Voxel-Based Analysis of MRI Detects Abnormal Visual Cortex in Children and Adults With Amblyopia

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Abstract: Amblyopia, sometimes called “lazy eye,” is a relatively common developmental visual disorder well characterized behaviorally; however, the neural substrates associated with amblyopia in humans remain unclear. We hypothesized that abnormalities in the cerebral cortex of subjects with amblyopia exist, possibly as a result of experience-dependent neuronal plasticity. Anatomic magnetic resonance imaging (MRI) and psychophysical vision testing was carried out on 74 subjects divided into two age ranges, 7–12 years and 18–35 years, and three diagnoses, strabismic amblyopia, anisometropic amblyopia, and normal vision. We report a behavioral impairment in contrast sensitivity for subjects with amblyopia, consistent with previous reports. When the high-resolution MRI brain images were analyzed quantitatively with optimized voxel-based morphometry, results indicated that adults and children with amblyopia have decreased gray matter volume in visual cortical regions, including the calcarine sulcus, known to contain primary visual cortex. This finding was confirmed with a separate region-of-interest analysis. For the children with amblyopia, additional gray matter reductions in parietal-occipital areas and ventral temporal cortex were detected, consistent with recent reports that amblyopia can result in spatial location and object processing deficits. These data are the first to provide possible neuroanatomic bases for the loss of binocularity and visual sensitivity in children and adults with amblyopia. *Hum Brain Mapp* 25:222–236, 2005. © 2005 Wiley-Liss, Inc.

Key words: VBM; neural substrate; plasticity; pediatric; strabismus; morphology; V1; striate cortex

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INTRODUCTION

The mammalian visual system has been studied for decades as a prime example of neuronal plasticity during development due to the significant role of postnatal visual experience in achieving the adult state [Hubel et al., 1977; Wiesel, 1982]. In particular, the development of binocular vision (i.e., balanced and coordinated input from both eyes) is extremely sensitive to visual experiences during the first months of life. If the vision of one eye is impaired due to either deprivation of light (e.g., monocular cataract), a deviated eye, or strong near- or farsightedness during infancy, then a permanent reduction in the quality of vision often results. This condition is called amblyopia or “lazy eye.” Studies of animals with induced amblyopia suggest that impaired physiologic input from one eye leads to abnormalities in visual portions of the brain because (1) the weaker eye cannot compete with the dominant eye to form normal monocular connections, or (2) because cooperative binocular connections do not form [e.g., Horton et al., 1997; Horton and Hocking 1998a; Tychsén, 1992]. Without early intervention, these losses persist into adulthood. The impact of the first factor is thought to be greatest for severe conditions such as monocular occlusion, compared to monocular blur, and especially eye deviation. [e.g., Hendrickson et al., 1987; Horton and Hocking, 1998b; Tychsén and Burkhalter, 1997].

Amblyopia is quite common in human subjects, and it offers an opportunity to explore how a history of abnormal sensory experience can affect the functional anatomy of the human cerebral cortex. At least 2% of the North American population suffers from amblyopia, and it is the most common form of visual impairment in children. Subjects have impaired vision in one eye that cannot be corrected with glasses. Some specialists distinguish *anisometropic* amblyopia, which refers to conditions of unequal refractive error between the two eyes, from *strabismic* amblyopia, which refers to reduced acuity through a deviated eye. The impaired eye in all types of amblyopia exhibits decreased visual acuity and reduced contrast sensitivity [e.g., Bradley and Freeman, 1981; Gsälder and Green, 1971; Hess, 1979]. Subjects with strabismic amblyopia typically show a less severe impairment in contrast sensitivity, along with an additional loss of accuracy for tasks that contain multiple elements, such as Vernier acuity [Hess et al., 1978; Levi and Klein, 1982], supporting the idea that strabismus may cause a type of amblyopia different from anisometropia. Finally, binocular integration, including stereoscopic depth perception, is disrupted severely in both types of amblyopia.

The treatments for amblyopia, such as patching of the weak eye, are typically effective during critical periods of neuroplasticity in the first 6–9 years of life [Park et al., 2004; Pediatric Eye Disease Investigator Group, 2004; Rahi et al., 2002]. Nevertheless, these treatments are usually only partially effective at restoring sight to the affected eye so amblyopia often persists into adulthood. Most psychophysical and all prior neuroimaging studies have used adult subjects, due to their availability and ability to carry out the experimental tasks. Nevertheless, it is important to study children

so that the development of amblyopia and the specific neurologic effects of treatment can be understood better.

Current understanding of amblyopia in animal models suggests that pathologic changes occur primarily at the cortical level, rather than more peripheral levels [e.g., Cleland et al., 1982; Gillard-Crewther and Crewther, 1988; see Hendrickson et al., 1987; reviewed in Asper et al., 2000; Hess, 2001]. Consistently, evidence for cortical dysfunction in human amblyopia has been provided by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies [e.g., Barnes et al., 2001; Demer et al., 1997, 1988; Goodyear et al., 2000]. However, the specific neural substrates that actually produce amblyopia in humans remain unclear. Most evidence comes from animals with induced amblyopia, particularly physiologic assessment of neurons in primary visual cortex (V1), where input from the two eyes is first combined. These studies have found that individual neurons show reduced visual acuity and contrast sensitivity and fewer binocular neurons are present [Crewther and Crewther, 1990; Kiorpes et al., 1998; Movshon et al., 1987; Smith et al., 1997]. There is mixed evidence with regard to monocular dominance bias and ocular dominance column structure but in general, more of these abnormalities are found for anisometropic than for strabismic cases [e.g., Hendrickson et al., 1987; Horton et al., 1997; Mower et al., 1982; Tychsén and Burkhalter, 1997]. Finally, a striking loss of binocular vision has been reported by animal studies of strabismus and amblyopia, including reduced disparity sensitivity and directional asymmetry in the ability to track a moving object [Kiorpes et al., 1996; Kumagami et al., 2000; Mori et al., 2002; Tychsén and Lisberger, 1986; Wong et al., 2000]. Abnormal binocular interaction has also been indicated by functional human neuroimaging [Lee et al., 2001].

In addition, evidence has accumulated to suggest that abnormalities in cortical processing are not limited to V1. For example, when the performance of amblyopic animals on contrast sensitivity and resolution tasks is compared carefully to the neurophysiologic abnormalities of neurons in V1, it is clear that these abnormalities do not account fully for behavioral loss [Crewther and Crewther, 1990; Kiorpes and McKee, 1999]. Moreover, in human subjects, behavioral measures recently have revealed impairments when the amblyopic eye is used for tasks such as spatial localization or face recognition, which depend heavily upon extrastriate cortex [Giaschi et al., 1992; Lerner et al., 2003; Sharma et al., 2000; see also Imamura et al., 1997].

To investigate potential neural substrates of human amblyopia, we used high-resolution anatomic MRI in combination with vision tests to compare a large group of children and adults with both anisometropic and strabismic subtypes of amblyopia to control subjects. Standard psychophysical tasks, including contrast sensitivity, were used to validate our diagnostic groups and support the generality of our findings. For analysis of anatomic brain images, we first employed a computational method called voxel-based morphometry that is automated and unbiased. This method

TABLE I. Subject information

Subject	n	Age (yr)	Education (yr)*	Patch (months)	Near acuity (logMAR)		Far acuity (logMAR)		Stereoaucuity (log arc-sec)
					Normal/fellow	Amblyopia	Normal/fellow	Amblyopia	
Child									
Control	17	10.2 (1.5)	16.5 (3.3)	—	0.07 (0.2)	—	−0.006 (0.06)	—	1.6 (0.05)
Strabismic	10	9.7 (1.5)	13.9 (2.5)	14 (12)	0.1 (0.1)	0.5 (0.5) ^{ab}	0.1 (0.1)	0.5 (0.4) ^{ab}	3.2 (0.7) ^c
Anisometropic	15	9.8 (1.2)	14.1 (3.0)	17 (16)	0.02 (0.08)	0.5 (0.4) ^{ab}	0.01 (0.05)	0.5 (0.4) ^{ab}	2.8 (0.9)
Adult									
Control	10	25.2 (3.8)	14.5 (2.5)	—	−0.002 (0.07)	—	−0.05 (0.05)	—	1.8 (0.4)
Strabismic	13	24.8 (5.5)	15.4 (3.0)	23 (26)	0.05 (0.08)	0.3 (0.3) ^{ab}	0.03 (0.09)	0.3 (0.3) ^{ab}	3.2 (0.7) ^c
Anisometropic	9	28.9 (6.5)	15.0 (3.3)	11 (16)	0.08 (0.1)	0.5 (0.3) ^{ab}	0.05 (0.13)	0.4 (0.2) ^{ab}	2.7 (0.6) ^c

Standard deviations of the means reported in parentheses. LogMAR, Log base 10 of minimum angle of resolution in arc minutes.

* Parental education used as an index for young subjects.

^a Amblyopic eyes significantly different from worse eye of control subjects.

^b Amblyopic eyes significantly different from fellow eyes.

^c Amblyopic subjects significantly different from control subjects.

makes statistical comparisons between subgroups and can be more sensitive than radiologic inspection. Secondly, a region-of-interest (ROI) approach was used for further analysis of individual subjects. We hypothesized that anatomic differences would be observed in primary visual cortex, the first point of binocular interaction. We further hypothesized that abnormalities might be detected in extrastriate areas of the parietal and temporal cortex, consistent with the prior literature. These hypotheses were confirmed by our results indicating that amblyopia in children and adults is associated with visual cortical morphologic abnormalities including but not limited to V1. This data has been presented previously in a preliminary form [Mendola et al., 2002].

MATERIALS AND METHODS

Subjects

We studied 74 subjects, either children (7–12 years of age) or adults (18–35 years of age) with a diagnosis of anisometropic amblyopia, strabismic amblyopia, or normal control. The children were recruited by physician referral from the pediatric ophthalmology service at West Virginia University Eye Institute. In contrast, adults (who rarely continue to attend eye clinics) were recruited by advertisements in surrounding regions of West Virginia, Pennsylvania and Maryland. All subjects provided informed consent (WVU Protocol #14788). Subject information is listed in Tables I–III. Any subject with known or suspected neurologic conditions were excluded from the study. Diagnosis of anisometropic amblyopia was assigned based on: (1) an interocular refractive difference of hyperopia $\geq +1.00$ diopter, astigmatism $\geq +1.00$ diopter, or myopia ≥ -2.50 diopter; or (2) a history of anisometropia but no history of strabismus or strabismus surgery. Diagnosis of strabismus was made based on strabismus or strabismus surgery, and no anisometropia (as defined above). In clinical practice, it is common to find that

some subjects with amblyopia present with a mixed anisometropic/strabismic diagnosis, although little consensus exists regarding additional subtypes. In our subject population, two strabismic children, three strabismic adults, three anisometropic children, and one anisometropic adult could be classified as mixed (Tables II and III).

In Caucasian populations of strabismic subjects, there is often a greater prevalence of subjects with inward eye deviations (esotropia) than outward deviations (exotropia) [Ing and Pang, 1974], and our sample was no exception. The number of strabismic children with esotropia was eight, whereas two were exotropic; for adult strabismics, nine were esotropic and four were exotropic. The direction of strabismic deviation was measured with the alternate-cover test, and the amount of deviation (either manifest or latent) was determined with prism testing for both near and distance viewing [von Noorden and Campos, 2002].

All but one of the amblyopic subjects, an adult exotropic strabismic subject, showed reduced clinical visual acuity in their amblyopic eye at the time of testing (Tables I–III). One other strabismic adult had amblyopia (i.e., impaired acuity) in both eyes. The distribution of strabismus in the left eye (OS) compared to right eye (OD) was fairly balanced in the children (5 OS; 5 OD) and in the adults was (9 OS; 4 OD). The proportions for anisometropic children and adults were 6 OS, 10 OD and 5 OS, 4 OD, respectively. Most subjects had a history of eye-patch treatment, i.e., a patch was worn to cover the dominant eye (which is called the *fellow* eye), and thus force the subject to use the weak eye for daily visual tasks for a portion of each day. Nevertheless, the presence of amblyopia at the time of testing showed that the deficit was never reversed completely (Table I).

Vision Testing

All subjects completed an ophthalmologic exam to confirm diagnosis that included tests of ocular motility, dilation,

TABLE II. Strabismic information

Subject	Dev	Y/N	Surgery		Curr dev (prism diopters)	Patch (months)	Ambly acuity (logMAR)	Interocular acuity difference (logMAR)		Interoc refract diff (diopters)*	Stereoaucuity (arc-sec)
			Number	Age (yr)				Near	Far		
Child 1	EX	N	—	—	20	0	0.30	0.20	0	2.0 ^a	100
Child 2	ES	N	—	—	0	14	0.30	0	0.20	0.25	3,500
Child 3	ES	N	—	—	3	6	0.50	0.10	0.10	0.25	3,500
Child 4	ES	N	—	—	5	2	0.54	0	0.45	0.25	700
Child 5	ES	N	—	—	16	12	0.10	0	0.10	1.75 ^a	3,500
Child 6	ES	Y	1	1	DVD	26	0.38	0	0.22	0.25	100
Child 7	ES	Y	1	1.5	0	36	1.10	1.0	0.90	0.25	7,000 ^b
Child 8	ES	Y	1	5.5	15	12	1.18	0.97	1.0	1.5 ^a	7,000 ^b
Child 9	ES	Y	1	8	3	24	0.50	0.50	0.50	0	800
Child 10	EX	Y	1	10	10	3	1.30	1.20	1.0	0	7,000 ^b
Adult 1	EX	N	—	—	0	12	0.50	0.30	0.40	0	800
Adult 2	ES	N	—	—	0	2	0.30	0.30	0.30	0.75	400
Adult 3	ES	N	—	—	6	?	0.01	0.01	0.19	?	200
Adult 4	ES	N	—	—	8	0	0.40	0	0.30	0	7,000 ^b
Adult 5	EX	Y	1	4	5	12	0.20	0.20	0.20	1.5	3,500
Adult 6	EX	Y	2	9, 19	0	1	0.30	0	0	0.25	100
Adult 7	EX	Y	2	0.7, 8	0	84	0.80	0.59	0.59	3.5 ^a	7,000 ^b
Adult 8	ES	Y	1	2	4	60	0.30	0.01	0.30	1.5 ^a	400
Adult 9	ES	Y	1	5	6	6	0.10	0	0.01	0.75	800
Adult 10	ES	Y	1	6	12	24	0.90	0.90	0.90	1.0	3,500
Adult 11	ES	Y	1	5	10	?	0.70	0.11	0.11	0.75	7,000 ^b
Adult 12	ES	Y	2	2, 13	25	36	0.40	0.40	0.40	2.75 ^a	7,000 ^b
Adult 13	ES	Y	3	0.5, 10, 18	6	24	0.20	0.20	0.30	0.75	7,000 ^b

Dev, eye deviation; EX, exotropic; ES, esotropic; Y/N, yes/no; Curr dev, current deviation; Ambly acuity, Snellen acuity for amblyopic eye, lowest of near or distance; LogMAR, Log base 10 of minimum angle of resolution in arc-minutes; Interoc refract diff, Interocular refractive difference; DVD, dissociated vertical deviation

* Spherical equivalent.

^a Presence of inter-ocular refraction difference consistent with mixed diagnosis.

^b A score of 7,000 arc-sec was assigned to subjects who scored incorrect on every trial.

fundus exam, autorefraction, Snellen visual acuity (Lombart Instrument, Norfolk, VA), Worth 4-dot, Titmus Stereo Test (Titmus Optical, Inc., Petersburg, VA), Ishihara color plates (Kanehara and Co., Ltd., Tokyo, Japan), and Lighthouse contrast charts (Lighthouse International, New York, NY). The results for visual acuity and the Titmus Stereo Test are given in Table 1. The raised-circles portion of the Titmus test consists of nine trials of increasing difficulty, associated with stereoacuity from 40 to 800 arc-s. Another section of this test assesses crude stereopsis with an image of a housefly. Perception of depth in this stimulus is associated with acuity of 3,500 arc-s. There were some subjects whose stereoacuity is so poor that they could not perceive the fly. For these subjects a (doubled) score of 7,000 arc-s was assigned, as an approximation (Tables II and III). As a result, in Table I the log transform of the arc-s scores was used so that the reported means and standard deviations would be less biased by the approximations. In addition, the nonparametric test, Wilcoxon/Kruskal-Wallis (rank sum), was used to compare

the diagnosis groups (Table I). The very low scores achieved by the amblyopic subjects indicate very limited binocular integration.

Additional psychophysical tests were administered in a laboratory setting. We determined the contrast sensitivity function, i.e., the inverse of the minimum contrast necessary to allow detection of a sinusoidal grating as a function of spatial frequency, for each eye of every subject. Subjects wore a translucent patch over one eye during the monocular testing. Five spatial frequencies were tested. The gratings subtended 6 degrees in size. This test was administered as a temporal two-alternative forced choice (2AFC) design (Fig. 1A). Each trial comprised two sequential epochs, one of which contained a grating (200-ms duration), and the other contained a blank screen. The entire trial lasted 2,400 ms and the intertrial interval was 1,800 ms. An interactive staircase algorithm found the threshold associated with 71% correct performance by using a 2 up-1 down procedure (i.e., increasing difficulty after two correct responses and decreasing

TABLE III. Anisometropic information

Subject	Patch (months)	Ambly acuity (logMAR)	Interocular acuity difference (logMAR)		Interoc refract diff (diopter)	Stereoacuity (arc-sec)
			Near	Far		
Child 1	12	0.30	0.30	0.30	4.25	400
Child 2	48	0.40	0.20	0.40	1.25	400
Child 3	12	0.50	0.18	0.60	5.5	400
Child 4 ^a	12	0.40	0.30	0.40	3.25	7,000 ^e
Child 5	8	0.30	0.20	0.30	2.25	200
Child 6	0	1	0.70	0.90	2.5	7,000 ^e
Child 7 ^b	48	0.70	0.70	0.60	2.25	7,000 ^e
Child 8	1.5	1.10	0.89	1	1.5	800
Child 9	24	0.80	0.60	0.70	2.4	3,500
Child 10	12	0.40	0.50	0.50	2.0	60
Child 11	24	0.20	0.01	0.11	1.75	50
Child 12 ^c	4	0.10	0	0	2.0	40
Child 13	15	0.30	0.30	0.30	0.5	100
Child 14	6	1.30	1.30	1.30	4.5	700
Child 15	36	0.30	0.18	0.30	1.0	800
Adult 1 ^d	48	0.30	0.19	0.01	0	400
Adult 2	2	0.60	0.50	0.50	3.5	800
Adult 3	12	0.40	0.20	0.30	1.75	400
Adult 4	2	0.20	0.20	0.01	1.5	800
Adult 5	0	0.80	0.80	0.40	0.75	100
Adult 6	12	1	0.70	0.60	0.5	7,000 ^e
Adult 7	1	0.60	0.60	0.60	2.25	800
Adult 8	1	0.20	0.01	0.20	2.75	100
Adult 9	24	0.30	0.30	0.20	2.0	800

Ambly acuity, Snellen acuity for amblyopic eye, lowest of near or distance; Interoc refract diff, Interocular refractive difference; logMAR, log base 10 of minimum angle of resolution in arc minutes.

^a ET16 prism diopter deviation developed subsequent to experiment.

^b ET8 prism diopter deviation.

^c Surgery at 1 year of age.

^d No strabismus or surgery, no current interoc refract diff, but patch treatment.

^e A score of 7,000 arc-sec was assigned to subjects who scored incorrect on every trial.

difficulty after one error). The first three reversals employed increments and decrements of 6 dB. The last four reversals used increments and decrements of 2 dB, and the mean of these last four reversals was used to calculate the threshold.

MRI Acquisition

Subjects were scanned in a General Electric 1.5-Tesla MRI scanner. Standard T₁-weighted high-resolution (~1 mm³) anatomic scans of fast spoiled grass (FSPGR) gradient echo type, optimized for contrast between gray and white brain matter, were obtained for each subject. Imaging parameters for the 3D FSPGR scan were as follows: fast IR prep (prep time = 300 ms); echo time (TE) = 9 ms; flip angle (FA) = 20 degrees; field of view (FOV) = 24 cm; matrix = 256 × 256; slice thickness = 1.2 mm; 124 slices; and 0.9375 mm in-plane resolution. Three such scans (7 min, 32 s each) were obtained. Scanner noise was attenuated with earplugs. Subject's head motion was restricted with soft padding around

the head, and the necessity of head stability was emphasized clearly to all subjects.

MRI Analysis

Head motion assessment

Before further analysis of the brain images, we confirmed by visual inspection that all datasets were uncontaminated with major head motion artifacts. To make sure that the diagnostic subgroups did not differ with respect to amount of head motion, the statistical parametric mapping (SPM)99 software package (<http://www.fil.ion.ucl.ac.uk/spm/>) was used to measure the amount of head motion that occurred during the duration of the scan session. This standard algorithm minimizes the sum of the squared discrepancies between two scans with a cost function implemented as a first-order Taylor series approximation of a rigid body transformation [Friston et al., 1995, 1996]. Specifically, motion across the three scans was extracted in the form of three

linear translation motion vectors (x , y , and z) and three rotation motion vectors (roll, pitch, and yaw). For each subject, a total motion index was created by summing the six motion components. To assess within-scan head motion, a separate blind, subjective analysis was carried out. Visual inspection of 20 slices that included the occipital pole was carried out, and the presence of any subtle but visible motion artifacts were determined using a three-point scale, for every subject.

Whole brain analysis

We analyzed the original SPGR brain images using the optimized version of the voxel-based morphometry (VBM) module within the widely used analysis software package SPM99 [Ashburner and Friston, 2000] using the Linux operating system. This tool is applied throughout the brain without a priori assumptions about locations of change. The VBM method has been used previously to document pathologic changes in many brain areas, and validated with other independent morphologic analyses such as blinded manual measures [e.g., Vargha-Khadem et al., 1998]. In the present study, the optimized VBM protocol outlined by Good et al. [2001] was used to avoid errors caused by misclassification of non-brain voxels. In addition, the analysis followed the volume correction protocol that compensates for any change in image intensity caused by the spatial normalization step that places the images of all the subjects in an age group in a common coordinate system [Good et al., 2001].

Data from individual subjects were organized into six groups according to our three diagnoses and two age groups (Table I). All three scans from each subject were reviewed and averaged together, after realignment via the motion correction algorithm in SPM99. In the case of two subjects with obvious motion artifacts in one of the three images scans, that scan was discarded, and the average was based on the remaining two. The subsequent analysis was carried out according to the following standard steps. For each subject, preliminary segmentation of the images into gray matter, white matter, cerebrospinal fluid (CSF), and other non-brain partitions was carried out. Any non-brain voxels in the gray matter images were cleaned further by erosion and conditional dilation. This prevents the contribution of non-brain voxels in the estimation of normalization parameters in the following step. Spatial normalization placed the gray matter partition of each subject in the same age group in a common coordinate system. This consists of minimizing the sum of squared differences between each data set and a template image (Montreal Neurological Institute [MNI] adult brain template in SPM99) according to the basis function approach [Friston et al., 1995]. The resulting normalization parameters were then applied to the original anatomic images (in native space) so that they were optimally normalized [Good et al., 2001]. Final segmentation of the optimally normalized images into gray matter, white matter, CSF, and non-brain partitions was then completed. Next, brain extraction was carried out to remove the non-brain voxels, and smoothing (12-mm full-width half-maximum

[FWHM] isotropic Gaussian kernel) was applied to the extracted normalized gray matter partition.

Finally, comparison between amblyopic and control groups was derived for the gray matter of the entire brain using parametric statistics. This provides an estimate of gray matter volume differences derived on a voxel-by-voxel basis in the form of t -maps. Similar to any group-level analysis using a general linear model (GLM), the results identify the brain voxels for which between-group variation is larger than is within-group variation. We chose an appropriately conservative statistical threshold for our current data set, verified by using a bootstrapping strategy that ensured that minimal areas were found to differ in a comparison in which the subject diagnosis was sorted randomly ($P < 0.005$). Almost all regions of change reported here exceeded this threshold substantially ($P = 0.0003$). Following the practice of previous studies with a priori hypotheses about regions of significant difference, P values were not corrected for multiple comparisons (Vargha-Khadem et al., 1998).

The anatomic location of the gray matter areas that differed between groups was ascertained via the standard statistical parametric t -maps as well as careful visualization of the parametric maps on the inflated cortical surface of each hemisphere of a normal subject in each age group. The inflated surface reconstruction of each subject's cerebral cortex was generated using FreeSurfer software, which has been well documented previously [Dale et al., 1999; Fischl et al., 1999] (<http://www.nmr.mgh.harvard.edu/freesurfer>). Dark gray areas on the cortical surface represent the sulci and light gray areas represent the gyri. This technique allows significant differences that are located inside sulci to be visualized easily. To relate the (group-based) statistical parametric maps to the cortical surface of a particular individual subject, the t -value maps were converted to $(-\log P)$ maps. The anatomic dataset of one normal subject in each age group was chosen randomly, and the $\log P$ maps were transformed from the normalized group space to the individual coordinates of that normal subject with an inversion of the normalization procedures in the VBM protocol. The statistical maps were thus coregistered with the individual cortical surface, and painted upon it.

Calcarine ROI Analysis

A second analysis of the MRI images was carried out to extract a separate estimate of gray matter intensity in the calcarine region of every subject. The software program MRICro (<http://www.psychology.nottingham.ac.uk/staff/cr1/micro.html>) was used to define a 3D calcarine ROI. The Brodmann cortical area system was used to define area 17, and the automated anatomical labeling system [Tzourio-Mazoyer et al., 2002] was used to define area V1. Although these two ROIs mostly overlap, small differences in definition occur. To ensure an ROI that would include V1 in all subjects (and probably small portions of V2) we created our calcarine ROI by taking the union of the area 17 and the area V1 ROI, as defined by the corresponding atlases included in MRICro. This calcarine ROI was applied to the segmented

Figure 1.

Contrast sensitivity test. **A:** Depiction of the stimuli. Detection of a centrally located grating was tested with a two alternative forced-choice paradigm for five spatial frequencies. Subjects discriminate between a grating stimulus (1) and a homogeneous screen (2). The threshold luminance contrast necessary for detection of the grating was determined for each eye separately. **B:** Performance is plotted as sensitivity (the inverse of threshold) for each diagnostic group as a function of spatial frequency. The amblyopic eyes are impaired relative to the control eyes and fellow eyes, especially for high spatial frequencies. Error bars show standard error of the mean.

gray matter images created by VBM. We carried out this analysis for unsmoothed gray matter images because they preserve anatomic detail. We repeated the analysis for the smoothed images because they form the input to the VBM program, and thus provide a direct link with the group analysis. By applying the ROI to each subject separately we extracted the mean voxel intensity within the calcarine region. These calcarine intensity values were then compared directly across diagnosis groups.

RESULTS

Psychophysical Tests

In the case of contrast sensitivity, we observed the expected inverted U-shaped function, with peak sensitivity at middle spatial frequencies for control eyes and most fellow eyes. For anisometropic and strabismic eyes, we observed an

Figure 2.

Localization of VBM gray matter reductions in visual cortex of children with anisometropia and strabismus compared to normal subjects. **A:** Statistically significant gray matter volume reductions for the anisometropic children compared to the control children is indicated by a blue color scale. The scale is shown in bottom right corner, and ranges from $P = 0.005$ to $P = 0.0003$. The statistical changes are plotted on three inflated views of both hemispheres. From top to bottom row, the lateral, ventral, and medial views of the brain are shown. Left column shows left hemisphere, and right column shows right hemisphere. Cortical gyri and sulci are uniformly light and dark gray, respectively. The cortical surface of one normal child is used for the display. TO, transverse occipital sulcus; CS, collateral sulcus; PO, parietooccipital sulcus; Calc, calcarine sulcus. **B:** The equivalent comparison is shown for the strabismic group compared to the control group, and all conventions are the same as in A. Ant, anterior pole; Pos, posterior pole.

overall reduction in sensitivity and a shift in peak sensitivity to lower spatial frequencies, consistent with many previous studies [e.g., Bradley and Freeman, 1981; Hess, 1979; Gstalder and Green, 1971] (Fig. 1). To test for significant impairments for both amblyopic subtypes, and spatial frequency dependence, analysis of variance (ANOVA) was carried out. Three factors were included in the ANOVA: diagnosis (control, strabismic, and anisometropic); spatial frequency (0.5, 1, 2, 4, and 8 cpd); eye tested (amblyopic or fellow). The amblyopic eye status was assigned to the eye with lower acuity in the control subjects or randomly if visual acuity was identical in both eyes.

For the pediatric groups, the variability between subjects was high for the amblyopic eye, although this was much less true for the fellow eye, indicating that children understood the task. The overall patterns of performance were as expected, with one exception. The children with strabismus showed slight impairments in the fellow, as well as amblyopic, eye. Although the fellow eye is often found to have normal visual acuity in amblyopia, several studies have reported impairments in the fellow eye, especially for more sensitive tests such as contrast sensitivity [e.g., Leguire et al., 1990; Giaschi et al., 1992]. ANOVA for the pediatric anisometropic versus control groups showed a main effect of diagnosis ($F = 8.7$; $P = 0.007$), a main effect of spatial frequency ($F = 6.9$; $P < 0.0001$), and of eye tested ($F = 19.7$; $P < 0.0001$). In addition, all possible (two- and three-way) interactions were significant ($P < 0.0001$). The comparison of strabismic versus control groups also showed a main effect of diagnosis ($F = 8.8$; $P = 0.007$) and of eye tested ($F = 28.4$; $P < 0.0001$), as well as an interaction between diagnosis and eye tested ($F = 28.0$; $P < 0.0001$).

For the adult anisometropic population, in addition to the expected main effect of spatial frequency ($F = 6.8$; $P = 0.0003$), we obtained significant interaction between diagnosis and spatial frequency ($F = 2.9$; $P = 0.02$). Also significant was the interaction between eye tested and diagnosis ($F = 6.1$; $P = 0.01$) and eye tested and spatial frequency ($F = 2.4$; $P = 0.05$). In contrast, when we compared the strabismic adults to their controls, we did not obtain a significant main effect of diagnosis. We found only a main effect of spatial frequency ($F = 2.7$; $P = 0.03$), but not an interaction with diagnosis. Despite the overall similarity in the pattern of results for adult anisometropic and strabismic subjects (and comparable variability in the two groups; Fig. 1), the impairment thus was less severe in the strabismic group, as has been reported by others [e.g., McKee et al., 2003]. Interestingly, the fellow eyes of strabismic adults showed slightly enhanced sensitivity compared to that in control subjects. This has also been witnessed and discussed in prior studies [McKee et al., 2003].

Head Motion During MRI

If the subjects with amblyopia produced more head motion during MRI than their control subjects did, then the comparisons of brain structure within VBM might be confounded by this factor. We quantified the total amount of

head motion that occurred for the duration of the scan session by summing the motion in the three cardinal translations (x, y, and z) and three cardinal rotations (roll, pitch, and yaw). We compared the diagnosis groups according to this total motion measure with t tests. Neither the anisometropic children ($t = 1.0$; $P = 0.3$) nor the strabismic children ($t = 0.3$; $P = 0.8$) differed significantly from control children. Similarly, neither the anisometropic adults ($t = 1.8$; $P = 0.1$) nor the strabismic adults ($t = 1.0$; $P = 0.3$) differed from control adults. We thus found no difference between diagnostic groups with regard to between scan head motion. To assess within scan head motion, we compared the blind, subjective three-point rating of motion artifact across diagnosis groups. For the adults, every subject obtained a score of 1 (no visible motion artifact) regardless of diagnosis, so the diagnostic groups were identical. For the young subjects, slight motion artifact was occasionally visible, but neither the anisometropic children ($t = 0.15$; $P = 0.8$) nor the strabismic children ($t = 1.3$; $P = 0.2$) differed significantly from control children.

Voxel-Based Morphometry

For the children, the results showed regions of lower gray matter volume in the visual cortex of both the anisometropic and strabismic groups relative to that in their control group (Fig. 2). Areas of statistically significant gray matter reductions were seen in four distinct neocortical regions: calcarine and paracalcarine cortex, the medial parietal-occipital junction, the lateral parietal-occipital junction, and the ventral temporal cortex. Slight differences in location of significant change between the anisometropic and strabismic children can be seen in Figure 2. Specifically, the areas of reduced gray matter volume were larger in the anisometropic subjects in the ventral temporal cortex. The medial parietal-occipital cortex was affected bilaterally in the anisometropic children, but only in the right hemisphere of the strabismic children. Areas of statistically reduced gray matter volume outside of the visual cortex were minimal.

For the adults, more restricted areas of change were seen. Similar to the children, an area of reduced gray matter volume in the calcarine cortex was detected for the anisometropic adults. Only a very small region of significant difference in calcarine cortex was detectable for the strabismic adults (Fig. 3). The VBM differences between adults and children are considered in the discussion.

Calcarine ROI Analysis

The ROI approach was adopted to evaluate further the significance of VBM differences detected in the region of the calcarine cortex and to allow variability at the individual subject level to be assessed more directly. Although the gray matter images used for VBM and for this analysis were the same, this analysis was independent of VBM in that the calcarine area was first identified based upon standard atlases, and then all voxels meeting the anatomic criteria were analyzed (Fig. 4). In this way, the mean voxel intensity of the gray matter partition in the calcarine cortex was extracted

Figure 3.

Localization of VBM gray matter reductions in visual cortex of adults with amblyopia compared to normal subjects. Statistically lower volume of gray matter for both anisometropic and strabismic groups are indicated by blue scale as in Figure 2. The cortical surface of one normal adult is used for the display. Other conventions are the same as for Figure 2.

for both hemispheres of every subject for the smoothed and unsmoothed images. The distribution of the smoothed right hemisphere values can be visualized directly within and between subgroups (Fig. 5). The subjects with amblyopia showed a consistent mean reduction in gray matter intensity of the calcarine region compared to that in control subjects. For the child population, the strabismic subjects had significantly lower intensity values than did control subjects ($t = 2.2$; $P = 0.04$). The anisometropic children also showed intensity reductions, but the difference missed significance ($t = 1.4$; $P = 0.18$). For the adult population, intensity values were reduced in strabismic subjects ($t = 2.0$; $P = 0.05$) and anisometropic subjects ($t = 2.5$; $P = 0.02$) compared to that in control subjects. The comparison between strabismic and anisometropic subjects was not significant for either children ($t = 1.6$; $P = 0.12$) or adults ($t = 1.2$; $P = 0.25$). When the same analysis was repeated for the unsmoothed images, the distributions looked very similar, and all the same trends were evident. The equivalent t tests for strabismic or anisometropic children versus control children yielded $P = 0.05$ and $P = 0.15$, respectively, and for strabismic or anisometropic adults versus control adults, $P = 0.09$ and $P = 0.01$, respectively. Finally, similar results were obtained for the unsmoothed left hemispheres of the strabismic children ($P = 0.02$) and anisometropic children ($P = 0.07$), but the left hemisphere differences were not significant for strabismic adults ($P = 0.4$) or anisometropic adults ($P = 0.1$).

It is notable that amblyopic subjects have amblyopia in either the left or the right eye (as defined by lower visual acuity). The effects of amblyopia might thus possibly be

different in the hemisphere contralateral as compared to that ipsilateral to the affected eye. The above analysis is mixed with respect to the laterality of the amblyopic eye. A second analysis was carried out to compare the mean voxel intensity values of the calcarine cortex contralateral and ipsilateral to the affected eye, using unsmoothed images. The results did not indicate a consistent laterality effect. For the contralateral hemisphere of strabismic or anisometropic children compared to that in control children, $P = 0.05$ and $P = 0.2$, respectively; the ipsilateral hemisphere yielded $P = 0.03$ and $P = 0.01$, respectively. The equivalent contralateral values for adults were $P = 0.03$ and $P = 0.09$, respectively, and for ipsilateral $P = 0.04$ and $P = 0.48$, respectively. Finally, for all the amblyopic groups, there were no significant differences between the contralateral and ipsilateral hemisphere, or between left and right hemispheres.

DISCUSSION

This study indicates for the first time that the visual cortices of adults and children with amblyopia are associated with morphologic changes. We found indications of reduced gray matter volume in several regions of visual cortex including V1. The brains of children with amblyopia also showed regional differences in extrastriate regions of the parietal-occipital and ventral temporal cortex. These findings are consistent with neurologic models of amblyopia that propose that abnormal visual experience during developmental critical periods and the resulting visual deficits are associated with structural changes in visual portions of the brain.

Before relating our results to the current understanding of amblyopia, we discuss limitations associated with the VBM analysis technique. It should be made clear that although the VBM tool aims to be sensitive to differences in gray matter volume, significant results can also arise from any pathology that causes systematic differences in the intensity of gray matter relative to white matter. For example, because of the inherent difficulty of segmentation of gray from white matter, segmentation is a potential source of error. Another source of uncertainty is the spatial normalization process that places all subjects in a common coordinate system. This morphing process removes most of the macroscopic differences between individual brains. VBM is thus most sensitive to a "mesoscopic" level of structure that is neither microscopic nor macroscopic [Ashburner and Friston, 2001]. Examples of cortical morphology that could conceivably show systematic differences at such a mesoscopic scale include cortical curvature or local position changes.

It thus remains possible that the abnormalities we report are a reflection of some real but undetermined neural pathology other than a simple reduction in neuronal number, neuron size, or density of neural connections. The results do provide compelling evidence that some systematic structural alterations characterize the brains of subjects with amblyopia at the group level. Moreover, the strength of our approach is that several regions of abnormality were detected with a completely unbiased technique requiring no

Figure 4.

The 3D calcarine region of interest (ROI) overlaid on one slice for individual subject. **A:** The unsmoothed gray matter partition of a representative slice is shown, and the right hemisphere 3D calcarine ROI is overlaid in yellow. The ROI was defined from standard atlases. **B:** The same slice is shown after smoothing. The mean intensity of all voxels in the ROI was determined for every subject for the unsmoothed and smoothed images. The left and right hemispheres were measured separately.

assumptions about extent or location of regional brain differences. The fact that the same standardized VBM method has been applied to many neurologic conditions including Alzheimer's Disease, Huntington's Disease, normal aging, and even in normal subjects who are highly trained musicians, and occipital changes do not occur, provides some indirect support for the specificity of our findings [Good et

al., 2001; Karas et al., 2003; Kassubek et al., 2004; Sluming et al., 2002]. Finally, our findings provide clear hypotheses regarding sites of abnormality in subjects with amblyopia that can be evaluated further with noninvasive functional imaging studies in humans and invasive electrophysiologic studies in animals.

Although it is becoming increasingly certain that the peripheral eye conditions in amblyopia are associated with anatomic and functional abnormalities in neurons in the central nervous system (CNS), it remains unclear if CNS effects are a result of visual deprivation or if these effects actually cause amblyopia. Amblyopia is thought to be produced by a defect somewhere in the visual system that triggers a cascade of other abnormalities during development. In attempts to unravel which symptoms represent causes and which represent effects, many accounts of amblyopia emphasize form deprivation and the competitive interactions between the two eyes as causative factors. The opposite perspective has also been voiced. For example, it has been proposed that cases of infantile strabismus could result from a CNS developmental impairment of motion perception that disturbs the eye alignment that normal infants achieve at ~3 months [Tychsen, 1992]. Descending as well as ascending input within the visual system hierarchy thus may play a role. Although we favor an experience-dependent interpretation of our current results, there may in fact be both eye-to-brain effects and brain-to-eye effects that interact in amblyopia.

We next comment on the fact that we obtained rather similar results for our strabismic and anisometropic groups. Past research has differentiated the performance of subjects

Figure 5.

Plot of right hemisphere calcarine gray matter intensity for all subjects. The results of applying the calcarine ROI to the smoothed images of both the adult and child groups are shown. Diagnosis subgroups are shown in an overlaid fashion, identified

with a color code. Within each diagnosis subgroup, the subjects have been sorted from highest to lowest intensity values for easier visual comparison. The consistently lower values for amblyopic subjects can be directly observed.

with the strabismic and anisometropic subtypes of amblyopia based on their performance on monocular position acuity tasks, with larger deficits often seen in strabismics [e.g., Hess et al., 1978; Levi and Klein, 1982]. A second source of evidence comes from the severe loss of binocular vision seen for both subtypes. A recent large study of 427 amblyopes suggests that the degree of binocularity loss may sometimes predict the degree of abnormality on monocular tasks better than clinical subtype [McKee et al., 2003]. Similar ideas have been proposed in animal studies of induced amblyopia [Crewther and Crewther, 1990; Kiorpes et al., 1998]. Both reduced acuity and loss of binocularity thus are recognized currently as important aspects of amblyopia. In the current study, almost all amblyopic subjects had impaired acuity as well as impaired binocular function. Similarly, in the case of Chan et al. [2004], who recently found gray matter reductions in strabismic adults very similar to our current results, eight of ten strabismics had amblyopia (as defined by reduced acuity in one eye). It is therefore not yet possible to isolate the contribution of acuity loss from that of binocularity loss. There are some human subjects (typically exotropic strabismics) who alternately fixate with either eye and never develop normal binocular vision, but do not suffer acuity loss. It would be informative to focus on these subjects in a future study.

Children With Amblyopia

To our knowledge, there have been no prior neuroimaging studies of children with amblyopia that employ statistical analysis. Although a few investigations relating clinical MRI to strabismus exist, they specifically studied subjects with preexisting abnormal MRI or neurologic conditions [e.g., Brodsky et al., 2002]. There is one study that was confined to neurologically normal infants (<2 years of age) selected based on infantile strabismus, although analysis relied on subjective radiologic inspection. In this case, only a nonsignificant trend was reported: 3 of 38 strabismic subjects showed some posterior periventricular abnormality compared to 0 of 27 control infants [Ohtsuki et al., 2000]. We used an automated analysis to detect abnormal anatomy that could not be identified easily with simple inspection. 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 the child comparisons; however, the evidence suggests that there is no significant difference in gross brain volume of children after age 5 [Giedd et al., 1996; Reiss et al., 1996] and that SPM normalization techniques are robust after age 6 [Muzik et al., 2000]. The mean age of our subjects was ~10 years of age, and no subject was younger than 7 years of age. Moreover, our control and amblyopic groups were very well matched for mean years of age, so our results should be specific to amblyopia. Finally, the areas that seem to show the most age-related variation are the parietal and frontal regions that barely overlap with our findings [Wilke et al., 2002].

The significant changes observed in the gray matter partition of the calcarine cortex are consistent with the func-

tional localization of area V1 in children [Conner et al., 2004] and adults [e.g., Sereno et al., 1995], and are discussed further in the next section. In addition to the abnormalities detected in and around V1, we found loss of gray matter in several regions of visual association cortex in the children. Cortex surrounding the medial parietooccipital sulcus was affected. It is known from studies of monkeys that neurons in extrastriate visual areas in medial parietal-occipital cortex code eye position signals [Nakamura et al., 1999]. The precise homologies between humans and monkeys are not clear in this parietal region, but our region of interest might correspond to the monkey area V6(A), thought to be a part of the network that controls visually guided movements [Battaglini et al., 2002]. Given that clinical human data suggests that in adults damage to this cortical territory is associated with optic ataxia [Perenin and Vighetto, 1988], there may be a connection between the abnormalities we found and the visuomotor symptoms of amblyopia. The posterior parietal cortex has also been implicated in the control of vergence eye movements [Kapoula et al., 2004], so binocularity may be another important factor.

The visual cortex abnormalities detected in the lateral occipital cortex adjacent to the transverse sulcus are more enigmatic. This region may correspond to the visual areas V3A or V7, which have been associated previously with motion sensitivity, and spatial visual attention [Tootell et al., 1997, 1998]. Interestingly, one psychophysical study of amblyopia has found that subjects show a mild form of visual neglect when briefly presented targets must be counted [Sharma et al., 2000]. These visual areas are also activated strongly in normal subjects during stereoscopic depth perception [Mendola et al., 1999; Rutschmann and Greenlee, 2004; Tsao et al., 2003], and this ability is impaired severely in our subjects with amblyopia.

Quite prominent gray matter loss was seen in the ventral temporal cortex. These cortical regions have been associated with pattern perception and object recognition in a large number of studies [e.g., Grill-Spector, 2003]. Although the traditional focus within the amblyopia field has been on V1 and V2, it is becoming increasingly clear that higher-level visual areas are likely to be abnormal. This claim is consistent with accumulating evidence that higher-level processing of visual form may be impaired in amblyopia. Examples include discrimination of patterns and shapes, and detection of contours in noise [Hess et al., 1999; Kovacs et al. 2000]. Moreover, a recent study showed with fMRI 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 [Lerner et al., 2003]. In that study, the two clinical subtypes were mixed together, with a preponderance of the strabismic type. Interestingly, there is also evidence that even short periods of binocular deprivation in the first years of life (e.g., due to binocular congenital cataracts that are removed) can lead to deficits in form and face processing as an adult [Le Grand et al., 2001; Lewis et al., 2002]. Our results suggest that ventral temporal cortex may also be abnormal in those subjects.

Adults with Amblyopia

Fewer regions of gray matter reduction were detected in our adult subjects than in our child subjects. There are several possible explanations for the difference between children and adults with amblyopia. First, the children and adults were not selected equivalently. The children were recruited by physician referral. In contrast, adults (who rarely continue to attend eye clinics) were recruited by advertisements in the local community. There thus may be a selection bias favoring mildly affected adults, who may be more willing to participate in research. Furthermore, it can be seen from Table I and Figure 1 that the visual deficits of the children, as measured with tests of visual acuity and contrast sensitivity, were more severe than they were for the adults. Third, it may be that the adults have benefited more than the children have from their treatment regimes and life-long visual experience. Fourth, there may be a transient marker of amblyopia, which is rendered invisible to our methods by subsequent brain development. Finally, we do not rule out the possibility that additional areas of change might be observed in future MRI studies of amblyopia as techniques gain even better spatial resolution and sensitivity.

A consistent finding across children and adults is the loss of gray matter in the calcarine region. This finding is supported further by a recent replication that applied the same VBM method in a separate group of adult exotropic strabismics compared to controls [Chan et al., 2004]. These gray matter reductions found in V1 might be due to competitive interactions between the monocular inputs that do not function properly when one eye becomes overly dominant. The fact that we found a reduction in gray matter volume in visual cortex indicates that any loss of neurons, neuropil, or synapses driven by the weak eye is not compensated for entirely by the effective connectivity of the dominant, fellow eye. Moreover, there may be a specific loss of binocular connectivity in subjects with amblyopia compared to that in control subjects. Consistently, it has been reported that adult animals with amblyopia show fewer anatomic binocular connections in V1 [Tychsen and Burkhalter, 1995], and reduced numbers of neurons physiologically driven by both eyes [Kiorpes et al., 1998; see also Horton and Hocking, 1998b]. Furthermore, the pattern of several anatomic markers was altered in histologic analysis of V1 in two individual animals with strabismic amblyopia, especially in the subcompartments associated with binocular integration [Fenstermaker et al., 2001].

A few prior anatomic studies of the visual system in human adults with amblyopia exist. These studies have not found significant change in the thickness of the retinal nerve fiber layer in amblyopic eyes [Baddini-Caramelli et al., 2001; Colen et al., 2000]; however, significant shrinkage of cell size in the lateral geniculate thalamic nucleus in one subject with strabismic amblyopia has been reported [von Noorden and Crawford, 1992]. The only information about adult visual cortex comes from two subjects for which histologic analysis did not find a change in the pattern of ocular dominance

columns. This might be explained by ocular dominance column development occurring before amblyopia onset or by the limited changes in ocular dominance in nondeprivation amblyopia [Horton and Hocking 1996; Horton and Stryker 1993]. Moreover, these authors did not rule out the possibility that other neural connections in V1 are perturbed.

Consistent with our anatomic suggestions, functional metabolic imaging of adults with amblyopia has shown reduced activity in visual cortex in response to visual stimulation [e.g., Barnes et al., 2001; Demer et al., 1997; Goodyear et al., 2000]. A particularly relevant study examined the resting metabolic levels in the brains of subjects with amblyopia while their eyes were open but no stimulus was presented [Choi et al., 2002]. Even for this resting baseline condition, reductions of activity compared to that in control subjects were observed in a broad expanse of visual cortex in Brodmann areas 18, 19, 37, and 7, including the calcarine cortex and medial parietal-occipital cortex. The present morphologic findings may provide an anatomic basis for such results.

Conclusions

Using MRI, we detected morphologic changes in the visual cortex of human subjects with a history of either strabismic or anisometropic amblyopia. We interpret the observed reductions in gray matter volume as a result of the subject's history of abnormal visual experience, consistent with the idea that amblyopia is associated with experience-dependent morphologic changes. These changes may relate to either the reduced visual acuity or the severely abnormal binocular vision (e.g., stereopsis) in amblyopia. The sites of morphologic changes are broadly consistent with known behavioral losses in visual sensitivity, localization ability, and depth perception. The striate and extrastriate sites of abnormality provide specific hypotheses to guide future physiologic studies in animals and humans with amblyopia. Eventually, with improved resolution, anatomic (as well as functional) brain imaging techniques may provide supplemental information to help design and monitor amblyopia treatments. In principle, an anatomic or physiologic index that requires limited behavioral compliance may be complementary to psychophysical techniques. Furthermore, we demonstrated the feasibility of using these techniques with children, who are likely to benefit most from treatment regimes.

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REFERENCES

Ashburner J, Friston KJ (2000): Voxel-based morphometry: the methods. *Neuroimage* 11:805–821.

- Ashburner J, Friston KJ (2001): Why voxel-based morphometry should be used. *Neuroimage* 14:1238–1243.
- Asper L, Crewther D, Crewther SG (2000): Strabismic amblyopia. Part 2. Neural processing. *Clin Exp Optom* 83:200–211.
- Baddini-Caramelli C, Hatanaka M, Polati M, Umino AT, Susanna R Jr (2001): Thickness of the retinal nerve fiber layer in amblyopic and normal eyes: a scanning laser polarimetry study. *J AAPOS* 5:82–84.
- Barnes GR, Hess RF, Dumoulin SO, Achtman RL, Pike GB (2001): The cortical deficit in humans with strabismic amblyopia. *J Physiol* 533:281–297.
- Battaglini PP, Muzur A, Galletti C, Skrap M, Brovelli A, Fattori P (2002): Effects of lesions to area V6A in monkeys. *Exp Brain Res* 144:419–422.
- Bradley A, Freeman RD (1981): Contrast sensitivity in anisometric amblyopia. *Invest Ophthalmol Vis Sci* 21:467–476.
- Brodsky MC, Fray KJ, Glasier CM (2002): Perinatal cortical and subcortical visual loss: mechanisms of injury and associated ophthalmologic signs. *Ophthalmology* 109:85–94.
- Chan ST, Tang KW, Lam KC, Chan LK, Mendola JD, Kwong KK (2004): Neuroanatomy of adult strabismus: a voxel based morphometric analysis of magnetic resonance structural scans. *Neuroimage* 22:986–994.
- Choi MY, Lee DS, Hwang JM, Choi DG, Lee KM, Park KH, Yu YS (2002): Characteristics of glucose metabolism in the visual cortex of amblyopes using positron-emission tomography and statistical parametric mapping. *J Pediatr Ophthalmol Strabismus* 39: 11–19.
- Cleland BG, Crewther DP, Crewther SG, Mitchell DE (1982): Normality of spatial resolution of retinal ganglion cells in cats with strabismic amblyopia. *J Physiol* 326:235–249.
- Colen TP, de Faber JT, Lemij HG (2000): Retinal nerve fiber layer thickness in human strabismic amblyopia. *Binocul Vis Strabismus Q* 15:141–146.
- Conner IP, Sharma S, Lemieux SK, Mendola JD (2004): Retinotopic organization in children measured with fMRI. *J Vis* 4:509–523.
- Crewther DP, Crewther SG (1990): Neural site of strabismic amblyopia in cats: spatial frequency deficit in primary cortical neurons. *Exp Brain Res* 79:615–622.
- Dale AM, Fischl B, Sereno MI (1999): Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 9:179–194.
- Demer JL, Grafton S, Marg E, Mazziotta JC, Nuwer M (1997): Positron-emission tomographic study of human amblyopia with use of defined visual stimuli. *J AAPOS* 1:158–171.
- Demer JL, von Noorden GK, Volkow ND, Gould KL (1988): Imaging of cerebral blood flow and metabolism in amblyopia by positron emission tomography. *Am J Ophthalmol* 105:337–347.
- Fenstemaker SB, Kiorpes L, Movshon JA (2001): Effects of experimental strabismus on the architecture of macaque monkey striate cortex. *J Comp Neurol* 438:300–317.
- Fischl B, Sereno MI, Dale AM (1999): Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage* 9:195–207.
- Friston KJ, Ashburner J, Frith CD, Poline JB, Heather JD (1995): Spatial registration and normalization of images. *Hum Brain Mapp* 2:165–189.
- Friston KJ, Williams S, Howard R, Frackowiak RS, Turner R (1996): Movement-related effects in fMRI time-series. *Magn Reson Med* 35:346–355.
- Giaschi DE, Regan D, Kraft SP, Hong XH (1992): Defective processing of motion-defined form in the fellow eye of patients with unilateral amblyopia. *Invest Ophthalmol Vis Sci* 33:2483–2489.
- Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey BJ, Kozuch PL (1996): Quantitative magnetic resonance imaging of human brain development: ages 4–18. *Cereb Cortex* 6:551–560.
- Gillard-Crewther S, Crewther DP (1988): Neural site of strabismic amblyopia in cats: X-cell acuities in the LGN. *Exp Brain Res* 72:503–509.
- Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS (2001): A voxel-based morphometric study of aging in 465 normal adult human brains. *Neuroimage* 14:21–36.
- Goodyear BG, Nicolle DA, Humphrey GK, Menon RS (2000): BOLD fMRI response of early visual areas to perceived contrast in human amblyopia. *J Neurophysiol* 84:1907–1913.
- Grill-Spector K (2003): The neural basis of object perception. *Curr Opin Neurobiol* 13:159–166.
- Gstalter R, Green DG (1971): Laser interferometric acuity in amblyopia. *Arch Ophthalmol* 8:251–265.
- Hendrickson AE, Movshon JA, Eggers HM, Gizzi MS, Boothe RG, Kiorpes L (1987): Effects of early unilateral blur on the macaque's visual system. II. Anatomical observations. *J Neurosci* 7:1327–1339.
- Hess RF (1979): Contrast sensitivity assessment of functional amblyopia in humans. *Trans Ophthalmol Soc U K* 99:391–397.
- Hess RF (2001): Amblyopia: site unseen. *Clin Exp Optom* 84:321–336.
- Hess RF, Campbell FW, Greenhalgh T (1978): On the nature of the neural abnormality in human amblyopia; neural aberrations and neural sensitivity loss. *Pflügers Arch* 377:201–207.
- Hess RF, Wang YZ, Demanins R, Wilkinson F, Wilson HR (1999): A deficit in strabismic amblyopia for global shape detection. *Vision Res* 39:901–914.
- Horton JC, Hocking DR (1996): Pattern of ocular dominance columns in human striate cortex in strabismic amblyopia. *Vis Neurosci* 13:787–795.
- Horton JC, Hocking DR, Kiorpes L (1997): Pattern of ocular dominance columns and cytochrome oxidase activity in a macaque monkey with naturally occurring anisometric amblyopia. *Vis Neurosci* 14:681–689.
- Horton JC, Hocking DR (1998a): Monocular core zones and binocular border strips in primate striate cortex revealed by the contrasting effects of enucleation, eyelid suture, and retinal laser lesions on cytochrome oxidase activity. *J Neurosci* 18:5433–5455.
- Horton JC, Hocking DR (1998b): Effect of early monocular enucleation upon ocular dominance columns and cytochrome oxidase activity in monkey and human visual cortex. *Vis Neurosci* 15: 289–303.
- Horton JC, Stryker MP (1993): Amblyopia induced by anisometropia without shrinkage of ocular dominance columns in human striate cortex. *Proc Natl Acad Sci USA* 90:5494–5498.
- Hubel DH, Wiesel TN, LeVay S (1977): Plasticity of ocular dominance columns in monkey striate cortex. *Philos Trans R Soc Lond B Biol Sci* 278:377–409.
- Imamura K, Richter H, Fischer H, Lennerstrand G, Franzen O, Rydberg A, Andersson J, Schneider H, Onoe H, Watanabe Y, Langstrom B (1997): Reduced activity in the extrastriate visual cortex of individuals with strabismic amblyopia. *Neurosci Lett* 225:173–176.
- Ing MR, Pang SW (1974): The racial distribution of strabismus—a statistical study. *Hawaii Med J* 33:22–23.
- Kapoula Z, Yang Q, Coubar O, Daunys G, Orssaud C (2004): Transcranial magnetic stimulation of the posterior parietal cortex delays the latency of both isolated and combined vergence-saccade movements in humans. *Neurosci Lett* 360:95–99.

- Karas GB, Burton EJ, Rombouts SA, van Schijndel RA, O'Brien JT, Scheltens P, McKeith IG, Williams D, Ballard C, Barkhof F (2003): A comprehensive study of gray matter loss in patients with Alzheimer's disease using optimized voxel-based morphometry. *Neuroimage* 18:895–907.
- Kassubek J, Juengling FD, Kioschies T, Henkel K, Karitzky J, Kramer B, Ecker D, Andrich J, Saft C, Kraus P, Aschoff AJ, Ludolph AC, Landwehrmeyer GB (2004): Topography of cerebral atrophy in early Huntington's disease: a voxel-based morphometric MRI study. *J Neurol Neurosurg Psychiatry* 75:213–220.
- Kiorpes L, Kiper DC, O'Keefe LP, Cavanaugh JR, Movshon JA (1998): Neuronal correlates of amblyopia in the visual cortex of macaque monkeys with experimental strabismus and anisometropia. *J Neurosci* 18:6411–6424.
- Kiorpes L, McKee SP (1999): Neural mechanisms underlying amblyopia. *Curr Opin Neurobiol* 9:480–486.
- Kiorpes L, Walton PJ, O'Keefe LP, Movshon JA, Lisberger SG (1996): Effects of early-onset artificial strabismus on pursuit eye movements and on neuronal responses in area MT of macaque monkeys. *J Neurosci* 16:6537–6553.
- Kovacs I, Polat U, Pennefather PM, Chandna A, Norcia AM (2000): A new test of contour integration deficits in patients with a history of disrupted binocular experience during visual development. *Vision Res* 40:1775–1783.
- Kumagami T, Zhang B, Smith EL 3rd, Chino YM (2000): Effect of onset age of strabismus on the binocular responses of neurons in the monkey visual cortex. *Invest Ophthalmol Vis Sci* 41:948–954.
- Lee KM, Lee SH, Kim NY, Kim CY, Sohn JW, Choi MY, Gyu Choi D, Hwang JM, Ho Park K, Lee DS, Suk Yu Y, Hyun Chang K (2001): Binocularity and spatial frequency dependence of calcarine activation in two types of amblyopia. *Neurosci Res* 40:147–153.
- Le Grand R, Mondloch CJ, Maurer D, Brent HP (2001): Early visual experience and face processing. *Nature* 410:890.
- Leguire LE, Rogers GL, Bremer DL (1990): Amblyopia: the normal eye is not normal. *J Pediatr Ophthalmol Strabismus* 27:32–39.
- Lerner Y, Pianka P, Azmon B, Leiba C, Loewenstein A, Harel M, Hendler T, Malach R (2003): Area-specific amblyopic effects in human occipitotemporal object representations. *Neuron* 40:1023–1029.
- Lewis TL, Ellemberg D, Maurer D, Wilkinson F, Wilson HR, Dirks M, Brent HP (2002): Sensitivity to global form in glass patterns after early visual deprivation in humans. *Vision Res* 42:939–948.
- Levi DM, Klein SA (1982): Differences in vernier discrimination for gratings between strabismic and anisometric amblyopes. *Invest Ophthalmol Vis Sci* 23:398–407.
- McKee SP, Levi D, Movshon JA (2003): The pattern of visual deficits in amblyopia. *J Vision* 3:380–405.
- Mendola JD, Dale AM, Fischl B, Liu AK, Tootell RB (1999): The representation of illusory and real contours in human cortical visual areas revealed by functional magnetic resonance imaging. *J Neurosci* 19:8560–8572.
- Mendola JD, Roy A, Conner IP, Chan ST, Kwong K, Fischl B (2002): Cortical morphologic changes characterize anisometropic and strabismic amblyopia. In: 8th International Conference on Functional Mapping of the Human Brain, Sendai, Japan.
- Mori T, Matsuura K, Zhang B, Smith EL 3rd, Chino YM (2002): Effects of the duration of early strabismus on the binocular responses of neurons in the monkey visual cortex (V1). *Invest Ophthalmol Vis Sci* 43:1262–1269.
- Movshon JA, Eggers HM, Gizzi MS, Hendrickson AE, Kiorpes L, Boothe RG (1987): Effects of early unilateral blur on the macaque's visual system. III. Physiological observations. *J Neurosci* 7:1340–1351.
- Mower GD, Burchfiel JL, Duffy FH (1982): Animal models of strabismic amblyopia: physiological studies of visual cortex and the lateral geniculate nucleus. *Brain Res* 281:311–327.
- Muzik O, Chugani DC, Juhasz C, Shen C, Chugani HT (2000): Statistical parametric mapping: assessment of application in children. *Neuroimage* 12:538–549.
- Nakamura K, Chung HH, Graziano MS, Gross CG (1999): Dynamic representation of eye position in the parieto-occipital sulcus. *J Neurophysiol* 81:2374–2385.
- Ohtsuki H, Yoshifumi K, Hasebe S, Kono R, Harada Y (2000): Comparative study of brain lesions detected by magnetic resonance imaging between strabismus and non strabismus in infancy. *Ophthalmologica* 214:105–110.
- Park KH, Hwang JM, Ahn JK (2004): Efficacy of amblyopia therapy initiated after 9 years of age. *Eye* 18:571–574.
- Pediatric Eye Disease Investigator Group (2004): A prospective, pilot study of treatment of amblyopia in children 10 to <18 years old. *Am J Ophthalmol* 137:581–583.
- Perenin MT, Vighetto A (1988): Optic ataxia: a specific disruption in visuomotor mechanisms. *Brain* 111:643–674.
- Rahi JS, Logan S, Borja MC, Timms C, Russell-Eggitt I, Taylor D (2002): Prediction of improved vision in the amblyopic eye after visual loss in the non-amblyopic eye. *Lancet* 360:621–622.
- Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB (1996): Brain development, gender and IQ in children: a volumetric imaging study. *Brain* 119:1763–1774.
- Rutschmann RM, Greenlee MW (2004): BOLD response in dorsal areas varies with relative disparity level. *Neuroreport* 15:615–619.
- Sereno MI, Dale AM, Reppas JB, Kwong KK, Belliveau JW, Brady TJ, Rosen BR, Tootell RB (1995): Borders of multiple visual areas in humans revealed by functional magnetic resonance. *Science* 268:889–893.
- Sharma V, Levi DM, Klein SA (2000): Undercounting features and missing features: evidence for a high-level deficit in strabismic amblyopia. *Nat Neurosci* 3:496–501.
- Sluming V, Barrick T, Howard M, Cezayirli E, Mayes A, Roberts N (2002): Voxel-based morphometry reveals increased gray matter density in Broca's area in male symphony orchestra musicians. *Neuroimage* 17:1613–1622.
- Smith EL 3rd, Chino YM, Ni J, Cheng H, Crawford ML, Harwerth RS (1997): Residual binocular interactions in the striate cortex of monkeys reared with abnormal binocular vision. *J Neurophysiol* 78:1353–1362.
- Tootell RB, Hadjikhani N, Hall EK, Marrett S, Vanduffel W, Vaughan JT, Dale AM (1998): The retinotopy of visual spatial attention. *Neuron* 21:1409–1422.
- Tootell RB, Mendola JD, Hadjikhani NK, Ledden PJ, Liu AK, Reppas JB, Sereno MI, Dale AM (1997): Functional analysis of V3A and related areas in human visual cortex. *J Neurosci* 17:7060–7078.
- Tsao DY, Vanduffel W, Sasaki Y, Fize D, Knutsen TA, Mandeville JB, Wald LL, Dale AM, Rosen BR, Van Essen DC, Livingstone MS, Orban GA, Tootell RB (2003): Stereopsis activates V3A and caudal intraparietal areas in macaques and humans. *Neuron* 39:555–568.
- Tychsen L, Lisberger SG (1986): Visual motion processing for the initiation of smooth-pursuit eye movements in humans. *J Neurophysiol* 56:953–968.

- Tychsen L (1992): Binocular vision. In: Hart W, editor. *Adler's physiology of the eye: clinical applications*. St. Louis: Mosby YearBook. p 773–853.
- Tychsen L, Burkhalter A (1995): Nasotemporal asymmetries in V1: ocular dominance columns of infant, adult, and strabismic macaque monkeys. *J Comp Neurol* 388:32–46.
- Tychsen L, Burkhalter A (1997): Neuroanatomic abnormalities of primary visual cortex in macaque monkeys with infantile esotropia: preliminary results. *J Pediatr Ophthalmol Strabismus* 32:323–328.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15:273–289.
- Vargha-Khadem F, Watkins KE, Price CJ, Ashburner J, Alcock KJ, Connelly A, Frackowiak RS, Friston KJ, Pembrey ME, Mishkin M, Gadian DG, Passingham RE (1998): Neural basis of an inherited speech and language disorder. *Proc Natl Acad Sci USA* 95:12695–12700.
- von Noorden GK, Campos EC (2002): *Binocular vision and ocular motility: theory and management of strabismus*, 6th ed. St. Louis: Mosby. 672 p.
- von Noorden GK, Crawford ML (1992): The lateral geniculate nucleus in human strabismic amblyopia. *Invest Ophthalmol Vis Sci* 33:2729–2732.
- Wiesel TN (1982): Postnatal development of the visual cortex and the influence of environment. *Nature* 299:583–591.
- Wilke M, Schmithorst VJ, Holland SK (2002): Assessment of spatial normalization of whole-brain magnetic resonance images in children. *Hum Brain Mapp* 17:48–60.
- Wong AM, Lueder GT, Burkhalter A, Tychsen L (2000): Anomalous retinal correspondence: neuroanatomic mechanism in strabismic monkeys and clinical findings in strabismic children. *J AAPOS* 4:168–174.