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fMRI Studies of Binocular Suppression in Human Amblyopic Subjects

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**Thesis submitted to the
School of Medicine
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Master of Science
in
Neurobiology and Anatomy**

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Abstract

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Binocular suppression has been previously studied psychophysically and with VEPs. Here we used functional magnetic resonance imaging (fMRI) to image activity in visual cortex while human subjects with normal vision or amblyopia experienced binocular integration. One eye viewed a dynamic grating pattern. The other eye viewed one of four stimuli, i.e., black mask, gray mask, or two grating masks that varied in contrast. We observed a luminance masking effect, that is, when the gray mask is compared to the black mask, there was less activation in many retinotopic areas (consistent with previous VEP studies). The result was observed in all control subjects. Interestingly, in the anisometropic subjects there was a pronounced asymmetry in the luminance masking effect, with the stronger effect seen when the fellow eye was masked. We conclude that interocular suppression studied with fMRI may be related to the concept of binocular suppression scotomata in subjects with amblyopia.

Dedication

My husband, Joe, has provided immeasurable support and encouragement throughout the process of my education in WVU, he inspires me and helps me work through life's ups and downs. I feel so lucky to have him in my life. I dedicate my achievements to him. We feel fortunate for our happiness, and look forward to great times ahead.

In addition, I want to thank to my parents, Yulan Lu and Fenting Sun, for their love and support. Their confidence in me has given me the strength to overcome obstacles in life and has brought me closer to fulfilling my life's goals.

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In addition, I would like to thank the members of my committee for their contributions and insights. Thanks to Dr. Odom for his valuable help in experimental design and suggestions in scientific writing. Thanks to Dr. Puce for her advice that helps me learn about and understand new advances in human vision research and for her help in improving my scientific writing.

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A. Introduction

1. Primate Visual System

The primate visual system is responsible for the transformation of photons of light into visual perception. As shown in Figure 1, the visual system is made up of both optical and neural components. The optical components of the eye (i.e., cornea and lens) refract and focus light through the eye onto the retina. The neural components begin with the photoreceptor layer in the retina, and in subsequent relays to the thalamus and visual cortex, they encode and transmit the light pattern into increasingly more processed neural signals.

Figure 1: The Primate visual system.

The retina, which lines the entire posterior chamber of the eye, contains the first neural elements of the visual system (Marcelja, 1979). The photoreceptors are comprised of two types, rods and cones. The rods are distributed throughout the retina except in the fovea (i.e., the central 2 degrees of the retina), are primarily responsive under low luminance levels and have only one photopigment. The cones are concentrated in the fovea, are most responsive under higher luminance levels, and possess one of three photopigments (Romavo & Raninen, 1990). The rods and cones transmit their signals toward the brain using similar pathways. Both photoreceptor types signal to bipolar cells, which in turn signal to retinal ganglion cells (RGCs). These connections essentially occur in a serial fashion, with pooling and refinement of receptive fields happening through the actions of the retinal interneurons, termed the horizontal and amacrine cells. The axons of the retinal ganglion cells form the optic nerve and provide the output from the retina to the next relay station, the lateral geniculate nucleus (LGN) of the thalamus.

As a result of the combination of inputs from many bipolar cells and the actions of the retinal neurons, the receptive fields (i.e., small regions of visual space that can influence activity levels) of the RGCs have a center-surround organization (Marcelja, 1979). Most RGC receptive fields have a center region which either increases or decreases the RGC firing rate with light stimulation, and a surrounding region which inhibits this response. This lateral inhibition enhances boundary features of visual stimuli, and represents the first major computation performed by the visual system.

Once the ganglion cell axons leave the retina, they travel through the optic nerve to the optic chiasm, a partial crossing of the axons. At the optic chiasm the left and right visual worlds are separated. After the chiasm, the fibers are called the optic tract. The optic tract wraps around the cerebral peduncles of the midbrain to get to the LGN.

The LGN contains six main layers (Fig. 2), which serve to segregate the inputs from parvocellular and magnocellular channels and to align the inputs from the two eyes while nonetheless keeping them physically segregated. The upper four layers (parvocellular) receive inputs from retinal midget cells, while the lower two (magnocellular) receive inputs from retinal parasol cells. There is an approximately 1:1 coupling between ganglion cells and LGN relay cells (Lee, Virsu, & Creutzfeldt, 1983). The interlaminar layers of the LGN have very small cells of a different type that also receive input from the retina; this rather heterogeneous group of cells are koniocellular. A recent study (Sinchich et al., 2004) has shown that there is a direct projection from LGN to cortical area MT in macaque monkey, and the constituent neurons are mostly koniocellular. The relationships between the numerous other RGC types and cell types in the LGN remain less certain.

The receptive field properties of cells in the parvocellular layers are similar to those of the midget ganglion cells; the receptive field properties of the magnocellular layers similar to those of the parasol cells. The parasol cells (a class of larger ganglion cells) have large receptive fields and respond transiently to sustained illumination. Due to this input, LGN magnocellular neurons also respond quickly but transiently to stimulation of their receptive fields. Furthermore, magnocellular neurons are not sensitive to color contrast, but are very sensitive to low luminance contrast ($\sim 2\%$ or less), and are optimally tuned for low spatial and high temporal frequencies (Hicks et al., 1983; Schiller

and Colby, 1983). The midget cells (a class of smaller ganglion cells) have small receptive fields and respond selectively to specific wavelengths. These cells project to the parvocellular layers. LGN parvocellular neurons are slowly adapting and have smaller receptive fields, capable of high resolution vision. These parvocellular neurons are sensitive to color contrast, are not sensitive to low luminance contrast ($< 10\%$), and are optimally tuned for high spatial and low temporal frequencies (Hicks et al., 1983; Schiller and Colby, 1983).

Figure 2: Histology of LGN. A. LGN 6 main layers; B. Each layer receives inputs from a different eye: 3 layers for the left eye and 3 layers for the right. Axons from one eye were labeled*. (retina.umh.es/Webvision/VisualCortex.html)

The optic nerve and LGN can be said to possess a “retinotopic map”. This is due to the fact that RGC axons carrying information from adjacent locations in the retina are located adjacent to each other, and the connections are orderly through the layers of the retina and out to the LGN (Marcelja, 1979; Schneider et al., 2004). The first major manifestation of this retinotopic organization divides visual space into left and right

hemifields. Axons carrying information from the left half of visual space (left nasal hemiretina and right temporal hemiretina) project to the right LGN, and axons carrying information from the right half of visual space (right nasal hemiretina and left temporal hemiretina) project to the left LGN.

Axons originating from the LGN maintain retinotopy in their projection to the ipsilateral primary visual cortex (area 17), also called V1 or striate cortex, located at the occipital pole of the brain (Nelson & LeVay, 1985). The distribution of these axons to ipsilateral V1 is divided by the horizontal meridian, such that axons carrying representations of the upper visual field project to visual cortex below the calcarine sulcus, while axons carrying representations of the lower visual field project to visual cortex above the calcarine sulcus.

Once the LGN axons reach V1, they terminate primarily in a single sub-layer of cortex (Blasdel & Fitzpathick, 1984). Axons arising from the magnocellular layers of the LGN terminate in layer 4Ca of V1, while axons from the parvocellular layers of the LGN terminate in layer 4Cb. V1 in humans contains six layers and is approximately 2 mm thick, with the input layer 4 the thickest. Recall that the receptive fields of both ganglion cells and LGN neurons were center-surround (Marcelja, 1979), and that they responded optimally to points of light (Ramoal, 1985). Neurons in the cortex, however, respond very poorly to points of light. The optimal stimulus for most cortical neurons turns out to be a bar of light, in a very specific orientation (Ramoal et al., 1985). This is believed to be due, in part, to ordered convergence of LGN axons on to cortical neurons.

V1 is organized into functional columns, including both orientation and ocular dominance columns (Malach et al., 1993; Horton & Hocking, 1996). This means that the cells in any given column have a preferential bias for a stimulus of a particular orientation presented to either the ipsilateral or the contralateral eye (Fig. 3). Orientation preference is mapped in an orderly fashion, encompassing all 360 degrees (deg). Ocular dominance columns alternate in V1. It is known that their relative widths can be altered in animals after monocular visual deprivation, such as induced amblyopia (Movshon et al., 1987; Kiorpes et al., 1998; Horton and Hocking, 1997, 1998; Horton et al., 1997, 1999).

Figure 3: The ocular dominance stripes. As the signal is transmitted to upper layers of cortex, the information from the two eyes is mixed and binocular vision is created, but here in 4C the two eyes are still entirely separate. Therefore, if the inputs from a single eye could be labeled, in 4C little columns of label would be seen. If layer 4C were cut tangentially (parallel to the surface) through, it would be seen that all those pillars line up next to each other and form tiger stripes. (retina.umh.es/Webvision/VisualCortex.html)

The staining patterns of the metabolic enzyme cytochrome oxidase (CO) have been used to demonstrate the magnocellular and parvocellular projections in V1 and V2 (Horton, 1984; Tootell et al., 1984; Livingstone and Hubel, 1987). There is evidence showing that magnocellular outputs from layer 4Ca travel through layer 4B of V1 to the CO thick stripes of V2 and area MT, conveying information about motion and stereo. The area MT is known to selectively process information about motion (Maunsell et al., 1983). For example, a very high proportion of MT neurons discriminate direction of motion within the visual field according to electrophysiological recordings in monkeys. It is thought that the sensitivity of motion stimuli in MT is related to the inputs that it receives from lower visual areas known to receive input from the magnocellular subcortical pathway (Maunsell et al., 1990). Most evidence in the past also indicated that in V1 layer 4C β projects to layer 2/3, where parvocellular input splits into two streams, patch and interpatches, segregating color and form signals that are propagated to V2 and subsequently to V4. Koniocellular input is added to the color stream in layer 2/3 patches. That was the projection pattern for the functional organization of V1 and V2 based on a tripartite framework developed by Livingstone and Hubel (1988), who described three

distinct pathways: magno to thick stripes, parvo to pale stripes, and konio to thin stripes (Fig. 4).

This model has been widely accepted until fairly recently. However, using retrograde tracers from V2 to V1, Figure 5 demonstrated that V1 output arises from just two sources: patch columns and interpatch columns (Sincich & Horton, 2002). Patch columns project to thin stripes; interpatch columns of V1, mainly from layers 2 and 3, project to both the thick stripes and the pale stripes of V2. These neurons seem specialized to encode either information about color or form features of visual stimuli. This new model predicts that the parvocellular pathway involving the CO patches from V1 to V2 remains fairly segregated, while the other magnocellular and parvocellular pathways are mixed. Nonetheless, visual areas at higher levels show selectivity for stimuli with characteristics more magnocellular or parvocellular in quality (Schein & Monasterio, 1987)

Figure 4: The tripartite model of the visual system. Livingstone & Hubel (1988)

Figure 5: Schematic model of projections from V1 to V2 in macaque monkeys showing that cells in layer 2/3, 4A, and 4B from patches project to thin stripes, whereas those from interpatches project to thick and pale stripes. Cells in layer 5/6 from patches and interpatches project to all stripe types. Sincich and Horton (2002)

Visual information is transmitted from V1 and V2 to other visual areas as well as to other regions of the brain. These extrastriate visual areas include V3, V3A, V4v, V4/V8, VP, and MT in humans. The connections between visual areas have a hierarchical organization so that more posterior areas can be considered “lower-tier” in relation to “higher-tier” anterior areas (the terms “lower-tier” and “higher-tier” to refer to general positions in the presumptive human hierarchy). Each extrastriate area contains another retinotopic map of visual space, processing increasingly more complex stimulus features with larger receptive fields with increasing separation from V1 (Desimone et al., 1985; Maunsell and Newsome, 1987). For example, studies of selective lesions to macaque V2 show that there is no effect on visual acuity (as is seen in V1 lesions), but that the animals instead have increased difficulty performing a task requiring detection of a set of collinear dots in a random dot background (Merigan et al., 1993). In a follow-up lesion study of macaque V4v, this group demonstrated an almost complete deficit for discriminating the orientation of collinear dots in a random dot background (Merigan, 1996). These findings illustrate the specificity with which primate visual areas process features of the visual scene, and suggest that these areas (V2 and V4v) are involved in form processing, likely relying on parvocellular inputs.

Beside the traditional retino-geniculo-striate visual pathway, it is hypothesized that alternate visual pathways exist which bypass striate visual cortex since some subjects deprived of primary visual cortex still have some residual visual functions, e.g., the

“blindsight” phenomenon. The proposed visual processing mechanisms subserving these residual visual abilities have been controversial (Boyer et al., 2004). It has been suggested that the residual vision without conscious awareness may be mediated by the superior colliculus via the extrageniculate retinotectal pathway (Ro et al., 2004), or a geniculo-extrastriate pathway (Stoerig & Cowey, 1989). The extrageniculate vision account received the strongest support from some studies of the theories of blindsight. After lesions to V1, monkeys frequently demonstrate preserved ability to make accurate saccades to stimuli in their blind field (Poppel, 1973), while a subsequent lesion to the superior colliculus abolished the effect in monkeys (Mohler & Wurtz, 1977). Furthermore, directionally sensitive cells in area MT fail to respond to stimuli in the monkey’s blind field after a lesion to the superior colliculus (Rodman et al., 1990). However, some studies do find evidence that support the geniculo-extrastriate account. The vast majority of projection neurons within the dorsal LGN die as a result of retrograde degeneration after lesion to V1 (Van Buren, 1963). In contrast, a small number survive, and at least a subset of these neurons project to extrastriate cortex (Cowey & Stoerig, 1989). The connections of the koniocellular layer to the superior colliculus and extrastriate cortex indicate that it might be involved in the alternate visual pathways. Koniocellular cells receive major ascending inputs from the superior colliculus in simian primates (Harting et al., 1991). In both macaques and other primates, koniocellular cells provide the major dorsal LGN inputs to the upper layers of the cytochrome oxidase blobs in V1. Functional heterogeneity in the koniocellular system in macaques is indicated by the observation that nonoverlapping populations within the koniocellular channel provide projections to the extrastriate cortex and the V1 blobs, respectively (Lysakowski et al., 1988).

Although anatomical and electrophysiological studies in primates and other animals have provided a great deal of information about the organization of the subcortical and cortical visual system, these techniques are largely unavailable for studying aspects of human vision. Until very recently, most information about the human cortical visual system has been inferred from studies of the effects of accidental lesions in patients’ brains. Fortunately, the data from this field is well supported by the animal literature. Human cortical lesion studies affecting individual visual areas offered the first

evidence that distinct human visual areas play separable roles in high-level aspects of vision. For example, motion agnosia and achromatopsia following lesions to small regions of lateral or ventral visual cortex, respectively, have been described in the literature and in clinical practice (De Renzi, 2000; Stasheff and Barton, 2001; Rizzo and Vecera, 2002). In addition, VEP studies have been performed clinically for around 25 years for clinical screening, assessment, and monitoring the effectiveness of occlusion therapy in amblyopic subjects (Henc-Petrinovic et al., 1993; Stevens et al., 1994; Simons et al., 2004; Weiss & Kelly, 2004). Information from these lesion studies and the VEP studies has proven invaluable, but it remains difficult to generalize the findings to the normal visual system. However, relatively recent advancements in functional neuroimaging techniques (i.e., fMRI) allow investigators to study normally functioning visual systems. The activity of many visual areas can be monitored simultaneously, and small areas of the brain can be sampled at relatively fast rates and physiologic recordings from human cortex can be made with good spatial resolution. The fMRI mechanism and its application in human visual system study will be discussed in the following sections.

2. Functional Magnetic Resonance Imaging (fMRI).

fMRI is a noninvasive technique that maps changes in brain hemodynamics that correspond to perceptual or mental operations which are reflected by patterns of neural activation, thus extending traditional anatomical imaging to include maps of human brain function (Ogawa et al., 1992; Bandettini et al., 1992; Kwong, et al., 1992). The fMRI signal is based on the increase in blood flow to the local vasculature that accompanies neural activity in the brain. It results in a corresponding local reduction in deoxyhemoglobin because the increase in blood flow occurs without an increase of similar magnitude in oxygen extraction (Roy and Sherrington, 1890; Fox and Raichle, 1988). The blood oxygenation level-dependent (BOLD) response, which arises when autoregulation of blood flow causes an increase in local blood volume in response to neural activity, is the basis of fMRI signal. Since deoxyhemoglobin is paramagnetic (being a substance in which an induced magnetic field is parallel and proportional to the intensity of the magnetizing field but is much weaker than in ferromagnetic materials), it

alters the T2* weighted magnetic resonance image signal (Ogawa et al., 1990a and b, 1992, 1993; Belliveau et al., 1990, 1991; Turner et al., 1991; Tank et al., 1992). The differential effect of deoxyhemoglobin relative to hemoglobin on the spinning of neighboring hydrogen atoms is detected by the MR scanner, and the image is reconstructed by two-dimensional Fourier analysis. Therefore, the intensity values of individual voxels in this data set represent the relative blood oxygenation level of the tissue encoded by those voxels. fMRI methods are optimized to acquire multiple images of the relevant volume very quickly, resulting in a four- dimensional (x, y, z, and t(time)) data set with reasonable spatial (2mm) and temporal resolution (1-2 s) (Turner, et al., 1998).

This new ability to directly observe brain function opens an array of new opportunities to advance our understanding of brain organization. Due to the ability to image the entire 3-dimensional volume of brain, fMRI is capable of isolating many simultaneous and coordinated brain events. fMRI methods are currently applied to identify brain structures involved with visual perception, thus, it enables us to learn quite a lot about the organization of human visual cortex. Functional neuroimaging experiments have been used to study aspects of vision ranging from very “low” level, such as luminance contrast dependence of the fMRI response in early visual areas, to “high” level studies of object and face recognition which highlight larger contributions from higher-tier areas (e.g., Tootell et al., 1995; Kanwisher et al., 1997). Retinotopic mapping of the borders of multiple visual areas and the use of brain flattening techniques provide for more accurate visualization of the retinotopic organization of visual cortex. The most fundamental finding has been the identification of approximate 30 visual areas and the demonstration of the retinotopic organization of these visual areas, including V1 (striate cortex) and extrastriate areas V2, V3, V3a, VP, and V4v (DeYoe et al., 1996; Engel et al., 1997; Sereno et al., 1995, 2001). These visual areas are comprised of normal representations of retinotopic visual field, and are thought to be responsible for more sophisticated analyses of visual stimuli that lead toward visual perception (Horton and Hoyt, 1991). The revealed retinotopic organizations in humans enable us to study its relationship to contrast perception.

3. fMRI Studies of Contrast Perception in Normal Subjects.

The relationship between luminance contrast perception and the fMRI BOLD response in visual cortex has been studied in normally sighted adults. It has been established that the separate visual areas are differentially tuned for contrast. V1 and extrastriate area MT are well-studied visual areas, and there is a robust differences in the contrast sensitivity between V1 and MT. Based on results from electrophysiological studies showing that the average contrast sensitivity in single units in V1 is much lower than that in MT, Tootell et al. (1995) expected to find a high contrast sensitivity in the fMRI response in human area MT, relative to that in V1. They demonstrated that stimuli of increasing log contrast produce a progressive increase in MRI amplitude in V1 across most of the visible contrast range. The MT response to the lowest contrast tested (1.6%) starts at a relatively high BOLD level, and then becomes saturated at contrasts higher than 6% contrast. Tootell et al. reported that half-amplitude fMRI signals in V1 and MT occurred at 15% and 1% contrast, respectively. According to the contrast gain function derived from averaged MR time courses, a stimulus alternating between 6% and 100% contrast produces robust MR modulation of V1, but no modulation in MT (Tootell et al., 1998). It is therefore hypothesized that the area MT may be optimized to detect targets at low contrast levels, while V1 is good for discriminating between objects at higher contrast levels. The contrast sensitivity in other visual areas is either similar to that in V1 or similar to that in MT. For example, the contrast sensitivity in human V2 was only slightly higher than that in V1, whereas the contrast sensitivity in V3 was indistinguishable from that in MT (Tootell et al., 1998). Goodyear and Menon (1998) also reported proportionality between increasing stimulus contrast and the magnitude of the fMRI response in V1, additionally they also found an increase in the spatial extent of activation with increasing stimulus contrast within V1, but no such trend was seen within extrastriate cortex. The findings suggest there is an increase in neuronal activity in V1 with increasing luminance contrast, consistent with much other physiological evidence.

According to these early fMRI studies of contrast perception, the BOLD response in V1 can be predicted by stimulus contrast, but recent studies suggest that *perceived* contrast is the predictor. Boynton et al. (1999) demonstrated that detection of a contrast

increment produces an increase in the fMRI responses of a criterion amount in V1, V2, V3, and V3A. The amplitude increase is dependent upon the perception of increased contrast over the pedestal contrast (Weber's law). Consistent with this, Ress et al. (2003) also reported that V1 response to stimuli presented near threshold is detectable for hits, but is also seen at a similar level for false alarms during a contrast-detection task. Hits and false alarms elicit significantly more cortical activity than did correct rejects and misses. That false alarms evoked more activity than misses suggests that activity in early visual cortex corresponded to the subjects' percepts, rather than to the physically presented stimulus.

However, some other provocative results do exist. Ress et al. (2000) argue that the responses measured in V1 might be actually dominated by attentional components, since the activity of V1 became greater when the subject was more likely to correctly discern the presence or absence of the pattern during a pattern-detection task. The stimulus-independent activity in V1 during the pattern detection performance indicates that the MR signal in early visual areas are more closely correlated with the subjects' response than visual input from presented stimuli (Ress et al., 2000). This finding complicates the interpretation of any study of MR responses to grating presentation near contrast threshold, including the study conducted by Boynton et al. in 1999. Presenting stimuli with suprathreshold contrast should be able to avoid this confound, since Ress et al. also showed that the MR signal in V1 falls for false alarms and rises for hits when stimuli presented at suprathreshold contrast level made the detection task easier.

Singh et al.(2000) studied suprathreshold contrast perception by monitoring the variation in fMRI response magnitude in multiple visual areas to sinusoidal gratings with 100% contrast over a range of spatial and temporal frequencies. The spatial frequency tuning curves for human visual areas at suprathreshold contrast levels demonstrated that visual areas thought to receive relatively large parvocellular input (e.g., V1, V2, and V4v) are tuned for higher spatial frequencies, while visual areas thought to be more magnocellular (e.g., V3A and MT) are tuned for lower spatial frequencies. The temporal frequency tuning curves in each visual area were shown to be more or less identical, the optimal temporal frequencies are around 8 Hz which would result in maximal response

with a 2 cycles per degree (c.p.d.) grating. The parametric modulation of spatial and temporal frequency also showed that the two dimensions are essentially separable.

These studies indicate that different visual areas are tuned to different contrast levels and different spatial frequency, with a dramatic difference between V1 (striate cortex) and MT (extrastriate cortex), other visual areas behave either more like V1 or more like MT. These hints from fMRI studies of contrast perception in normals are relevant to understanding the neural basis of amblyopia from electrophysiological and psychophysical studies.

4. Definition of Amblyopia.

Amblyopia, often referred to as “lazy eye,” is a common developmental disorder of vision with an average incidence of 2-4% of the general population in U.S. It is defined as abnormally low visual acuity (acuties most frequently range from 20/30 to 20/60), even with optical refractive correction and in the absence of any obvious retinal or central pathology. Reduced visual acuity in the affected eye usually coexists with normal acuity in the patient’s other eye (i.e., the fellow eye). In fact, partial or full suppression of the amblyopic eye’s input by the fellow eye can occur when both eyes are open. As a result, depth perception relying on binocular disparity cues is almost universally absent, forcing the patient to use other depth cues (e.g., texture, shading, and superposition) for navigating in space. Clinical experience indicates that amblyopia is caused by form vision deprivation and/or abnormal binocular interaction in early childhood (Von Noorden, 1973; Von Noorden, 1975; Kiorpes et al., 1998). It develops during maturation of the visual pathway and is reversible during the first seven to eight years of life. This is known as the 'critical period'. In some situations the critical period may be extended (Simmers, 1999).

Amblyopia is usually classified into three categories. The most common two types of amblyopia are those secondary to strabismus or unequal refractive errors (Von Noorden, 1967; McKee et al., 2003). **Anisometropic** amblyopia results from unequal refractive error between the two eyes (a difference of more than or equal to 0.75 diopter is generally thought to be significant). Because one eye is always out of focus with respect to the other, the visual system learns to ignore one image in preference to the

other. The eyes may be myopic to different extents or hyperopic to different extents. Uncorrected myopic anisometropes (one eye significantly myopic) frequently rely upon the less myopic eye for distance viewing and the other (more myopic) eye for near viewing. In uncorrected hyperopic anisometropes, however, the less hyperopic eye has a more clearly focused retinal image when viewing both distance and near targets, and thus the other eye is chronically blurred. Amblyopia is frequently associated with hyperopic anisometropia, but rarely found in myopic anisometropes (Eggers & Blakemore, 1978; Townshend et al., 1993; McKee et al., 2003).

Strabismic amblyopia is defined by a congenital misalignment of the ocular axes (McKee et al., 2003; Michaelides & Moore, 2004). This may occur due to defective development of the extraocular muscles, their cranial nerve innervation, and/or the feedback mechanism involving proprioception which normally aligns the eyes during infancy. The deviating eye can turn inward (esotropia) or outward (exotropia). Strabismus can be intermittently or constantly present, and the angle of deviation may be stable or variable. In addition, the eye that deviates may alternate. In humans, constant esotropia is frequently associated with the presence of amblyopia. Exotropic deviations, on the other hand, are often intermittent or alternating and do not frequently coexist with amblyopia.

Deprivation amblyopia can result from any physical obstruction to normal vision, such as lid suture, dense unilateral cataract, corneal opacification, or palpebral hemangioma (a benign childhood tumor on the eyelid). It causes exclusion of all visual information other than diffuse light (Von Noorden GK, 1978). In humans, congenital cataract leads to amblyopia unless cataract surgery is performed in early life. This form of amblyopia arises much less often than the amblyopia associated with strabismus or anisometropia since the obstruction is usually obvious upon physical inspection and is typically treated as soon as possible after birth.

Untreated amblyopia can have a negative impact in adult life. Some career choices have specific visual acuity requirements. The number of careers barred to adults with reduced vision increases with the severity of the deficit (Adams, 1999). A minimum lifetime risk of 1.2% of serious visual impairment through loss of acuity in the eye with the better vision, for example from trauma, age-related retinal changes (ARMD) and

circulation problems (retinal vein and artery occlusions etc.) has been estimated (Rahi, 2002). A national surveillance study in 2002 in the UK found that only 35% (36/102) of people who lost the vision in their non-amblyopic eyes were able to continue in paid employment (Rahi, 2002).

The ultimate goal of an amblyopia study is to understand the neural basis of amblyopia, which will help develop better treatment methods that either allow treatment later in life or provide better detection and treatment early in life. Treatment for amblyopia broadly consists of a combination of spectacle correction and patching or 'penalization' of the better-seeing eye (Mittelman, 2003). The most common types of penalization are eye patching and the use of atropine drops to induce cycloplegia (pupillary dilation and paralysis of accommodative focus). Early detection is a key factor in successful treatment by a pediatric ophthalmologist. However, compliance with standard ophthalmological treatments remains problematic. Many children do not comply with their treatment schedule because patches may be viewed as uncomfortable or socially embarrassing (Dixon-Woods et al., 2006). Since the stronger eye is patched, children must learn to cope with poorer vision in the weaker eye until it becomes stronger (Holmes et al., 2003). For these reasons, combined with the fact that amblyopia often goes undetected in childhood, many children become adults with amblyopia. By the time a person with a lazy eye reaches adulthood, there is generally significant vision loss, resulting from years of the brain ignoring signals from the weaker eye. For these reasons, new approaches to amblyopia research are needed to pioneer more effective therapies.

5. Definition of Visual Acuties and the Contrast Sensitivity Function.

The spatial resolution of the visual system is usually assessed using a simple measure of static visual acuity. A typical visual acuity test consists of a number of high contrast, black-on-white targets of progressively smaller size. The smallest target that one can successfully read denotes one's visual acuity. In real life situations, human beings detect and identify spatial forms that vary widely as a function of target size, contrast, and spatial orientation. A simple assessment of visual acuity often does not predict an individual's ability to detect objects of larger size (Ginsburg et al., 1982; Watson et al.,

1983). Contrast sensitivity testing complements and extends the assessment of visual function provided by simple acuity tests. Contrast sensitivity measurements yield information about an individual's ability to see low-contrast targets over an extended range of target size (and orientation).

Contrast sensitivity tests use sine-wave gratings as targets instead of the letter optotypes typically used in tests of acuity. Sine-wave gratings possess useful mathematical properties and researchers have discovered that early stages of visual processing are optimally "tuned" to such targets (Watson, et al., 1983). A contrast sensitivity assessment procedure consists of presenting the observer with a sine-wave grating target of a given spatial frequency (i.e., the number of sinusoidal luminance c.p.d. of visual angle). The contrast of the target grating is then varied while the observer's contrast detection threshold is determined. Typically, contrast thresholds of this sort are collected using vertically oriented sine-wave gratings varying in spatial frequency from 0.5 (very wide) to 32 (very narrow) c.p.d. of visual angle. It measures the least amount of contrast needed to detect a visual stimulus (i.e. a grating on an otherwise homogeneous and isoluminant gray background) and gives us a more complete quantization of subjects' visual capabilities.

A person's contrast sensitivity, defined as the inverse of the contrast threshold, is typically plotted over a range of spatial frequencies. This is known as the contrast sensitivity function (CSF). Humans with normal vision are best at seeing mid-range spatial frequencies (1 – 4 c.p.d.). This range represents the peak contrast sensitivity range, but must be distinguished from the grating acuity. The grating acuity of an observer is defined as the highest resolvable spatial frequency at full contrast, i.e., the x-intercept of the CSF. Figure 6 demonstrates a representative contrast sensitivity function (Campbell, 1983). Note that the peak contrast sensitivity for this observer is for 2 c.p.d. stimuli, and that the grating acuity is extrapolated to 50 c.p.d.

At many visible contrast levels it is possible to increase or decrease the spatial frequency of a stimulus to a point where it can no longer be discriminated by the human visual system. Figure 7 illustrates the spatial frequency and contrast dependence of the CSF (Campbell & Robson, 1968). Choosing a set contrast level near the center of the y-

axis, it becomes apparent that regions to the left (low spatial frequencies) and right (high spatial frequencies) cannot be discriminated from homogeneous gray regions.

Figure 6: The CSF for a human adult. This curve defines the adult's window of visibility. The human visual system is capable of discriminating stimuli that appear under the curve. (Campbell, 1983)

Figure 7: Spatial frequency and contrast dependence of the CSF. The spatial frequency of the grating is increasing from left to right, and the contrast is increasing from top to bottom. The bars in the middle are easiest to see at the bottom, with the bars at both sides being harder to see all the way to the top. (Campbell and Robson, 1968)

The CSF reveals much about the function of the visual system, but it does not predict performance on all visual tasks. Some tasks have been classified as examples of “hyperacuity” because the resulting measures of visual acuity are underestimated by the contrast sensitivity function. Vernier acuity is the most commonly described hyperacuity, in which the visual system can detect an spatial offset of two line stimuli at a resolution much finer than the highest grating acuity level (Levi & Klein, 1982a; Levi & Klein, 1982b). Vernier acuity is also often described as a positional acuity task, since it is dependent upon the relative positions of multiple components within the visual field. The human visual system can discriminate differences in the spatial position very precisely, about five times less than the distance between nearby photoreceptors in the fovea. Such high-resolution performance suggests additional interpolation processes occur in visual cortex (Westheimer, 1979).

The CSF and other acuity tasks not only provide a good measure of the functional range of the human visual system, but also provide quantification of the abnormal patterns associated with visual system disease. Amblyopia, for example, is characterized by several functional abnormalities in spatial vision (Hess, 1990; Ciuffreda, 1991), including reductions in visual acuity, CSF, and vernier acuity. In addition, abnormal spatial interactions (Polat et al., 1997; Levi et al., 2002) and impaired contour detection (Hess et al., 1997; Kovacs et al., 2000) are found, and some subjects report spatial distortions (Sireteanu, 1993). The CSF in amblyopia will be discussed further in the following section.

6. Contrast Sensitivity Function in Amblyopia.

The contrast sensitivity in amblyopia is generally described as being depressed, especially at high spatial frequencies, with the peak sensitivity shifted to lower spatial frequencies (Fig. 8). Gstalder and Green (1971) first published the contrast sensitivity function of amblyopic human subjects, confirming predictions surmised from the animal literature (Gstalder and Green, 1971). Performance of the fellow eye is typically unaffected relative to normal eyes of subjects without amblyopia. It can be inferred that the amblyopic loss most strongly affects the neural units with small receptive fields that

are thought to detect patterns with high spatial frequency content. The parvocellular visual pathway that supports perception of high spatial frequencies may be selectively deficient in amblyopic subjects (Hess and Anderson, 1993; Kubova et al., 1996; Demirci et al., 2002).

Figure 8. The amblyopic contrast sensitivity function shows that the CSF for the amblyopic eye is depressed at high spatial frequencies, and that the peak sensitivity is shifted toward the left (Gstalder and Green, 1971).

Studies have shown that the CSF in amblyopia is dependent upon several stimulus characteristics beyond spatial frequency and contrast, such as stimulus luminance, size, and retinal location. Volkers et al. (1987) report that the contrast sensitivity is strongly dependent on the width of the stimulus and utilize the discrepancy between the high-frequency cut-off ('grating acuity') and the Snellen acuity to help differentiate amblyopia from optic nerve degeneration. In optic nerve degeneration a decrease in contrast sensitivity is found at low spatial frequencies. Decreased contrast sensitivity at high

spatial frequencies is atypical but occurs in those disorders associated with decreased Snellen visual acuity, such as amblyopia. Figure 9 shows that as the level of light decreases from daylight (photopic) to twilight (mesopic), visual sensitivity drops primarily at high spatial frequencies; lower frequencies are little affected. But when the light falls to extremely low levels (night time/ scotopic), sensitivity decreases even at low frequencies (Hess & Howell, 1977). Hess and Howell (1978) report that the contrast sensitivity deficit for strabismic amblyopes decreases at all spatial frequencies as stimulus luminance decreases from photopic levels (3 to 30 cd/m²) to scotopic levels (0.3 to 0.003 cd/m²). Katz et al. (1984) demonstrated that CSFs vary with stimulus field size, retinal location and degree of amblyopia. The peak contrast sensitivity of amblyopic eyes increased markedly with increasing field size for stimuli presented foveally, it can reach the same magnitude as that of the fellow eye for centrally presented stimuli subtending 20 deg of visual angle. Peripherally, peak contrast sensitivity remained lower in the amblyopic eyes for all field sizes examined. High spatial frequency cut-offs were reduced both centrally and peripherally with all field sizes in the amblyopic eyes (Katz et al., 1984). The effect of increasing field size on the cut-off acuity is smaller than the effect on peak contrast sensitivity. Amblyopic eyes benefit to a greater extent from increased stimulus field size than non-amblyopic eyes in terms of peak contrast sensitivity (Katz et al., 1984). This might be due to fewer and/or less sensitive cortical neurons being driven by the amblyopic eye in humans with naturally occurring amblyopia. These data indicate that stimuli must be used with luminance levels in the range of 2 to 30 cd/ m² and that stimuli must not be too large (< 8 deg visual angle) in order to expose the deficit.

Figure 9: CSFs measured at three different light levels.
(Hess & Howell, 1977)

Although the conditions associated with strabismus and anisometropia may seem quite different (one being an oculomotor anomaly and one being an interocular difference in refraction), amblyopes associated with these conditions share many visual characteristics. Both groups show reduced sensitivity to contrast, particularly at higher spatial frequencies, and markedly degraded positional acuity (Ciuffreda et al., 1991). Furthermore, a recent investigation has revealed that a significant proportion of both groups experience perceptual distortions when viewing simple grating patterns with the affected eye (Barrett et al., 2003).

Despite this behavioral overlap, some differences do exist for certain visual tasks, including CSF. The psychophysical difference between these two amblyopic subtypes will be discussed more specifically in the following sections.

Anisometropic amblyopia is produced by optical defocus. Optical defocus is generally the result of refractive errors, which selectively remove high spatial frequencies

from the retinal image. The resulting CSF for the weak eye is depressed most markedly at high spatial frequencies. Although both strabismics and anisometropes exhibit similar contrast sensitivity losses at high spatial frequencies, the anisometropes are more likely to suffer additional deficits at low spatial frequencies as a linear function of depth of amblyopia (Levi and Harwerth, 1977; Sjostrand, 1981; Campos et al., 1984). Optotype acuity and grating acuity are decreased in proportion to the severity of the contrast sensitivity deficit (Levi and Klein, 1982a; Levi, 1988). In addition, the extent of the contrast sensitivity deficit across the visual field also appears to differ between the two subtypes (Hess and Pointer 1985). Nearly the entire visual field is affected for anisometropes and, unlike strabismic amblyopia, there are no asymmetries between the nasal and temporal fields (Yu et al., 1998).

Strabismic amblyopia results from misalignment of the visual axes. Strabismus deprives the visual cortex of the synchronous firing provided by simultaneous correlated images from the two retinal foveas, resulting in binocular rivalry and suppression of one eye's inputs at the level of the striate cortex (Hubel, 1982). The primary contrast sensitivity deficit is in the high spatial frequency range, although a few strabismic individuals have been reported with essentially normal contrast sensitivity functions (Hess et al., 1978). The grating acuity deficit observed in strabismic amblyopes is proportional to the contrast sensitivity loss, but other acuities related to positional localization such as vernier acuity are affected to a greater degree (Levi and Klein, 1982b). Strabismic individuals are distinguished by the decoupling of vernier and grating acuity, with vernier falling off faster than grating acuity (Levi and Klein, 1985). This additional deficit in vernier (spatial localization) acuity suggests that strabismic amblyopes suffer from a topological disorganization of cortical connections. The contributing factor might be the fact that the deviated eye is aligned with an eccentric point away from the fovea, and the retino-cortical connections from this point are paired during development with foveal connections from the other eye.

Another important consideration for strabismic amblyopia may be the strong suppression of input from the deviated eye to avoid diplopia. This reduction of input may leave the deviated eye unable to support accurate form perception. As Figure 10 shows, Hess et al. (1978) illustrated the nature of the strabismic perception. In this case, the

contrast of the gratings was increased to suprathreshold levels. The strabismic subjects reported perception of geometrically distorted gratings, regardless of their contrast sensitivity deficit. This abnormal perception existed even in subjects with normal contrast sensitivity functions. Consistent with these findings, strabismic individuals report systematic distortions of visual space when viewing with their amblyopic eye (Bedell and Flom, 1981).

Figure 10. The distorted perception of grating for a strabismic subject with normal CSF in both eyes. The subject was asked to draw what he perceived. Notice the misperception persisted even at high contrast levels (Hess et al., 1978).

7. Psychophysical Studies of Suprathreshold Contrast Perception in Amblyopia.

Suprathreshold contrast perception may well be more relevant for the activities of daily living, since all visible stimuli or objects are, by definition, at or above threshold. One way to measure suprathreshold contrast perception is with a task in which a subject presses a button as soon as possible after a stimulus is seen. Levi and others (Levi et al., 1979a; Loshin and Levi, 1983) have investigated suprathreshold contrast perception in

strabismic and anisometropic amblyopes using such a reaction time paradigm. Reaction time increased as the grating contrast decreased for every spatial frequency. The reaction times for the amblyopic eye were prolonged compared to the nonamblyopic eye at all spatial frequencies and contrast values, but most markedly at high spatial frequencies. The contrast vs. reaction time function for the amblyopic eyes was biphasic in the middle range of spatial frequencies, indicating that two separate mechanisms detect gratings at high and low contrast levels. Interestingly, the mechanism operating at high contrast levels was relatively more impaired in the more severe amblyopes.

Loshin and Levi also examined perceived contrast with a subjective matching paradigm (Loshin and Levi, 1983). Using a staircase procedure to determine the point of equivalent contrast for a test stimulus viewed with the fellow eye and a reference stimulus viewed with the amblyopic eye, this study showed no systematic impairment in contrast perception for the amblyopic eye. However, the contrast matching paradigm used in this study requires subjects to compare their perception using different eyes, a difficult task that may introduce bias. The conclusion of no impairment was based on average data from six amblyopic subjects with a range of diagnoses and subtypes.

Hess and Bradley (1980) also used a matching paradigm to study suprathreshold perception. The study showed that contrast matching data for a representative strabismic amblyope and an anisometropic amblyope are very different. For the strabismic amblyope, the amblyopic eye perceives the target correctly once the target contrast is slightly above its detection threshold, independent of the spatial frequency of the target. However, for the anisometropic amblyope, contrast perception is degraded over a substantial suprathreshold range (0.5 Log Unit (LU) – 1 LU). This range is dependent on the spatial frequency of the pattern. In this study, results indicate that strabismic amblyopes maintain normal suprathreshold contrast perception, behaving as though the contrast range had been truncated to a different extent for each spatial frequency. However, other studies of suprathreshold perception in amblyopia show apparently discrepant results. Ciuffreda et al. (1987) demonstrated impairment of contrast discrimination in strabismic amblyopic eyes, and the deficit was present at each of the spatial frequencies tested. Kiper and Kiorpes (1994) also report abnormalities in processing contrast information at suprathreshold level in experimental strabismic

monkeys. The strabismic monkeys exhibit elevated thresholds for a wide range of spatial frequencies in their increment threshold contrast detection experiment, with the deviated eyes showing marked deficit at medium and high spatial frequency. The different conclusions reached by Hess and Bradley (1980) and Ciuffreda et al. (1987) might be due to different measures used in these studies. Kiper and Kiorpes (1994) and Ciuffreda et al. (1987) used similar increment threshold procedures in measuring contrast increment threshold, and their results are consistent with each other.

Impairment of contrast detection and discrimination in amblyopic eyes is presumably due to early abnormal visual experience, involving visual cortical neurons sensitive to contrast (Campbell et al., 1972). The abnormal visual experience includes abnormal binocular interactions due to constant strabismic suppression (Burian et al., 1974) or mild monocular form deprivation due to uncorrected anisometropia (Bradley et al., 1981). Binocular interactions in amblyopia observed through psychophysical measurements (including visual acuity, contrast sensitivity and contrast increment threshold) will be discussed in the following section.

8. Psychophysical Studies of Binocular Interactions in Amblyopia.

Given that amblyopia begins with unbalanced input from the two eyes, the fact that subjects with amblyopia develop abnormal binocular interactions is not surprising. Several lines of evidence suggest amblyopic subjects have reduced binocular integration. Amblyopic individuals suffer from binocular abnormalities such as impaired stereoacuity, and clinical tests for stereopsis reveal stereoblindness in many subjects with amblyopia. Binocular function can be assessed at even more basic levels using careful psychophysics. For example, measurement of the CSF for observers with normal vision reveals improved contrast sensitivity in one eye with subthreshold or threshold stimulation of the other eye. This is understood to be a result of binocular summation. On the other hand, when a stimulus is presented to one eye above the threshold contrast, normal observers show a threshold elevation effect in the other eye as a result of interocular inhibition (Legge, 1979). These effects are specific for orientation and spatial

frequency of the stimulus, and represent binocular combination of visual information in visual cortex.

Interocular inhibition has been reported intact for amblyopic subjects in contrast to impaired binocular summation. Levi et al. (1979a) performed dichoptic masking experiments to study binocular interactions for grating patterns in humans with normal binocular vision and in humans with amblyopia via comparison of monocular and binocular contrast thresholds. Results showed that a suprathreshold masking grating presented to one eye elevated the contrast threshold for gratings presented to the fellow eye. This masking effect was similar in subjects with normal and abnormal binocular vision. On the other hand, a masking grating with subthreshold contrast presented to one eye reduced the contrast threshold for detection of a similar grating presented to the fellow eye in normal subjects, but no such subthreshold summation is evident in the amblyopic observers. They conclude that while strabismic amblyopia disrupted the normal excitatory interactions between the two eyes, cortical inhibitory binocular connections were not disrupted, which is consistent with their previous results showing that binocular summation was absent at all contrast levels, but binocular occlusion was evident at high contrast levels for amblyopic observers (Levi, 1979b).

Although dichoptic masking is present in subjects with amblyopia, newer work shows that the degree of interocular inhibition is not always normal. Also using dichoptic masking, Harrad and Hess (1992a) found unequal masking in subjects with abnormal binocularity, arguing that suppression in amblyopia could not be simply accounted for by dichoptic masking observed in normals. In the study, seven strabismic subjects show unequal masking and in five of these subjects the masking function of the normal eye shows less than expected threshold elevation while the amblyopic eye shows more than expected threshold elevation. Unequal masking was always seen when the difference in contrast sensitivity between the two eyes is greater than 8dB. For anisometropic amblyopes tested at spatial frequencies where there was a large contrast sensitivity difference between the two eyes, either normal masking or a small degree of unequal masking was observed. Possible reasons that Levi et al. (1979a) did not find unequal masking include the use of only a low contrast mask of 0.5 LU above threshold, and the fact that only three subjects were studied and one of the three was an anisometropic

amblyope who would not necessarily show unequal masking. These psychophysical findings show that binocular interactions in the amblyopic visual system are abnormal.

Some additional psychophysical studies have specifically focused on binocular suppression. One of the early studies assessed the monocular luminance detection threshold of a green square in amblyopic eyes and the elevation of the detection threshold under binocular conditions (adjacent, non-overlapping red square presented to the other eyes) as a measure of interocular suppression in 6 normal subjects, 9 strabismic amblyopes (all esotropes) and 2 anisometropic amblyopes (Sireteanu and Fronius, 1981). They found that visual acuity is selectively impaired in the central part of the visual field of the deviated eye in human strabismic amblyopes as well as anisometropic amblyopes. With fellow eye open (binocular condition) the detection threshold is elevated in amblyopic eye due to interocular suppression for both groups of subjects. Holopigian (1989) further studied the effects of stimulus strength on the depth of suppression in 6 amblyopic subjects (3 anisometropic and 3 strabismic amblyopes) and 4 normal observers. For both clinical suppression in amblyopic subjects and binocular rivalry suppression in normal subjects, the depth of suppression was constant, regardless of the alterations made to the contrast, the luminance, and the spatial frequency of the inducing stimulus. Freeman and Jolly (1994) later observed similar results by measuring visual acuity. Normal acuity under monocular viewing was reduced to its lowest during the suppressive phase of binocular rivalry in 5 normal subjects, and was reduced less when the fellow eye viewed a contoured nonrivalrous stimulus. In 9 strabismic subjects, by contrast, acuity was markedly reduced in going from monocular to binocular viewing independent of the inducing stimulus. Their interpretation was that the acuity loss when an amblyopic subject goes from monocular to binocular viewing was attributable to interocular suppression. The degree to which interocular suppression depends on the stimulus parameters presented to the other eye is still an open question that we will assess in this project.

These psychophysical findings in human amblyopia are correlated with and supported by animal studies. Animal models studies will be discussed in detail in the following section.

9. Animal Models of Amblyopia.

Animal models of amblyopia provide valuable insights into the role of early visual experience on the structure and function of the human brain. In animal investigations, anisometropia can be generated by optical means (e.g., by inserting –10D contact lens in front of one eye) or by unilateral installation of atropine (Von Noorden, 1978). Strabismus can be generated by using prisms or by sectioning the extraocular muscles (Mower et al., 1982; Hubel and Wiesel, 1965). Form deprivation can be produced by lid suture, patching, or monocular enucleation during development, and these studies are frequently referred to as monocular deprivation studies (Wiesel and Hubel, 1963). The effects are typically measured in adults through electrophysiology, metabolic staining, or behavioral performance.

Hubel and Wiesel conducted the pioneering neurophysiological and neuroanatomical studies initially on cats and then later on monkeys in the 1960s, which won them a Nobel Prize. They studied the effects of monocular deprivation (produced by lid suture) (Wiesel and Hubel, 1963; Hubel et al., 1977; LeVay et al., 1980) and surgically-induced strabismus (Hubel and Wiesel, 1965) on the structure and function of the visual system. Their results established that a site of deficit in amblyopia is at the level of the primary visual cortex (striate cortex, V1, Brodmann's area 17), and demonstrated the dramatic changes in cortical function following abnormal visual experience during a critical period early in life. They proposed a developmental mechanism that depends critically on a competitive interaction between the cortical afferents from the two eyes, which was supported by the finding that the adverse effects of monocular deprivation were much greater than those produced by binocular deprivation. This developmental mechanism provided guidance for the following amblyopia studies, and is further developed into a principle of current amblyopia doctrine.

Based on this early work, debate over the neural basis and probable site for the amblyopia deficit continues today. In general, animal models show reduced visual acuity and contrast sensitivity in individual neurons in V1 driven by the amblyopic eye, which is consistent with the psychophysical findings. They also show decreased numbers of

binocular neurons in the striate cortical output layers, and substantial shifts of ocular dominance column toward the fellow eye in relatively severe animal models (Movshon et al., 1987; Kiorpes et al., 1998; Horton and Hocking, 1997, 1998; Horton et al., 1997, 1999). Specifically, Movshon et al. (1987) studied the effect of early unilateral blur by daily instillation of atropine in 5 macaque monkeys. Results showed that neurons driven by the treated eyes tended to have lower optimal spatial frequencies, poorer spatial resolution, and lower contrast sensitivity than neurons driven by the untreated eyes, and that the more affected animals had substantial eye dominance shifts and larger interocular differences. Kiorpes et al. (1998) also report reduced cortical binocularity in macaque monkeys with experimental strabismus or anisometropia according to electrophysiological measurements. Specifically, they studied 3 monkeys with surgically induced esotropic strabismus in the first weeks of life and 3 monkeys with anisometropia induced by wearing -10 diopter contact lenses in one eye during the first months of life. They have compared single-unit recording from V1 cortex with CSFs in the monkeys. Both amblyopic subtypes have fewer cortical binocular neurons than normal monkeys. The severity of the amblyopia, as indicated from CSFs, was related to the degree of shifts in cortical eye dominance (only for 3 seriously affected monkeys: one strabismic and two anisometropic). Unfortunately, this study did not have enough subjects to draw a clear picture about the relationship between shifts in eye dominance and spatial contrast sensitivity functions in monkeys with strabismus or anisometropia. Crawford et al. (2004) reported that the relative widths of the ODCs having input from amblyopic eyes were reduced in proportion to the age of onset and the duration of the early visual abnormality, and that the relative losses in contrast sensitivity were in ordinal agreement with the losses in cortical afferents as reflected by the reduction in width of the respective ODCs in V1.

In more severe models using eyelid-sutured macaques, the column shrinkage was more extensive in layer 4Cb (parvocellular input) than in 4Ca (magnocellular input) (Horton and Hocking, 1997). Furthermore, Horton and Hocking (1998a; 1998b) described functional metabolic effects in layer 4C (i.e., the primary input layer) of striate cortex in macaques related to the severity of the amblyopia-inducing technique (i.e., enucleation or lid suture). In normally sighted monkeys, layer 4C of striate cortex stains

homogeneously for CO. In contrast, monocularly enucleated monkeys exhibited sharp borders between alternating ODC of approximately the same width, demonstrating greater metabolic activity in neurons driven by the intact eye. Cortical sections from lid-sutured primates showed a lower contrast pattern of thin and thick columns alternating with more diffuse border strips, which indicates a metabolic deficit in the column core zone driven by the sutured eye and a relative deficit in the binocularly driven border zones. In addition, Horton et al. (1999) revealed a pattern of thin dark alternating with wide pale columns in striate cortex in strabismic macaques with strong fixation preference, resulting from reduced CO activity in both eye's binocular border stripes and one eye's monocular core zone. Finally, CO staining showed a pattern of thin dark bands in layer 4 in a macaque monkey with naturally occurring anisometropic amblyopia, and Horton et al. (1997) interpret the pattern as the result of relative loss of CO activity along the borders of the ocular dominance columns, regions specialized for binocular processing.

The data from animal models are useful when predicting human amblyopia. One would expect CO staining patterns in V1 in human strabismus or anisometropia to exhibit a pattern of thin dark bands in layer 4 similar to that found in naturally occurring anisometropic macaques, with relatively dark column centers for the unimpaired eye and pale column centers for the impaired eye, alternating with diffuse border zones which are driven by both eyes. Results obtained from monkey amblyopia describe the anatomical and metabolic effects of monocular deprivation as a model for amblyopia. It is clear that there is a reduction in the number of binocular neurons and a shift in ocular dominance toward the unimpaired eye in striate cortex.

Harrad and Hess (1992b) proposed that the %age of surviving binocular neurons (the degree of binocularity) is inversely related to the depth of binocular suppression in amblyopia. This notion is supported by the electrophysiological finding from single cell recording in surgically induced strabismic cats that striate neurons with balanced inputs from the two eyes show binocular interactions closest to normal at least in terms of their orientation selectivity, whereas the majority of neurons with weak or no direct input from one eye exhibit profound interocular suppression (Sengpiel et al., 1994). Furthermore, this physiological study supports the notion that the primary visual cortex is indeed a site

of interocular suppression in strabismus. Finally, the suppressive interactions observed at the neuronal level in the strabismic cats (Sengpiel et al., 1994) resemble pathological suppression in strabismic humans in their relative independence of stimulus parameters (Holopigian et al., 1988).

One disadvantage of most of the animal studies of amblyopia is that they focus on V1 alone. So it is difficult to correlate with human psychophysical data where it is not possible to know which visual or nonvisual cortical areas may be contributing to a specific behavior. Physiological studies of human amblyopia incorporated the findings from animal studies and investigate the behavior of V1 as well as other visual areas in amblyopia.

10. Physiological Studies of Contrast Perception in Amblyopia.

Physiological studies of human amblyopia have been done to assess contrast perception and binocular interactions. Previously, electroencephalogram (EEG), i.e., visually-evoked potential (VEP) was the major technique utilized in electrophysiological experiments in human amblyopia. Neuroimaging techniques, such as fMRI as well as positron emission tomography (PET), have been largely applied in studying human amblyopia since they provide better spatial resolution. VEPs are still in use in amblyopia studies to provide corroborating findings with the neuroimaging techniques as fMRI cannot resolve time courses or top-down versus bottom-up processes.

Attempts to study the neural deficit of human amblyopia were previously made by using PET and SPECT neuroimaging techniques. Kabasakal et al. (1995) reported that SPECT images were found to have a lower activity rate (change of regional cerebral blood flow increase) in the visual cortex for the stimulated amblyopic eye relative to normal eye stimulation in every patient. Consistent with the SPECT result, three PET studies of amblyopia report decreased signal in visual cortex with amblyopic eye stimulation compared to fellow eye stimulation (Demer et al., 1988; Demer et al., 1997; Mizoguchi et al., 2005) regardless of amblyopic subtype; while one PET study observed that reduced activity solely in the extrastriate visual cortex (i.e. ipsilateral Brodmann's areas 18,19) of 8 individuals with strabismic amblyopia following amblyopic eye stimulation with similar activation of V1 (BA 17) after stimulation through either eye

(Imamura et al., 1997). In general, the reduced metabolic rate in visual cortex following amblyopic eye stimulation is consistent with the staining pattern changes of the metabolic marker CO in macaque striate cortex with amblyopic models that were reported by Horton et al. (1995).

One drawback of PET scan is that because the radioactivity decays rapidly, it is limited to monitoring short tasks. Thus, it is impossible to employ visual tasks like those used to measure the CSF in a PET scan. With spatial resolution of two or three millimeters at present and relatively good temporal resolution, fMRI has largely superseded PET for the study of brain activation patterns that might be helpful in revealing binocular suppression in human amblyopia.

Contrast perception in human amblyopia is well addressed in the first fMRI study, which was conducted by Goodyear et al. (2000) in four strabismic amblyopia subjects and six control subjects. Vertical sinusoidal gratings were presented at a fixed suprathreshold contrast (22%), and at six spatial frequencies (0.5, 1, 2, 4, 8, and 12 c.p.d.). The experiment was comprised of individual trials using an event-related design, where the six spatial frequencies were presented in random order three times each for 3 seconds each trial. Region of interest (ROI) analysis was used to interpret the fMRI response; the ROI was defined as the intersection of an anatomically-defined area including V1 and some of V2, and a functional mask which is composed from the union of all voxels with significant fMRI responses from any stimulus functions as a filter so that “junk voxels” can be excluded in an effort to have more sensitive analysis specific to the stimuli presented. The visibility of gratings at a fixed contrast decreases for low and high spatial frequencies, even in normal subjects (CSF curve that defines window of visibility). Thus, psychophysical experiments were conducted to measure the perceived contrast of the 22% contrast grating at each spatial frequency by adjusting the contrast of a reference grating at a fixed spatial frequency (1 c.p.d. for amblyopes and 4 c.p.d. for normals) until it appeared of equivalent contrast to the subject. The perception of 22% contrast as a function of spatial frequency was found to be in an inverse U shape curve for both eyes of both normal and amblyopic subjects. Only one of the four amblyopic subjects showed suppressed contrast perception for the amblyopic eye at the high spatial frequencies. The magnitude of the fMRI response to the stimuli also show an inverse U

shape, which covaried with the perceived contrast of the grating in all subjects, regardless of spatial frequency. Interestingly, the subject who had suppressed contrast perception at the high spatial frequencies for the amblyopic eye showed lower MR signal for those stimuli, so it still held true that the BOLD signal magnitude can be predicted by the reduced perceived contrast. Results also showed that for all amblyopia subjects significantly “activated” fMRI voxels were fewer during amblyopic stimulation than during fellow eye stimulation. These findings suggest that the extent of cortical activity is less from amblyopic eye stimulation.

Most previous VEP studies present stimuli monocularly. However, a few studies have employed dichoptic methods (separate stimuli shown to each eye) to study binocular interactions. These studies are reviewed below.

11. Physiological Studies of Binocular Suppression in Amblyopia.

Binocular interaction in amblyopia has been addressed in some VEP studies. Based on the fact that dynamic dichoptic luminance stimulation produces nonlinear (difference) beats in stereonormal adults, the beats are thought to represent nonlinear processing of the stimuli by binocularly responding neurons in visual cortex. However, these beats are absent or diminished in stereoblind subjects (Baith and Levi, 1988). Thus, the dichoptic VEPs give a quantitative assessment of cortical binocularity. Baith and Levi (1989) reported that the strabismic amblyopes demonstrate severe reductions in the amplitude of these beats compared with normal observers. This electrophysiologic evidence confirms that strabismic amblyopes indeed have abnormal binocular interactions and is supported by later studies. Stevens et al. (1994) recorded dichoptic luminance beats VEPs from 20 children (ages 7 months to 8 years) with abnormal binocular ability secondary to strabismus and compared this to a control group. Stereoblind children were found to generate significantly lower dichoptic signal-to-noise ratios than stereonormal children. Apkarian et al. (1981) also studied the monocular and binocular VEPs of strabismic amblyopes to investigate electrophysiological correlates of abnormal binocular function. They reported that VEP spatial frequency tuning functions of amblyopic eyes tend to show a reduced cutoff spatial frequency in relation to the

fellow eye, and that binocular interactions depend upon the spatial frequency and contrast of the stimulus, and may vary from inhibition to summation. In this study, the binocular interactions in strabismic amblyopes were similar to those of normal subjects, which suggest that some binocular function may be preserved in the cortex of patients with strabismic amblyopia, therefore, caution should be taken when the steady-state VEP is used to differentiate normal from abnormal binocular vision.

Binocular *suppressive* interactions have become a focus for some studies of amblyopia since binocular suppression is thought to be underlying neural deficits of amblyopia. Some individuals with severe amblyopia can be unaware of the visual information from part of the visual field of the non-fixing eye under binocular conditions. The term suppression scotoma is used to define the area in which visual information is not perceived. Wright et al. (1986) examined the effect of amblyopic suppression on P-VEP in five anisometropic amblyopes with scotomas and four normal subjects by comparing responses generated under monocular conditions with monocular responses under binocular viewing conditions (suppression). P1 amplitudes in patients with large suppression scotomas were found not to be recordable under binocular viewing conditions; the P1 amplitudes in patients with small suppression scotomata were greatly reduced under binocular viewing. Their data indicate that the P1 component of the transient P-VEP is greatly diminished when stimuli are presented within the area of suppression.

In another effort to reveal mechanisms of binocular suppression, Norcia et al. (2000) monitored cortical activity by using VEP recordings in five normal observers and five stereo-deficient observers, and dichoptic masking of 9.5 Hz reference gratings by a 7.4Hz swept contrast grating. Results showed that contrast response functions measures at 7.4 Hz were increased under monocular viewing, and that contrast threshold was elevated when a dichoptic masker (20% contrast 9.5 Hz, vertical grating) was presented to the other eye. Consistent with psychophysical experiments, the dominant eye exerts a greater suppressive effect on the non-dominant eye in the dichoptic masking of contrast experiment.

In addition to contrast, the role of luminance in binocular suppression has also been documented in human physiological studies. Interocular suppression due to

luminance had a considerable effect on VEP amplitude measured at 200 msec in subjects with normal binocular vision. When a constant stimulus in one eye changes from darkness to diffuse light, VEP amplitude driven by a dynamic stimulus in the other eye measured at 200 msec was reduced in 6 human subjects (Harter et al., 1980). In another study the same luminance suppressive effect were duplicated, changing a dichoptic mask from darkness to diffuse light reduced the VEP amplitude at 60 msec and 220msec in eight adults with good monocular acuities (Odom and Harter, 1983).

Neural mechanisms of binocular suppression are still poorly understood in amblyopia. VEP studies provide higher temporal resolution, but offer limited spatial resolution. fMRI cannot resolve the time course of the suppression effect, but provide good spatial resolution. No fMRI study has yet directly investigated binocular suppression. Our experiments specifically addressed binocular suppression in amblyopia using fMRI as a tool. We tested whether the suppression on one eye is dependent on the parameters of stimuli presented to the other eye and assessed the degree of asymmetry of the suppressive effect between the two eyes that has been demonstrated previously by psychophysical and physiological studies.

12. Current experiment

We had two major aims in these experiments:

Aim 1. Measure the monocular contrast sensitivity function and dichoptic masking psychophysically

The contrast sensitivity function is one of the tests that define the depth of amblyopia. Therefore, it was crucial that we carefully assess the contrast sensitivity of both eyes in all subjects, since this allowed us to create individualized stimuli for the fMRI experiment. Both vertical and horizontal gratings were used to test the monocular contrast sensitivity function in one eye with a gray screen presented to the other eye, and it was expected to observe reduced contrast sensitivity for amblyopic relative to fellow eyes.

Certain binocular interactions in strabismic amblyopia have been shown, using psychophysics and VEP physiology, to be abnormal relative to healthy vision, and

perhaps even relative to anisometropic amblyopia. Binocular inhibition is present in amblyopic subjects, but abnormal in strength and symmetry between the two eyes. In this project, the original findings of Levi and colleagues (Levi et al., 1979) were tested with a larger subject pool. Monocular contrast detection in one eye was tested with a masking stimulus presented to the other eye. We expected unequal masking effects with more suppression of the amblyopic eye by the fellow eye and weaker suppression of the fellow eye by the amblyopic eye. The results for vertical and horizontal gratings were compared. We expected to see smaller interocular suppression with horizontal grating stimuli compared to the effect with vertical grating stimuli because vertical orientations engage stereoscopic mechanisms known to be abnormal in our subjects. We incorporated into the fMRI experiments similar stimuli that are known to cause psychophysical inhibition (i.e., medium contrast dichoptic mask). We assessed the degree to which presence or absence of psychophysical inhibition across subjects may correlate with these fMRI measures.

Aim 2. Correlate psychophysical deficits with fMRI activation pattern of binocular suppression.

The current experiment was the first neuroimaging study to compare the fMRI response to stimuli of defined spatial frequency and contrast shown under monocular or dichoptic binocular conditions. We obtained novel data from control subjects. Furthermore, we observed abnormalities in the extent and magnitude of cortical activation in subjects with amblyopia under binocular conditions compared to control subjects.

We presented to the non-tested eye stimuli with defined contrast over a suprathreshold range that is known to exert a suppressive effect on the tested eye, with the tested eye viewing a dynamic suprathreshold grating with either vertical or horizontal orientation (two eyes view stimuli with same orientation). Specifically, the experiment was performed with single-trial grating stimuli (4Hz, 1.0 LU above threshold) presented to one eye and a blocked design of mask modulation (black background, gray background, stimuli 0.5 LU above threshold, stimuli 1.0 LU above threshold) presented to the other eye.

Our experiments were built upon the findings of Goodyear et al.(2000), that the BOLD signal is proportional to perceived contrast, by controlling the stimuli for perceived contrast. Our experiments also had larger subject groups, promoting more statistical power. We expected to find a modulation of response that is dependent on contrast of the gratings presented to the non-tested eye and asymmetry of suppression between the two eyes. In another word, the hypothesis was that with an increase in luminance or contrast for stimuli in one eye, greater suppression effects would be observed on the signal strength of dynamic stimuli in the other eye; with vertical stimuli the unequal masking effect might be greater.

The animal literature suggests a difference between anisometropic and strabismic amblyopes in terms of binocularity. For example, Kiorpes et al.(1998) studied six monkeys with either surgically induced esotropic strabismus or refractively induced anisometropia (3 of each) in order to investigate the binocularity and ocular dominance effects. Both amblyopic subtypes were found to have fewer cortical binocular neurons than control monkeys, especially strabismics (control: 70-85%; anisometropic: 15-50%; strabismic: 18-27%). Analogously, the fMRI study by Lee et al. (2001) reported a decreased index of binocularity in strabismic relative to anisometropic amblyopia. However, this study did not directly investigate interactions between the two eyes with dichoptic stimulation. We specifically compared subjects with anisometropic amblyopia to subjects with strabismic amblyopia and observed a clear difference between etiological groups.

This research is innovative because it applied a noninvasive technique (fMRI) to better understand binocular suppression in human subjects with amblyopia. Interocular suppression was measured with a better combined spatial and temporal resolution than had been achieved in the past. In the future, these methods may provide objective feedback information about the course of treatment and lead to improved outcomes for many amblyopic patients.

B. General Methods

Subjects

We studied 17 adult volunteers aged 25 to 43 years (11 female, 6 male). Six were control subjects (CTL), six had previously been diagnosed with anisometropic amblyopia (ANISO), and five had previously been diagnosed with strabismic amblyopia (STRAB). It is important that our experimental strategy allows both individual and group analyses that allow inter-ocular comparisons for each subject, as well as group analysis. Written informed consent was obtained from all subjects (fMRI of Human Visual Perception and Amblyopia, WVU IRB protocol # 14788), in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Any subject with known or suspected neurological conditions was excluded from the study.

Our subject groups were matched for mean age (CTL = 31.0 ± 4.2 , ANISO = 34.5 ± 6.3 , STRAB = 32.6 ± 7.6) and mean years of education (CTL = 15.0 ± 2.9 , ANISO = 15.7 ± 2.5 , STRAB = 15.0 ± 3.3). Most subjects with amblyopia have a history of patch treatment, i.e., a patch was worn to cover the dominant eye (which is called the *fellow eye*), and to force the subject to use the weak eye for daily visual tasks for a portion of each day. Nevertheless, the presence of amblyopia at the time of testing shows that the deficit was never completely reversed (Table 1). Three of the six strabismic subjects also reported surgical correction of their deviation in childhood.

All subjects completed a full ophthalmologic exam at the WVU Eye Institute to confirm their diagnosis. Diagnosis of anisometropic amblyopia was assigned on the basis of:

- 1.) Interocular refractive difference of hyperopia $\geq +1.0$ diopter, astigmatism $\geq +1.0$ diopter, or myopia ≥ -2.5 diopters; or
- 2.) History of anisometropia but no history of strabismus or strabismus surgery.

Diagnosis of strabismus was made on the basis of a history of strabismus or strabismus surgery, but no anisometropia. The direction and magnitude of strabismic deviation in our subjects was determined with cover-uncover, alternate cover, and prism testing. All five of our strabismic subjects showed inward eye deviations (esotropia) rather than outward deviations (exotropia). Congenital esotropia and exotropia are commonly associated with amblyopia, although it has previously been shown that esotropia has the greater prevalence in Caucasian populations (Ing and Pang, 1974). Latent deviations for our subjects ranged from 0-12 prism diopters (Table 1).

All the experiments were conducted in a darkened MRI room using the dual-eyepiece fiber optic Avotec system. This was calibrated to ensure predictable, linear outputs. Monocular contrast sensitivity and dichoptic masking experiments were first administered prior to scanning; fMRI activation task stimuli were then administered using the Avotec system and stimuli tailored to the monocular contrast sensitivity values obtained for each eye.

Table 1. Subject Characteristics

CTL (N=6)	NDE	OS	OD	Age at Diagnosis (years)	Patch Duration (months)	Age at surgery (years)	Current Deviation prism (Diopter)	Stereo- acuity (arc-sec)
LW	Right	20/20	20/25	-	-	-	-	40
CA	Right	16/20	20/20	-	-	-	-	40
KE	Left	20/25	20/20	-	-	-	-	40
DW	Left	16/20	20/20	-	-	-	-	80
LK	Right	20/20	20/20	-	-	-	-	40
AZ	Right	16/20	16/20	-	-	-	-	40
ANISO (N=6)	AE	OS	OD	Age at Diagnosis (years)	Patch Duration (months)	Age at surgery (years)	Current Deviation prism (D)	Stereo- acuity (arc-sec)
JL	Right	20/25	20/50	5	12	-	-	400
LyW	Left	20/63	16/20	8	1	-	-	800
JW	Left	20/32	20/20	5.5	24	-	-	800
AG	Left	20/63	20/20	9	2	-	-	800
RW	Right	16/20	20/32	9	1	-	-	100
KT	Right	20/40	20/160	9	12	-	-	> 3500
STRAB (N=5)	AE	OS	OD	Age at Diagnosis (years)	Patch Duration (months)	Age at surgery (years)	Current Deviation prism (D)	Stereo- acuity (arc-sec)
DS	Left	20/25	16/20	0.5	?	-	6	> 3500
MY	Right	20/25	20/50	10.5	0	-	8	200
CL	Left	20/40	20/20	2	60	2	4	800
KH	Right	16/20	20/32	3	24	0.5,10,18	6	> 3500
BC	Left	20/160	20/20	4	24	6	12	3500

NDE – non-dominant eye; AE – amblyopic eye

OS – left eye; OD – right eye

The NDE in normals is assigned based on which eye has lower Snellen acuity. If Snellen acuity is same in both eyes then grating acuity was taken as the assessment criterion.

Alignment of stimuli

Since our experiments assessed binocular interaction, stimuli must be correctly aligned in each eye. We achieved correct alignment of stimuli in the two eyes with two complementary methods: Nonius cues and an alternate-cover-test.

Nonius cues rely on a subject's perceptual report. In order to align the stimuli and fixation points in the two eyes we relied on the fact that eye piece positions can be controlled independently by Avotec hardware. In addition, we can adjust the location of stimuli with our presentation software for each eye. During the alignment procedure, the subjects viewed nonius cues comprised of separate pattern elements shown to each eye that form a simple shape (square) when fused. We used multiple cues located at the center of gaze as well as in the periphery. Green shapes were presented to left eye and red shapes are presented to the right eye (Fig. 11). As expected, some amblyopic subjects that possess constant suppression of the fovea were never able to achieve fusion of the central cue. We took a stepwise approach to align the stimulus. We began by adjusting the eye pieces under the guidance of eye tracking cameras, in order to bring each eye approximately in the center of the screen. Subjects' perception of monocular and binocular alignment of the nonius cue was determined, and based on these perceptual reports fine adjustment of the stimulus was performed with software itself. This procedure was repeated until the stimuli were aligned as closely as possible (until the two shapes form squares in the subject perception).

The second part of the alignment process involved alternate cover testing. The alternate-cover-test relies on the motor reflex that causes subjects to foveate sudden-onset, monocular stimuli. As illustrated in Figure 12, the principle of this test is that when one eye is covered, it assumes a resting position as there is no visual input to guide fixation. When the cover is removed the eye moves from the resting position in order to take on fixation. In our setting, the experimenter started by switching-off one of the screens (screen turned black). This process was alternated for both the eyes. In strabismic and anisometric subjects heterotropia or heterophoria is sometimes present depending on the severity. The deviation (if present) was noted in the eye-tracking monitors. Based on the degree and direction of deviation the stimulus in the weak eye was moved via

software control. This process is analogous to using prisms as part of the clinical cover test. The best attempt was made to reach the ‘end point’ where no movement occurs in the eye. This end point is the point where stimulus location effectively compensates for the eye deviation.

Figure 11: Nonius cues. Ideal alignment is achieved when the two shapes form squares in the subject perception.

Dual Eye Tracking

Figure 12: Alternate cover test

The resulting stimulus locations were noted and compared to those derived from the perceptual reports described previously. In most cases the magnitude of movement of stimuli was identical to that of previous value and in all subjects it was similar. This implies that alignment by cover test and by visual perception is the same. Thus, at this particular value of alignment the stimulus is presented at the fovea.

All psychophysical tests were administered with optical correction. All tests included practice trials to ensure that the stimuli were visible and the task instructions were adequately understood. Figure 13 illustrates the dichoptic arrangement in our experiment.

Figure 13: Schematic illustration of experimental set-up.

Psychophysical Testing

Monocular contrast sensitivity

The stimuli were generated using the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997) and MATLAB 5 for Macintosh OS 9 on a PowerMac G4 computer with dual SVGA display drivers (output resolution = 832 x 624 pixels, 30 deg horizontal x 23 deg vertical).

Each eye was tested separately in the dichoptic setup. Monocular functions were assessed as the non-tested eye viewed mean level gray screen. Each trial lasted 2.7 seconds. The preparation time, showtime and accept time were 400, 300 and 2000 msec respectively. A cross was used as a fixation point. We used a sinusoidal pattern at 2 c.p.d, either horizontal or vertical orientation. We expected control subjects have near peak response at 2 c.p.d, while the strabismics and anisometropes might show impaired performance. For each trial, the subject viewed two temporally sequenced epochs identified by audible tone, containing either a horizontal sinusoidal grating (subtending 8 deg of visual angle) or an isoluminant gray screen. The subject was required to identify the epoch that included the grating; even if the subject is not certain and needed to guess (the two-alternative forced choice paradigm, 2-AFC). An interactive staircase procedure (2-up, 1-down) was used to approach the contrast detection threshold (the 71% correct level), terminating after seven reversals. The first three reversals used a step size of 6 dB to rapidly approach threshold, while the last four reversals used a 2 dB step size. The contrast threshold was taken as the geometric mean of the last four reversals. The threshold data thus obtained was used to compute supra-threshold stimuli for the other experiments. Specifically, we calculated the contrast corresponding to 0.5 LU and 1.0 LU above the detection threshold obtained for each orientation and for each eye.

Dichoptic masking

Dichoptic masking was measured separately for two different masking stimuli, either a vertical or horizontal 2 c.p.d. grating at 1.0 LU above threshold. Amblyopic eye and fellow eye were both tested. This experiment utilized a 2-alternative temporal forced-choice contrast matching paradigm. For each trial, the eye being tested viewed one temporally sequenced vertical or horizontal sinusoidal grating stimulus. The non-testing eye viewed a masking grating in both epochs. If we take the “Test OS” conditions as an example, the contrast values of the masking gratings are set to 1.0 LU above OD’s monocular threshold (Fig. 14). The test grating was shown to OS in one of the two epochs, randomly chosen and a staircase procedure found the test grating contrast that was detectable in the presence of and the mask. For each trial the subject made a

perceptual decision as to which grating stimulus possesses the most contrast (“which has more contrast”). A starting contrast of 40% was used. The preparation time, show time and inter-trial interval were 400, 300 and 2000 msec respectively. A central cross was used as a fixation point. An interactive staircase procedure (2-up, 1-down) was used to approach a value (percent contrast), terminating after seven reversals. This entire test was then repeated for the “Test OD” condition that reverses the above description of the two eyes.

Figure 14. Schematic stimuli for dichoptic masking. OS represents left eye; OD represents right eye.

Functional Magnetic Resonance Imaging

Visual Stimuli

During the MR imaging experiments, the visual stimuli were generated using the same optics used for the previous psychophysics. fMRI experiments were conducted dichoptically, explicitly stimulating both eyes to examine binocular integrations (Fig. 15). One eye viewed a dynamic grating pattern (pattern onset/offset at 4 Hz) with spatial frequency of 2 c.p.d and contrast set at 1 LU above the threshold of that eye. The other eye was shown one of 4 different masking stimuli, i.e., black homogeneous screen, gray homogeneous screen, static 2 c.p.d grating with contrast of 0.5 LU above that eye’s threshold, or static 2 c.p.d grating with contrast of 1.0 LU. Note either vertical or horizontal stimuli are presented to both eyes.

Figure 15: Paradigm schematic for visual stimuli in fMRI experiments.

Attention to the stimulus was assessed during fMRI experiments using interactive stimuli (e.g., decisions based on the state of the fixation mark). A central fixation arrow was present at all times for all stimuli. Subjects were clearly instructed to maintain fixation on this arrow at all times throughout the functional scanning. Subjects were also instructed to perform a task monitoring the appearance of the fixation arrow to aid fixation stability. The fixation target was a small arrowhead (0.8 deg) pointing in one of four directions (i.e., up, down, left, or right) which randomly changed direction with an inter-trial interval of approximately 6 s during the course of an fMRI scan. Subjects used a fiber-optic button pad with four buttons configured in a cross shape to report arrow orientation. The subjects pressed one of four keys to match the orientation of the arrow.

Accuracy of fixation was measured through an eye movement tracking system (Sensomotorics, Inc.), which works because there is an infrared camera house in the Avotec stimulus delivery system. Eye movements were monitored using the Sensomotorics iView system in order to ensure fixation stability during the functional scans. The iView system was used to measure gaze position in the stimulated eye, and was calibrated using a nine-point display at a screen resolution of 832 x 624 pixels, subtending approximately 30 deg horizontal x 23 deg vertical of visual angle. Accurate

calibration was not achieved in a minority of subjects (see Results), but most subjects were able to have eye tracking throughout both experiments. The sampling rate of the iView camera was 60 Hz.

fMRI scanning procedure

All experiments require the subjects to lie supine in an MR scanner while fixating the center of visual stimuli. MR imaging is exquisitely sensitive to head motion. Head movement (within and between scans) was minimized by the use of foam packing. We monitored either the non-dominant eye or dominant eye for movements to ensure adequate fixation during the functional scans. Our use of the button-box tasks also helps to minimize translational eye position drifts.

All subjects have previously undergone anatomical MRI scanning in an earlier session. The purpose of these anatomical scans is to allow us to create computer-based reconstructions of individual subjects' cortical surfaces. Whole brain high resolution SPGR scans are collected using a standard quadrature head coil, and the entire cortical surface is reconstructed as an unfolded and flattened map of the cortex (Dale and Sereno, 1993). This is accomplished using a semi-automated software suite (FreeSurfer) which segments gray from white matter and the pia surface from the surrounding cerebrospinal fluid (Fischl et al., 1999; Dale et al., 1999; Fischl et al., 2001). Within-plane resolution for the axial SPGR scans was 0.9375 mm in the phase and frequency-encoded directions with a slice thickness of 1.2 mm. Subsequent to the flattening procedure, all experimental data can be displayed either on the individual subject's inflated cortical surface or on flattened "patches" of the occipital pole. This allows individual cortical organization to be more intuitively visualized.

fMRI was performed in a 3.0 Tesla General Electric MR scanner using spiral-out imaging techniques. After a sagittal localizing scan was obtained, a T1-weighted inversion recovery sequence (TR = 400 ms) was used to acquire 20 interleaved 4-mm slices with 0.86×0.86 mm in-plane resolution, oriented perpendicular to the calcarine sulcus (Glover and Lai, 1998). These anatomical scans were later used to register the functional scans to the FSPGR slices that defined the cortical surface. The next step was

to acquire multiple functional scans using the same slice prescription selected in the anatomical scans, but with 3.44×3.44 mm in-plane resolution. Functional images were acquired every 2 seconds (s) in each slice using a quadrature receive-only surface coil. The surface coil is designed to maximize the signal-to-noise ratio (SNR) from the occipital pole of the subject's brain. Functional signals reflecting neural activity via local oxygen consumption and blood flow were acquired (Kwong et al., 1992; Ogawa et al., 1992) using a spiral-out gradient echo sequence (TE = 40 ms, TR = 4000 ms, flip angle = 65 deg, FOV = 22 cm, matrix 64×64 (Glover, 1999). For all the subjects, each functional scan had a duration of 4 min and 56 s, and 144 time points were collected from each slice in all scans. Eight scans of this type were administered in one session, four scans for vertical stimuli and four scans for horizontal stimuli. The entire scanning procedure typically lasted about 1 hr.

The stimuli were displayed in the scanner using the Avotec SilentVision dichoptic eyepieces. Subjects viewed the images with both eyes open by looking straight ahead into the eyepieces, which were placed approximately 1 cm in front of their eyes. Subjects used the eyepieces' built-in optical correction. For each functional scanning run, one eyepiece displayed a dynamic sinusoidal grating and fixation target to the tested eye while the other displayed fixation target and 4 different masks in a randomized order to the masked eye. Left and right eye testing was alternated during each experiment.

Statistical analysis

fMRI experiments were analyzed as a blocked design using the FS-FAST (MGH) software package for fMRI analysis. All data sets were first linearly detrended and motion-corrected to match the acquisition in the temporal center of the first run. Then intensity normalization of the data was performed. The next step was sorting data according to different categories between which we need to make comparisons, such as sorting data according to left eye-tested and right eye-tested or according to stimulus orientation (Horizontal or Vertical), or combining all 8 scans for each subject together as one set of data in order to obtain greater signal compared to either left eye-tested or right eye-tested analysis in control group. So for each subject, we had 5 analyses (Mixed-averaging all 8 scans, L-left eye-tested, R-right eye-tested, H-horizontal, V-vertical). The

next step is selective averaging in which hemodynamic response functions during each block were compared.

We had five binocular conditions in each scan, 0 = fixation point on gray screen to both eyes, 1= dynamic grating + black mask, 2 = dynamic grating + gray mask, 3 = dynamic grating + 0.5 LU grating, 4 = dynamic grating + 1.0 LU grating.

The comparisons we made between these conditions are as follows: The first comparison we made was between the 4 different masks and baseline (1-0, 2-0, 3-0, 4-0). The second comparison we made was between three masks and the black mask (2-1, 3-1, 4-1). We called the results of these comparisons the *luminance* masking effect, because the main difference between the subtracting conditions is the luminance of the mask. Next, we made a comparison between the two grating masks and gray mask (3-2, 4-2), we called this the *pattern* effect, since the main difference between the subtracting conditions is the presence of a grating. The last comparison we made is between the two grating masks (4-3). We called this the *contrast* effect when 0.5 LU grating is directly subtracted by 1.0 LU grating because the main difference between the subtracting conditions is the difference in contrast.

After these comparisons, statistical significance was measured via an F-test ($p = 0.05$ to $p = 0.00001$). Maps of statistical significance were painted onto the inflated cortical surface representation for overall visualization, which specifically test how visual areas were differentially affected as a function of stimulus condition and which eye (amblyopic or normal) is viewing the stimulus.

C. Results

Psychophysical results

Monocular contrast sensitivity

First we compared the data between groups. Contrast sensitivity in the amblyopic eyes of both groups was depressed compared with control eyes at 2 c.p.d., consistent with the commonly reported higher spatial frequency deficit in amblyopia (Hess and Howell,

1977; Bradley and Freeman, 1981; McKee et al., 2003). When comparing anisometropes AE with control subjects' NDE using t-test, we observed worse performance from anisometropes AEs, and this was significant for vertical ($p = 0.013$). Although there was also increased contrast threshold for horizontal gratings, the larger variability within the group's performance resulted in a statistical trend towards significance ($p = 0.053$). In contrast, when we compared the performance of strabismic AE with controls' NDE, we did not obtain any statistically significant difference ($p = 0.467$ for horizontal stimuli; $p = 0.306$ for vertical stimuli). Higher variability between subjects for the strabismic eyes made the comparison with NDEs of control subjects less significant.

We also made interocular comparisons within each group using t-tests. For control subjects, when comparing NDE versus DE performance, there were no observed difference ($p = 0.737$ for horizontal stimuli; $p = 0.889$ for vertical stimuli) (Fig. 16). For anisometropic group, we obtained a significant difference between AE versus FE for horizontal stimuli ($p = 0.021$) and for vertical stimuli ($p = 0.026$). Anisometropic eyes performed less accurately. For strabismic subjects, results showed that contrast threshold for AE do not differ significantly from that for FE ($p = 0.441$ for horizontal stimuli; $p = 0.3$ for vertical stimuli), although a clear trend for reduced sensitivity in AE was observed.

Figure 16: Log contrast threshold. Contrast sensitivity in the amblyopic eyes of both groups was depressed compared with control eyes. Error bar represents standard deviation of the mean, which tells the extent of variability between subjects.

Dichoptic masking

We tested binocular contrast integration, with a dichoptic masking paradigm that measures the effect on the tested eye when a mask was presented to the other eye (1.0 LU supra-threshold of that eye's contrast threshold). Both eyes were tested in sequence for each subject.

We first compared the masking effect of FEs on AEs for amblyopic subjects to that of DEs on NDEs for control subjects. As shown in Figure 17, when comparing the AE of anisometropic subjects to NDE of control subjects, there was a stronger masking effect on AEs compared to the masking effects on NDEs from DEs, and it is statistically significant when vertical grating masks were used ($p = 0.004$). The effect was not significant for horizontal grating mask ($p = 0.193$) although there was a similar trend. For strabismic subjects, we observed similar results. A significantly stronger masking

effect on AEs compared to the masking effects on NDEs from DEs was observed, and it is statistically significant when vertical grating masks were used ($p = 0.016$), but not for horizontal grating masks ($p = 0.686$).

We next made interocular comparisons within each group using t-tests. In control subjects, no asymmetry in interocular masking effects were observed between NDE and DEs ($p = 0.286$ for horizontal stimuli; $p = 0.166$ for vertical stimuli). However, we observed an abnormal contrast integration pattern in anisometropic and strabismic subjects. There was an asymmetry in the masking effects between AE and FE. Anisometropic eyes had weaker than normal masking effect on FEs, while FEs exerted stronger than normal masking effect on AEs. The asymmetry is significant for horizontal gratings ($p = 0.013$), and there was a clear asymmetry for vertical gratings ($p = 0.06$). For strabismic subjects, there was also an asymmetry observed between AEs and FEs. The AEs exerted weaker masking effects on FEs, while FEs had stronger masking effects on AEs. The asymmetry is statistically significant when vertical grating masks were used ($p = 0.042$), and approached significance when horizontal grating masks were used ($p = 0.055$).

One concern is that for amblyopic subjects with very low sensitivity, a stimulus that is 1.0 LU above threshold could require a contrast greater than 100 %. In other words, we may run out of dynamic range for some subjects. Nevertheless, our preliminary study showed that only with a 1.0 LU mask presented to the masked eye where obvious masking effects obtained, so we included this stimulus condition. Exceeding dynamic range did in fact occur for subjects with severe suppression on the weak eye (four anisometropic and one strabismic subject, and one control subject), so we compensated by using 100 % contrast as the mask if 1.0 LU above threshold of that eye is greater 100 %.

Figure 17: Dichotic masking effect. There was an asymmetry in the masking effects between AE and FE in amblyopic subjects.

fMRI experiment

Fixation Stability

We wanted to determine if there were differences in fixation behavior between the amblyopic and control subjects, as poor fixation could possibly confound the interpretation of any fMRI differences. To achieve this goal, we used the iView eye tracking system to measure ocular movements during each fMRI scan. However, we were not able to collect fixation data from three strabismic subjects and one control subject, primarily due to failures in the eye tracker calibration software. In these cases, we used the fixation task accuracy to assess fixation and attention.

We calculated the standard deviation of horizontal gaze position for each subject to provide a single index of fixation during each scan. Vertical gaze position was also

measured, but was less variable between subjects, consistent with the known pattern of fixational eye movements in amblyopes (Westall and Aslin, 1984; Ciuffreda et al., 1980). Three anisometropic subjects and two strabismic subjects had AE tracked and three anisometropic subjects had FE tracked. For control subjects, two had NDE tracked and three had DE tracked. Mean gaze position variance was calculated from the raw eye position data after filtering for blinks. The means did not significantly differ between amblyopic and control eyes (CTL-NDE-tracked = 3.2 deg, ANISO-AE-tracked = 3.4 deg, STRAB-AE-tracked = 2.9 deg), although more variability existed between subjects in the amblyopic groups than in the control group (Fig. 18). Also, only strabismic groups displayed a trend for less fixation stability than controls. Interestingly, anisometropic subjects had overall better fixation stability when viewing with their amblyopic eyes than control NDEs. Review of fixation stability data confirmed that there were no significant differences between the groups ($p = 0.922$ CTL-NDE versus ANISO-AE; $p = 0.655$ CTL-NDE versus STRAB-AE).

Figure 18: Eye tracking data segregated according to tracked eye being AE versus FE within each group. Results indicate that there were no significant differences in accuracy between the groups. We did not obtain STRAB-FE-tracked data.

We also considered any differences for whether the tracked eye was being tested versus being masked, but results showed there were no significant differences between the two statuses for the tracked eye within each group and no significant differences between groups (Fig. 19) (ANISO-tested versus masked $p = 0.95$; STRAB-tested versus masked $p = 0.905$; CTL- tested versus masked $p = 0.468$; CTL-masked versus ANISO-masked $p = 0.543$; CTL-masked versus STRAB-masked $p = 0.947$).

Figure 19: Eye tracking data segregated according to tracked eye being tested versus being masked within each group. There were no significant differences in accuracy between the two conditions for the tracked eye within each group and no significant differences in accuracy between groups.

Fixation discrimination task

Subjects were instructed to maintain fixation on a central “arrowhead” target. We monitored subject’s attention by recording their button-press each time the arrowhead changed orientation. As Figure 20 shows, there were no statistically significant differences in accuracy between NDE of control subjects versus AE for anisometropic

subjects ($p = 0.104$); and no statistically significant differences in accuracy between DE v FE ($p = 0.256$). Also there were no significant differences in accuracy between DE of control subjects versus FE of strabismic subjects ($p = 0.077$). However, AE of strabismic subjects was less accurate at the discrimination task compared to NDE of control subjects ($p = 0.016$). Nevertheless, all subjects performed at better than 89% accuracy.

Figure 20: Fixation discrimination task.

Visual stimuli

During the fMRI scans, a dynamic grating was presented to the tested eye with one of 4 different masks presented to the other eye in a randomized order. The 4 masks were a black screen, gray screen, 0.5 suprathreshold grating and 1.0 suprathreshold grating. In addition, a baseline condition consisting of fixation point on gray screen for both eyes was used to begin and end each scan. For amblyopia subjects, the dynamic grating was presented to AE while 4 different masks were presented to FE when AE was tested; the dynamic grating was presented to FE while 4 different masks were presented

to the AE when FE was tested. Control subjects were tested analogously with respect to their DE and NDE.

We have plotted the physical contrast of the stimuli shown to each eye of all subject groups (Fig. 21). This represents our strategy to show equal perceived contrast to both eyes. The issue of running out of dynamic range for some subjects was applicable to four anisometropes and one strabismic subject. We used both 0.5 LU grating and 1.0 LU grating as masks in fMRI functional scans. For one anisometropic subject and one strabismic subject, both 0.5 LU above threshold and 1.0 LU above threshold of the weak eye were greater than 100%, we compensated by choosing 60% /100% or 80%/ 100% as masks according to the range of the values we obtained by calculating exact 0.5 LU and 1.0 LU above threshold. For three anisometropic subjects, 0.5 LU above threshold of the weak eye was under 100% and only 1.0 LU was greater than 100%, so we used exact value of 0.5 LU above threshold of that eye as one mask and 100% grating as a second mask when we tested FE.

Figure 21: Physical contrast of the stimuli presented to the masked eye.

fMRI data

Control subjects

We first consider the BOLD signal elicited by each binocular masking condition compared to baseline. For control subjects, we began by looking at the data separately for the left eye tested and right eye tested conditions, but no difference was observed between the two eyes in 5/6 subjects (Fig. 22). In one control subject (DW) there was moderately more activation in all mask conditions versus baseline for the right eye (Dominant Eye-DE) than for the left eye (NDE).

In all six control subjects, we observed that there was greater BOLD signal in the black mask condition compared to the three other mask conditions. When the black mask condition is directly subtracted from the three other mask conditions, a reduction in BOLD signal is present on the flattened brain map. We called this the luminance masking effect, i.e., BOLD signal is reduced when luminance of mask is increased. The difference between black and gray mask conditions is plotted for all control subjects (Fig. 23). In 5 out of 6 control subjects, the luminance effect is more apparent for the gray mask condition compared to the two grating mask conditions. In one control subject (CA) it was 1.0 LU supra-threshold grating mask condition that showed largest luminance effect. Also in 5 out of 6 control subjects, the gray mask condition was equivalent to 0.5 LU supra-threshold grating mask condition, only in one subject (DW) the luminance effect is greater in gray mask condition than in 0.5 LU supra-threshold grating mask condition. Most importantly, there is no difference between the luminance effect in gray mask condition from NDE-tested versus DE-tested data.

Figure 22a: NDE-tested versus DE-tested base conditions for one representative control subject. Subjects' initial followed by a letter indicating which eye was tested is in left upper corner of the image. Each of the four major columns represents each different mask condition versus baseline. Baseblack – black mask versus baseline, basegray – gray mask versus baseline, base05 – 0.5 LU grating mask versus baseline, base10 – 1.0 LU grating mask versus baseline. Lh - left hemisphere, rh - right hemisphere. Note there is no difference in BOLD signal between the DE-tested and NDE-tested conditions. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Figure 22b: NDE-tested versus DE-tested base conditions for one representative control subject. Note there is no difference in BOLD signal between the DE-tested and NDE-tested conditions. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

As already indicated above, when the two grating mask conditions were directly compared to the gray mask condition, no reliable differences were visible in any of the six subjects. Finally, when the 1.0 LU supra-threshold grating mask condition was directly compared to 0.5 LU supra-threshold grating mask condition, no differences were seen in any subject. Thus we observed no pattern or contrast effect, as defined in the methods section.

We also looked at the data separately for the horizontal versus vertical grating conditions. The data from each orientation condition is the result of averaging 2 scans from NDE-tested condition and 2 scans from DE-tested condition. Five out of Six control subjects showed greater activation for all four mask conditions versus baseline when horizontal gratings were presented during scans. One exception (CA) showed greater activation for the 4 different masks versus baseline when vertical gratings were presented during scans.

Figure 23. Luminance effect in gray mask condition for all 6 control subjects. Lumgray represents gray mask being subtracted by black mask. Note there is no difference between the DE-tested and NDE-tested conditions. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Anisometropia subjects

The results from the anisometropic subjects differed from those obtained from control subjects in that there was a clear difference between the results obtained when the AE was tested versus FE-tested conditions. Overall, all 6 subjects showed greater BOLD signal for the 4 different masks versus baseline in AE-tested than in FE-tested conditions (Fig. 24). In AE-tested conditions the black mask condition versus baseline elicited the greatest amount of signal compared to the three other mask conditions. This applies to every anisometropic subject, and is similar to the pattern obtained from both the DE and NDE of control subjects.

Figure 24a: AE- tested versus FE-tested base conditions for one representative ANISO subject. Note ANISO AE-tested base conditions had greater BOLD signal compared to ANISO FE-tested base conditions. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Figure 24b: AE- tested versus FE-tested base conditions for one of two representative ANISO subject. Note ANISO AE-tested base conditions had greater BOLD signal compared to ANISO FE-tested base conditions. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Interestingly, in 5/6 anisometropia subjects, the luminance effect observed in the AE-tested condition was clearly more pronounced than that in control subjects. There was one exception (RW) who did not show a luminance effect in AE condition (Fig. 25), which might be explained by the fact that RW is a mild anisometropes with good stereo-acuity (Table 1). In the five anisometropia subjects with luminance effect observed from AE, the luminance effect was approximately equal strength regardless of mask type compared to the black mask. The results obtained from the anisotropic subjects when FE was tested were quite different. There was no luminance effect observed.

We also looked the data separately for the horizontal versus vertical grating condition. The data from each orientation condition is the result of averaging 2 scans from AE-tested condition and 2 scans from FE-tested condition. Five out of six anisometropia subjects showed greater activation for the 4 different masks versus baseline conditions when horizontal gratings were presented during scans, which is consistent to the data from control subjects.

Similar to the results obtained from control subjects, when the two grating mask conditions were directly compared to the gray mask condition, no reliable differences were visible in anisometropic subjects. Finally, when the 1.0 LU supra-threshold grating mask condition was directly compared to 0.5 LU supra-threshold grating mask condition, no differences were seen in any subject.

Figure 25: Luminance effect in gray mask condition for all 6 ANISO subjects. Note there is a significant difference between the AE-tested and FE-tested conditions. Greater luminance effects were seen in AE-tested gray mask conditions in 5 out of 6 ANISO subjects. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Strabismic subjects

For strabismic subjects, the data obtained were much more variable than that obtained from control or anisometropic subjects (Fig. 26; Fig. 27). We were able to observe a clear difference between AE and FE in only 2/5 strabismic subjects (MY and KH). For these two subjects, we again observed greater BOLD signal for the 4 different masks versus baseline in AE-tested than in FE-tested conditions. Also, for both AE and FE, black mask elicited greatest amount of signal compared to the three other masks. However, unlike the anisometropic subjects, the luminance effects was greater for FE compared to the data from AE, also greatest in the gray mask condition. There was greater BOLD signal for the 4 different masks versus baseline from vertical stimuli data compared to horizontal data, so the luminance effect was greater for vertical gratings than for horizontal gratings.

For the other 3 strabismic subjects (DS, CH, BC), there were differences in BOLD signal between AE-tested versus FE-tested conditions, but it did not always deviate toward one direction. For BC, there were greater BOLD signal for the 4 different masks versus baseline from AE-tested data than from FE-tested data. But for DS, greater BOLD signal were seen from FE-tested data. For CH, equivalent signal was seen for AE-tested versus FE-tested conditions. For these three subjects, there was approximately an equivalent amount of BOLD signal regardless of mask type in both FE-tested and in AE-tested conditions. No luminance effects were observed in FE-tested condition (Fig. 28), while from AE-tested data, we observed a subtle luminance effect. Similar to the other 2 strabismic subjects, there was greater BOLD signal for the 4 different masks versus baseline from vertical stimuli data compared to horizontal data.

When the two grating mask conditions were directly compared to the gray mask condition, no reliable differences were visible in strabismic subjects. Only in BC we observed a subtle effect in AE data when the two grating mask conditions were directly compared to the gray mask condition. Finally, when the 1.0 LU supra-threshold grating mask condition was directly compared to 0.5 LU supra-threshold grating mask condition, no differences were seen in any subject.

Figure 26a: AE-tested base conditions for 5 STRAB subjects. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Figure 26b: AE-tested base conditions for 5 STRAB subjects. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Overall, we obtained consistent results in anisometropia subjects showing that there was a significant asymmetry in luminance effect in gray mask condition between AE being tested versus FE being tested. In order to describe the anatomical locations of the changes in BOLD signal, we made a comparison between one representative control

subject NDE condition versus one representative anisometropia subject AE condition (Fig. 29). The locations and the amount of activation in black mask versus baseline, and the locations and the extent of the luminance effect can be seen on an inflated brain model. For the control subject, activation is widely spread over the occipital lobe, from lateral, medial and ventral views. Intra-parietal sulcus, posterior superior temporal sulcus also had subtle BOLD signal. Activation can also be seen in lateral temporal lobe. The control subject showed luminance effect in visual areas along the calcarine sulcus and in medial occipital lobe. In contrast, the anisometropia subject had much greater amount of activation, and it distributed over occipital lobe, extending toward parietal lobe. Activation is also seen in intra-parietal sulcus, posterior superior temporal sulcus, ventral temporal lobe. The magnitude of the luminance effect is clearly larger than for the control subject. The anatomical locations of luminance effect for the anisometropia subject are roughly the same as where we can see activation for black mask versus baseline. It indicates that the luminance effect we observed in gray mask condition for anisometropic subjects is the result of greater extent of activation that black mask can elicit.

STRAB-FE-tested

Figure 27a: FE-tested base conditions for 5 STRAB subjects. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

STRAB-FE-tested

Figure 27b: FE-tested base conditions for 5 STRAB subjects. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

Figure 28: Luminance effect in gray mask condition for 5 STRAB subjects. Note differences in luminance effect were observed only in 2/5 STRAB subjects between the AE-tested and FE-tested conditions. Greater luminance effects were seen in FE-tested gray mask conditions. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

One control subject

Figure 29a: Anatomical locations of activation for one representative control subject, with NDE-tested condition in black mask versus baseline above the luminance effect in NDE-tested gray mask condition. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

One ANISO subject

Figure 29b: Anatomical locations of activation for one representative anisotropic subject, with AE-tested condition in black mask versus baseline above the luminance effect in AE-tested gray mask condition. Color scale at bottom right of each image indicates a positive difference in signal by going from $p = 0.00001$ (yellow) to $p = 0.05$ (gray), and a negative difference in signal by going from $p = 0.00001$ (cyan) to $p = 0.05$ (gray).

D. Discussion

We obtained interesting findings from both the psychophysical results and fMRI results, although some of the results do not exactly match our expectations. First, we observed depressed contrast sensitivity for AEs for anisometropia subjects, while most strabismic subjects have preserved contrast sensitivity. Second, there was an asymmetry between the two eyes for amblyopic subjects in dichoptic masking, with AEs being under more suppression from FEs. We expected to see smaller interocular suppression with horizontal grating stimuli compared to the effect with vertical grating stimuli, but no such effect was observed. Last, as we expected, the fMRI experiment revealed a pronounced asymmetry between the luminance masking effect when the amblyopic eye was masked versus when the fellow eye was masked, with stronger effect seen when the fellow eye was masked in anisometropic subjects. But we did not obtain a modulation of response that is dependent on contrast of the gratings presented to the non-tested eye, and we did not see orientation modulation effect in binocular suppression, contrary to our expectations. I will discuss our findings systematically, firstly psychophysical findings followed by fMRI results, and finally the correlation between the two.

Psychophysical findings

Amblyopia is well known as a disorder of spatial vision, which is characterized by decreased visual acuity and compromised contrast sensitivity for amblyopic eyes (Hess, 1990; Ciuffreda, 1991). In our monocular contrast sensitivity testing, the amblyopic eyes of both groups exhibited depressed contrast sensitivity compared with control eyes at 2 c.p.d., consistent with the commonly reported higher spatial frequency deficit in amblyopia (Bodis-Wollner, 1980; Hess and Howell, 1977; Bradley and Freeman, 1981; McKee et al., 2003). The compromised contrast sensitivity we observed in human amblyopic subjects might well be explained by animal literature which reported that both amblyopic subtypes have fewer cortical binocular neurons than normal by comparing single-unit recording from V1 cortex with CSFs in monkeys (Kiorpes et al., 1998), and that the relative losses in contrast sensitivity were in ordinal agreement with the losses in cortical afferents as reflected by the reduction in width of the respective ODCs having

input from amblyopic eyes in V1 of monkeys (Crawford et al., 2004). For our anisometropic group, we obtained a significant difference between AE versus FE as well as impairment compared to control subjects. For strabismic subjects, contrast threshold for AE, while reduced compared to FE or control NDE, was not significantly different, indicating that the acuity of AE are relatively preserved, consistent with the report of a strabismic subject with distorted perception of grating but normal CSF in both eyes (Hess et al., 1978) and other studies that show less severe and consistent decrease in sensitivity for strabismic eyes (Levi and Harwerth, 1977; Sjostrand, 1981; Campos et al., 1984). One possible explanation might be because some strabismic subjects alternately fixate, that is, either eye can be the deviated eye such that the deviated eye is not under constant suppression from the fixating (dominant) eye. The different performance observed in this project between the two groups well reflects the reports from previous studies that although both strabismics and anisometropes exhibit similar contrast sensitivity losses at high spatial frequencies, the anisometropes are more likely to suffer additional deficits at low spatial frequencies as a linear function of depth of amblyopia (Levi and Harwerth, 1977; Sjostrand, 1981; Campos et al., 1984).

The spatial separation of the two eyes leads to differences, or disparities, between the retinal images of binocularly viewed objects that do not lie at the fixation point. Vertical orientation can convey the horizontal disparity—a critical factor for Wheatstone (classic) stereopsis—but horizontal orientation *per se* lacks horizontal disparity information (Yazdanbakhsh et al., 2004). So the eyes are more likely to be exposed to spatial disparity in stereoptic fusion process at vertical orientation. However, when we compared the results obtained with vertical versus horizontal gratings, we did not obtain a significant difference between the two orientations. Anisometropes AEs significantly differ from NDE of control subjects for vertical orientation stimulation but not for horizontal grating, the larger variability within the group's performance have made it just miss statistically significance. This slight difference between gratings with different orientation might seem align with our expectation since vertical orientations engage stereoscopic mechanisms known to be abnormal in our subjects. However, the performance of control subjects for horizontal orientation was slightly worse and noisy

compared to that for vertical stimulation, and this contributing factor was not part of the hypothesis.

In addition to contrast sensitivity findings, we observed an abnormal contrast integration pattern in anisometropic and strabismic subjects with our dichoptic masking paradigm, as we expected. The masking effect of FEs on AEs for both amblyopic groups is significantly stronger than that of DEs on NDEs for control subjects. And there was a significant asymmetry in the masking strength between AEs and FEs in both amblyopia groups compared to the interocular difference in control subjects. The asymmetry is consistent with previous reports that the masking function of the fellow eye shows less than expected threshold elevation while the amblyopic eye shows more than expected threshold elevation in five of the seven subjects (Harrad and Hess, 1992a). This was also seen in one of our previous studies which reported unequal masking in both anisometropic and strabismic subjects (Agrawal et al., 2006). This unequal dichoptic masking can find its anatomical support in animal studies showing that there is a reduction in the number of binocular neurons and a shift in ocular dominance toward the unimpaired eye in striate cortex (Horton & Hocking, 1997; Horton et al., 1997).

The asymmetry between anisometropic eyes and FEs is significant for horizontal gratings, and there was a similar trend for vertical gratings. Anisometropic eyes are also depressed relative to NDE of control subjects, significantly for vertical gratings. Strabismic subjects also showed an asymmetry between AE and FE, significantly for vertical gratings, as well as impairment relative to control NDE in this condition. Again, it seems that vertical gratings caused somewhat greater interocular suppression compared to horizontal grating stimuli for both groups. This was our expectation since vertical orientations could cause more spatial disparity compared to horizontal gratings for the two eyes. But after taking a careful look at the performance of NDEs in control subjects, we are not convinced that this subtle effect is reliable because the data from NDEs of control subjects at horizontal orientation were worse and noisy compared to that at vertical orientation. Direct comparisons between vertical and horizontal conditions here are not significant, so additional data would be needed to draw firm conclusions about the role of orientation in dichoptic masking.

fMRI findings

Since it has been reported that fixation stability can be impaired in amblyopia (Westall and Aslin, 1984), it is a potential confound. However, we did not find significantly impaired fixation stability in our amblyopes according to the eye-tracking data. Furthermore, the fixation discrimination task showed only a mild loss of accuracy for strabismic subjects. Interestingly, our subject with the poorest amblyopic eye acuity and poorest stereo-acuity, BC (Table 1), also had the lowest fixation task accuracy for amblyopic eye viewing. Nevertheless, all subjects performed at better than 89% correct, supporting our claim that fixation and attention were maintained throughout the experiment. These measurements suggest that impaired fixation is not a significant confound in this study. Our fMRI paradigm is not particularly sensitive to eye movements. Moreover, our psychophysical results are comparable to previous studies and eye movements are minimized with the use of briefly presented stimuli.

In all six control subjects, we observed that there was greater signal in the black mask condition compared to the three other mask conditions. The extent of mis-match between the two eyes is greatest in the black mask condition, which might be an important explanation for why the black mask elicits the greatest BOLD signal compared to the other three mask conditions. This is a novel fMRI observation for control subjects. The role of luminance in binocular suppression has been documented in VEP studies in human subjects with normal monocular acuities (Harter et al., 1980; Odom & Harter, 1983). Our interpretation according to “mis-match” is similar to the explanation that Odom and Harter offered for their results. VEP amplitude elicited by monocular patterns flashed to one eye were suppressed by increases in contraocular luminance, which resembles the process of going from black mask to gray mask reducing the extent of “mis-match” in our fMRI experiment, subsequently reducing the signal elicited.

Five out of six control subjects showed greater activation for all four mask conditions versus baseline when horizontal gratings were presented during scans. Consistent with the data from control subjects, 5 out of 6 anisometropia subjects also showed greater activation for the 4 different masks versus baseline conditions at horizontal grating stimulation. The simplest explanation would appear to be that the horizontal stimuli shown to the tested eye was a more effective stimulator and/or less

prone to interocular suppression. However, this horizontal bias in fMRI data does not correlate with psychophysics results in a simple way. Both eyes of the control group performed worse for horizontal stimulation in contrast sensitivity test while NDE did better for vertical stimulation in dichoptic masking with DE relatively well at horizontal stimulation. The issue of stimulus orientation remains provocative but unsettled.

The main and interesting new finding in 6 anisometropic subjects is that there was a clear difference between the results obtained when the AE was tested versus FE-tested conditions. All 6 subjects showed greater BOLD signal for the 4 different masks versus baseline in AE-tested than in FE-tested conditions. In addition to this overall effect, the difference between AE-tested and FE-tested conditions is particularly pronounced for black mask condition. During AE-tested scans, FEs can differentiate the 4 different masks in a very sensitive way such that the higher visual cortices recognized the extent of mis-match between the two eyes fully. According to psychophysical results, AE is under suppression from FE (Sireteanu and Fronius, 1981), and FEs exert stronger masking effect on the AEs (Harrad and Hess, 1992a). During FE-tested scans, AEs were not able to discriminate the 4 different masks as sensitive as FEs could do, so the extent of mis-match between the two eyes could not be recognized in the higher visual cortices. In other words, these subjects appear to have adapted to their condition in such a way that mis-matched stimuli shown to the AE are readily ignored, and do not generate normal signals. The same logic could explain why in AE-tested conditions all 6 subjects showed greater BOLD signal for the 4 different masks versus baseline compared to FE-tested data.

In AE-tested conditions the black mask condition versus baseline elicited the greatest amount of signal compared to the three other mask conditions. Therefore, we observed a pronounced asymmetry between the luminance masking effect when the amblyopic eye was masked versus when the fellow eye was masked, which is also supported by VEP studies that reported the dominant eye exerts a greater suppressive effect on the non-dominant eye with a mask of 20% contrast in their dichoptic masking experiment (Norcia et al., 2000). In another VEP study, results showed that P-VEP amplitudes were greatly reduced under binocular viewing compared to monocular condition (Wright et al., 1986). In that study the result can be related to our “luminance

effect” because the monocular condition could be considered similar to a black mask being presented. In both cases the increased stimulation of the FE caused a reduction in signal evoked from AE. Our study is the first to use fMRI to demonstrate a luminance effect in binocular suppression in amblyopia subjects.

In the case of strabismic amblyopia, we were able to observe a clear difference in the luminance masking effect between AE and FE in only 2/5 subjects. Although there was an interocular asymmetry observed, it is opposite to the data from anisometropic subjects. For the other 3 strabismic subjects, there was an equivalent amount of BOLD signal for the four masks regardless of which eye being tested. One interpretation is that the weak eye was under constant severe suppression, which is supported by an electrophysiological finding from single cell recording reporting that the majority of neurons with weak or no direct input from one eye exhibit profound interocular suppression in primary visual cortex of the strabismic cats (Sengpiel et al., 1994). Therefore, the visual areas had roughly same amount of BOLD signal no matter what mask was presented to the eyes since the extent of mis-match between the two eyes could not be recognized. It is well reflected in our result that the suppressive interactions observed at the neuronal level in the strabismic cats (Sengpiel et al., 1994) resemble pathological suppression in strabismic humans in their relative independence of stimulus parameters (Holopigian et al., 1988). The actual magnitude of BOLD signal that we obtained for all masking conditions did differ widely across subjects and may reflect, in part, a heterogeneous response to our baseline condition.

Finally, we did not obtain any results that demonstrate a role of contrast modification in binocular suppression, contrary to some of our expectations from fMRI results with normal subjects. Nevertheless, this negative result is consistent with previous psychophysical studies of amblyopia, which reported that the depth of suppression was constant, regardless of the alterations made to the contrast, the luminance, and the spatial frequency of the inducing stimulus (Holopigian, 1989), and that the acuity loss when an amblyopic subject goes from monocular to binocular viewing is independent of the inducing stimulus (Freeman and Jolly, 1994). We still need further work in order to draw a firm conclusion about the degree to which interocular suppression depends on the stimulus parameters presented to the other eye.

The lack of contrast modification in binocular suppression in our fMRI experiment might also be related to the physical contrasts we used as the stimuli for fMRI. We compared the values we obtained from dichoptic masking (which means how much contrast the tested eye needs to be able to tell there is a grating on the top of 1.0 LU above threshold masking presented to the masked eye) to the physical contrasts we used as the test stimuli in fMRI (1.0 LU masked were presented to the masked eye in both dichoptic masking and fMRI). It seems that physical contrasts we used as the test stimuli in fMRI are higher than the contrasts the eye needed to detect a grating on top of a 1.0 LU mask for both eyes for each group. The test stimuli set at 1.0 LU above threshold might be at a saturating level of contrast stimulation for the eyes, so the two masking stimuli (0.5 LU and 1.0 LU) did not make any difference in BOLD signal being driven by the tested eye. This might explain why we did not obtain the modifying influence of contrast on binocular suppression. It would be worthwhile to consider similar fMRI experiments with lower contrast stimuli in the future.

We looked into the correlation between our psychophysics results and fMRI findings. As we observed from dichoptic masking there was a significant asymmetry in the masking strength between AE and FE. The psychophysical results indicate that binocular suppression is not equal in strength between AEs and FEs in amblyopia subjects, which is also reflected in our fMRI activation patterns. Since we observed novel patterns of activation in anisometropic subjects, including a pronounced asymmetry between the luminance masking effect when the amblyopic eye was masked versus when the fellow eye was masked. We have examined whether those subjects that showed greatest luminance masking effect also have the greatest extent of psychophysical dichoptic masking, but there is no clear correlation. This is presumably due to the many differences between stimuli and task for psychophysics and fMRI.

In conclusion, we obtained consistent findings from our anisometropic subjects: abnormal patterns of activation in subjects with amblyopia, including a pronounced asymmetry between the luminance masking effect when the amblyopic eye was masked versus when the fellow eye was masked. We conclude that interocular suppression can be studied with fMRI and may be related to the concept of binocular suppression scotomata in subjects with amblyopia.

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