BOLD fMRI Response of Early Visual Areas to Perceived Contrast in Human Amblyopia

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Goodyear, Bradley G., David A. Nicolle, G. Keith Humphrey, and Ravi S. Menon. BOLD fMRI response of early visual areas to perceived contrast in human amblyopia. J Neurophysiol 84: 1907–1913, 2000. In this study, we used a temporal two-alternative forced choice psychophysical procedure to measure the observer’s perception of a 22% physical contrast grating for each eye as a function of spatial frequency in four subjects with unilateral amblyopia and in six subjects with normal contrast. Contrast thresholds were also measured using a standard staircase method. Additionally, blood-oxygenation-level–dependent (BOLD) functional magnetic resonance imaging (fMRI) was used to measure the neuronal response within early visual cortical areas to monocular presentations of the same 22% physical contrast gratings as a function of spatial frequency. For all six subjects with normal vision and for three subjects with amblyopia, the psychophysically measured perception of 22% contrast as a function of spatial frequency was the same for both eyes. Threshold contrast, however, was elevated for the amblyopic eye for all subjects, as expected. The magnitude of the fMRI response to 22% physical contrast within “activated” voxels was the same for each eye as a function of spatial frequency, regardless of the presence of amblyopia. However, there were always fewer “activated” fMRI voxels during amblyopic stimulation than during normal eye stimulation. These results are consistent with the hypotheses that contrast thresholds are elevated in amblyopia because fewer neurons are responsive during amblyopic stimulation, and that the average firing rate of the responsive neurons, which reflects the perception of contrast, is unaffected in amblyopia.

INTRODUCTION

Amblyopia, clinically defined as a reduction in visual acuity in an otherwise healthy and properly corrected eye, is found in 1–4% of the North American population (Ciuffreda et al. 1991; Daw 1995; Kiorpes and Movshon 1996; von Noorden 1990). The study of amblyopia in humans has almost entirely been by psychophysical means, where it has been demonstrated that the disorder produces a variety of spatial vision abnormalities (Carkeet et al. 1996; Hess et al. 1978; McKee et al. 1992; Reed et al. 1996; von Noorden 1990). A ubiquitous finding is a reduction in contrast sensitivity, the reciprocal of the contrast required to visually detect a target (for review, see Ciuffreda et al. 1991), which for the normal eye is maximum between 2 and 5 cycles per degree (cpd) (see for example, Blakemore and Campbell 1969; Campbell and Robson 1968). For the amblyopic eye, the reduction in contrast sensitivity becomes more pronounced at higher spatial frequencies, and in some cases, maximum sensitivity occurs at a spatial frequency lower than that for the normal eye (Bradley and Freeman 1981; Hess and Pointer 1985; Hess et al. 1978).

Contrast detection thresholds are dependent on the “noise” present in the visual system (e.g., Graham 1989). That is, neural firing rates (integrated over some region) must exceed some signal-to-noise ratio threshold in order for a contrast pattern to be detected by the observer. In the case of amblyopia, more contrast may be required to surpass this threshold signal-to-noise ratio. Decreases in signal could result from fewer responsive neurons during stimulation of the amblyopic eye or from neural firing rates that are less during amblyopic eye stimulation than during normal eye stimulation, while increases in noise could result from a loss of neural connections or a rearrangement of connections within the cortex (for discussion see Kiorpes and McKee 1999; Kiorpes et al. 1999; Levi 1991). In contrast-matching studies performed well above threshold, targets viewed separately with the amblyopic and normal eye appear to have the same contrast (Hess and Bradley 1980; Hess et al. 1983; Loshin and Levi 1983). A possible interpretation of this result is that once the detection signal-to-noise ratio of neural activity has been surpassed, the perception of contrast may depend on the average firing rate of the responsive neurons, which appears not be impaired in amblyopia (Levi 1991).

Animal models have demonstrated that neural substrates of amblyopia are evident in visual areas as early as primary visual cortex (area V1) in the form of a reduction in binocular processing and/or a marked shift in the ocular dominance of neuronal response away from the amblyopic eye (Crawford and von Noorden 1979, 1980; Hubel et al. 1977; Kiorpes et al. 1998; LeVay et al. 1980; Movshon et al. 1987). Although studies of human amblyopia have also demonstrated a decrease

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in binocular interaction (Holopigian et al. 1986; Horton et al. 1997; Levi et al. 1979), histological studies of human visual cortex demonstrate no shift in ocular dominance away from the amblyopic eye (Horton and Hocking 1996; Horton and Stryker 1993). However, this may have been due to the onset of amblyopia being after the critical period of ocular dominance column development (Daw 1995).

Neuroimaging studies of human amblyopia in living human brain using positron emission tomography (PET) (Demer et al. 1988) and single photon emission computed tomography (SPECT) (Kabasakal et al. 1995) have demonstrated that the measured signal within V1 during monocular stimulation of the amblyopic eye is less than that measured during monocular stimulation of the normal eye. However, no psychophysical assessment of the perception of contrast was determined. Magnetoencephalography (MEG) has demonstrated interocular differences in the magnitude of evoked magnetic responses to chromatic gratings throughout the entire occipital cortex of amblyopes; however, no correlation with contrast sensitivity of the amblyopic eye was found (Anderson et al. 1999). Because the magnitude of the measured signal within an image voxel during functional magnetic resonance imaging (fMRI) is thought to be correlated with the amount of neural activity within that voxel (for a review, see Ogawa et al. 1998), fMRI is well suited for noninvasively measuring the neuronal response to suprathreshold contrast. We investigated the perception of contrast using both psychophysical and fMRI measurements to understand the relationship between these very different methods and to determine the neural correlates of contrast perception in human amblyopia.

METHODS

Subjects

Four subjects with unilateral amblyopia developed during early childhood or infancy were recruited through the Department of Ophthalmology at the London Health Sciences Center, London, Ontario, Canada. Each subject had been previously evaluated clinically by orthoptic assessment. During all experiments, each subject’s vision was optically corrected using their existing prescription lenses. Six subjects with no known visual deficits were recruited from the academic environment of the University of Western Ontario (UWO) to act as control subjects. All subjects had no previous experience with MR imaging. The experimental protocol was approved by the UWO Human Subjects Review Board.

Visual stimuli

Visual stimuli consisted of stationary vertical sinusoidal gratings at six spatial frequencies [0.5, 1, 2, 4, 8, 12 cycles per degree (cpd)] and at a mean luminance of 20 cd/m². Contrast and luminance of each grating were measured using a Minolta CS-100 Chroma Meter (Minolta Camera, Osaka, Japan). Contrast was defined in the usual way as the maximum luminance of the grating minus the minimum luminance, divided by twice the mean luminance. Each grating was confined to a circle that subtended 12° of visual angle, and was reduced in contrast near the edge of the circle to eliminate sharp edges. The surrounding area of the display was gray at a luminance of 20 cd/m². Visual stimuli were presented on a projection screen that was mounted onto the patient bed of the MR scanner, and was located 1.25 m from the subject’s eyes. An angled mirror, positioned above the subject’s eyes, provided a full view of the screen. Subjects wore a pair of liquid crystal shutter glasses (Milgram 1987), allowing the experimenter to control when each eye viewed the stimulus.

Psychophysical measurements of the perception of contrast

Psychophysical experiments were conducted while the subject lay in the MR scanner. The perception of 22% physical contrast was measured at each spatial frequency by obtaining the matching contrast of a standard reference frequency (1 cpd for amblyopes, 4 cpd for normals) using a temporal two-alternative forced choice procedure shown by example in Fig. 1A. The eye being tested was presented with one of the six test frequency gratings for 500 ms at 22% contrast with the eyepiece of the shutter glasses opaque for the fellow eye. This was followed by a 500-ms presentation of an isoluminant gray screen matched in mean luminance to the grating. Finally, the reference frequency, which did or did not differ in physical contrast from that of the test frequency, was presented for 500 ms. The subject was then asked to choose the grating that contained the greater contrast. This was repeated to obtain five responses at each of five contrasts of the
reference frequency (12, 17, 22, 27, and 32%; this range of contrasts was selected on the basis of pilot studies), which were paired with the test frequency in random order. For one-half of the trials, the order of presentation was reversed. The perceived contrast of the test frequency grating was then obtained as the matching contrast of the reference frequency by taking the 50% point on a fitted curve to the corresponding response function (see Fig. 1B) (Wetherill and Levitt 1965). This procedure was then repeated for the remaining test frequencies, and then the whole procedure was repeated for the other eye.

An additional measurement of the perception of contrast was made to compare contrast perception across eyes. To accomplish this, the 22% contrast reference frequency was presented to one of the eyes for 500 ms. The eyepiece of the glasses was then made opaque for that eye. Then, the fellow eyepiece was made transparent, and the reference frequency was shown to the other eye at one of the above five contrasts for 500 ms. The same forced-choice procedure was then used to match the contrast of the 22% reference frequency as seen with one eye to that seen with the other eye.

Finally, contrast threshold measurements were taken for each eye separately using a standard staircase method. Because contrast perception measurements were obtained as matches to a fixed reference frequency, only the contrast threshold of the reference frequency was measured.

fMRI

All imaging experiments were performed on a Varian/Siemens Unity INOVA 4 Tesla whole-body MR scanner, equipped with 25 mT/m whole-body gradients. An 8-cm-diameter quadrature radio frequency (RF) surface coil was placed at the back of the subject’s head and the subject’s head was immobilized using a well-padded head vice that maintained the high-frequency noise. The 8-cm-diameter quadrature radio frequency signals, and maintained the high-frequency noise. The magnetic field (B0) produced by the whole-body gradients. An 8-cm-diameter quadrature radio frequency (RF) surface coil was placed at the back of the subject’s head and the subject’s head was immobilized using a well-padded head vice that kept the head and head pad in place throughout the duration of the experiment. Hence, to relieve subject boredom and reduce subject motion, we used a 15° head-occluder and a 0.25- to 2.25- prism diopter correction.

Anatomical localizer scans, anatomical reference scans, and a short sequence of the standard frequency within trials of the experiment, were also obtained to ensure that the subject was in phase with the presentation of the visual stimuli (as well as all lower spatial frequencies). The hemodynamic response.

Table 1. Classification of amblyopia for each subject by orthoptic assessment

<table>
<thead>
<tr>
<th>Subject, Age (yr), Sex Eye</th>
<th>Refractive Correction</th>
<th>Strabismus</th>
<th>Strabismus/Anisometropia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sphere (diopters)</td>
<td>Cylinder (diopters)</td>
<td>Axis, deg (rotation)</td>
</tr>
<tr>
<td>DA, 42, M, R</td>
<td>+0.50</td>
<td>+0.00</td>
<td>+1.00</td>
</tr>
<tr>
<td>MA, 56, F, R</td>
<td>+6.00</td>
<td>+1.00</td>
<td>97</td>
</tr>
<tr>
<td>CB, 51, F, R</td>
<td>+5.25</td>
<td>+0.75</td>
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<table>
<thead>
<tr>
<th>Subject, Eye</th>
<th>Refractive Correction</th>
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<tr>
<td></td>
<td>Sphere (diopters)</td>
<td>Cylinder (diopters)</td>
<td>Axis, deg (rotation)</td>
</tr>
<tr>
<td>L.L., 39, F, R</td>
<td>+6.00</td>
<td>+1.00</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>+3.75</td>
<td>+1.00</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>+1.50</td>
<td>+2.00</td>
<td>180</td>
</tr>
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L.XT., left exotropia; R.XT., right exotropia; R.ET., right esotropia.

Using a box-car input correlation function incorporating a temporal delay to allow for the intrinsic delay of the hemodynamic response, one functional map of image voxels showing a significant increase (P < 0.01) in MR signal above baseline was created for each and every spatial frequency. These maps were combined as a “logical OR” to create one map of voxels to be used in further analyses. The average fMRI response over the three presentations for each spatial frequency was then computed for each voxel (expressed as a percent increase in MR signal above baseline).

To estimate the noise in the fMRI signal during visual stimulation, the magnitude of the MR signal as a function of time (i.e., the MR time course) for each image voxel within our selected ROIs (all voxels in the raw image data, not merely the resulting functional map) was Fourier transformed, multiplied by a high-pass frequency filter, and inverse-Fourier transformed to regain the MR time course. This method removed components of the MR signal that were in phase with the presentation of the visual stimuli (as well as all lower frequency signals), and maintained the high-frequency noise. The standard deviation of the magnitude of the MR signal for time points corresponding to stimulation of the eye was then computed as an estimate of the noise during stimulation of the eye.

RESULTS

The orthoptic assessment for each subject with amblyopia is summarized in Table 1. The severity of anisometropic and strabismic amblyopia varied across subjects, ranging from 4 to 50 prism diopters of strabismic deviation and a 0.25- to 2.25-diopter difference in either spherical or cylindrical refractive correction. Only subjects with a spherical or cylindrical correction >1 were considered anisometropic.

Figure 2 shows each subject’s perception of 22% contrast as a function of spatial frequency measured with each eye for the four subjects with unilateral amblyopia as well as the average perception for the six subjects with normal vision. All subjects correctly perceived the reference frequency as a 22% contrast grating (i.e., physical contrast agreed with perceived contrast). One subject, subject CB, was unable to detect the 12 cpd target with the amblyopic eye. Thus data for subject CB at this spatial frequency was not analyzed. The curves in each graph (and in all subsequent similar graphs) were obtained by fitting the data, S, to a function of the form

\[ S = a\exp(-k) \]
where $\omega$ is spatial frequency, and $a$, $b$, and $c > 0$ are fitted parameters. For all subjects with normal vision and for three of the four subjects with amblyopia, the perception of contrast was the same for both eyes. For each of these three amblyopes, there was little or no difference in the location of the maximum of the curve fitted to the data for each eye [DA: 2.5 cpd (preferred eye), 2.1 cpd (amblyopic eye); MA: 2.5, 1.9; CB: 1.8, 1.8]. Subject LL exhibited a decrease in the perception of contrast measured with the amblyopic eye, and this decrease was more pronounced at higher spatial frequencies. In this case, the maximum of the curve fitted to the amblyopic eye data occurred at 0.8 cpd, and at 2.4 cpd for the preferred eye data.

Figure 3 shows that the magnitude of the fMRI response as a function of spatial frequency was the same for each eye for the subjects with normal vision and for the same three amblyopes that exhibited the same psychophysical response for each eye. However, the magnitude of the fMRI response to stimulation of the amblyopic eye for subject LL was smaller at higher spatial frequencies compared with the fMRI response to stimulation of the normal eye, in agreement with the psychophysical data.

Figure 4 correlates the results of Figs. 2 and 3 to show that regardless of spatial frequency the magnitude of the fMRI response reflects the perception of contrast and is the same for each eye, even for subject LL. That is, even though the perception of 22% contrast measured with the amblyopic eye of LL was reduced, the correlation between perceived contrast and fMRI response was the same as that for the preferred eye. This remarkable relationship between fMRI response and level of contrast is consistent with a model that predicts a power relationship between the two at low contrast levels (Boynton et al. 1999), i.e., fMRI response $\propto$ (perceived contrast)$^b$, where $b$ is usually >2. For our data, $b$ was 4.54 ($R^2 = 0.9$).

The threshold contrast of the reference frequency for the amblyopic eye was elevated for all subjects [DA: 2.4% (amblyopic eye), 1.5% (preferred eye); MA: 2%, 1.2%; CB: 1.9%, 1.5%; LL: 3%, 2.2%]. Figure 5A shows the perceived contrast as a function of spatial frequency expressed in multiple units of the threshold contrast for each eye (i.e., the perceived contrast divided by threshold contrast), averaged over the four subjects with amblyopia. Figure 5B shows the number of activated voxels that were in each of the functional maps created at each spatial frequency before they were combined. Although Fig. 4 suggests that the magnitude of the fMRI response reflects the perception of contrast and is not dependent on the presence of amblyopia, Fig. 5 suggests that the total neural activity within early visual areas contributing to a detectable fMRI response is not the same for each eye. The reduction in the number of activated voxels resulting from amblyopic eye stimulation was...
significant ($P < 0.05$, paired $t$-test at each spatial frequency across subjects) only at higher spatial frequencies, but the trend is quite clear. In support of the finding that the total neural signal in early visual areas is smaller during stimulation of the amblyopic eye than it is during stimulation of the normal eye, it was found that on average across subjects with amblyopia, the underlying high-frequency noise in the fMRI signal during stimulation of the amblyopic eye was $0.20 \pm 0.04\%$ (mean $\pm$ SE). This did not differ from the noise during stimulation of the good eye ($0.22 \pm 0.03\%$), nor different from the noise during stimulation of either eye of those subjects with normal vision ($0.19 \pm 0.02\%$).

**DISCUSSION**

Using brief stimuli ($<4$ s) is key in high spatial resolution fMRI studies to maintain the spatial specificity of the fMRI response by preventing saturation of the local hemodynamic response (Das and Gilbert 1995; Maloney and Grinvald 1996) and minimizing adaptation effects. We have previously shown that an unsaturated hemodynamic response is also more likely to maintain proportionality between neural activity and blood-oxygenation-level–dependent (BOLD) fMRI signal (Menon and Goodyear 1999). Our findings may not have been possible using a sustained visual stimulus, as any linearity between neural activity and fMRI response would have likely been lost. As well, relatively minor changes in the perceived contrast of the stimulus would have been masked by the spreading of the vascular response to areas unassociated with the neural activity.

Previous fMRI analysis techniques for the study of contrast response include preselecting voxels for analysis on the basis of functional localizer experiments using high stimulus contrasts (Boynton et al. 1999). This removes any statistical biases toward any one contrast level by fully saturating the fMRI response. In our study, however, only 22% physical contrast was used. We did not use the reference frequency to preselect voxels for analysis because this would have favored that spatial frequency. Our approach of creating a statistical map for each and every spatial frequency and then combining the maps to create one group of voxels for further analysis removes any bias toward one spatial frequency.

Of the four subjects shown in Table 1, subject LL demonstrated the most severe case of anisometropia (i.e., the greatest difference between the eyes in terms of refractive correction). The reduction in contrast perception for subject LL as measured with the amblyopic eye is consistent with previous findings (Hess and Pointer 1985) demonstrating that in moderate-to-severe cases of anisometropia, there is an elevation in the level of contrast at which the performance of the two eyes equalizes. Hence, our visual stimulus parameters may have been such that a 22% contrast was not sufficient for subject LL to overcome the perceptual deficits of the amblyopic eye at low contrast. This was shown as well in the fMRI response to stimulation of the amblyopic eye with the same visual targets (Fig. 3). These data, as well as the data for the other three amblyopes and for the subjects with normal vision, clearly demonstrate that fMRI response, and consequently the level of neural activity, in early visual areas of the visual cortex correlates with the perception of contrast. These results are consistent with previous work showing that the fMRI response reflects the level of contrast (Goodyear and Menon 1998; Tootell et al. 1998), and further that the coding of contrast within monocular neurons is spared in amblyopia (Kiorpes et al. 1998; Movshon et al. 1987). Of course, the voxels of our MR images contain many types of neurons, not merely monocular neurons. Hence we cannot claim that the measured fMRI response is a direct measure of the activity of neurons that respond preferentially to stimulation of one eye. The exact contribution of the activity of these types of neurons to the fMRI signal is difficult to determine given the practical spatial resolution restrictions of fMRI. Nonetheless, our results show that magnitude of neural activity per image voxel is not impaired in the presence of amblyopia when contrast is sufficient to equate the perception of that contrast across the eyes.

Figure 5B illustrates, however, that there is a reduction in the number of “activated” image voxels in the presence of amblyopia. Because no change in image signal-to-noise is expected across the eyes during the experiment, the presence of fewer voxels in our functional maps for stimulation of the amblyopic eye as a function of spatial frequency cannot be explained as an imaging artifact. Statistical methods of generating functional maps depend on signal magnitude and signal variance to determine whether a voxel is significantly “active.” Our analysis has shown that the underlying high-frequency noise in the fMRI signal during visual stimulation is unchanged in the presence of amblyopia, and thus a difference in signal magnitude must be responsible for a difference in the number of activated voxels. A reduction in the number of voxels in our functional maps for amblyopic eye stimulation is consistent with low spatial resolution results obtained from PET (Demer et al. 1988), SPECT (Kabasakal et al. 1995), and MEG studies (Anderson et al. 1999), since total (or pooled) activity measured by these methods in the visual cortex would be related to the product of the magnitude of our fMRI signal change and the number of voxels in our maps. A reduction in pooled neural activity within the visual cortex has also been proposed as a possible mechanism for elevated contrast thresholds in amblyopia (for a review, see Levi 1991). When perceived contrast is expressed in multiple units of the threshold contrast (Fig. 5A),
our data seem to suggest that the number of detectable voxels at some statistical threshold using fMRI (Fig. 5B) may be related to the difference in threshold contrast between the normal and amblyopic eyes since that difference is signal-to-noise dependent. This is merely suggestive, of course, since our measurements are made at 22% contrast, not at threshold. However, we speculate that at all perceptually equivalent contrasts, the relative number of detectable voxels during stimulation of the normal and amblyopic should remain approximately constant. Once the perception of contrast becomes reduced for the amblyopic eye, the relative number of image contrasts, the relative number of detectable voxels during stimulation since we expect that the magnitude of the fMRI signal will decrease for the amblyopic eye (as for example, subject LL in Fig. 3) as contrasts approach threshold. The direct investigation of contrast threshold response versus suprathreshold contrast response using fMRI will require stimuli restricted to the fovea or to a narrow range of eccentricities since unlike psychophysical judgements of suprathreshold contrast (see for example, Cannon and Fullenkamp 1988), contrast threshold judgements are heavily eccentricity dependent (e.g., Robson and Graham 1981; Savoy and McCann 1975).

Further elucidation of the neural substrates governing contrast sensitivity loss in amblyopes must be performed with detailed retinotopy (Tootell et al. 1998) to examine the contribution of areas outside V1. In addition, fMRI at higher spatial resolution will allow an evaluation of the response of the ocular dominance columns (Menon and Goodyear 1999) to stimulation of amblyopic eye. Nonetheless, our current results clearly demonstrate that fMRI of the visual cortex of amblyopes is sensitive to the reduction in pooled neural activity in response to stimulation of the amblyopic eye as a function of spatial frequency. As well, fMRI is also sensitive to neural populations whose contrast coding seems to be spared at suprathreshold contrast, even in the presence of amblyopia. Although, based on the results of this study, we cannot specify the neural mechanisms underlying amblyopia, we have demonstrated that fMRI is a useful noninvasive tool in the investigation of amblyopia at the cortical level.

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