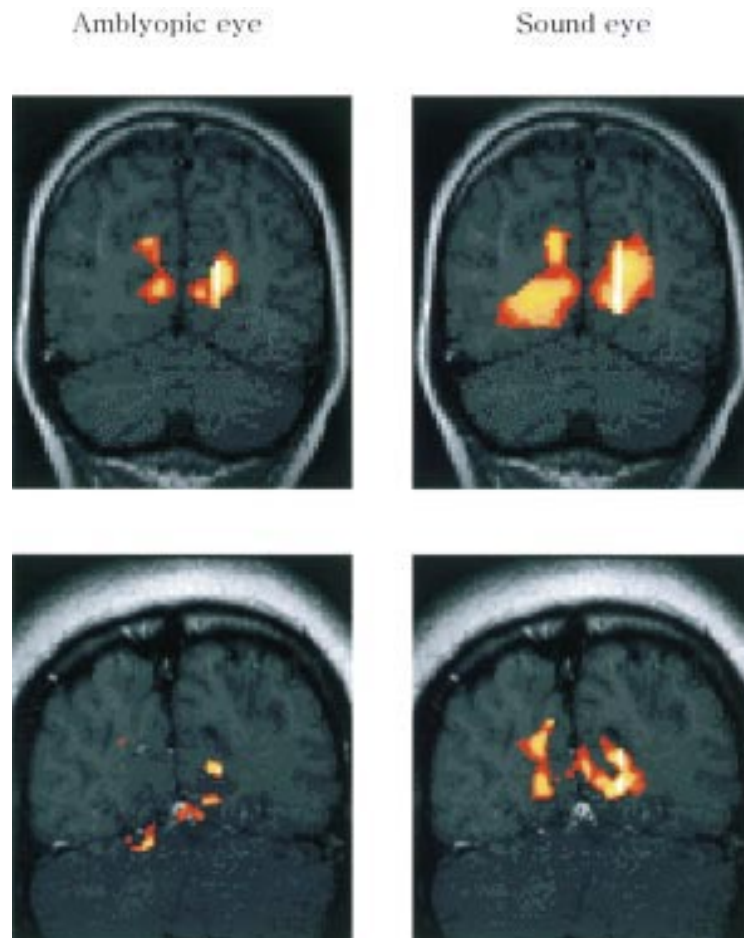


Figure 1 Examples of calcarine activation are shown for an anisometropic patient (upper) and a strabismic patient (lower), in which the activation by amblyopic eyes was found to be significantly less than that for the sound eyes. Images are vertically aligned along the calcarine fissures with occipital lobes. During visual stimulation, local increases in signal intensity were detected in the medial-posterior regions of the occipital lobes along the calcarine fissures. Yellow coloured regions represent a more activated state than the red coloured regions.

using a gradient echo 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 from 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 1996 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 (aniso-hypermetropic amblyopia in seven, anisomyopic 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

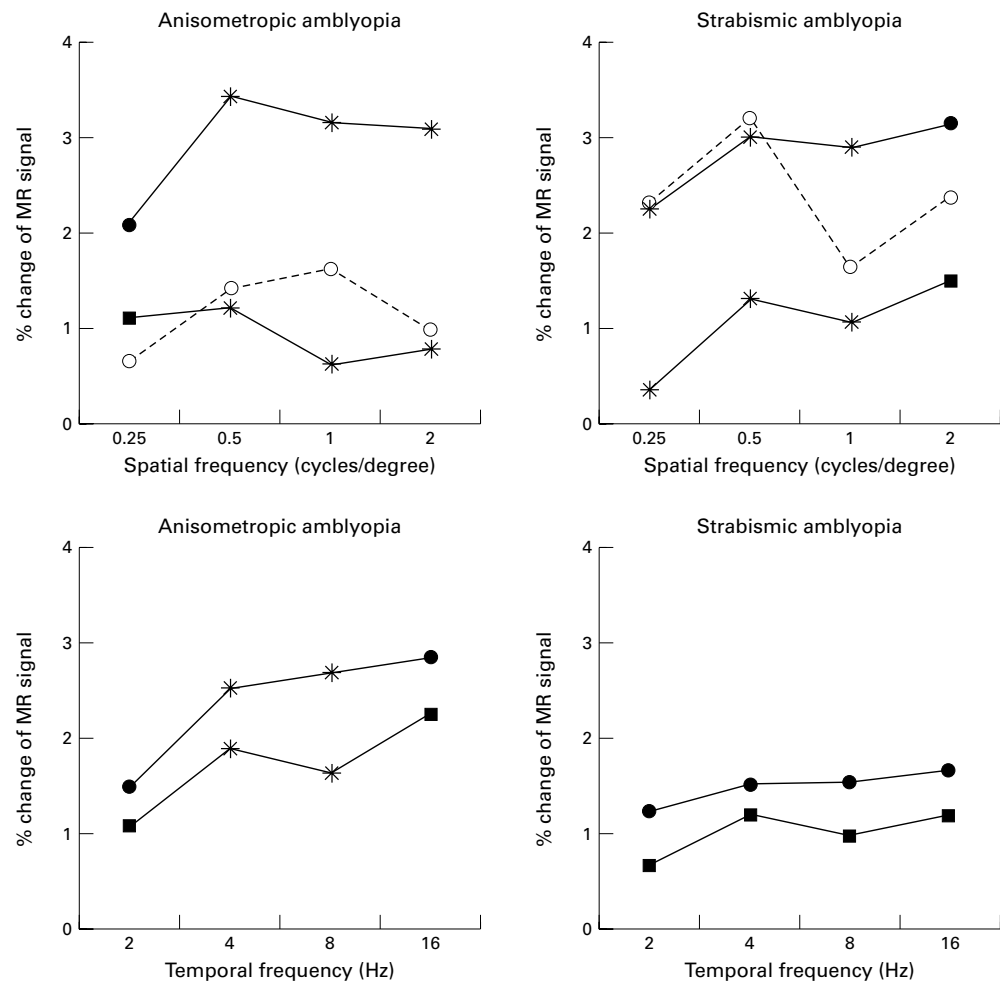


Figure 2 The average percentage change of functional MRI signal in anisometropic and strabismic amblyopias. Solid circles represent the sound eyes, open circles show the defocused response in sound eyes, and rectangles represent amblyopic eyes. Asterisks mean that the difference between the two responses was statistically significant ( $p < 0.05$ ).

( $p < 0.05$ , Fig 2, Table 2). 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).

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

		Spatial frequency (cycles/degree)			
		0.25	0.5	1	2
Anisometropic amblyopia	Amb and sound	0.12	0.01	0.01	0.01
	Defocused and sound	0.37	0.43	0.07	0.45
Strabismic amblyopia	Amb and sound	0.03	0.01	0.01	0.08
	Defocused and sound	0.11	0.08	0.32	0.24
		Temporal frequency (Hz)			
		2	4	8	16
Anisometropic amblyopia	Amb and sound	0.06	0.02	0.02	0.06
Strabismic amblyopia	Amb and sound	0.17	0.24	0.24	0.28

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.

## 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.<sup>14</sup> 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.<sup>14</sup> 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.<sup>15</sup> 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

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.<sup>14</sup>

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.<sup>12</sup> 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.<sup>26</sup> 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.<sup>27–29</sup> 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.<sup>30–31</sup> 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.<sup>13</sup> 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.<sup>32</sup> 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.<sup>23</sup>

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 cortical activation during visual perception.<sup>23–33</sup> Therefore, the age related difference should be considered in evaluating calcarine activation. However, we did not have age matched control subjects, because intrasubject 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, extrastriate 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 temporospatial 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.

Supported in part by a grant from the Seoul National University Hospital Research Fund (03-98-050).

Presented in part at the annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, FL, 9 May 1999.

- 1 Von Noorden GK. Mechanisms of amblyopia. *Adv Ophthalmol* 1977;34:93–115.
- 2 Hendrickson AE, Movshon JA, Eggers HM, *et al*. Effects of early unilateral blur on the macaque's visual system. II. Anatomical observations. *J Neurosci* 1987;7:1327–39.
- 3 Von Noorden GK, Middleditch PR. Histology of the monkey lateral geniculate nucleus after unilateral lid closure and experimental strabismus: further observations. *Invest Ophthalmol Vis Sci* 1975;14:674–83.
- 4 Headon MP, Powell TPS. Cellular changes in the lateral geniculate nucleus of infant monkeys after suture of the eyelids. *J Anat* 1973;116:135–45.
- 5 Von Noorden GK. Histological studies of the visual system in monkeys with experimental amblyopia. *Invest Ophthalmol Vis Sci* 1973;12:727–38.
- 6 Kiorpes L, Kiper DC, O'Keefe LP, *et al*. Neuronal correlates of amblyopia in the visual cortex of macaque monkeys with experimental strabismus and anisometropia. *J Neurosci* 1998;18:6411–24.
- 7 Von Noorden GK, Crawford MLJ, Levacy RA. The lateral geniculate nucleus in human anisometropia amblyopia. *Invest Ophthalmol Vis Sci* 1983;24:788–90.
- 8 Von Noorden GK, Crawford MLJ. The lateral geniculate nucleus in human strabismic amblyopia. *Invest Ophthalmol Vis Sci* 1992;33:2729–32.
- 9 Horton JC, Stryker MP. Amblyopia induced by anisometropia without shrinkage of ocular dominance columns in human striate cortex. *Proc Natl Acad Sci USA* 1993;90:5494–8.
- 10 Horton JC, Hocking DR. Pattern of ocular dominance columns in human striate cortex in strabismic amblyopia. *Vis Neurosci* 1996;13:787–95.
- 11 Goodyear BG, Nicolle DA, Humphrey GK, *et al*. fMRI response of early visual areas to perceived contrast in human amblyopia. *J Neurophysiol* 2000;84:1907–13.
- 12 Imamura K, Richter H, Fischer H, *et al*. Reduced activity in the extrastriate visual cortex of individuals with strabismic amblyopia. *Neurosci Lett* 1997;225:173–6.
- 13 Anderson SJ, Holliday IE, Harding GF. Assessment of cortical dysfunction in human strabismic amblyopia using magnetoencephalography. *Vis Res* 1999;39:1723–38.
- 14 Demer JL, von Noorden GK, Volkow ND, *et al*. Brain activity in amblyopia. *Am Orthopt J* 1991;41:56–66.

- 15 Kabasakal L, Devranoglu K, Arslan O, et al. Brain SPECT evaluation of the visual cortex in amblyopia. *J Nucl Med* 1995;36:1170-4.
- 16 Norcia AM, Harrad RA, Brown RJ. Changes in cortical activity during suppression in stereoblindness. *Neuroreport* 2000;11:1007-12.
- 17 Sireteanu N, Tonhausen N, Muckli L, et al. Cortical site of the amblyopic deficit in strabismic and anisometric subjects, investigated with fMRI. [ARVO Abstract] *Invest Ophthalmol Vis Sci* 1998;39:S909 (Abstract no 4186).
- 18 Blamire AM, Ogawa S, Ugurbil K, et al. Dynamic mapping of the human visual cortex by high-speed magnetic resonance imaging. *Proc Natl Acad Sci USA* 1992;89:11069-73.
- 19 Ogawa S, Tank DW, Menon R, et al. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci USA* 1992; 89:5951-5.
- 20 Schneider W, Noll DC, Cohen JD. Functional topographic mapping of the cortical ribbon in human vision with conventional MRI scanners. *Nature* 1993;365:150-3.
- 21 Belliveau JW, Kennedy DN, McKinstry RC, et al. Functional mapping of the human visual cortex by magnetic resonance imaging. *Science* 1991;254:716-9.
- 22 Sereno MI, Dale AM, Reppas JB, et al. Borders of multiple visual areas in humans revealed by functional magnetic resonance imaging. *Science* 1995;268:889-93.
- 23 Born P, Leth H, Miranda MJ, et al. Visual activation in infants and young children studied by functional magnetic resonance imaging. *Pediatr Res* 1998;44:578-83.
- 24 Demer JL, von Noorden GK, Volkow ND, et al. Imaging of cerebral blood flow and metabolism in amblyopia by positron emission tomography. *Am J Ophthalmol* 1988;105:337-47.
- 25 Barns GR, Hess RF, Pettre V, et al. FMRI responses to sinusoidal grating stimuli in strabismic amblyopes. [ARVO Abstract] *Invest Ophthalmol Vis Sci* 1999;40:S644 (Abstract no 3391).
- 26 Cascairo MA, Mazow ML, Holladay JT, et al. Contrast visual acuity in treated amblyopia. *Binoc Vis Qtrly* 1997;12:167-74.
- 27 Campos EC, Prampolini ML, Gulli R. Contrast sensitivity differences between strabismic and anisometric amblyopia: objective correlate by means of visual evoked response. *Doc Ophthalmol* 1984;58:45-50.
- 28 Sjostrand J. Contrast sensitivity in children with strabismic and anisometric amblyopia. A study of the effect of treatment. *Acta Ophthalmol* 1981;59:25-34.
- 29 Loeffler M, Wise JS, Gans M. Contrast sensitivity letter charts as a test of visual function in amblyopia. *J Pediatr Ophthalmol Strabismus* 1990;27:28-31.
- 30 Mentis MJ, Alexander GE, Grady CL, et al. Frequency variation of a pattern-flash visual stimulus during PET differentially activates brain from striate through frontal cortex. *Neuroimage* 1997;5:116-28.
- 31 Fox PT, Raichle ME. Stimulus rate dependence of regional cerebral blood flow in human striate cortex, demonstrated by positron emission tomography. *J Neurophysiol* 1984;51:1109-20.
- 32 Demer JL, Grafton S, Marg E, et al. Positron-emission tomographic study of human amblyopia with use of defined visual stimuli. *J AAPOS* 1997;1:158-71.
- 33 Levine BK, Beason-Held LL, Purpura KP, et al. Age-related differences in visual perception: a PET study. *Neurobiol Aging* 2000;21:577-84.