Detection of Abnormal Visual Cortex in Children With Amblyopia by Voxel-Based Morphometry

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- PURPOSE: To detect the abnormalities of gray matter in children with amblyopia by voxel-based morphometry (VBM).
- DESIGN: Prospective, nonrandomized clinical trial.
- METHODS: Thirteen children with amblyopia and 14 normally sighted children underwent magnetic resonance (MR) examination. The two groups were age-matched with a mean age of 5.8 years. In the amblyopia group, five children had strabismus amblyopia, and eight had anisometropic amblyopia. We analyzed the original 3-dimensional T1 brain images using the VBM module within the widely used analysis software package SPM2 (Welcome Department of Cognitive Neurology, London, United Kingdom). After normalization, segmentation, and smoothing of the images, comparison between amblyopic and control groups was derived for the gray matter of the entire brain using parametric statistics.
- RESULTS: The results of VBM analysis indicated that the amblyopic group had decreased gray matter density in the middle frontal gyrus, parahippocampal gyrus, fusiform gyrus, inferior temporal gyrus of the left hemisphere, and the bilateral calcarine cortices. The radii of these regions ranged from 12 to 36 voxels. These abnormalities were consistent with morphologic changes in brain regions related to visual function.
- CONCLUSIONS: Using MR and VBM analysis, we detected morphologic changes in the visual cortex of children with amblyopia, which may indicate developmental abnormalities of visual cortex during the critical growth period. (Am J Ophthalmol 2007;143:489–493. © 2007 by Elsevier Inc. All rights reserved.)

The neural basis of human amblyopia represents the role of early experience on the structure and function of the human brain. Neurophysiologic studies have provided a great deal of evidence regarding the functional effects of vision deprivation on the visual cortex.1–3 Primary visual cortex was considered as the principal site of vision deficit, whereas some extrastriate cortex was also found to be responsible for abnormalities of special visual function.4–6 However, neuroanatomic investigations for these abnormalities remain limited in human subjects, especially in children with amblyopia. Until recently, a voxel-based morphometry (VBM) study that focused on the cortical change was addressed, indicating that adults and children with amblyopia have decreased gray matter volume in visual cortical regions.7 VBM is an approach that gives a even-handed and comprehensive assessment of anatomic differences throughout the whole brain. It involves a voxel-wise comparison of the local concentration of gray matter between two groups of subjects.8 Because it is an automated analysis technique, VBM has been widely used to identify abnormal anatomy in some neurodegenerative diseases.9 However, one caveat is that the VBM tools use an adult brain template to reach a solution during spatial normalization, so this could be a source of error for comparing measurements in children. In the VBM study of children with amblyopia, no subject younger than 7 years of age was included, and the mean age of the subjects was approximately 10 years.7 However, they were relatively older in comparison to the critical period of the visual system, which is around the onset age of stereopsis. In this study, we tried to use VBM to investigate the morphologic change of gray matter in young children with amblyopia and to identify the developmental abnormalities related to amblyopia.

METHODS

- SUBJECTS: Fourteen pediatric patients with amblyopia (six males, eight females; age range, 4 to 8 years; mean age,
5.8 years) and 14 normally sighted children (10 males, four females; age range, 3.5 to 9 years; mean age, 5.8 years) were recruited in this study. Informed consent for participation was obtained from every subject’s parents, and the ethics committee at Beijing University approved all study protocols. The children with amblyopia were recruited by physician referral from the pediatric ophthalmology service at Peking University First Hospital. First, the children with vision problems completed an ophthalmologic exam that included tests of ocular motility, dilation, fundus exam, autorefraction, and visual-evoked potentials. After the diagnosis of amblyopia was confirmed, they were referred to magnetic resonance (MR) scanning prior to amblyopia treatment. Patients with a known organic brain disorder or with specific clinical evidence of neurologic dysfunction were excluded from this series. Five children had strabismus amblyopia and nine had anisometropic amblyopia. Their results were summarized in Table 1. Controls were recruited from the children who underwent magnetic resonance imaging (MRI) examination for other purposes unrelated to vision problems. Four of them were volunteers, five of them were referred to MR scanning because of headache, two children were scanned for trauma, one child was scanned for dwarfism, and the other two were scanned for febrile convulsions. They were confirmed to have normal visual acuity and no neurologic conditions. The two groups were matched for age.

**DATA PROCESSING:** For gray matter analysis, 3-dimensional SPGR images were processed with the method of standard VBM using SPM2 (Welcome Department of Cognitive Neurology, London, United Kingdom). Because the adult brain template provided by SPM2 is different from our subjects in the aspects of brain configuration and image contrast, we built our own template based on the data sets from all subjects for spatial normalization. An affine and a nonlinear spatial normalization of the raw MRI sets were performed, and they were segmented to extract the gray matter tissue. The extracted gray matter sets across subjects to the overall grand mean. The threshold used for selection of voxels was 40% of the grand mean value. In addition, the proportional scaling routine was used, which normalized the gray matter sets across subjects to the overall grand mean. This ancillary analysis was meant to assess the relative distribution of gray matter reduction in amblyopes compared to controls. Implicit masking was used to ignore zeros, and

### TABLE 1. Information of Amblyopic Subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Amblyopia</th>
<th>Distance Acuity (logMAR)</th>
<th>Refractive Error</th>
<th>Eye Deviation, Arc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Child 1</td>
<td>Anisometropia</td>
<td>0.6</td>
<td>1.0</td>
<td>+3.50</td>
</tr>
<tr>
<td>Child 2</td>
<td>Strabismic</td>
<td>1.2</td>
<td>0.1</td>
<td>+1.50</td>
</tr>
<tr>
<td>Child 3</td>
<td>Strabismic</td>
<td>0.1</td>
<td>0.5</td>
<td>+9.00</td>
</tr>
<tr>
<td>Child 4</td>
<td>Strabismic</td>
<td>0.2</td>
<td>1.0</td>
<td>+6.50</td>
</tr>
<tr>
<td>Child 5</td>
<td>Anisometropia</td>
<td>0.6</td>
<td>1.0</td>
<td>+1.00</td>
</tr>
<tr>
<td>Child 6</td>
<td>Anisometropia</td>
<td>0.4</td>
<td>0.5</td>
<td>+5.00</td>
</tr>
<tr>
<td>Child 7</td>
<td>Anisometropia</td>
<td>1.0</td>
<td>0.7</td>
<td>+6.00</td>
</tr>
<tr>
<td>Child 8</td>
<td>Anisometropia</td>
<td>0.7</td>
<td>0.7</td>
<td>+2.50</td>
</tr>
<tr>
<td>Child 9</td>
<td>Strabismic</td>
<td>0.4</td>
<td>1.0</td>
<td>+3.50</td>
</tr>
<tr>
<td>Child 10</td>
<td>Anisometropia</td>
<td>0.4</td>
<td>0.4</td>
<td>+0.50</td>
</tr>
<tr>
<td>Child 11</td>
<td>Anisometropia</td>
<td>1.0</td>
<td>0.1</td>
<td>+1.50</td>
</tr>
<tr>
<td>Child 12*</td>
<td>Anisometropia</td>
<td>0.6</td>
<td>0.6</td>
<td>+0.50</td>
</tr>
<tr>
<td>Child 13</td>
<td>Strabismic</td>
<td>0.3</td>
<td>1.0</td>
<td>+5.25</td>
</tr>
<tr>
<td>Child 14</td>
<td>Anisometropia</td>
<td>0.1</td>
<td>1.0</td>
<td>+5.00</td>
</tr>
</tbody>
</table>

*This child’s data were excluded because of motion artifact contamination.
RESULTS

THERE WAS NO STATISTICAL DIFFERENCE IN AGE BETWEEN the two groups (5.8 years ± 1.4 for children with amblyo-

opia, 5.8 years ± 1.9 for controls). The amblyopic group consisted of five children with strabismus amblyopia and eight children with anisometropic amblyopia (clinical data are shown in Table 1).

The statistical parametric map showed several voxel clusters of reductions in gray matter volume in the amblyo-

pic group relative to controls. Areas of significant gray matter reductions were seen in the following distinct neocortical regions: middle frontal gyrus, parahippocampal gyrus, fusiform gyrus, inferior temporal gyrus of the left hemisphere, and the bilateral calcarine cortices (Table 2). They were all significant at the uncorrected $P < .001$ cluster level. After correction for multiple comparisons, these clusters remained significant at the level of $P < .05$ (Figures 1 and 2).

DISCUSSION

NEUROPHYSIOLOGIC STUDIES IN THESE YEARS HAVE proved that primary visual cortex is the principal site for the visual loss, yet there are definitely additional deficits at higher levels of the visual pathways. As an automatic way to evaluate differences in brain morphology between pa-

tient groups, VBM provides voxel-wise estimates of inter-

group differences in gray matter volume in a standardized space.8 With this method, we detected significant reduc-

tion of gray matter density in regions of visual cortex, which is consistent with previous studies.7,11 Histologic studies conducted on animal models have revealed the

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**TABLE 2.** Regions of Significantly Reduced Gray Matter Volume in the Brains of Children With Amblyopia Relative to Age-Matched Controls Revealed by Voxel-Based Morphometry

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Number of Voxels</th>
<th>$P$ value of Cluster Level (Corrected)</th>
<th>Coordinates X, Y, Z</th>
<th>Peak Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral calcarine cortices</td>
<td>14</td>
<td>0.042</td>
<td>-1, -37, 8</td>
<td>3.24</td>
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<tr>
<td>Left parahippocampal gyrus and fusiform gyrus</td>
<td>36</td>
<td>0.035</td>
<td>-25, -2, -24</td>
<td>3.80</td>
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<tr>
<td>Left inferior temporal gyrus</td>
<td>12</td>
<td>0.027</td>
<td>-60, -25, -24</td>
<td>3.31</td>
</tr>
<tr>
<td>Left middle frontal gyrus</td>
<td>16</td>
<td>0.029</td>
<td>-45, 33, 23</td>
<td>3.68</td>
</tr>
</tbody>
</table>

Children with amblyopia, n = 13; age-matched controls, n = 14.

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FIGURE 1. Voxel-based morphometry (VBM) revealed the foci of reduced gray matter volume in visual cortex of children with amblyopia relative to normal subjects. Left parahippocampal gyrus and fusiform gyrus (arrowhead), left middle frontal gyrus (black arrow), left inferior temporal gyrus (red arrow).

FIGURE 2. Voxel-based morphometry (VBM) revealed the significant cluster of reduced gray matter volume in bilateral calcarine cortices of children with amblyopia (crossed line).

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global calculation was based on the mean voxel value.10 Voxel-by-voxel between-group comparisons of gray matter density were investigated at a statistical threshold of $T > 3.42$ ($P < .001$, uncorrected for multiple comparisons) using the Student t test.
effects of visual deprivation on the ocular dominance stripes in layer IVc of the striated cortex.12 Yet direct evidence from human amblyopia concerning neuroanatomic changes remains unavailable. By using VBM, our data revealed the morphologic changes of the primary visual cortex in children with amblyopia, which might be related to the underlying histologic changes.

The fusiform gyrus and inferior temporal gyrus may correspond to the visual areas V4, which have been associated with analysis of form and color.13 The higher visual functions, such as shape discrimination, pattern perception and feature detection, were proved to be abnormal in patients with amblyopia.14,15 However, these visual deficits cannot be simply attributed to neural undersampling or disarray in primary visual cortex; higher visual cortical areas are likely to be involved in the neural dysfunction. A recent study of functional magnetic resonance imaging (fMRI) showed that the ventral temporal cortex is very poorly activated when adults with amblyopia view face stimuli with their weak eye, despite normal activation in other conditions.16 The prominent gray matter loss in the ventral temporal cortex of amblyopic children may provide neuroanatomic evidence of ventral visual pathway impairment.

An enigmatic finding in our results is the reduction of gray matter density in the middle frontal gyrus, which is consistent with the location of frontal eye fields (FEFs). Among the visual output of primary visual cortex, the FEFs are also reached. The FEFs triggers intentional saccades to visual targets in the environment, to target locations, or to the location where it is predicted that the target will appear.17 The abnormality of FEFs seemed to be related to the presence of strabismic amblyopia; however, the gray matter volume in the FEFs was found to be reduced in our amblyopic group, whereas it was increased in the previous report of Chan and associates.11 The investigators argued that the greater gray matter volume in FEFs was compatible with a hypothesis of plasticity in the oculomotor regions to compensate for the cortical deficits in the visual processing areas. Perhaps the age difference between the two studies induced the emergence of significant discrepancy. It will be interesting to further investigate the process of neuroplasticity with respect to age.

It is perhaps of note that our significant results were mostly gathered in the left hemisphere, except for regions of calcarine cortex. The cluster of calcarine cortex was located in the middle of two hemispheres and extended into both sides, so we believed that it represented abnormalities of bilateral calcarine cortices. In the previous VBM study, the gray matter loss of various visual cortices in the amblyopes was almost symmetrical.7 It is possible that the small sample in our study hindered the detection of abnormalities in the right hemisphere. Another possibility is that laterality exists in the development of extrastriate regions of visual cortex. Data from a previous study revealed that age-related differences in sylvian fissure asymmetry were significant.18 Age-related increases in local gray matter proportion bilaterally in the tempoparietal cortices are anatomically and temporally related to the sulcal asymmetries. These extrastriate regions may also grow faster in the left hemisphere than in the right hemisphere, so the anatomic difference between amblyopic children and normal children was more prominent in the left side.

Although voxel-based analysis can automatically reveal abnormalities of the entire brain, it is not without limitations and problems.8 The templates provided by SPM are from normal adults and are potentially less suitable for children. For this reason, we built our own template to conduct spatial normalization. By using this template, we obtained reasonable results in our study, while our subjects’ young age seemed to cause no major problem of misregistration. The extension of clusters in our results was relatively local in comparison to that of the previous study,7 however. It may be attributed to the low sensitivity of our method, or the young age of our groups, or the mild severity of amblyopia.

In conclusion, our results show gray matter reduction within visual cortices in the brains of amblyopic children, including primary visual cortex and some extrastriate cortices. These findings may indicate developmental changes of visual cortex during the critical period, as well as the genesis of visual dysfunction in amblyopic children. The VBM method can be reliably used in the analysis of MRI data sets of children, and it will be helpful in providing supplemental information to clinical evaluation.

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REFERENCES