The amblyopic deficit and its relationship to geniculo-cortical processing streams

Robert F. Hess¹, Benjamin Thompson¹,², Glen A Gole³ and Kathy T. Mullen¹

¹. McGill Vision Research, Dept. of Ophthalmology, McGill University, Montreal, Canada
². Department of Optometry and Vision Science, University of Auckland, Auckland, New Zealand
³. The Wesley Hospital Research Institute, Dept. of Ophthalmology, University of Queensland, Brisbane, Australia

Corresponding author: Robert F. Hess
McGill Vision Research (H4.14)
Department of Ophthalmology
687 Pine Ave West, Montreal
Quebec, Canada H3A 1A1
Tel: 514 843 1690

Email: robert.hess@mcgill.ca

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Abstract

Amblyopia or lazy eye is the most common cause of uniocular blindness in adults and is caused by a disruption to normal visual development as a consequence of unmatched inputs from the two eyes in early life, arising from a turned eye (strabismus), unequal refractive error (anisometropia), or form deprivation (e.g. cataract). Using high-field fMRI in a group of human adults with amblyopia, we previously demonstrated that reduced responses are observable at a thalamic level, that of the lateral geniculate nucleus (LGN) (Hess et al, 2009 EJN 29, 1064-70). Here we investigate the selectivity of this deficit by using chromatic and achromatic stimuli that are designed to bias stimulation to one or other of the three ascending pathways (the parvocellular, magnocellular and koniocellular). We find the greatest LGN deficit is for stimuli modulated along the chromatic, L/M cone opponent axis of colour space, suggesting a selective loss of parvocellular function in the LGN. We also demonstrate a cortical deficit that involves all the visual areas studied (V1, V2, V3, VP, V3A, V4), and we find this is greatest for the two chromatic responses (S cone opponent and L/M cone opponent) versus the achromatic response, as might be expected from a loss of segregation of chromatic pathways in the cortex.

Keywords: Vision, color vision, lateral geniculate nucleus (LGN), visual cortex,
amblyopia, fMRI, L/M cone opponent, parvocellular, koniocellular.
Introduction

Amblyopia (incidence 3%) is a disorder affecting visual development in humans that results in a uniconal visual loss, in which individuals have impaired visual performance using one eye (the “amblyopic eye”) and a normal “fixing” eye. Although in human amblyopia it has been known for some time that the visual deficit originates post-retinally (Hess and Baker 1984; Hess et al. 1985), it has only recently been shown using functional MRI that the lateral geniculate nucleus (LGN) has reduced responses when driven by the amblyopic eye, indicating a functional deficit at the thalamic level (Hess et al. 2009b). This result in human vision is striking because the physiological origin of the deficit in animal models has been extensively investigated using single cell neurophysiology and the current consensus is that the neural responses of both the retina (Cleland et al. 1982; Cleland et al. 1980) and LGN are normal (Blakemore and Vital-Durand 1986; Derrington and Hawken 1981; Levitt et al. 2001; Sasaki et al. 1998) but see (Chino et al. 1994; Ikeda and Tremain 1978; Levitt et al. 2001; Sherman et al. 1975; Yin et al. 1997), even though the LGN layers that receive input from the affected eye exhibit histological abnormalities (Eimon et al. 1978; Guillery 1972; Tremain and Ikeda 1982; von Noorden and Crawford 1992). Anomalous single cell responses are first found in layer 4c of striate cortex (cytoarchitectonic area 17 in cat and area V1 in primate).

Previously we revealed a functional LGN deficit that is common to all types of amblyopia by using a flickering checkerboard stimulus with combined modulation of
luminance and color contrast, mean luminance and chromaticity (Hess et al. 2009b). The spatio-temporal broadband stimulus used was chosen to maximize the overall activity of the LGN and allow comparisons of monocular activation between eyes, but its disadvantage is that it cannot be used to assess the selectivity of the deficit for the different processing streams that relay information through the LGN. The LGN receives input from at least three distinct retinal pathways: the parvocellular pathway originating from the midget retinal bipolar cells (Derrington and Lennie 1984; Lee et al. 1990; Merigan et al. 1991), the magnocellular pathway emanating from the parasol retinal ganglion cells (Derrington and Lennie 1984; Kaplan and Shapley 1982; Lee et al. 1990; Solomon et al. 1999), and the koniocellular pathway receiving from specialized ganglion cells driven by short wavelength (S cone) photoreceptors (Chatterjee and Callaway 2003; Dacey and Packer 2003; Martin et al. 1997). All three cellular populations in the LGN are potentially activated by the checkerboard stimulus described above. In this study, we use spatio-temporal narrowband stimuli whose contrast is modulated along different cardinal axes in color space to bias activation to each of the above three LGN processing streams. For normal human vision, a previous study of the LGN has compared responses obtained to all three types of cardinal stimuli at equivalent cone contrasts and demonstrated that robust BOLD responses to Ach, L/M and S cone opponent modulation (Mullen et al. 2008) can be obtained. In the cortex, strong BOLD responses for chromatic stimuli modulated along both cardinal axes (activating L/M opponent and S cone opponent pathways) can be seen that involve all areas in the ventral pathway, with chromatic preferences revealed in areas
V1 and VO (Brewer et al. 2005; Engel et al. 1997; Hadjikhani et al. 1998; Liu and Wandell 2005; McKeefry and Zeki 1997; Mullen et al. 2008; Mullen et al. 2007; Wade et al. 2002; Wandell et al. 2005).

In this paper we make simultaneous fMRI recordings from the LGN and cortex in a group of amblyopic subjects to investigate any selectivity of the LGN and cortical anomalies. Our results suggest that the LGN anomaly in amblyopia is greatest for L/M cone opponent stimuli, indicating that it is selective for parvocellular function. We also find a substantial cortical deficit affecting both striate and extra-striate areas, and we show that this is greater for chromatic as opposed to achromatic stimuli in the ventral pathway. These effects are consistent with a selective parvocellular deficit at the level of the LGN, where parvo, magno, and konio-cellular pathways are segregated, but which translates into a more general deficit for chromatic stimuli as a consequence of the mixing of the information from the two afferent chromatic pathways at the cortical level.

Methods

Subjects and stimuli

We studied 7 amblyopes selected to cover a range of etiologies including 3 strabismic, 1 mixed anisometropic-strabismic, 1 anisometropic, and 2 form-deprivation amblyopes, as detailed in Table 1. We measured the region of the retina used for fixation in all
subjects using visuoscropy (Table 1) and we monitored the fixation eye-movements of all
amblyopic subjects while they were viewing the stimulus in a control experiment run
outside of the scanner using an in-house video monitoring of the pupil with subsequent
off-line analysis of the variability of fixation. All subjects fixated on the central fixation
mark provided, although the amblyopic eye was less steady than the fellow fixing eye
(Table 1). The degree of unsteadiness was small, however, compared to the field size
used (12°). All experiments were undertaken with the understanding and written
consent of each subject. The study conforms to ‘The Code of Ethics of the World
Medical Association (Declaration of Helsinki)’, printed in the *British Medical Journal* (18
July 1964).

Table 1 about here

Two different types of stimuli were used: a spatio-temporal broadband checkerboard
stimulus with chromatic and achromatic contrast modulation (check size = 0.5°,
squarewave modulation =16Hz, contrast of 80%, field size of 10° height x12° width) as
illustrated in figure 1A, or a narrowband ring stimulus sinusoidal in space and time
(spatial frequency = 0.5c/d, temporal frequency = 2 Hz), presented in a Gaussian
temporal envelope (sigma = 125ms). For the latter stimulus, as illustrated in figure 1B,
there were three different types (RG, BY and Ach) that isolated L/M cone opponent, the
S cone opponent or the achromatic (luminance) post-receptoral mechanisms
respectively (Mullen et al. 2007). The cone contrasts were set to high suprathreshold
levels of 11% (Ach), 4% (RG) and 30% (BY). The circular stimulus was viewed as 16° (full width) by approximately 12° (full height), since stimulus height was limited top and bottom by the subject’s placement in the bore of the magnet.

Figure 1 near here

Experimental protocols

For the broadband checkerboard stimulus a standard block design was used, as previously described (Hess et al. 2009b), composed of alternate presentations of the stimulus and blank (zero luminance) intervals (18 seconds of stimulus presentation, 18 seconds of blank, 10 blocks per run, 2 scanning runs). The checkerboard was presented in a 2AFC paradigm within a 3 second cycle; each stimulus presentation was for 800ms with an inter-stimulus interval of 200ms and 1.2 seconds for response. Sinusoidal ring stimuli were presented in a 2AFC paradigm within a 3 second cycle; each stimulus was within a 500ms time window in a temporal Gaussian contrast envelope (sigma=125ms) with an inter-stimulus interval of 500ms and 1.5 seconds for the response, repeated 6 times for each condition (18 seconds). A roving baseline design was used whereby each block consisted of four conditions, the 3 types of ring stimuli (Ach, RG, BY) and a blank (mean luminance) interval with a fixation dot, as previously described (Mullen et al. 2007). The presentation order of these four conditions was pseudo-randomized from block to block with each block being presented 10 times in each of two scanning runs.
To control for attentional modulation known to affect cortical and subcortical structures (O'Connor et al. 2002), subjects performed a 2AFC contrast discrimination task during all experiments that involved discriminating detectable differences in the contrast of pairs of stimuli within a stimulus cycle and responding with a button press (Hess et al. 2009b; Mullen et al. 2007). During the fixation (blank) epochs for the checkerboard stimuli, dummy button presses were made. For the ring stimuli during the fixation epoch, a similar contrast discrimination task was performed on a small white annulus surrounding the black fixation spot (Mullen et al. 2007). During scanning sessions feedback on the task was not given and % correct data were not recorded. The contrast difference between stimulus pairs was large enough to be distinguishable by a normal eye (>90% correct on average). In a dummy scanning session, we measured the psychophysical performance using the checkerboard stimuli for the fixing and amblyopic eyes and responses were above 90% correct for both fixing and amblyopic eyes (Hess et al. 2009b). For the ring stimuli, data collected on a group of normal subjects (n=5) show that the contrast discrimination task was in the > 90% correct range for Ach, RG and BY stimuli, with no significant difference between these three conditions. During all experimental paradigms participants viewed the central fixation mark monocularly and a tight-fitting eye patch was used to occlude the other eye. The same stimuli were presented to both amblyopic and fellow eyes and both the subject’s eyes were tested in the same scanning session.

Magnetic resonance Imaging
All magnetic resonance images were acquired using a 4T Bruker MedSpec system at the Centre for Magnetic Resonance, Brisbane, Australia. A transverse electromagnetic (TEM) head coil was used for radiofrequency transmission and reception (Vaughan et al. 2002). For the checkerboard stimulus, 256 T2*-weighted gradient-echo echoplanar images (EPI) depicting blood oxygen level dependent (BOLD) contrast (Ogawa et al. 1990) were acquired in each of 24 planes with TE 30 ms, flip angle =90°, TR 1500 ms, in-plane resolution 3.1 x 3.1 mm and slice thickness 3 mm (0 mm gap). For the sinewave ring stimuli, 240 T2*-weighted gradient-echo echoplanar images (EPI) depicting blood oxygen level dependent (BOLD) contrast were acquired in each of 36 planes with a TE of 30 ms, TR 3000 ms, in-plane resolution 3.6x3.6 mm and a slice thickness of 3 mm (0.6 mm gap). These parameters were also used for the binocular LGN localization scans (see below). All slices were taken parallel to the calcarine sulcus and arranged to include the anatomical location of the LGN. Two to three fMRI scans were performed in each session. Head movement was limited by foam padding within the head coil. In the same session, a high-resolution 3D T1 image was acquired using an MP-RAGE sequence with TI 1500 ms, TR 2500 ms, TE 3.83 ms, and a resolution of 0.9 mm³.

Table 2 near here

LGN localization

Left and right LGNs were localized in each participant using both anatomical and functional data. LGN localization data were acquired in a separate scanning session.
conducted under binocular viewing conditions. During scanning, participants viewed alternating blocks of the high contrast squarewave checkerboard and the blank intervals with a small dim fixation dot, as described above (see ‘Stimuli’) but with binocular rather than monocular viewing. Localization was based on the average of 2 scanning runs. Data were analyzed for each individual participant using a GLM analysis and statistical maps of t-values were visualized at the FDR corrected (Benjamini and Hochberg 1995) level of q < 0.001. LGNs were defined as a stimulus responsive region in the appropriate anatomical location (Kastner et al. 2004). Regions of interest (ROIs) were created by first identifying the peak voxel (ie. the voxel whose activity was most reliably correlated with the presentation of the stimulus) within the LGN region, then a cube of 1000 mm$^3$ (10mmx10mmx10mm) was centered on the peak voxel and the region of interest was defined as all voxels within the cube contiguous with the peak voxel whose activity in response to the checkerboard stimulus was above threshold (q < 0.001). The Talairach coordinates of all the LGNs are given in Table 2.

**Identification of cortical visual areas**

Retinotopic mapping was performed using standard techniques (Dumoulin et al. 2003). Both polar angle and eccentricity maps were visualized on flattened representations of the cortical surface to allow the boundaries between visual areas to be defined. Only voxels within each cortical area that were activated significantly (FDR corrected q < 0.001) during binocular viewing of the LGN localization stimulus (see above) were included in the cortical ROIs to ensure that non-responsive voxels were excluded.
Data analysis

Data analysis was conducted with the commercially available Brain Voyager analysis package version 1.9.10 (Brain Innovations, Maastricht, The Netherlands). Functional scans were high-pass filtered and motion corrected using subroutines within Brain Voyager. They were then aligned to each subject’s high resolution anatomical images (resampled at 1mm\(^3\)) and transformed to Talairach space (Talairach and Tournoux 1988). Time series data were extracted from the LGN region of interest for each individual participant using an event related averaging paradigm. For checkerboard stimuli, time series data were normalized to the preceding 2TRs (when the subject was viewing the blank) to provide a baseline for the %BOLD change measure. Average %BOLD change was calculated as the average %BOLD values within a temporal window starting 4TRs (6 seconds) after the onset of the stimulus and ending 4TRs after the offset of the stimulus. For the sinusoidal ring stimuli, %BOLD change for each stimulus type was calculated by normalizing to the last 4TRs of the fixation blocks. Average %BOLD change was then calculated using the same approach described above with the exception that the averaging window was lagged by 1TR (3 secs) for this protocol as stimuli were not separated by a fixation interval.

For the checkerboard stimuli, %BOLD change data were analyzed using paired t-tests to compare activation generated by fellow eye stimulation with that generated by amblyopic eye stimulation for the LGN and each cortical area separately. For the
sinewave ring stimuli, within subjects ANOVAs (degrees of freedom adjusted for sphericity using the Huynh-Feldt correction) with factors of Eye (amblyopic vs. fellow) and Chromaticity (Ach vs. RG vs. BY) were used to test for a differential pattern of responses between the activity generated by each eye for the different stimuli, as indicated by a significant interaction between Eye and Chromaticity. This analysis was performed separately for the LGN and V1. Data from extra-striate visual areas were analyzed together in the first instance using an AVOVA with factors of Eye, Chromaticity and Visual Area (V2, V3, VP, V3A and V4). As this analysis gave significant effects, separate ANOVAs were then conducted on each extra-striate area separately. ANOVAs were followed up by post-hoc paired t-tests (2-tailed) that were conducted on the different chromaticity conditions within each eye separately, in order to identify the differing patterns of responses. For ANOVAs in which a significant interaction was present a critical p-value of \( p < 0.05 \) was employed for each post-hoc paired t-test.

Results

Comparison of responses in the LGN

Figure 2 near here
Figure 2 shows the responses in the LGN between the amblyopic and fellow fixing eyes for a narrowband spatio-temporal stimulus modulated along the three different cardinal axes in colour space (figure 2). Previously reported results in the LGN (Hess et al. 2009b; figure 2) for a broadband stimulus containing both luminance and chromatic contrast have shown a significantly stronger response to fellow fixing eye stimulation compared with amblyopic eye stimulation ($t(5) = 4.79$, $p = 0.005$). For the spatio-temporal narrowband stimulus (figure 2) the LGN shows a differential pattern of response to the Ach, RG and BY stimuli depending on whether the stimulus is presented to the fixing or amblyopic eye. The fellow fixing eye responds best to the RG stimulus whereas the amblyopic eye does not. A within subjects ANOVA with factors of Eye (fellow vs. amblyopic) and Chromaticity (Ach vs. RG vs. BY) confirmed that this interaction between Eye and Chromaticity is significant ($F(2,12) = 5.05$, $p = 0.026$). Post-hoc paired t-tests revealed that this interaction is driven by a significantly higher response to the RG stimulus than to the Ach ($t(6) = 3.41$, $p = 0.014$) or the BY stimuli ($t(6) = 3.07$, $p = 0.022$) when the fixing eye was stimulated, with no such advantage occurring for the RG stimulus when the amblyopic eye is stimulated ($p < 0.05$). This suggests a selective loss of L/M cone opponent responses in the LGN when driven by the amblyopic eye.

**Comparison of responses in the cortex**

Figure 3 near here
We first show a voxel-based analysis of visual cortex on the group data to illustrate the distribution of preferential activation between the fellow and amblyopic eyes for the chromatic versus the achromatic stimuli, or vice versa (figure 3). In this figure we show the average group data for all seven subjects with separate representations for fellow eye stimulation (upper panels) and amblyopic eye stimulation (lower panels). Data are represented on computationally flattened representations of the left and right occipital lobes of one participant (MLT). The medial side of each panel represents the primary visual cortex that has been “cut” along the cortical representation of the horizontal meridian and the positions of the border locations based on MLT’s retinotopic map dividing the early visual areas V1, V2, V3, VP, V3A and hV4 are marked by black lines. As these boundary positions are based on the retinotopic mapping data of one of our subjects, they are for illustration only.

This illustration is the result of a GLM analysis using a z-transformation based on the baseline components of the stimulus paradigm. Multiple comparisons were corrected using a false discovery rate of $q < 0.05$. The $t$-values represent the differences between the responses to the RG and Ach stimuli (figure 3A) or BY and Ach stimuli (figure 3B) with the red–yellow scale indicating a significantly greater response to the chromatic than achromatic stimuli and the blue–purple scale indicating a significantly greater response for achromatic than chromatic stimuli. The illustration shows quite strikingly in the fellow eye representation the presence of discrete visual cortical regions that
respond preferentially to the isoluminant chromatic stimuli (RG or BY) over the achromatic stimuli (red-yellow scale), demonstrating the responses that are quite typical of a normal eye (Engel et al. 1997; Hadjikhani et al. 1998; Hess et al. 2009b; Liu and Wandell 2005; Mullen et al. 2007; Wade et al. 2002). In the amblyopic eye representations, however, these regions have almost completely disappeared and instead we see some preference for Ach over RG stimuli (blue-purple scale) (figure 3A), or in the case of BY, mainly balanced responses for chromatic and achromatic stimuli (figure 3B). It is clear that the greater activation produced by the chromatic stimuli when the cortex was driven by the fixing eye (figure 3, top panels of A and B) is lost during amblyopic eye activation (figure 3, bottom panels of A and B) leaving a chromatic response that is now weaker than, or equal to, the achromatic one.

In order to provide an objective and quantitative analysis of the illustration depicted in Figure 3 and to separate the responses according to the different stimulus types, chromatic and achromatic contrast, and visual area, we used a region of interest (ROI) analysis for the different cortical areas. Results for area V1 are shown in Figure 4. The broadband checkerboard stimulus (figure 4A) produced a significantly weaker response to amblyopic eye stimulation than to fixing eye stimulation ($t(6) = 3.41$, $p = 0.014$. For the narrowband ring stimulus (figure 4B), a significant difference between the relative
responses to the Ach, RG and BY stimuli was observed for the fixing versus amblyopic eyes in V1 \( (F(2,12) = 11.07, p = 0.002) \). This interaction was driven by a significantly higher response to the two chromatic stimuli than to the Ach stimulus (Ach vs. RG, \( t(6) = 4.94, p = 0.003 \), Ach vs. BY, \( t(6) = 3.40, p = 0.015 \)) when the fixing eye was stimulated with no such preference for chromatic stimuli when the amblyopic eye was stimulated. For the amblyopic eye responses are similar across Ach and chromatic conditions and hence these results demonstrate a selectively greater loss for chromatic stimuli.

Figure 5 shows a similar interocular comparison of fMRI responses for the broadband checkerboard (left panels) and spatio-temporal narrowband stimuli (right panels) for the extra-striate visual areas V2, V3, VP, V4 and V3A. Responses to the broadband checkerboard stimulus (figure 5, left panels) exhibited no interaction between Eye and Visual Area \( (F(4,24) = 1.64, p = 0.2) \) indicating a consistent and significant deficit in the amblyopic eye response compared with that of the fixing eye across all extra-striate visual areas. Paired t-tests confirmed that this difference between the fixing and fellow amblyopic eye is statistically reliable in all areas \( (p < 0.05) \) with the exception of V3A. For the spatio-temporal narrowband stimuli (figure 5, right panels), the results in extra-striate visual cortex show a change in the relative responses to the Ach, RG and BY
stimuli between the two eyes ($F(2,12) = 12.19, p = 0.001$, figure 5, right panels), similar to
that found in the striate cortex. This difference in the pattern of activation between
fixing and amblyopic eyes is due to a greater deficit for the two chromatic stimuli
compared to the Ach stimulus for amblyopic eye activation. This characteristic
cromatic loss occurs across all areas and does not depend on whether the fixing eye
exhibits a significantly greater response to colour (as in V2 and V4) or not (as in V3, VP
and V3A). The result is that the amblyopic eye is driven best by achromatic stimuli in all
extra-striate areas except V4.

Figure 6 near here

Figure 6 summarizes the different types of dependencies found for fixing and fellow
amblyopic eyes for the spatio-temporal narrowband stimulus modulated along
different axes in colour space. The error bars are SDs although it should be noted that
within subject statistical evaluation was undertaken for which SEMs are the more
relevant indicator of variability. In the LGN, the best response for the fixing eye is to the
L/M cone opponent modulation, whereas this produces the poorest response for the
amblyopic eye. In striate and extra-striate cortex, the fixing eye stimulation produces
the best response to chromatic stimuli whereas for the amblyopic eye the opposite is
ture; the best response is to the achromatic stimulus (figure 3). Thus in the cortex, the
selective loss includes both types of chromatic response rather than just the L/M cone
opponent response.
In a previous investigation we showed, using a broadband checkerboard stimulus with combined luminance and colour contrast, that there was reduced activation for amblyopic eye stimulation in the LGN (Hess et al. 2009b). Here we first demonstrate that this loss extends to both striate and extra-striate cortex. Second, we have investigated the selectivity of these losses by comparing fixing and amblyopic eye responses to spatio-temporal narrowband stimuli that were defined by either luminance, L/M cone opponent or S cone modulation. The thalamo-cortical pathway is composed of three separate projections, namely the magnocellular, parvocellular and koniocellular projections, each responding preferentially to achromatic (Derrington and Lennie 1984; Kaplan and Shapley 1982; Lee et al. 1990; Solomon et al. 1999), L/M cone opponent (Derrington and Lennie 1984; Lee et al. 1990; Merigan et al. 1991) or S cone isolating stimuli (Chatterjee and Callaway 2003; Dacey and Packer 2003; Martin et al. 1997) respectively. Thus to assess whether the information carried by all three projections is affected equally in amblyopia or whether there is a selective deficit, we compared the fixing and amblyopic eye performance to each of these three diagnostic stimuli. Previous studies of normal subjects using fMRI have highlighted important features of the response to chromatic stimuli in both LGN and cortex. In the LGN, robust responses are found to red/green, achromatic and blue/yellow stimuli at high contrasts (Mullen et al. 2008). In the cortex, there is also a robust response to colour in
V1 and in extra-striate areas of the ventral stream (Engel et al. 1997; Hadjikhani et al. 1998; Liu and Wandell 2005; McKeefry and Zeki 1997; Mullen et al. 2007; Wade et al. 2002). In addition, there is a relative boost of the response to S cone isolating stimuli in the cortex compared to the LGN (Mullen et al. 2008) consistent with the mixing of the parvo-and konio-cellular geniculate inputs at the cortical level (Conway and Livingstone 2006; De Valois et al. 2000; Horwitz et al. 2007; Johnson et al. 2004; 2001; Lennie et al. 1990; Solomon and Lennie 2005; Wachtler et al. 2003). Robust responses to colour, typical of the normal visual system, are also seen in the response of the fellow fixing eye of the amblyopes studied here (see figure 3). Our results indicate quite strikingly that fMRI deficit exhibits a chromatic selectivity and that this varies from the thalamus to the visual cortex. In the LGN, there is a selective loss of function in the L/M cone opponent response shown by the fact that for the fixing eye, L/M cone opponent stimuli produce best activation, yet for the amblyopic eye, these stimuli produce the least activation. In the striate and extra-striate cortex, the activity driven by the amblyopic eye exhibits a selective chromatic deficit for both L/M cone opponent and S cone responses. At neither site do we find a significant correlation between the fMRI deficit and the visual acuity; more severe subjects did not necessarily exhibit larger losses.

These findings have a number of implications. First, they suggest that at the level of the LGN where the parvocellular, magnocellular and koniocellular pathways are physiologically separate (Derrington and Lennie 1984; Martin et al. 1997; Solomon et al. 2000).
there may be a selective loss of parvocellular function because L/M cone opponent responses are mediated by parvocellular-cells. Second, the fact that the deficit at the cortical level includes S cone as well as L/M cone opponent responses may be explained on the basis of the known mixing of parvo-and konio-cellular cortical inputs. The population of cells in the LGN, as measured by single cell electrophysiology, exhibits a bimodal chromatic tuning reflecting the separate parvo-and konio-cellular contributions, whereas in the cortex this becomes unimodal due to the presumed mixing of parvo-and konio-cellular information (Conway and Livingstone 2006; De Valois et al. 2000; Horwitz et al. 2007; Johnson et al. 2004; 2001; Lennie et al. 1990; Solomon and Lennie 2005; Wachtler et al. 2003). Thus in terms of the current neurophysiology, the forms of both the lateral geniculate and cortical deficits are consistent with a primary loss of parvocellular geniculate function. Third, the fact that the LGN deficit is different from that found in striate cortex suggests a component of the LGN loss that cannot be solely due to feedback from striate cortex, implying a primary deficit in the LGN. This may be the result of less responsive cells or fewer cells (due to retrograde degeneration) responding to the input from the deprived eye.

Could the apparent loss of chromatic relative to achromatic sensitivity in the cortex driven by the amblyopic eye be a consequence of a loss affecting mainly central vision? The L/M cone opponent response is more confined to central vision, whereas the achromatic and S cone opponent response exhibit a more gradual fall-off with eccentricity measured both psychophysically (Mullen 1991; Mullen and Kingdom 2002;
Mullen et al. 2005) and in terms of V1 BOLD activation (Mullen et al. 2007; Vanni et al. 2006). Hence a cortical deficit confined to central vision might produce a selective L/M cone opponent loss in an ROI analysis. Two findings argue against this. First, the selective chromatic cortical loss reported here occurs equally for L/M cone opponent and S-cone isolating stimuli, ruling out an explanation based solely on the regional nature of the deficit. Second, even though one previous study showed that the fMRI deficit in amblyopia is more centrally located (Li et al. 2007), this has not been a consistent finding (Conner et al. 2007).

The present study is the first to compare fMRI activation for stimuli whose contrast is defined by modulations in cardinal directions in colour space designed to optimally activate magnocellular, parvocellular and koniocellular projections and to conclude that the deficit at the level of the LGN may be selective to parvocellular function while that at the cortex is selective for chromatic processing. We do not conclude that the cortical deficit is limited parvocellular function only. A number of studies have argued that parvocellular-driven cortical function is selectively compromised for the amblyopic eye input; for example, Miki et al. (2008) using fMRI argued for a selective loss for the parvocellular stimulus for one anisometropic amblyope. Mizoguchi et al. (2005), using PET, and Shan et al. (2000), using evoked potentials, came to a similar conclusion based on the response to different spatio-temporal stimulation. Hess et al (2009a) reported a selective cortical fMRI deficit to high contrast stimuli when driven by the amblyopic eye and model this in terms of deficient parvocellular function at the level of the LGN.
Although these studies use stimuli of different spatio-temporal or contrast composition in an effort to separate parvo-from magno-driven cortical function, this may not be ideal for two reasons; first there is evidence that parvo-and magnocellular geniculate input is mixed in cortical areas beyond 4C a & b (Lachica et al. 1992; Levitt et al. 1994; Martin 1992; Merigan and Maunsell 1993; Nealey and Maunsell 1994; Sawatari and Callaway 1996; Sincich et al. 2002; Vidyasagar et al. 2002) and second, suprathreshold stimuli of different spatio-temporal composition may not selectively activate each system (Merigan et al. 1991; Merigan and Maunsell 1990). Using a psychophysical approach, Grounds et al. (1983) report a selective loss in amblyopes of a spatio-temporal filter tuned to high spatial and low temporal frequencies claimed to reflect X-cell or parvocellular function. Davis et al (2006) reported a colour selective loss of psychophysical performance in the amblyopic eyes of late-onset strabismics, as well as a chromatic selective VEP latency deficit (Davis et al. 2008) that they attribute to a selective loss of parvocellular function. One limitation of the study by Davis et al (2006) was the use of a 3.2c/d stimulus for the chromatic thresholds, as this stimulus spatial frequency is likely to have significant luminance artefact (Faubert et al. 2000). A previous MEG study highlighted reduced power and longer latency in the cortical response to L/M cone opponent stimuli of low-mid spatial frequency (1-2 c/d) for amblyopic eye stimulation (Anderson et al. 1999). Interestingly, this did not have a direct threshold psychophysical correlate, since thresholds for low-mid spatial frequency stimuli (0.5-2 c/d) were normal, being slightly reduced only at a higher spatial frequency (4c/d).
Strabismic and anisometropic amblyopia has been traditionally defined psychophysically in terms of deficient luminance contrast sensitivity (Gstalder and Green 1971; Hess and Howell 1977; Levi and Harwerth 1977) and little is known about the sensitivity to isoluminant chromatic stimuli in amblyopia. One study suggests that the threshold deficit is similar for chromatic and achromatic stimuli (Mullen et al. 1996) whereas another (Davis et al. 2006) suggests that the chromatic thresholds are more raised than achromatic thresholds. Amblyopic eyes also exhibit a greater positional deficit for chromatic stimuli (Mullen et al. 1996). The fact that there is little psychophysical loss of chromatic sensitivity may be because psychophysical thresholds are determined by relatively small numbers of neurons and may not reflect the type of suprathreshold processing by large neural populations that underlies fMRI measures. The present finding using fMRI, based on a mass neuronal response, may have an advantage in revealing suprathreshold effects. Future psychophysical research comparing suprathreshold rather than threshold chromatic and achromatic contrast processing may be successful in revealing a behavioural correlate of the BOLD colour loss that we find in humans with amblyopia.

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thank all of our subjects for giving up their time. We are particularly indebted to Dr Joanne Wood of the Department of Optometry and Institute of Health and Biomedical Innovation, Queensland University of Technology, for the recruitment and scheduling of the patients at the onset of the study and for her help in obtaining ethics approval and to Ann Webber for patient recruitment.

Abbreviations

2AFC- two alternate forced choice; BOLD – blood oxygen level dependent; fMRI - functional magnetic resonance imaging; cpd – cycles per degree; FDR – false discovery rate; K - koniocellular; LGN – lateral geniculate nucleus; M- magnocellular; P- parvocellular; TR- repetition time; V – visual area; ROI – region of interest. GLM- general linear model; FDR- false discovery rate.
<table>
<thead>
<tr>
<th>Subject type of amblyopia</th>
<th>Refraction</th>
<th>Acuity</th>
<th>Eye alignment</th>
<th>Fixation centration</th>
<th>Fixation Variance</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>JLK strabismic</td>
<td>+0.75D</td>
<td>6/5</td>
<td>3° LET</td>
<td>2° eccentric</td>
<td>±0.74° ±2.7°</td>
<td>Large LET patching age 2yrs, surgery age 5yrs.</td>
</tr>
<tr>
<td></td>
<td>+0.765D</td>
<td>6/48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB strabismic</td>
<td>+0.50/-0.50x160</td>
<td>6/5</td>
<td>5° LET</td>
<td>central</td>
<td>± 0.39° ±0.52°</td>
<td>Surgery to correct large angle eso age 7</td>
</tr>
<tr>
<td></td>
<td>+1.00/-0.25x180</td>
<td>6/600</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF strabismic</td>
<td>-2.75D</td>
<td>6/6</td>
<td>3° XT, 10pd hypoT</td>
<td>4° eccentric</td>
<td>±0.10° ±0.39°</td>
<td>L ET and surgery in infancy and age 25yrs</td>
</tr>
<tr>
<td></td>
<td>-3.00D</td>
<td>6/240</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLG Aniso/strab</td>
<td>R -2.00/-2.75 x 15</td>
<td>6/7.5</td>
<td>6° LXT</td>
<td>1° eccentric</td>
<td>±0.15° ±0.36°</td>
<td>Anisometropia first detected in childhood, no surgery.</td>
</tr>
<tr>
<td></td>
<td>L -15.00/-2.25 x 180</td>
<td>CF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SJH anisomet.</td>
<td>+7/-3.00 x 150</td>
<td>6/30</td>
<td>ortho</td>
<td>central</td>
<td>±0.38° ±0.35°</td>
<td>First Rx at age 19yrs</td>
</tr>
<tr>
<td></td>
<td>+2.50/-1.25 x 80</td>
<td>6/4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DJL deprivation</td>
<td>+8.25/-1.00x90</td>
<td>CF</td>
<td>3° ET</td>
<td>6° eccentric</td>
<td>±3.1° ±0.18°</td>
<td>2 ops for ET age 9</td>
</tr>
<tr>
<td></td>
<td>+0.25D</td>
<td>6/6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MLT Deprivation</td>
<td>-2.00D</td>
<td>6/6</td>
<td>19° XT</td>
<td>2° eccentric</td>
<td>±0.42° ±1.8°</td>
<td>Cataract surgery age 7 yrs</td>
</tr>
<tr>
<td></td>
<td>-1.50D</td>
<td>CF</td>
<td></td>
<td></td>
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</tbody>
</table>

**Table 1.** Clinical details for the seven amblyopic participants. The following abbreviations have been used: strab for strabismus; aniso for anisometrope; deprv for deprivation; R for right eye; L for left eye; ET for esotropia; XT for exotropia; HT for...
hypertropia; ortho for orthotropic alignment; D for dioptre sphere; FIX for monocular fixation; L for left; R for right; CF count fingers.

Table 2

<table>
<thead>
<tr>
<th>Participant</th>
<th>Talairach Coordinates</th>
<th>Vol (mm³)</th>
<th>Talairach Coordinates</th>
<th>Vol (mm³)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>X Y Z</td>
<td></td>
<td>X Y Z</td>
<td></td>
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<tr>
<td>BB</td>
<td>-23 -26 -3</td>
<td>155</td>
<td>25 -25 2</td>
<td>86</td>
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<tr>
<td>CRF</td>
<td>-21 -27 -3</td>
<td>905</td>
<td>20 -27 -1</td>
<td>852</td>
</tr>
<tr>
<td>DJL</td>
<td>-19 -27 -3</td>
<td>91</td>
<td>18 -27 -1</td>
<td>147</td>
</tr>
<tr>
<td>JLK</td>
<td>-21 -24 -3</td>
<td>542</td>
<td>23 -22 -2</td>
<td>612</td>
</tr>
<tr>
<td>SJH</td>
<td>-23 -22 -4</td>
<td>243</td>
<td>25 -24 -2</td>
<td>283</td>
</tr>
<tr>
<td>MLT</td>
<td>-19 -28 1</td>
<td>518</td>
<td>20 -27 0</td>
<td>655</td>
</tr>
<tr>
<td>DLG</td>
<td>-19 -27 -3</td>
<td>91</td>
<td>18 -27 -2</td>
<td>147</td>
</tr>
<tr>
<td>Mean</td>
<td>-21 -26 -2</td>
<td>409</td>
<td>22 -25 -1</td>
<td>439</td>
</tr>
<tr>
<td>SD</td>
<td>2 2 2</td>
<td>306</td>
<td>3 2 1</td>
<td>310</td>
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</table>

Table 2. The LGN coordinates (mm) and volumes (mm³) located in stereotaxic space (Talairach & Tournoux, 1988) for the seven subjects.


Lachica EA, Beck PD, and Casagrande VA. Parallel pathways in macaque monkey striate cortex: Anatomically defined columns in layer III. *Proceedings of the National


Martin PR, White AJ, Goodchild AK, Wilder HD, and Sefton AE. Evidence that blue-on


Mullen KT, Dumoulin SO, and Hess RF. Color responses of the human lateral geniculate nucleus: selective amplification of S-cone signals between the lateral geniculate nucleus and primary visual cortex measured with high-field fMRI. *Eur J*


Sawatari A, and Callaway EM. Convergence of magno- and parvocellular pathways in


Figure 1. Example of the two types of stimuli used; a) a broadband multicoloured checkerboard presented abruptly in time flickering at 16Hz, and b) spatio-temporal narrowband radial gratings sinusoidally modulated in space (0.5c/deg) and time (2 Hz), calibrated to activate the achromatic (Ach), L/M cone opponent (RG) or S cone opponent (BY) processing streams, respectively.

Figure 2. Comparison of % BOLD signal in the LGN for fixing and amblyopic eyes for a spatio-temporal narrowband stimuli modulated along different axes in colour space (Ach: grey bars; RG: red bars, BY: blue bars). The asterisks denote statistical significance (P<0.05). Error bars show average within subjects SEM.

Figure 3. The results of a group contrast between (A) the RG-Ach conditions and (B) BY-Ach conditions for the fellow fixing (top) and amblyopic eyes (bottom). Positive t values (orange) represent a significantly greater activation of visual cortex by the chromatic than the achromatic stimuli, negative values (blue) represent the reverse. The flattened visual cortical surface of one participant (MLT) is used for the purposes of illustrating the group data. The medial edges of the flattened areas correspond to the representation of the horizontal meridian within the calcarine sulcus for this subject. The left hemisphere is shown on the reader’s left and the right hemisphere on the right. The boundaries of the visual areas are based on MLT’s retinotopic map and are presented only as a guide for interpretation of the group data.

Figure 4. Comparison of % BOLD signal in V1 for fixing and amblyopic eyes for (A) broadband checkerboard stimuli, and (B) spatio-temporal narrowband stimuli modulated along different axes in colour space (Ach: grey bars; RG: red bars, BY: blue bars). The asterisks denote statistical significance (P<0.05). Error bars show average within subjects SEM.
Figure 5. Comparison of % BOLD signal in extra-striate visual areas (V2, V3, VP, V4, V3A) for fixing and amblyopic eyes for the broadband checkerboard stimuli (left panels) and spatio-temporal narrowband stimuli modulated along different axes in colour space (Ach: grey bars; RG: red bars; BY: blue bars) (right panel). The asterisks denote statistical significance (P<0.05). Error bars show average within subjects SEM.

Figure 6. Summary of the different dependencies for fixing and fellow amblyopic eye for the spatio-temporal narrowband stimulus in LGN and cortical visual areas as marked. The error bars are SD but note that a within subject analysis was used for which SEM is the relevant indicator of variability.
a) Broadband Checkerboard

b) Spatio-temporal Narrowband Grating
LGN

!![Graph]

- Fellow Eye
- Amblyopic Eye

Legend:
- ACH
- RG
- BY

% BOLD Change
Figure 4

Bar graphs showing %BOLD change in V1 for fellow and amblyopic eyes.

**A**
- Fellow Eye: Average %BOLD change is 2.5.
- Amblyopic Eye: Average %BOLD change is 1.5.

**B**
- Fellow Eye: ACH, RG, and BY conditions show %BOLD changes of 1.2, 1.0, and 0.8, respectively.
- Amblyopic Eye: ACH, RG, and BY conditions show %BOLD changes of 0.6, 0.4, and 0.4, respectively.

Significance indicated by asterisk (*)
Figure 5
The diagram illustrates the response patterns of different brain areas in response to visual stimuli.

### LGN
- **Fellow Eye** (solid line)
- **Amblyopic Eye** (dashed line)

### V1
- % BOLD Change
- ACH, RG, BY

### Extrastriate
- % BOLD Change
- ACH, RG, BY