

Cortical Deficits in Human Amblyopia: Their Regional Distribution and Their Relationship to the Contrast Detection Deficit

Xingfeng Li, Serge O. Dumoulin, Behzad Mansouri, and Robert F. Hess

PURPOSE. The understanding of the site and nature of the cortical processing deficit in human amblyopia awaits the resolution of three fundamental questions about which there is, at present, much controversy: First, *is area V1 affected* as the present animal models would predict, but some imaging studies argue against? Second, *how extensive is the loss of extrastriate function, and does it simply follow as a consequence of an impaired V1 input?* Third, *does the brain imaging deficit, be it striate or extrastriate, correlate with the well-documented psychophysical loss?*—a fundamental issue on which previous brain imaging studies are divided.

METHODS. A spatially broadband stimulus was used to determine the functional MRI responses from the different retinotopically identified visual cortical areas in a group of normal ($n = 6$) and a group of amblyopic ($n = 11$) observers. Responses were compared between the amblyopic and fellow fixing eyes of amblyopes and between the dominant and nondominant eyes of normal subjects, in central and peripheral parts of the visual field. Psychophysical acuity and contrast sensitivity was also measured and its correlation with the brain imaging deficit determined.

RESULTS. V1 was affected in most but not all cases; the brain-imaging deficit involved extensive regions of extrastriate cortex and, at least with the stimuli used in the study, correlated with the V1 loss, suggesting a strong V1 influence; and neither the striate nor the extrastriate deficits correlated with the psychophysical contrast threshold losses at either high or low spatial frequencies.

CONCLUSIONS. The results suggest that there are significant suprathreshold processing deficits that are not a consequence of the well-known threshold deficit. Our preoccupation over the past 30 years with the contrast detection deficit in amblyopia limited to the processing within a circumscribed part of V1 may have to be modified to include not only processing deficits for high-contrast stimuli but also the involvement of multiple extrastriate areas. (*Invest Ophthalmol Vis Sci.* 2007;48:1575–1591) DOI:10.1167/iovs.06-1021

Amblyopia is a condition found in up to 3% of humans and is characterized by reduced, often irreversible, loss of vision in one eye due to a disruption of normal visual development because of strabismus, anisometropia, or pattern deprivation. Its site is not retinal^{1–4} but is thought to be cortical. Our understanding of the exact nature and site of the cortical deficit is not well developed, although there is a rich body of literature from psychophysical studies of human amblyopes and single-cell neurophysiology of animals made artificially amblyopic.

The psychophysical picture of human amblyopia is mainly in terms of contrast sensitivity. Amblyopes detect low-spatial-frequency stimuli with the same sensitivity as in normal subjects but require more contrast to detect higher spatial frequency stimuli.^{5–7} In strabismic amblyopia, this detection deficit involves mainly the central field.^{8,9} Contrast perception is normal however, even at high spatial frequencies, well above their raised detection thresholds.¹⁰ Thus, the visual problem in amblyopia on which our present animal models are based is limited to low contrast and high spatial frequencies and the central visual field.

Animal models of amblyopia have shown that strabismus or anisometropia produce a loss of binocularity of cortical cells,¹¹ an upset in the excitatory-inhibitory balance of binocular cells,^{12,13} reduced strength of cellular interactions¹⁴ and reduced mean sensitivity and mean spatial resolution of the foveal V1 cellular population as a whole.¹⁵ These latter findings have led to the widely held belief that the contrast sensitivity or visibility deficit can be explained in terms of the V1 response, although it has been acknowledged that the V1 losses may not be the whole story; they are quantitatively less than the behavioral deficit across a group of amblyopic animals, and there is a great deal of interanimal variability.¹⁶ There is still some controversy concerning whether the number of cells driven by the amblyopic eye is different from that driven by the fellow fixing eye. In general it is not,^{11,14,17–20} but there is evidence that in the case of severe convergent strabismus there may be fewer cells driven by the deviated eye,^{11,15,17,18,21,22} and also it has been reported, in a subset of animals, that there may be less foveal V1 neurons driven by the amblyopic eye.^{15,23} Granted we are not at the stage where we can go from cellular responses in V1 to perception even in normal animals, but there is still a demonstrated correlation between the visibility defect in amblyopia as assessed by spatial resolution and peak contrast sensitivity and the responses of cells in V1 driven by the amblyopic eye. There is also a realization, however, that there could be more to the story either by a consideration of V1 processing beyond that of isolated cells or by considering responses in visual areas other than V1.

Brain imaging in humans with amblyopia has the potential of providing additional information on the site of the deficit. It has the advantage of not requiring the intermediate animal model stage and reflecting a population measure of cortical

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TABLE 1. Clinical Data for the Study Subjects

Subj	Age/Sex	Type	Refraction	Acuity	Grating Acuity	Contrast Sensitivity at 1 cyc/deg	Squint	History, Stereo	
DV	23/F	LE	+0.25 DS	20/20	35.6	0.01	ET 3°	Detected age 5–6 y, patching for 6 mo; no surgery	
		mixed	+2.75/–1.25	175°	20/40	26.0			
EF	56/M	LE	+2.00/+1.00	180°	20/32	23.5	0.023	ET 6°	Detected age 6y, patching for 1–2 y; no surgery
		strab	+2.00/+1.00	130°	20/250	17.0	0.021		
GN	30/M	RE	+5.00/–2.00	120°	20/70	22.5	0.028	ET 8°	Detected age 5 y, patching for 3 mo, no glasses tolerated, two strabismus surgeries on RE at age 10–12 y
		mixed	+3.50/–1.00	75°	20/20	28.4	0.021		
HP	33/M	LE	–2.0/+0.50 DS		20/25	33.9	0.011	ET 5°	Detected at age 4 y, patching for 6 mo; surgery at age 5 y.
		strab			20/63	30.0	0.016		
LM	20/F	RE	+1.0/–0.75	90°	20/80	31.2	0.016	ET 6°	Detected at age 5 y, patching for 2 y
		mixed	–3.25 DS		20/25	37.2	0.015		
MB	50/M	RE	–1.00 DS		20/32	27.9	0.009	ET 3°	No surgery, first glasses at age 32 y
		strab	+1.00 DS		20/80	28.0	0.014		
MG	30/F	RE	–0.50 DS		20/100	20.0	0.015	ET 1°	Detected age 4 y, patching for 6 mo, no surgery
		strab	+0.50 DS		20/15	52.0	0.011		
OA	21/M	RE	+4.50/–5.00	30°	20/120	18.6	0.016	ET 5°	Detected at age 3 y, Rx and patching given at 3 y, no surgery
		mixed	–1.75/–1.75	150°	20/32	33.6	0.011		
VE	69/M	LE	–1.75/–1.75	150°	20/25	32.1	0.012	ET 5°	Detected age 10 y, no treatment
		mixed	+4.5/–5.00	30°	20/80	10.8	0.014		
XL	31/F	RE	–2.75/+0.75	110°	20/400	11.3	0.033	ET 15°	Detected age 13 y, no treatment
		strab	–2.50 DS		20/20	31.9	0.015		
YC	31/M	LE	+2.00 DS	DS	20/15	55.5	0.007	ET 10°	Detected age 2 y, patching for 4 y, glasses for 16 y
		strab	+2.00 DS	DS	20/40	42.3	0.008		

M, male; F, female; LE, left eye; RE, right eye; strab, strabismic amblyopia; DS, diopter sphere; entries for grating acuity, contrast sensitivity, correction, and fixation columns are quoted for right and left eyes, respectively.

function. Its main disadvantage is that it is a specific and indirect measure of neural function.²⁴ The results to date have been inconsistent; some studies have argued for normal V1 function, with the disturbance being exclusively restricted to the extrastriate cortex (Sireteanu R et al. *IOVS* 1998;39:ARVO Abstract 4186).^{25–27} Others have argued that V1 activation is reduced.^{28–37} Of the studies that report reduced V1 activation, some report a pattern of inactivation that is consistent with the psychophysical loss,^{30,34} whereas others argue that this is not the case.^{31–33} In particular, Anderson et al.,³¹ using magnetoencephalography (MEG), and Barnes et al.,³³ using fMRI, argue that the reduced activation does not correlate with the contrast sensitivity loss. In both of these studies, not only was reduced activation found at low spatial frequencies where contrast sensitivity is rarely affected in amblyopia, but also any reduced activation found at high spatial frequencies did not correlate with the loss in contrast sensitivity. Another finding in most imaging studies is that the extrastriate cortex is also affected (Sireteanu R et al. *IOVS* 1998;39:ARVO Abstract 4186),^{25–27,33,35} though the extent of the deficit is unknown, as is whether it simply follows from the V1 loss.

There are three questions that must be resolved to further our understanding of the cortical deficit in humans with amblyopia. First, *is V1 affected?* Animal studies suggest it is,^{15,23} but some recent brain imaging studies argue that it is not (Sireteanu R et al. *IOVS* 1998;39:ARVO Abstract 4186).^{25–27} Second, *how extensive is the loss of extrastriate function, and does it simply follow as a consequence of an impaired V1 input?* Animal studies have traditionally been limited to V1^{15,23}

and provide little elucidation. Brain imaging studies suggest that there is extrastriate dysfunction (Sireteanu R et al. *IOVS* 1998;39:ARVO Abstract 4186)^{25–36} but it is presently unresolved how extensive this is and, more important, whether it simply follows as a simple consequence of the V1 loss. If the extrastriate losses are shown to correlate with the V1 loss, then it suggests that the main site of dysfunction in amblyopia may be in V1. If the extrastriate losses are shown not to correlate with the V1 loss, then it suggests that there are independent, primary deficits in the extrastriate cortex. Third, *does the brain imaging deficit, be it striate or extrastriate, correlate with the well-documented psychophysical contrast detection loss described earlier?*—a fundamental question on which previous brain imaging studies are divided. Previous studies that have used spatially and temporally narrowband (i.e., sinusoidal grating) stimuli have suggested no correlation between reduced activation and either contrast sensitivity or grating acuity, whereas the studies that provide support for the relationship have used spatially and temporally broadband stimuli.^{30,34} This in itself is intriguing because one would have thought the opposite to be the case: The more spatially narrowband the stimuli, the greater the correlation with the loss of only a subset of cells. Its importance is that it raises the intriguing question as to whether there are processing deficits not related solely to detection (i.e., deficits for well detectable, high-contrast stimuli).

In this investigation, we set out to answer these important questions by an assessment of the magnitude and extent of the cortical deficit in retinotopically mapped regions of the striate

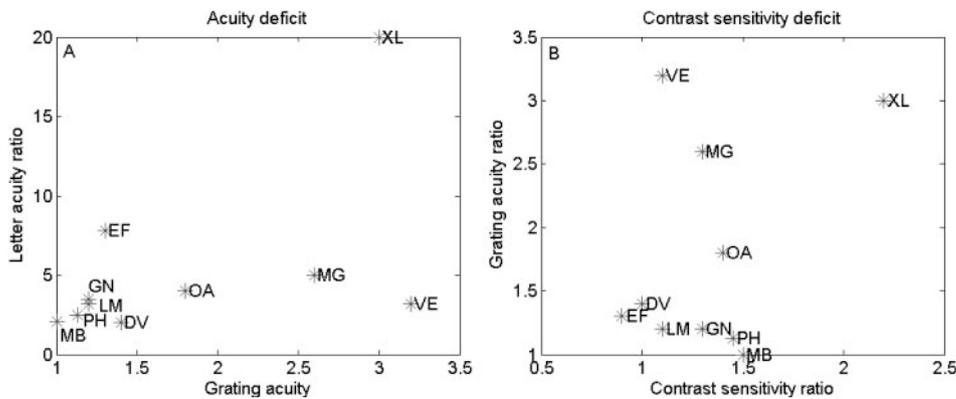


FIGURE 1. The psychophysical loss. (A) The acuity deficit was typically worse for letters than for gratings. (B) The contrast sensitivity deficit involved high and low spatial frequencies (1 cyc/deg).

and extrastriate cortex in humans with strabismic amblyopia. We used a spatially and temporally broadband mapping stimulus to assess whether any reduced cortical dysfunction correlates with the visibility loss, because the only evidence suggesting such a correlation has involved the use of broadband stimulation.^{30,34} Such a mapping approach allows the assessment of the regional extent (i.e., visual field) of the functional deficit in different visual areas as well as the extent to which the extrastriate deficit is correlated with either the visibility loss or the VI loss. The picture that emerges is one of a relatively consistent, correlated striate and extrastriate functional loss for suprathreshold spatiotemporal broadband stimuli that cannot be simply explained by the known psychophysical contrast detection deficit. We conclude that the cortical processing deficit in amblyopia is extensive, involving large regions of extrastriate cortex and is not simply explicable in terms of the known contrast detection deficit. Current notions that only high-spatial-frequency contrast processing in area V1 is disrupted in amblyopia should be revised.

METHODS

Subjects

Table 1 shows the clinical data for the 11 amblyopic subjects (average age, 34 ± 15 years) enrolled in the study. Clinically, amblyopia in humans can be subdivided into pure strabismus without anisometropia, pure anisometropia without strabismus and a mixed form where strabismus and anisometropia coexist. Six of our subjects had strabismic amblyopia, five had mixed strabismic-anisometropic amblyopia. During both the fMRI and psychophysics sessions, subjects wore non-magnetic spectacles to give them corrected acuity based on refraction. A control group of six normal subjects (average age, 29.8 ± 4 years) was also tested. During the scanning sessions, subjects monocularly viewed a stimulus back-projected into the bore of the scanner and viewed through an angled mirror. The eye not being stimulated was occluded with a black patch that excluded all light from the eye. All studies were performed with the informed consent of the subjects and

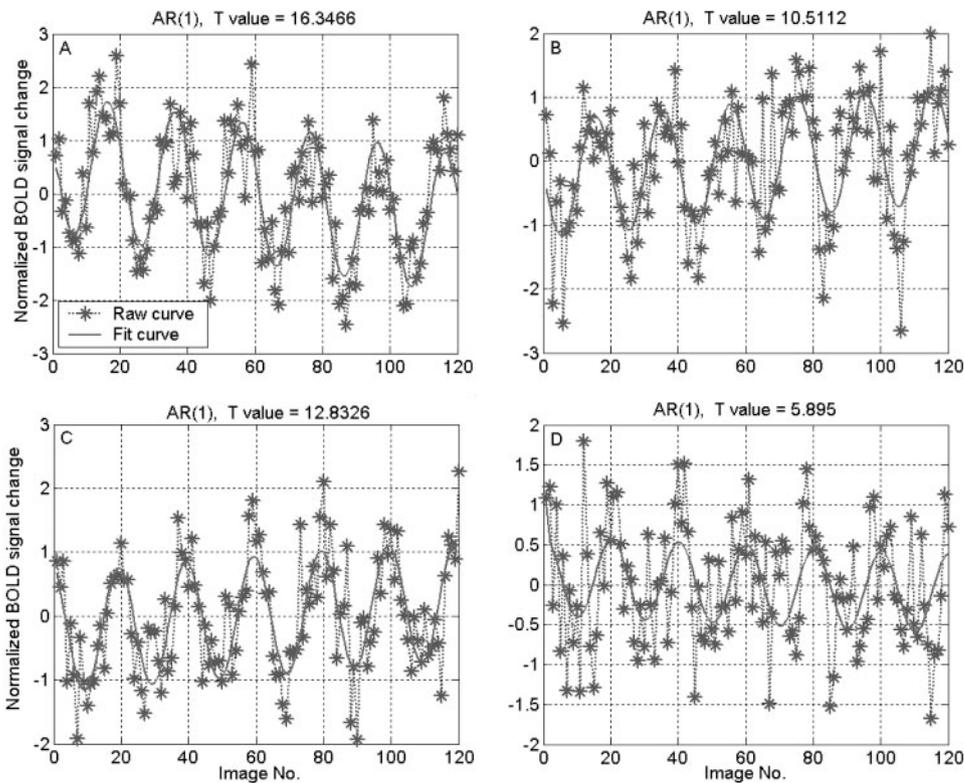


FIGURE 2. One example of BOLD signal change and *t* statistic calculation in our amblyopic subjects. (A, B) Time series from one eccentricity (clockwise order) run (fixing and amblyopic eye response time course, respectively), the fundamental component is of reduced amplitude when the cortex is driven by the amblyopic eye. (C, D) shows the time series from one polar angle (clockwise order) run (fixing and amblyopic eye response time course, respectively). All responses are at the same pixel position.

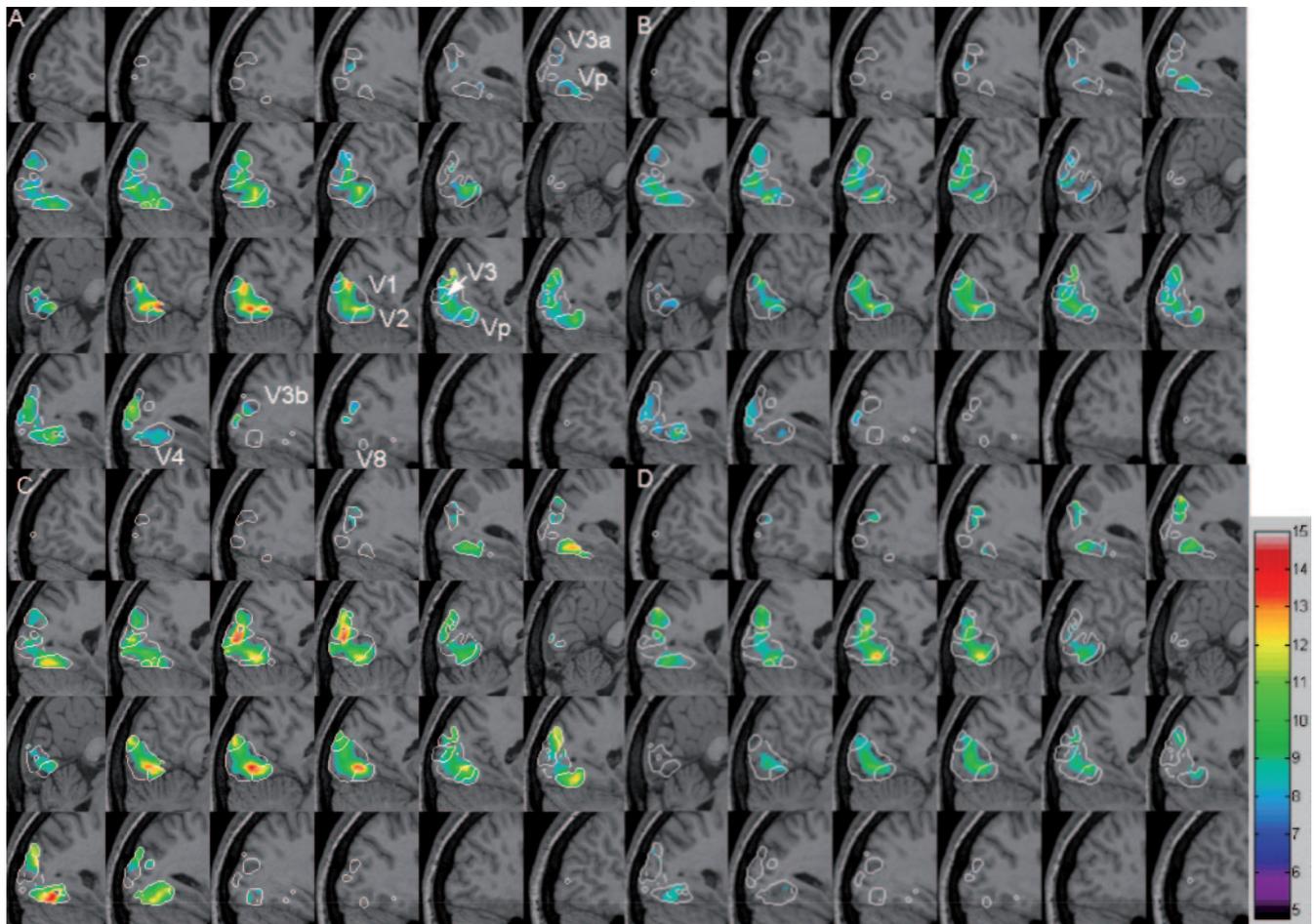


FIGURE 3. An example (subject LM) of the brain activation region for fixing (A, C) and amblyopic (B, D) eye stimulation in response to expanding, eccentricity (A, B) and clockwise, polar angle stimuli (C, D). Note the similar patterns of activation when each eye is stimulated, as well as the reduced activation when the amblyopic eye was stimulated. Color bar, the t statistic; threshold is $t > 4.79$.

the approved by the Montreal Neurologic Institute Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki.

Stimuli

The stimuli in this experiment were standard retinotopic wedge and annulus checkerboard sections used for retinotopic mapping.^{38,39} The abruptly alternating radial square wave checkerboard alternated at 8 Hz. The fundamental circumferential spatial frequency of the checks varied from 1.0 cyc/deg centrally to 0.1 cyc/deg peripherally. Both stimuli completed a full cycle in 12 time frames (0.03 Hz) giving a total of six cycles per scanning run (see Fig. 2). The checkerboard had a contrast of 80%. The wedge subtended 90°. The radial checkerboard contained 20 radial spokes and 10 concentric bands and subtended a visual angle of 34°.

Stimuli were presented in alternating runs between the left and right eyes of normal subjects or the fixing and amblyopic eyes of amblyopic subjects while the subject attended to a fixation spot and performed a visual task designed to control for attention. This task involved the detection of a coherent patch of checkerboard within the checkerboard stimulus as a whole that appeared at random times and positions. The responses were recorded via an optically isolated mouse to monitor the subject's attentive state, acquisitions were used only in sessions in which the percentage of correct responses was above 80%. All stimuli were back-projected onto a translucent screen by a gamma-corrected LCD video projector (model 820; NEC, New York, NY).

Using an automatic volumetric analysis,^{40,41} we defined separately the VFS map for fixing and fellow amblyopic eye stimulation. We then defined the common boundaries of different visual areas by combining the VFS information. We compared the signal strength within these commonly defined visual areas for dominant and nondominant eye stimulation of normal subjects and for fixing and fellow amblyopic stimulation of amblyopes. Eccentricities between 1.3° and 17° were tested.

Image Acquisition

A 1.5-T (Magnetom; Siemens, Madison, WI) scanner was used to collect both anatomic and functional images. Anatomic images were acquired with a rectangular coil (14.5 × 6.5 in.), head coil (circularly polarized transmit and receive), and T_1 -weighted sequence (TR [recovery time] = 22 ms; TE [echo time] = 10 ms; flip angle = 30°), giving 176 sagittal slices of 256 × 256-mm³ image voxels. Functional scans for each subject were collected with a surface coil (circularly polarized, receive only) positioned beneath the subject's occiput. Each functional imaging session was preceded by a surface coil anatomic scan (identical with the head coil anatomic sequence, except that 80 × 256 × 256 sagittal images of 2-mm slice thickness were acquired) to coregister the data later with the more homogeneous head coil image. Functional scans were multislice T_2^* -weighted, gradient-echo, planar images (TR = 3.0 seconds, TE = 51 ms, flip angle = 90°). Image volume consisted of 30 slices orthogonal to the calcarine sulcus. The field of

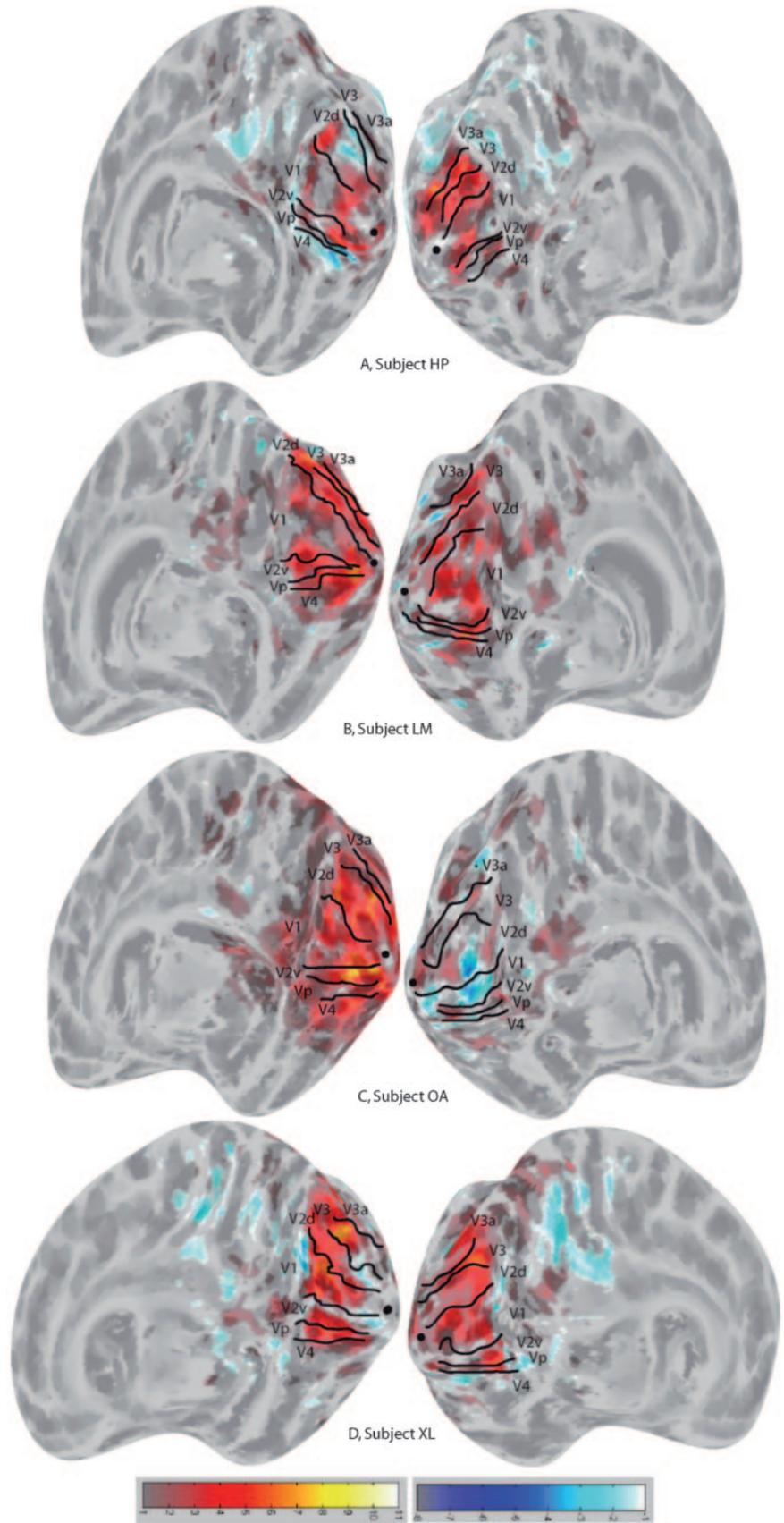


FIGURE 4. For illustrative purposes only, an oblique view of the unfolded cortex for four randomly selected amblyopes with the reduction in activation ((combined runs using good eye) – (combined runs using amblyopic eye)); quantified in terms of the *t* statistic) for amblyopic eye activation indicated. Reduced activation occurs not only in V1 but also extends to other retinotopic extrastriate visual areas (i.e., V2, V3, Vp, V3a, and V4). The hemispheric asymmetry apparent for one subject (OA) was not a general finding (see Fig. 12).

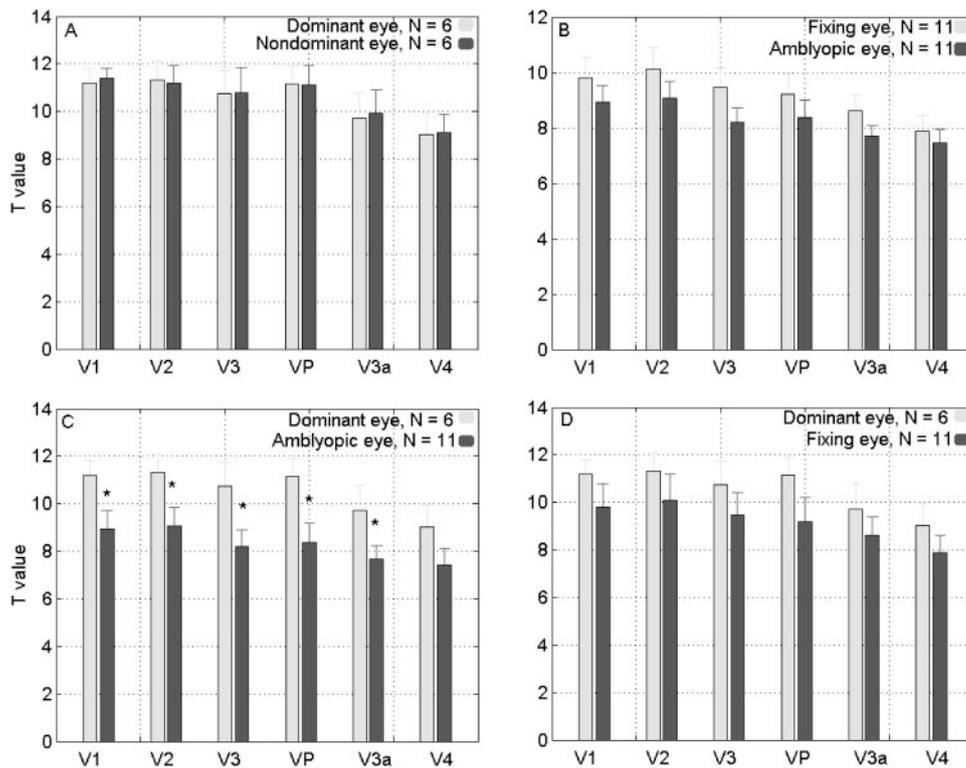


FIGURE 5. Group analysis. The mean and SEM of the *t* statistic from different runs for each VOI analysis for each subject group. There were 6 normal subjects and 11 amblyopes.

view was 256×256 mm, the matrix size was 64×64 with a thickness of 4 mm, giving voxel sizes of $4 \times 4 \times 4$ mm. Each experiment consisted of four acquisition runs for each eye (two eccentricity, two polar angle, two clockwise order, and two counterclockwise) each of 128 image volumes acquired at 3-second intervals for either the left and right eye of normal subjects or the fixing and amblyopic eye of amblyopes. Runs were alternated between the eyes in each case while the subject was performing the described task.

Data Analysis

Anatomic Images. The global T_1 -weighted anatomic (a)MRI scans were corrected for intensity nonconformity⁴² and automatically registered⁴³ in a stereotaxic space⁴⁴ using a stereotaxic model of 305 brains.⁴⁵ The surface coil aMRI, acquired in the same session as the functional images, was aligned with the head coil aMRI, thereby allowing an alignment of the functional data with the head coil MRI and subsequently the stereotaxic space. This method was validated in a previous study.⁴¹ The aMRIs were classified into gray matter, white matter, and cerebrospinal fluid (CSF), after which the four (gray matter, white matter, and left and right hemispheres) cortical surfaces for all subjects were simultaneously reconstructed at the inner and outer edges of the cortex.^{46–48} All processing steps were completely automatic, and all the data are presented in a stereotaxic space.

Functional Images. Dynamic motion correction for functional image time series for each run and for different runs were realigned at the same time by using `fmr_preprocess` (provided in the MINC software package: <http://www.bic.mni.mcgill.ca/software/>) with three-dimensional Gaussian low-pass filtering of time series data. The first eight scans of each functional run were discarded due to start-up magnetization transients in the data. The *t* statistic was calculated based on the fundamental frequency effects of the Fourier transformation (see the Appendix; The software, BrainVision, used for the fMRI data analysis is available in the public domain at: <http://www.mvr.mcgill.ca/Li/software.htm>). The design matrix was formed by combining fundamental frequency effects and the low-frequency drift effects. A general linear model (GLM) method was used to quantify the brain activation. Inference for the effects was developed according to the *t* statistics. Different runs for each eye were combined in a mixed model.^{49,50} The paired *t* statistic was used to test the significance of the activation ($t_{(117)} > 1.98$; $P < 0.05$, two-tailed *t*-test) between eyes.

Psychophysics

Eye Occlusion and Dominance. To test monocular function, we occluded either the fixing or fellow amblyopic eye with a black patch designed to exclude all light. This test was used for both the psychophysical testing and for the brain imaging. Under these condi-

TABLE 2. Multiple Paired *t*-Test Results of Group Analysis (Assume Equal Variance)

	<i>df</i>	V1	V2	V3	Vp	V3a	V4
Dom vs. nondom	10	0.257	0.105	0.024	0.035	0.157	0.061
Dom vs. fix	15	1.266	0.979	1.110	1.654	1.019	1.160
Dom vs. amb	15	2.484	2.315	2.558	2.756	2.142	1.676
Nondom vs. fix	15	1.516	0.893	1.106	1.603	1.288	1.313
Nondom vs. amb	15	2.891	2.226	2.494	2.676	2.554	1.881
Fix vs. amb	20	0.947	1.052	1.466	0.849	1.292	0.578

Data are results of paired *t*-test. Bold denotes statistically significant difference ($P < 0.05$). Dom, dominant eye; Fix, fixing eye; Amb, amblyopic eye; Nondom, nondominant eye; *df*, degree of freedom.

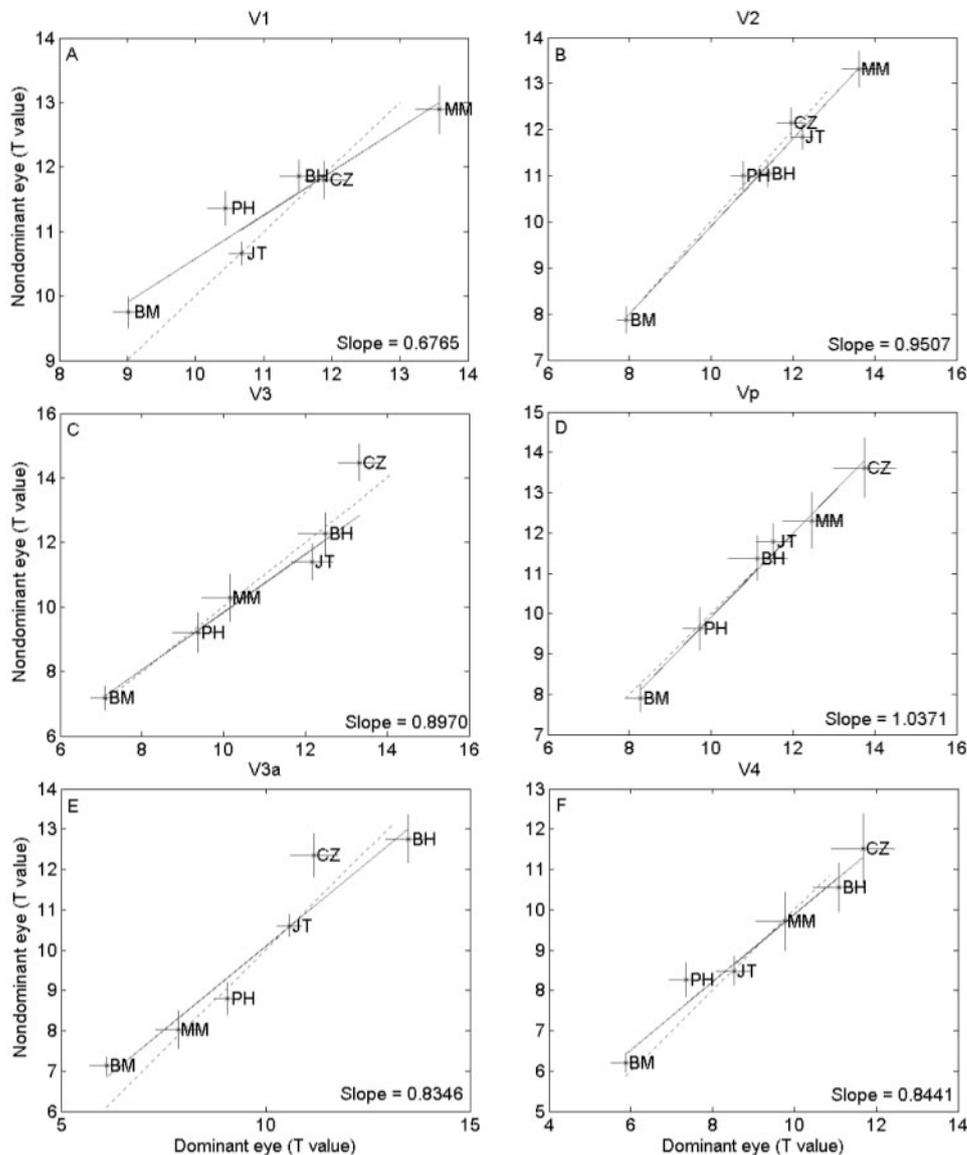


FIGURE 6. Brain activation (*t* statistic) from dominant versus nondominant eye stimulation in normal subjects; the slope of the *dashed line* is 1; the slope of the *solid line* is estimated by using robust regression method to the data. Slope is defined as the regression slope in $y = ax + b$.

tions there is no binocularly mediated suppression of the amblyopic eye because all pattern vision in the good eye has been abolished⁵¹ and thus our estimates of the reduced activation in the brains of amblyopes is a conservative one, as it does not include a binocular suppressive component. Eye dominance was assessed with a standard sighting test.⁵²

Eye-Movement Measurement. The fixation eye movements for the normal and fellow amblyopic eyes were measured separately for each subject with a video eye tracker (Cambridge Research Systems, Cambridge, UK) which sampled fixations at 50 Hz. Total viewing duration ranged from 3000 to 5000 ms. The subject was asked to fixate on a central fixation mark similar to that used in the scanner.

Grating Acuity. For each subject, grating acuity for optically corrected subjects was established by using a method of constant stimuli and a two-alternative, forced-choice (2AFC) paradigm with a VSG 3/4 card and a gamma-corrected monitor (Multisync XP 17; NEC). The two intervals were presented, one containing a static vertical grating of 80% contrast and the other, just mean luminance. The subject's acuity (corresponding to 82% correct identification) was measured with a staircase method over fixed spatial frequency steps from 2 to 52 cyc/deg. The field size had a diameter of 10°. A central fixation mark was provided.

Low-Spatial-Frequency Contrast Sensitivity. Contrast sensitivity (1 cyc/deg) measures were made with the VSG 3/4 card and gamma-corrected monitor (NEC). Contrast sensitivity was measured with a 2AFC paradigm and a staircase algorithm. Contrast sensitivity thresholds corresponding to 82% correct were determined for each eye of each amblyope. The field size was 10° diameter, and a central fixation mark was provided.

RESULTS

The Psychophysical Picture

The psychophysical loss exhibited by anisometric and strabismic amblyopes is now well established.⁵⁻⁷ It involves a letter acuity recognition deficit and a contrast sensitivity deficit. In terms of acuity, the deficit for recognizing letters is usually greater than it is for detecting gratings.⁵³ In terms of contrast sensitivity, higher contrast thresholds (i.e., reduced contrast sensitivity) are found at high spatial frequencies. The contrast threshold abnormality for some amblyopes is limited to high spatial frequencies, thresholds at low spatial frequencies being normal or in some cases better than normal. Others

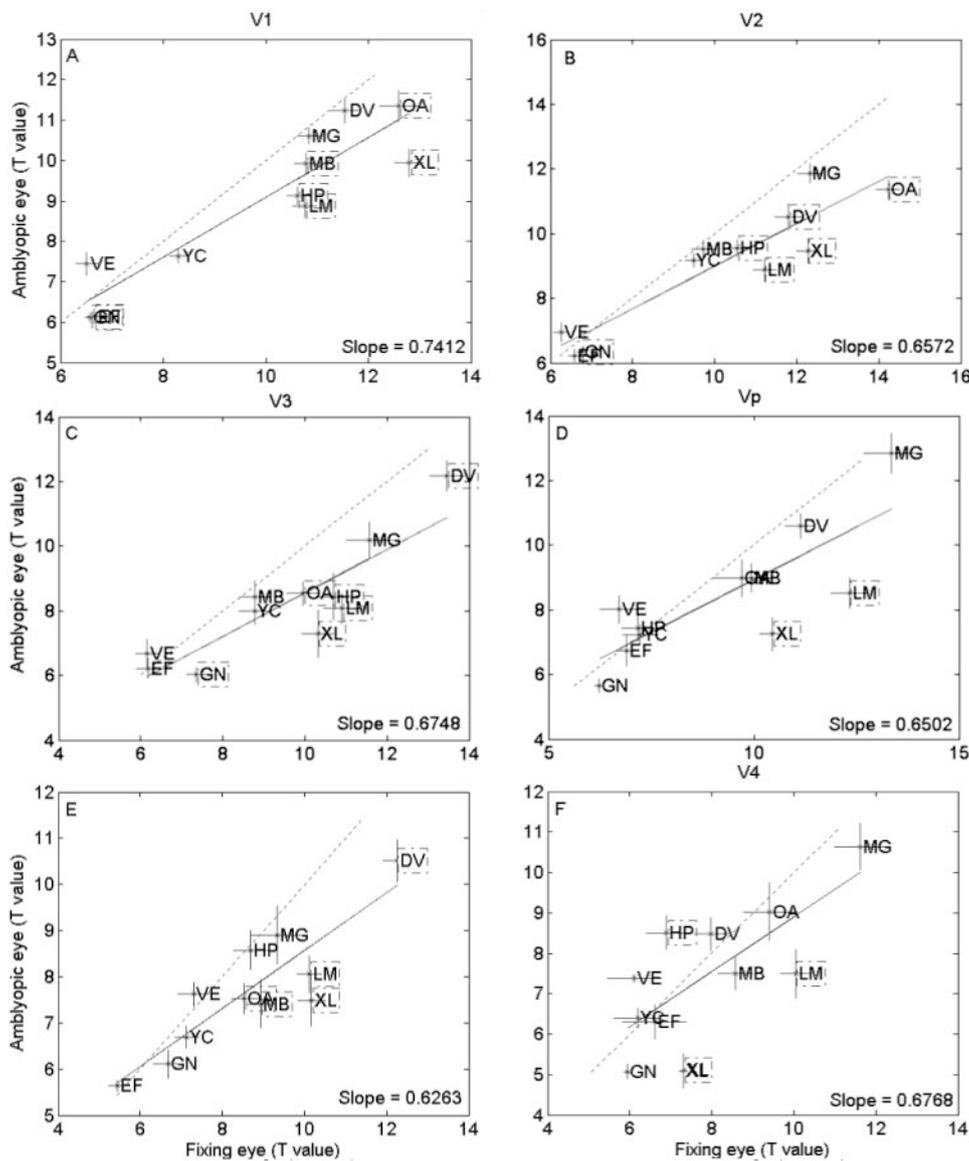


FIGURE 7. Brain activation (t statistic) for fixing versus amblyopic eye in amblyopic subjects; Data from subjects whose initials are within *dashed squares* are significant ($T > 1.960$; $P < 0.05$, two tailed t -test). *Dashed line*: represents equal activation; *solid line*: the robust fit to the amblyopic data as a whole. Slope is the regression slope in $y = ax + b$.

have a small residual, but constant, deficit at low spatial frequencies (1 cyc/deg and lower). Because no cases have been reported in which contrast deficits increase at low spatial frequencies, we have conservatively estimated the contrast threshold deficit in the low-spatial-frequency range by using a 1 cyc/deg stimulus. Above the raised threshold, amblyopes perceive the contrast of stimuli normally with the amblyopic eye.^{10,54,55} Furthermore, amblyopes can discriminate differences in contrast with normal accuracy at low spatial frequencies,⁵⁵ but at high spatial frequencies, there is evidence of a slight reduction in sensitivity.⁵⁵ A similar suprathreshold deficit has been reported in animals made artificially amblyopic and shown to be unrelated to the detection threshold deficit.⁵⁶ Thus, the picture that emerges is one of reduced function for low contrast stimuli of high spatial frequency.

In Figure 1 the psychophysical deficits are seen for our group of amblyopes. This figure shows a comparison of the acuity deficits (in **A**, grating and letter acuity; ratio of fixing eye/amblyopic eye) and the contrast sensitivity deficits (in **B**, grating acuity and contrast sensitivity at 1 cyc/deg). Grating acuity involved detection, and letter acuity involved recognition (linear acuity, each line containing six letters, five of

which had to be correctly identified). In each case, we saw the expected result. The acuity deficit was greater for letters, and the contrast sensitivity deficits involve high and low spatial frequencies.

The fMRI Picture

Figure 2 shows typical data (stars and dotted lines) for the fixing and amblyopic eyes of one of our amblyopic subjects (LM) for one voxel for the eccentricity (Figs. 2A, 2B) and polar angle (Figs. 2C, 2D) stimuli. The solid continuous curve represents the fitted response for calculation of the t statistic. The amblyopic response has a reduced t statistic but the difference is not large. We used the t statistic, because we wanted a measure that reflected response variance as well as amplitude.⁴⁹ For example, the Fourier amplitudes derived from the data displayed in Figures 2B and 2C are similar, but the higher t statistic for the data in Figure 2C stems from the better correlation of the signal with the sinusoidal variation of the stimulus. Although the use of the t statistic to quantify the fidelity of the response is superior to using just an amplitude measure,⁴⁹ we subsequently verified that our conclusions did

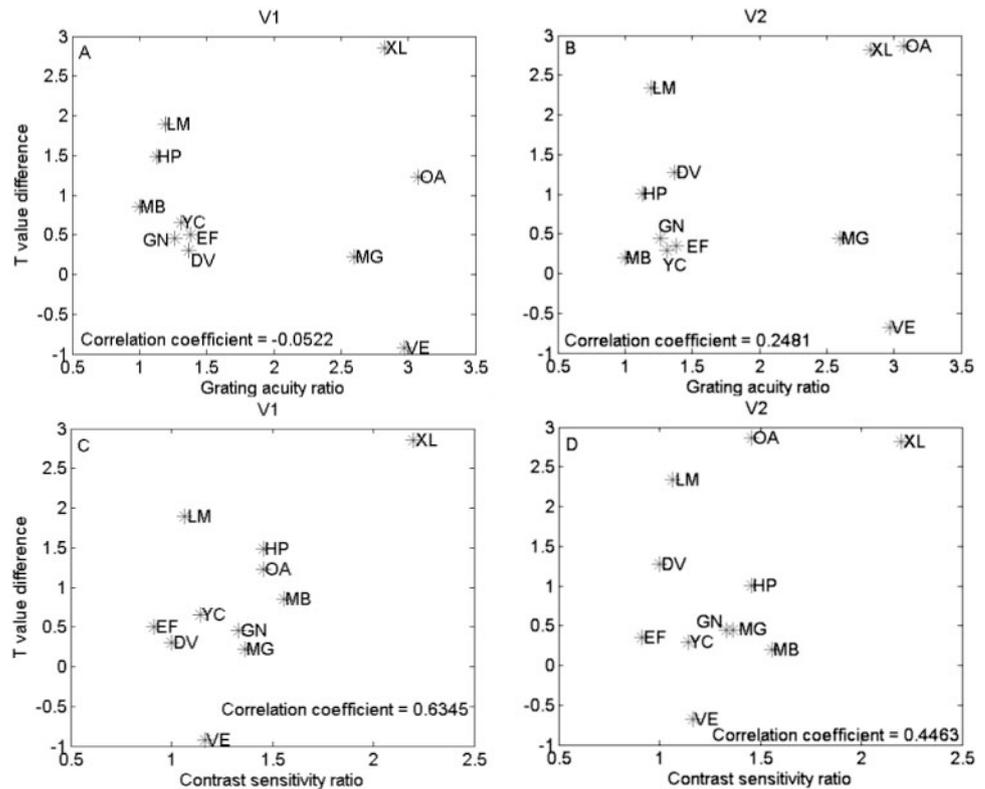


FIGURE 8. Grating acuity and contrast sensitivity ratios for each amblyope versus the reduced cortical activation; y-axis is the *F* statistic for the fixing eye minus the *t* statistic for the amblyopic eye; x-axis is the ratio of contrast sensitivity or grating. The respective correlation coefficients are shown.

not critically depend on our use of the *t* statistic. Similar results were obtained when using Fourier magnitude analysis.

Figure 3 shows an example of the regional brain activation by the mapping stimuli, for the fixing eye (Figs. 3A, 3C) compared with the fellow amblyopic eye (Figs. 3B, 3D) of one of the subjects (LM). A comparison of Figures 3A and 3B shows stronger activation of the fixing eye by the eccentricity stimulus where as Figures 3C and 3D illustrate the stronger activation of the fixing eye by the polar angle stimulus. Figure 4 shows a comparison of the regional reductions in activation for the combined runs (two polar angle and two eccentricity) for four randomly selected amblyopes (fixing versus amblyopic eye activation) for the retinotopically mapped cortical areas (solid black lines). In general, we found good correspondence between the VFS maps for fixing and fellow amblyopic eye stimulation, such that the commonly defined boundaries of different visual areas, used for our later analysis, were similar for fixing and fellow amblyopic eye stimulation. The reductions involved both striate and extrastriate cortical areas, and both hemispheres were affected. Only amblyope OA showed a strong hemispheric asymmetry and a better response with the amblyopic eye in V1-V2).

The *t* statistic for a standard volume-of-interest analysis of the mapped visual areas (i.e., V1, V2, V3, Vp, V3a, V4) of the dominant and nondominant eyes of six normal observers is displayed in Figure 5A. There is no significant activation difference between the eyes of our normal group for any individual area or for all the areas combined. A similar comparison between the fixing and amblyopic eyes of our amblyopic group is displayed in Figure 5B, and it shows consistently reduced cortical activation in these different cortical areas when driven by the amblyopic eye. Owing to the large group variance within any one area (i.e., not consistently exhibited by each subject; see Figure 7), this reduced activation of individual cortical areas did not reach significance (see Table 2 for results of a multiple paired *t*-test). Figure 5C shows that a similarly

consistent reduction of cortical activation was evident when the responses of the amblyopic eyes of amblyopes and the dominant eye of our normal observers were compared; significant differences were obtained in areas V1, V2, V3, Vp, and V3a (Table 2). Similar significant differences were obtained (data not shown) between the amblyopic eye and the non-dominant eye of normal observers (Table 2). The differences between the activation of fellow fixing eyes of amblyopes and the dominant eyes of normal observers were fractionally larger than a similar comparison between the amblyopic and fellow fixing eyes of amblyopes, suggesting that the cortex driven by the fellow fixing eye may not be identical to that driven by the eyes of normal observers. Such a comparison (dominant eyes of normal subjects versus fellow fixing eyes of amblyopes) is shown in Figure 5D where a small but consistent reduction of activation is seen across all cortical areas for the fellow fixing eyes of amblyopic observers, although with the large intersubject variance, none of these reductions are significant at the $P = 0.05$ level (two-tailed *t*). The extent of the amblyopic dysfunction is illustrated in Figure 4 where color-coded maps of the reduced activation are shown for four amblyopes along with the retinotopically mapped visual areas. Significant reductions occurred in striate as well as extrastriate areas.

Figures 6 and 7 illustrate these comparisons of functional activation in different cortical areas for normal subjects and amblyopes in a way in which the contribution from individual subjects can be identified. In Figure 6, the *t* statistic for the activation of the dominant and nondominant eyes of normal subjects is plotted against each other. On each of these and subsequent figures, the data for each subject are identified by his or her initials. The solid line is the best-fitting line to the population as a whole and, in all brain areas investigated, its slope (Fig. 6, bottom right of each panel) was not statistically different from unity (dotted line). A similar comparison is shown in Figure 7 for the amblyopic population, with the data for each amblyope indicated by initials. The best-fitting line to

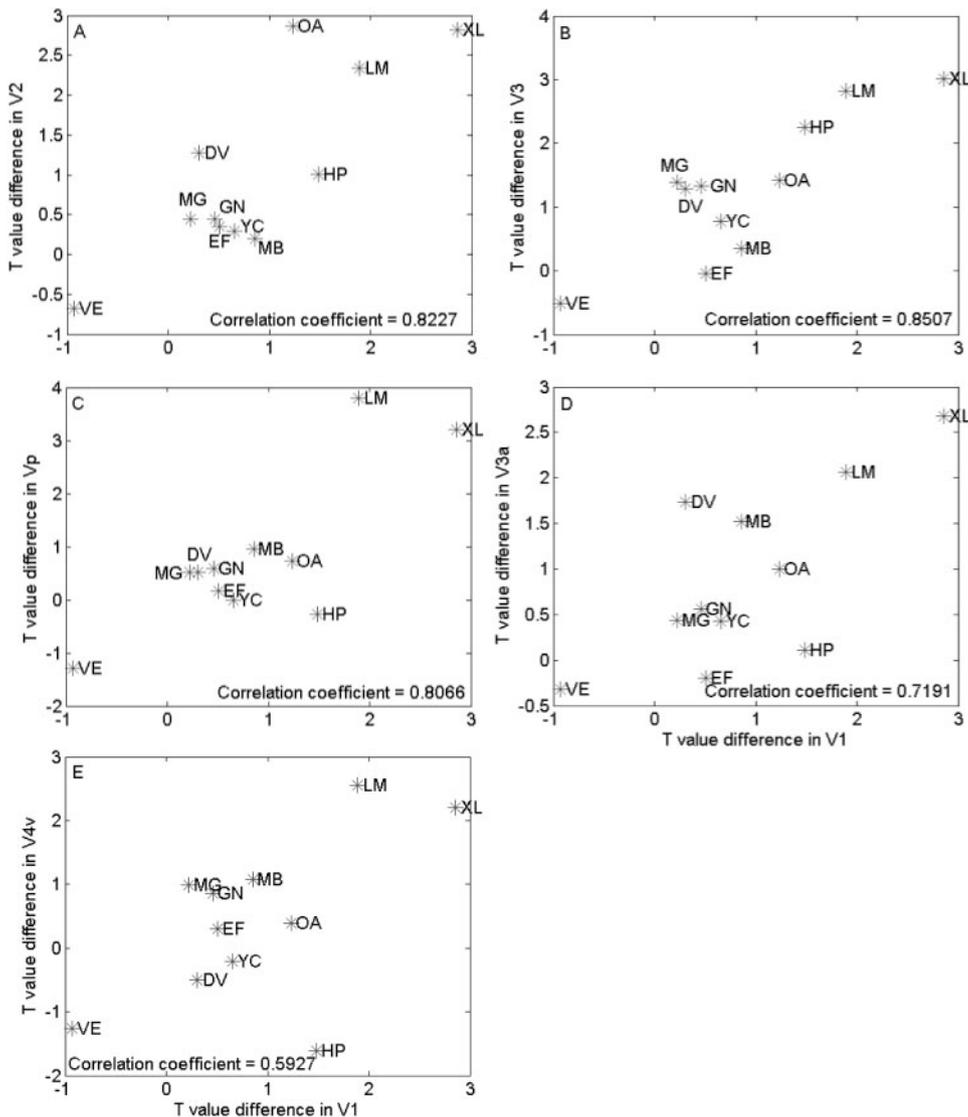


FIGURE 9. The correlations between the reductions in striate and extrastriate cortex are shown by plotting the *t* statistic differences for V1 against those for other visual areas. The respective correlation coefficients are shown.

the amblyopic population (solid line) can be compared with the unity prediction (dashed line) found for normal subjects (Fig. 6). The slope values are shown in Figure 7 (bottom right).

Because the large intersubject variability evident in Figures 6 and 7 for the normal and amblyopic populations limits the sensitivity of the group comparisons (i.e., either in terms of the means in Figure 5 or the slopes in Figure 7), we assessed the significance (volume of interest [VOI] paired *t*-test; fixing versus amblyopic eye, $P < 0.05$) of the reductions in cortical activation for each amblyopic subject separately using the fellow fixing eye as the control. The advantage of such a comparison is that each subject can act as his or her own control, with a subsequent reduction in variability. The disadvantage is that the fellow fixing eye's activation may be slightly reduced below that of the dominant eye of a normal observer (suggested by the results in Fig. 5C but not statistically significant in the group data) and as a consequence, any activation difference found between the amblyopic and normal fixing eye would underestimate the extent of the amblyopic dysfunction. In Figure 7, for each visual area, we have indicated the subjects (by enclosing initials in a dashed box) whose reduced cortical activation when driven by their amblyopic eye was statistically significant.

To estimate the extent to which any reduction in cortical activation that is evident in the amblyopic population (i.e., Fig. 7) correlates with the contrast sensitivity deficit, we compared, on a subject-by-subject basis, the difference in *t* statistics between the fellow fixing and amblyopic eyes with both the ratio of grating acuities (Figs. 8A, 8B) and contrast sensitivities (Figs. 8C, 8D). We made this comparison for visual areas V1 (Figs. 8A, 8C) and V2 (Figs. 8B, 8D) because the cells in these areas are thought, on the basis of their spatial properties, to make the main contribution to behavioral contrast sensitivity.^{57,58} We found no significant correlation (Fig. 8, bottom right of each panel) for either area V1 or V2 between either visibility measure and the reduced activation associated with responses from the amblyopic eye, although the correlation between the V1 loss and the contrast sensitivity ($r = 0.60$) loss bordered on significance. A similar correlation analysis (data not shown) between fMRI and psychophysics for areas V3, Vp, and V3a also yielded insignificant correlations.

Because it appears that several visual cortical areas that we mapped have reduced activation if driven by the amblyopic eye, we wondered to what extent the extrastriate loss correlates with the striate loss. The reduced V1 activation, as quantified by the *t* statistic difference between activation of fellow

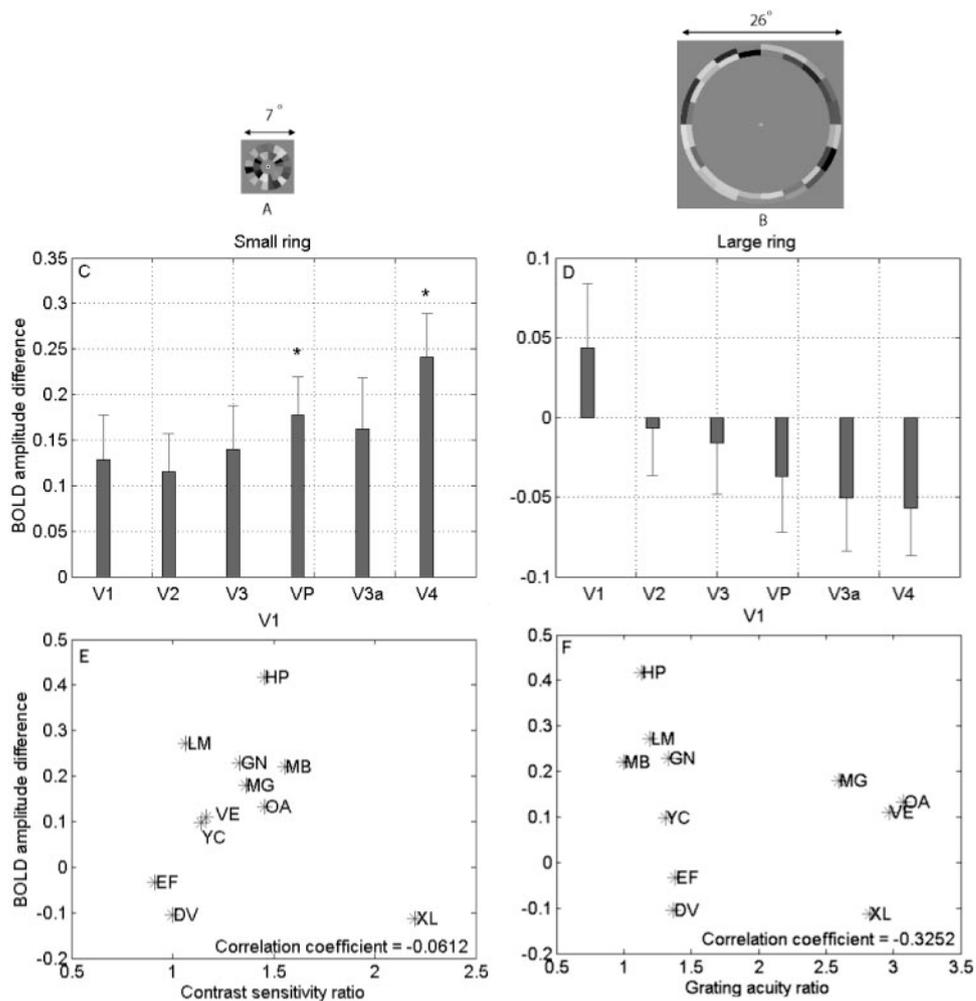


FIGURE 10. The difference in percentage of BOLD signal between the fixing and fellow amblyopic eyes (C, D) of our amblyopic group for centrally located (A) and peripherally located (B) stimuli. (E, F) Group correlations are shown for the reduced V1 activation for centrally located stimuli and the contrast sensitivity and grating acuity deficits for the same subjects.

fixing and amblyopic eyes, is plotted against the reduced activation in other visual cortical areas (i.e., V2, V3, Vp, V3a, and V4) in Figure 9. The correlation coefficients are shown, and these suggest that the striate and extrastriate losses are significantly correlated in all mapped areas.

Up to this point, we have compared cortical activation corresponding to topologically mapped visual areas without consideration for the visual field location subserved by different parts of the cortex. We now address the question of the visual field locus of the reduced cortical activation in amblyopia. Our mapping stimulus allows us to examine just the cortical responses that subserve central (inner radius 1.3°, outer radius 3.5°, fundamental spatial frequency 0.76 cyc/deg) as opposed to peripheral (inner radius 10.8°, outer radius 13°, fundamental spatial frequency 0.14 cyc/deg) parts of the visual field (Figs. 10A, 10B). These results, for different visual areas, for the amblyopic group as a whole are shown in Figures 10C and 10D for the central and peripheral visual fields, respectively. The difference in the percentage of blood oxygenation level-dependent (BOLD) signal between the fixing and fellow amblyopic eye is plotted for each visual area and shows that the reduced activation mainly affects the central field. The central field responses from the extrastriate areas, Vp and V4, exhibited significantly VOI reductions when driven by the amblyopic eye (Table 3) whereas none of the cortical areas subserving the peripheral field are significantly reduced (Table 4). The scatterplots shown in Figures 10E and 10F assess whether this reduction in activation for central field stimula-

tion correlates with either the contrast sensitivity loss or the grating acuity loss. The low correlations found reinforce the conclusions reached previously for the whole field stimulation (Fig. 8)—namely, that there is no strong correlation between the reduced activation and either the contrast sensitivity (Fig. 10E) or grating acuity (Fig. 10F) in human amblyopes, at least for the broadband stimuli used in the current study. It is apparent that the poor correlation found in Figure 10E between the reduced activation and the contrast sensitivity deficit is strongly influenced by the results of XL. Without XL's data, the correlation rises to 0.66, which just reaches significance (a correlation coefficient of 0.63 is significance at the 5% level). However, we could find no valid reason to exclude XL's data. Even though he has the greatest acuity deficit, his clinical profile, contrast sensitivity loss, and activation loss are not exceptional in any way.

Another way of illustrating that the functional activation deficit in strabismic amblyopia involves mainly central vision is to plot the difference in the percentage of BOLD signal obtained from fixing and fellow amblyopic eye stimulation for the central versus the peripheral stimulus for each subject (Fig. 11). The solid line is the veridical prediction, that is the prediction that the reduced activation affects central and peripheral visual field loci equally—in other words, if the fMRI deficit were equally distributed over the visual field. The data for most subjects lie above this equality line, indicating a greater central contribution to the functional defect in all visual areas.

TABLE 3. Multiple Paired *t*-Test Results for Small Ring, Central Response

	<i>df</i>	V1	V2	V3	Vp	V3a	V4
Dom vs. nondom	10	0.688	0.540	0.498	0.570	0.253	0.484
Dom vs. fix	15	0.525	0.158	0.563	1.397	2.142	2.237
Dom vs. amb	15	2.040	1.841	0.781	3.373	3.909	4.150
Nondom vs. fix	15	0.337	0.422	1.211	0.475	1.967	1.443
Nondom vs. amb	15	0.968	0.848	0.022	2.131	3.829	3.268
Fix vs. amb	20	1.713	1.584	1.849	2.513	1.665	2.751

Data are results of paired *t*-test. Bold denotes statistically significant at $P < 0.05$. Abbreviations as in Table 2.

To assess the previous claim that activity is reduced in areas V1 and V2 ipsilateral to the amblyopic eye,²⁵ we undertook a separate comparison of activation in areas V1 and V2 for the two hemispheres. Figure 12 shows a hemispheric comparison for the dominant and nondominant eyes of normal subjects and the fixing and fellow amblyopic eyes of amblyopes. We found no difference in the reduced amblyopic activation in either of these cortical areas. There is no indication of any hemispheric asymmetry within our amblyopic population when the cortex is driven by the amblyopic eye.

DISCUSSION

The V1 Loss

When a spatiotemporal broadband stimulus of suprathreshold contrast was used, our amblyopic subjects, as a group, did not show enhanced or even normal activity for amblyopic eye stimulation in area V1, contradicting a recent report.²⁶ The responses of amblyope OA (Fig. 4C) show a localized enhancement of activation in V1/V2d, but this was not a common finding. Generally, we found small but consistent *reductions* in activation in area V1 when driven by the amblyopic eye compared with that of the fellow fixing eye. Small but consistent differences also occur between the V1 response when driven by the fellow fixing eye of amblyopes compared with the dominant eye of normal subjects but not, as far as we could see, between the eyes of normal observers. As a group, these differences become significant in areas V1, V2, V3, Vp, and V3a. This suggests that V1 responds abnormally when driven by either the fellow fixing eye or the amblyopic eye, although the reduced activation is greater for the latter. Some amblyopes within our sample exhibited greater reductions than others in their V1 response, but we found no correlation with the contrast detection loss across our amblyopic group. It should be pointed out that our approach was one that would tend to give a conservative estimate of the magnitude of reduced functional activation in amblyopia: we completely covered the nonstimulated eye, thereby excluding any suppressive influences; and to reduce the intersubject variability, we compared amblyopic

responses to those of the fellow fixing eye in the same subjects, notwithstanding the possibility that the fellow fixing eye might itself be slightly reduced in its activation. We choose the two key psychophysical measures that have been shown to capture the variability in the visibility loss in a large sample of human amblyopes,⁵⁹—namely, the grating acuity and contrast sensitivity. Neither measure strongly correlated with the reduced functional activation in V1 or any other extrastriate area. This finding is at odds with conclusions in two previous studies^{30,34} that the reduced functional activation in amblyopia to spatiotemporal broadband stimuli, similar to that used in the current study, correlates with the psychophysical loss. However, it is consistent with two previous studies^{26,33} in which spatiotemporal narrowband stimuli were used and showed no group correlation with either of these two key psychophysical measures. We also show that the reduced activation selectively involves central field stimulation, a finding recently reported by Conner and Mendola³⁶ and Muckli et al.,²⁶ who report enhanced amblyopic peripheral responses. Our finding of a larger activation deficit for central vision may be because of visual field locus per se or because the deficit selectively involves the processing of higher spatial frequencies that are represented only in the more central parts of the field and selectively stimulated by our radial checkerboard stimulus. What is surprising if this latter explanation is correct is that there is not a better correlation between the functional central field deficit and psychophysics (i.e., grating acuity). Amblyopes exhibit larger saccadic fixation eye movements than do normal subjects, but we do not believe that these played a significant role in the reduced activations that we report. First, it has been shown that eye movements less than 3° have little effect on fMRI response,^{60,61} and none of our amblyopes had fixation eye movements exceeding 1.8°. Second, it is known that the magnitude of the eye movement abnormality in amblyopia correlates highly with the visual acuity^{62,63} yet our reduced activations did not show any significant correlation with visual acuity for either full field or central stimulation. All our subjects had strabismus, but five also had anisometropia. Although we did not observe any difference in the responses of subjects

TABLE 4. Multiple Paired *t*-Test Results for Large Ring, Peripheral Response

	<i>df</i>	V1	V2	V3	Vp	V3a	V4
Dom vs. nondom	10	0.905	0.796	1.091	1.101	0.812	1.485
Dom vs. fix	15	1.484	0.773	0.231	2.843	3.099	2.558
Dom vs. amb	15	2.020	0.724	0.383	2.192	2.303	2.033
Nondom vs. fix	15	0.056	0.408	1.471	0.852	1.823	0.741
Nondom vs. amb	15	0.565	0.519	1.474	0.449	1.196	0.234
Fix vs. amb	20	0.762	0.138	0.226	0.558	0.686	0.632

Data are results of paired *t*-test. Bold denotes statistically significant at $P < 0.05$. Abbreviations as in Table 2.

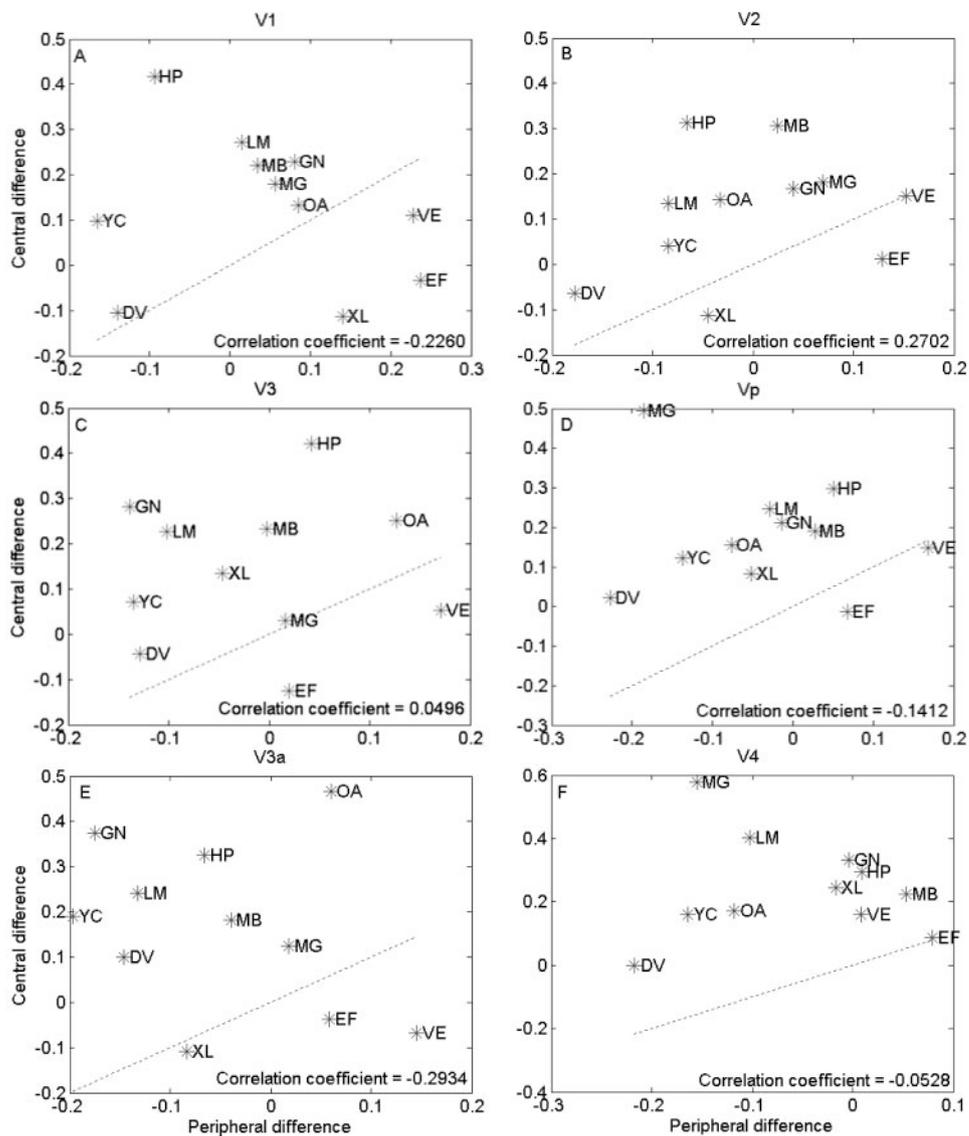


FIGURE 11. The central versus peripheral reduction (%BOLD signal change in fixing eye – %BOLD signal change in amblyopic eye) in functional activation for individual amblyopic subjects. *Dashed line:* equal reduction for central and peripheral stimulation, whereas responses above the *dashed line* indicate larger differences between fixing and amblyopic eye activation for central stimulation.

with or without anisometropia, a much greater sample would have been needed to resolve the question.

Currently, it is believed that the neural loss in V1 underlies the contrast sensitivity deficit in animals with amblyopia because of the strong correlation between the behavioral loss measured in terms of contrast sensitivity and grating acuity and comparable single-cell measures on the same animals.¹⁵ However, the current results and those of several previous studies of brain imaging^{26,31,33} in humans suggest no strong correlation between the reduced cortical activation and these two key measures of the psychophysical visibility loss: contrast sensitivity and grating acuity. This is either because human amblyopia is unlike its animal model or because the measures of cortical function that can be used in humans are fundamentally different from the single-cell measures used in animal studies. It is possible that the behavioral measures reflect the activity of just a few relevant cortical cells and therefore are reflected in the physiology, whereas fMRI and MEG represent more mass activity and as a consequence correlate less well. Furthermore, fMRI is an indirect measure of neural activity in V1, being more to do with its synaptic input and intracortical processing than the spiking activity of output cells,²⁴ and it may provide a quite different reflection of V1 processing. MEG (e.g., Anderson et

al.³¹), provides a more direct measure of the magnetic fields associated with neural activity but is susceptible to cancellation effects from multiple sources as a consequence of cortical geometry. Another issue involves the type of stimuli used for functional studies. These may not be optimal for making the link with the psychophysics or with single-cell studies. Ideally, one should use well-localized (i.e., foveal) spatiotemporal narrowband stimuli of low contrast to try to isolate the responses of just the subset of the V1 neurons that the animal models suggest are the most vulnerable.^{15,23,64-66} The approach taken by Muckli et al.,²⁶ in which they compared grating acuity as assessed psychophysically in an fMRI event-related design, speaks directly to this issue. They found not only a dissociation between the psychophysics and fMRI, using what would appear to be appropriate, narrowband stimuli, but they also report either no deficit or enhanced amblyopic activation for areas V1 and V2. Having said that, the type of stimuli remains an important issue that future studies should try to resolve, because the reduced activation documented in this and previous studies (Sireteanu R et al. *IOVS* 1998;39:ARVO Abstract 4186),^{25,28-35} using high-contrast narrow and broadband stimuli, suggest that there are significant V1 deficits in functional activation for suprathreshold, low-spatial-frequency stimuli.

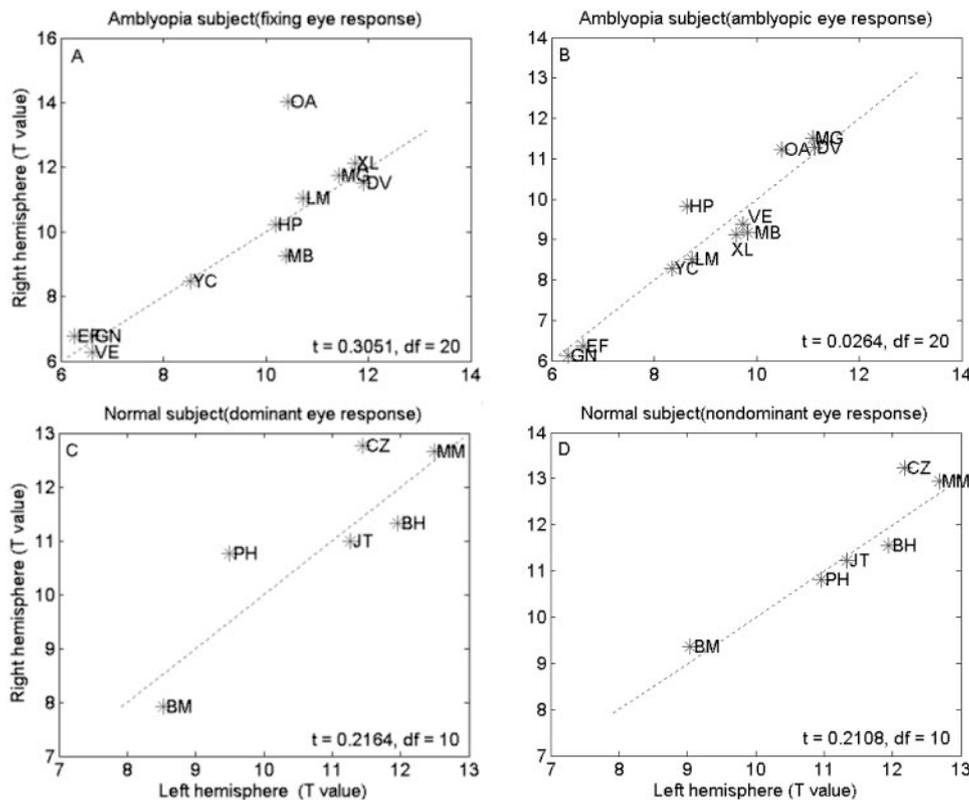


FIGURE 12. Hemisphere difference comparison (same stimuli, same eye). *Dashed line:* the equality line (i.e., right hemisphere response = left hemisphere response), t represents the derived t statistic, and df is degree of freedom.

The Extrastriate Contribution

One of the purposes of this study was to assess the degree of extrastriate involvement, as a number of previous studies (Sireteanu R et al. *IOVS* 1998;39:ARVO Abstract 4186)^{25–27,33,36} have suggested that the extrastriate cortex is either solely affected or also affected, and yet we know so little about extrastriate function in animals made artificially amblyopic. We show that the dysfunction may extend to many of the extrastriate areas that can be topographically mapped (i.e., V2, V3 Vp, and V3a exhibited significant reductions across our amblyopic group). The reduced activation in striate and extrastriate areas mainly involves the central field representation—true of the amblyopic sample as a whole and in most, but not all, individual amblyopes (Fig. 7). The lack of a strong correlation between the reduced cortical activation when driven by the amblyopic eye and the psychophysical deficit appears to hold for V2 as well as V1 and for central as well as whole-field stimulation. The reduced activation found in extrastriate areas showed a strong positive correlation with that found in V1 and this correlation was particularly strong in areas V2, V3, and Vp and weakest in area V4.

The Fellow Fixing Eye's Response

There is psychophysical evidence that the fellow fixing eye of amblyopes is not completely normal. This evidence involves form as well as global motion.^{67–72} Our functional data are suggestive of reduced function, although the group activation levels were not significantly different between the dominant eyes of normal subjects and the fixing eyes of amblyopes. Such a reduction in function could be due to a reduction in the number of binocularly activated neurons in these cortical areas. Although much is known about the loss of binocularity of V1 neurons as a result of a strabismus,¹¹ there are also reports of decreased numbers of binocular extrastriate cells.^{18,73} Even

though we suspect that the fellow fixing eye is reduced in its function compared with the dominant eye of normal subjects, we have still made all the comparisons between the response driven by the amblyopic and fellow fixing eyes to help combat the sometimes large intersubject differences in functional activation. In so doing, we are almost certainly underestimating the reduction in functional cortical activation produced by stimulation of the amblyopic eye.

We provide the following answers to the three posited questions: (1) *Is V1 affected?* The answer is yes, it is affected in most amblyopes. We found one case (OA) in which V1 and V2 were spared. (2) *How extensive is the loss of cortical function, and does it simply follow as a consequence of an impaired V1 input?* The answer is, there is extensive extrastriate loss, most of which correlates with the V1 loss, suggesting a primary site in V1, at least for the stimuli we used. (3) *Does the brain imaging deficit, be it striate or extrastriate, correlate with the well-documented psychophysical loss?* The answer is no, suggesting additional processing deficits for well detectable stimuli of low spatial frequency.

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APPENDIX: GENERAL LINEAR MODEL

The time series of BOLD signal change was normalized

$$\dot{Y}(i) = \frac{S(i) - \bar{S}}{\sigma}$$

where $\dot{Y}(i)$ is the normalized BOLD-fMRI time series, $S(i)$ is the raw fMRI time series, i is the sampled time point (image frame), \bar{S} is the mean value of the series, and σ is the SD of the time series.

Based on general linear model(GLM)^{49,50}

$$\dot{Y}(i) = \dot{X}\dot{\beta} + \varepsilon = [\dot{X}_1(i), \dot{X}_2(i), \dots, \dot{X}_k(i)] \begin{bmatrix} \dot{\beta}_1 \\ \dot{\beta}_2 \\ \vdots \\ \dot{\beta}_k \end{bmatrix} + \varepsilon \quad (A1)$$

where $\dot{\beta}$ is the regression parameters, ε is random error, and X_i is the $n \times k$ design matrix. We formed $\dot{X}_1(i)$ as:

$$\dot{X}_1 = \text{real}(\text{iff}(f)) = \cos(\omega)$$

where f is the fundamental frequency that was obtained by fast Fourier transformation of the response time series; iff is inverse Fourier transformation (the frequency of the stimuli was 6 in our study); real is the real part of the number. This is based on Euler's equation: $e^{j\omega} = \cos(\omega) + \sin(\omega)j$, where $j = \sqrt{-1}$ is the imaginary part of the inverse Fourier transformation. To eliminate the low frequency drift, we added polynomial drift $\dot{X}_2(i), \dots, \dot{X}_k(i)$ in the design matrix (we set the order of the polynomial equal to 1; $k = 3$, that is we add the $\dot{X}_2(i) = 1$ and slope drift $\dot{X}_3(i) = i$ in the design matrix).

The noise was modeled according to the first-order autoregressive model (AR(1))

$$\varepsilon_i = \rho\varepsilon_{i-1} + \xi$$

$$\text{where, } \xi \sim N(0, \sigma_\xi^2) \quad \rho = \frac{\sum_{i=2}^n \varepsilon_i \varepsilon_{i-1}}{\sum_{i=2}^n \varepsilon_{i-1}^2}$$

$$X_1 = \sqrt{1 - \rho^2}\dot{X}_1, \quad X_i = \dot{X}_i - \rho\dot{X}_{i-1}$$

$$Y_1 = \sqrt{1 - \rho^2}\dot{Y}_1, \quad Y_i = \dot{Y}_i - \rho\dot{Y}_{i-1}$$

so that the model becomes

$$Y_i = X_i'\beta + \xi_i \quad (A2)$$

The least-squares method was adopted to solve the equation (A2). We wanted to test $H: A\beta = c$, A is $p \times q$ matrix, statistic for this is: $A\hat{\beta} - c$; $\hat{\beta}$ is the estimated β ; H will be rejected if $A\hat{\beta}$ is sufficiently different from c . The residual sum of squares (RSS) is calculated:

$$\text{RSS} = \|Y - X\beta\|^2$$

where $\|\cdot\|$ is the norm, and

$$\hat{\beta} = X^+ Y$$

$\hat{\sigma}^2 = RSS/\nu$, where $\nu = n - \text{rank}(X)$

$$E = A\hat{\beta} \quad (A3)$$

the estimated SD is

$$S = \|AX^+\|\hat{\sigma}$$

The t statistic is calculated as

$$T = \frac{E}{S}$$

In this study, because we were interested in fundamental frequency effects, we set $c = 0$ and contrast $A = (100)$ in equation A3.

The mixed-effects model was used to combine runs of each eye:

$$E_j = Z_j' \gamma + \eta_j$$

where $j = 1, \dots, n$, n is the total number of runs; Z is the design matrix (average the effects using covariates $Z_j = 1$); η_j is normally distributed with 0 mean and variance $S_i^2 + \sigma_{\text{random}}^2$ independently for j , σ_{random}^2 is estimated using the expectation maximum algorithm. The threshold was estimated using Tstat_threshold; $P = 0.05$, $df = 117$, $t = 4.79^{(49,50)}$.