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Bridging the Gap: Understanding Eye Movements and Attentional Mechanisms is Key to Improving Amblyopia Treatment

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Abstract

Bridging the Gap:
Understanding Eye Movements and Attentional Mechanisms is Key to Improving Amblyopia Treatment

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Amblyopia is a developmental visual disorder resulting in sensory, motor and attentional deficits, including delays in both saccadic and manual reaction time. It is unclear whether this delay is due to differences in sensory processing of the stimulus, or the processes required to dis-engage/shift/re-engage attention when moving the eye from fixation to a saccadic target. In the first experiment we compare asymptotic saccadic and manual reaction times between the two eyes, using equivalent stimulus strength to account for differences in sensory processing. In a follow-up study, we modulate RT by removing the fixation dot, which is thought to release spatial attention at the fovea, and reduces reaction time in normal observers. Finally, we discuss the implications for these findings on future amblyopic treatment, specifically dichoptic video game playing. Playing videogames may help engage the attentional network, leading to greater improvements than traditional treatment of patching the non-amblyopic eye. Further, when treatment involves both eyes, fixation stability may be improved during the therapeutic intervention, yielding a better outcome than just playing a video game with a patch over the non-amblyopic eye.

Dedication

This thesis is dedicated to my grandfather, Rocco Gambacorta, who's lifelong passion for learning had a large impact on me at an early age. I'd also like to thank Shmuel Link for his patience and healthy cooking, and my rabbit, waffles, who encouraged me to share treats during writing breaks.

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Contents

Chapter 1

Introduction

1.1 What is amblyopia?

The human visual system has the remarkable challenge of taking correlated input from two independent sources and creating one rich three-dimensional world. Maps of visual space develop through waves of excitation across our retinae in utero, but it is not until we are born and begin interacting with the world around us that the neural pathways carrying these important visual signals become refined.

In 1981 Hubel and Wiesel won the Nobel prize for demonstrating the importance of visual input to the process of cortical development. By disrupting the sensory input in young kittens through an occlusion to one of the two eyes, they produced a physical change in the ocular dominance columns of primary visual cortex (Wiesel and Hubel, 1963). Further work showed that this brain restructuring was irreversible – normal input in adulthood could not undo the abnormalities that developed in response to these early disruptions (Wiesel and Hubel, 1965). They dubbed this stage of heightened neural plasticity the *critical period*.

These findings have had a profound impact on our understanding of this disorder as it naturally occurs in people, called amblyopia. While the form most similar to Hubel and Wiesel's experiments, deprivation amblyopia due to cataracts, is fairly rare, there are two additional forms that are much more common. In anisometropic amblyopia, a difference in the refractive power between the two eyes causes one image to be perceived as blurrier than the other, even when the refractive error has been fully corrected. The brain cannot process detailed information from this eye, but in a significant proportion of cases, stereoscopic processing remains intact. In another form, called strabismic amblyopia, the two eyes do not align properly, resulting in a different image falling onto each of the two foveae. In this case, the brain must determine which image to "see" and which to ignore or suppress. These patients often have little to no stereoacuity. McKee and colleagues (2003) provided further evidence for differences in the pattern of visual loss for these two groups, mainly along the dimensions of acuity and contrast sensitivity. It is possible that there are several other important factors involved in these patterns of loss, and we aim to further disambiguate these two clinical subgroups in this work.

1.2 Clinical significance

Amblyopia is a leading cause of visual impairment in younger individuals, occurring in approximately 2-4% of the general population (Birch, 2013; Drover et al., 2008; Friedman et al., 2009; McKean-Cowdin et al., 2013; Multi-Ethnic Pediatric Eye Disease Study (MEPEDS) Group, 2009; Williams et al., 2008). While Hubel and Weisel's groundbreaking experiments showed that early abnormal visual experience leads to changes of the response characteristics and connectivity of neurons in the primary visual cortex, many of the recently observed deficits, such as those in counting (Sharma et al., 2000) and multiple object tracking (Ho et al., 2006; Secen et al., 2011), cannot be fully explained by low-level processing capabilities, implicating involvement in a wider array of brain areas and networks.

Individuals with amblyopia frequently mention that more concentration or mental effort is required when doing a task with the amblyopic eye, as compared to the fellow eye, even when stimulus features are equally distinguishable in both eyes. There are several potential reasons for why this might be. However, before exploring this in greater depth, it's helpful to break down the processes that are essential to fast and accurate performance on these visual tasks.

1.3 Vision as an active process

When performing a visual task in an experiment, participants often begin a trial by looking at a fixation stimulus. While the act of fixation may seem simple at first glance, there are actually several important motor functions that are at play to maintain accurate fixation. Although the world appears stable to an observer maintaining a steady gaze, his or her eyes are making small quick eye movements called microsaccades, usually defined as less than a degree, roughly once or twice per second. Between these small jumps, the eyes are slowly drifting within the area of foveated interest (Wang et al., 2016). It is thought that these small eye movements help prevent the image from fading on the retina (Riggs and Ratliff, 1952), and may even serve to boost our spatial resolution (Rucci and Desbordes, 2003).

A common way to quantify these small eye movements made while fixating is called a bivariate contour ellipse area (BCEA, Steinman, 1965; Timberlake et al., 2005). The BCEA is a measure of the area for a specified proportion of eye positions in a given distribution and is defined as:

$$\mathrm{BCEA} = \pi\chi^2\sigma_x\sigma_y\sqrt{}(1-\rho^2)$$

where σ_x and σ_y are standard deviation of the horizontal and vertical eye position, ρ is the Pearson's correlation between the horizontal and the vertical eye positions during the trial and $\chi2 = 2.291$ is the chi-square value (2 degree of freedom) corresponding to a probability value of 0.682(i.e.±1SD). Smaller BCEA values indicate better fixation stability.

Recently, Chung et al (2015) measured fixational eye movements in amblyopic patients. They found larger BCEAs in the amblyopic eye, compared to the fellow eye, and both eyes of normal observers. This measure was also significantly greater in the strabismic patients compared to those with anisometropic amblyopia. A majority of the variance in BCEAs (68.57%) could be attributed to the error magnitude of microsaccades, visual acuity, amplitude and frequency of the saccades.

Returning to our theoretical experiment, after initial fixation the visual display is updated and participants may then explicitly or implicitly make one or more quick eye movements, known as saccades, to bring the area of visual space that is of interest to fall on the fovea. This process requires the engagement of the attentional networks to select which point(s) of interest to inspect more carefully. Implicit (known information about a scene) or explicit (an external cue) information is used to guide these processes.

During a trial, a participant may also be asked to make a manual response, by pressing a button on a keyboard that corresponds to detecting or making a decision about a particular stimulus. To meet this end, a decision based on one's perception of the available visual information must be made and a motor signal must be sent to press the correct key.

1.4 Reaction times are longer when viewing with the amblyopic eye

Many patients with amblyopia take longer to initiate a saccade to the abrupt appearance of an unpredictable target with the amblyopic eye (Mackensen, 1958; Ciuffreda, Kenyon & Stark, 1978ab; Niechwiej-Szwedo, Goltz, Chandrakumar, & Wong 2010; Niechwiej-Szwedo, Chandrakumar, Goltz & Wong, 2012). Once started, however, the saccade appears otherwise normal (Ciuffreda et al., 1978ab; Ciuffreda, Levi & Selenow, 1991; Niechwiej-Szwedo, Goltz, Chandrakumar, Hirji & Wong, 2010; Niechwiej-Szwedo et al, 2012). Another intriguing finding is that when the appearance of the target is predictable, as in the case of square wave targets, this latency difference between the two eyes disappears (Schor, 1975).

There is some evidence that this delay may reflect a higher-level cortical disruption. For example, patients with amblyopia are also slower at responding to targets when asked to make a manual response (Levi et al., 1979, Hamasaki and Flynn, 1981). Broadly speaking, there are many things that could affect reaction time. Previous studies have found that the magnitude of delay correlates with the reduction in visual acuity in amblyopic patients (Hamasaki and Flynn, 1981; McKee et al, 2016). Perhaps then, reaction time is delayed because amblyopic subjects simply can't see the stimulus as well in their amblyopic eye. From the reaction time literature, we know that stimulus strength can have a large effect on reaction time.

1.5 Piéron's law

Studies using a variety of sensory modalities have shown that we respond to stimuli in a mathematically predictable manner. For a given scenario, we will respond slowly to stimuli that are at the threshold of our detection. From there, as the intensity of the stimulus increases, our reaction time decreases following a negative power function, until it reaches an asymptote.

This law, known as Piéron's law (Piéron, 1914; 1952), can be written as follows:

$$RT = \alpha C - \beta + \gamma$$

where α and β describe the shape of the slop and γ sets asymptotic floor. The shape of this function can help us understand where and how reaction times differ between subjects, or in the case of patients with amblyopia, differences in reaction time between the two eyes. So for example, if two functions have the same overall shape, but one is displaced laterally, that tells us that the effective stimulus strength is different for the two individuals or for the two eyes in the case of an amblyopic patient. Alternatively, one function may be shifted vertically with respect to the other, i.e. has a higher or lower asymptote. This indicates that the minimum reaction for one individual (or eye) is intrinsically slower or faster than the other.

1.6 Aim 1 Overview

These known reaction time behaviors point to the need for a systematic study of amblyopic reaction time to exclude the possibility that the delay is purely due to a difference in visibility. To determine whether the temporal delay in amblyopia is due to lower level and/or higher level disruptions in visual processing, we will measure saccadic latency and manual reaction time at several contrasts levels that have the same effective stimulus strength, due to the fact that they are normalized by the threshold for that eye.

Using these results, we can fit a function which is a variant of Piéron's original equation, to the data, and compare reaction time between the two eyes in amblyopia without the confound of different sensory strengths (Burr et al., 1998).

The Burr function:

$$R = \alpha /(\log(\chi/\tau)) + R\infty$$

describes reaction time as a function of contrast threshold (τ), and is dependent upon two factors: α determining the slope, and R∞ determining the asymptote. It has several important advantages over the Piéron function that will be described in greater detail in the next chapter.

We know that both saccadic latency and manual reaction times in normal observers depend on contrast, and that both of these measures are delayed in the amblyopic eye. The main goal of Aim 1, is to systematically compare the reaction time differences at different contrast threshold units (CTUs) in both response modalities. Relating manual and saccadic response time delays can provide useful information when trying to understand the mechanism(s) responsible for these observed effects.

Our second goal is to compare the subtypes of amblyopia to assess whether they are related to different patterns in the resulting reaction time functions. As strabismic and anisometropic amblyopia are driven by different etiological factors, and visibility is only one of the many variables that can cause an increase in reaction time, we expect that there may be subgroup differences in our results for this study. For example, as anisometropic amblyopes frequently have reduced contrast sensitivity in their amblyopic eye but better oculomotor control than strabismic amblyopes, equating the stimulus to have the same effective contrast could reduce the amount of the delay in the anisometropic AE. Equating the contrast might have a lesser effect in the strabismic group, as increased fixation stability could still cause a delay compared to the non-amblyopic eye, even when the target is highly visible.

In sum, the findings from Aim 1 will allow us to assess how much of the between eye reaction time differences can be accounted for purely by equalizing the stimulus strength in the two eyes, and to what extent other factors play a role. Isolating the role of stimulus strength will allow us to assess if there is more than one main cause of reaction time delay in amblyopia and if this differs between subgroups.

1.7 Fixation and foveal attention may be disrupted

Why are amblyopic patients slower at responding to targets in the periphery, a part of the visual field that should be relatively normal in patients with amblyopia? As mentioned before, fixation stability is poorer when viewing with the amblyopic eye and in particular, microsaccades account for a large percentage of this increase (Chung et al., 2015). Mechanistically, we know that microsaccades are very similar in behavior to larger saccades (Otero-Millan et al., 2008). From a purely statistical point of view, if saccadic motion is greater it is more likely that the amblyopic eye is in a refractory state, it could lead to the increased saccadic reaction time observed in the first experiment. Studies have shown that microsaccades at fixation can slow reaction time to peripheral targets (Rolfs et al., 2006; Hafed & Krauzlis, 2010; Sinn & Engbert, 2011) and that the frequency of fixational saccades decreases as observers prepare to make a saccadic response (Watanabe et al., 2013).

The refractory period hypothesis does not account for the previously observed increase in manual reaction time, however. So, perhaps there is another component of the increased microsaccadic rate that is driving this multi-modal response delay. One potential explanation is a weaker ability to focus attention when fixating with the amblyopic eye.

A recent study by Yuval-Greenberg et al. (2014) has provided evidence that microsaccades are proceeded by small shifts in attention. In the study by Yuval-Greenberg et al. (2014), eye movements were monitored continuously and visual stimuli were triggered by spontaneous microsaccades. The stimulus, a ring of eight tilted gabors, was randomly presented at 5.5 to 6.5 degrees in the periphery. After presentation of the gabor ring, participants were instructed to make a discrimination regarding the orientation of the gabor that was in the same direction (congruent) or opposite direction (incongruent) of the direction of the microsaccade. Accuracy on the task was statistically higher for the congruent condition, providing evidence that small attention shifts related to spontaneous microsaccades can actually boost performance in a measurable way. If small eye movements at fixation can influence the response to peripheral targets, it becomes necessary to investigate what is going on at fixation, and to specifically modulate this component of the reaction time paradigm to understand its impact on reaction time.

1.8 Introducing the gap

One classical way of measuring the effect of fixation stability on reaction time is with the gap effect, first described by Salsow in 1967. Removing the fixation target just before the appearance of a peripheral target reduces one’s response time to the target, with the largest effect of about 50-60ms occurring with a 200ms gap. The mechanism(s) responsible for this robust reduction in latency are still unknown but several theories are often proposed.

It has been shown that a general warning effect, by changing the appearance of the stimulus target (Jin and Reeves, 2009) or even adding auditory cues (Machado and Rafal, 2000) signaling that a target will soon appear can account for a small percentage of the reaction time decrease, however, this cannot account for the entire effect.

Other components may include a fixational offset mechanism, in which the removal of visual input to the oculomotor fixation region prior to target onset facilitates an oculomotor release from the active fixation process occurs in the superior colliculus (Munoz & Wurtz, 1992). Alternatively, the gap effect could be related to higher-level processing mechanisms, such as attentional disengagement at the fovea, which could facilitate shifting attention to the periphery.

Given what we know about fixational eye movements, it's quite possible that they are also implicated in the gap effect, and Saslow even hypothesized this in his original paper describing the phenomenon. His predictions are listed below:

1. *The refractory period due to microsaccades should lead to longer saccadic latencies on trials with microsaccades than on those without.*

2. *More microsaccades should occur on overlap than on gap trials.*

3. *If only trials without microsaccades are considered, the gap effect should be reduced or eliminated.*

Despite the hundreds of papers investigating the mechanisms of the gap effect since 1967, and the dozens that refer to the positive relationship between microsaccades and latency in their conclusions, only two have explicitly measured these eye movements in relationship to the gap effect, and they reached opposing conclusions.

Kingstone et al., 1995 first studied this, with four subjects and a Purkinje eye tracker. They chose 3 conditions, overlap (no fixation offset), no-gap (fixation was extinguished at the same time the peripheral target appeared), and gap (fixation was extinguished 200ms before target onset), and measured the percentage of trials with microsaccades during a critical window, defined as the 200ms prior to target onset.

One major drawback of this study was that of 1,090 trials including data from all subjects, only 40 microsaccades occurred within the critical window. This 200ms time period is very small, given the frequency of microsaccades of approximately 1-2 times per second (Martinez-Conde et al., 2009). And the authors themselves speculate that "*any effect of the fixation point on the frequency of microsaccades may simply not have had time to manifest itself*."

Further, of the four subjects, only one subject greatly contributed to this count, yet all reported findings were an average of all subjects, by comparing the mean reaction time in the three conditions with and without the 40 trials including microscaccades. They use this null finding (no significant difference in reaction time for any of the three conditions), to conclude that microsaccades do not impact the gap effect. When they do look at the one individual with the largest number of microsaccades, they do find that he has a larger percentage of these eye movements on overlap vs. gap trials, and that the mean saccadic latency was reduced with comparing trials with and without microsaccades (233ms vs 224ms for overlap, and 206ms vs 188ms in the gap condition). Despite these very inconclusive findings, it was reported for almost two decades that fixational eye movements did not impact the gap effect.

Recently, another group challenged this finding (Watanabe et al, 2014). Noting that there is significant variability in both saccadic reaction time and microsaccades within the normally-sighted population, they enrolled 45 participants into their study, and measured fixation and saccadic reaction time in overlap and gap (200ms) conditions. They report that fixational saccades significantly increased saccadic reaction times by an average of 13.0ms ($p<0.001$), and further, they found a significant interaction of fixational saccades and gap SL ($p<0.01$), where fixational saccades reduced the size of the gap effect by an average of 7.9ms. This interaction effect is smaller than the previously found gap difference with and without microsaccades in the subject reported by Klinestone et al. (1995).

Further exploration revealed that most subjects generated more microsaccades on overlap than gap trials and that the frequency of these saccade decreased during the gap period. In some subjects, however, this decrease was immediately followed by a rebound period, which diminishes the overall effect of microsaccades in the reduced saccadic latency with the gap. The authors propose that the variation in the fixational saccade rebound might be "*explained by different responsiveness to foveal visual events*". Although they do not directly point to amblyopia, a population with impaired foveal perception, they do note the importance of studying the effect of microsaccades on the gap effect "*in a population with abnormal fixational saccade behavior, such as Parkinson's disease and attention deficit hyperactivity disorder*". Indeed, many groups have investigated the gap effect in special populations, including patients with Parkinson's disease, ADHD, autism, and dyslexia but, fixational eye movements have not been included in the analysis of these studies. Further, no groups have looked at how the gap effect might be affected by amblyopia.

One relevant study, however, includes a longitudinal sampling of children, aged 6-18, who had two assessments, approximately 19 months apart (Klein et al, 2011). Saccadic reaction time in the overlap condition decreased with increasing age, from about 385ms in the youngest observers, to 250 in the oldest observers. In the gap condition, the reaction times from the youngest participants were 325ms and decreased until about 225 in the oldest participants. Variability also decreased with increasing age, overall standard deviations went down by about 50ms for the means mentioned above. Interestingly, these effects did not appear to stabilize until 14 years or older. This shows that these mechanisms are still developing well into adolescence, and thus could be affected by abnormal visual development, as occurs in amblyopia. Another interesting finding is that the gap effect is actually larger for the younger observers. The authors speculate that this could be due to the fact that younger children have a harder time actively disengaging attention when there are competing visual stimuli. To the extent that the amblyopic visual system remains in an immature state (Levi & Carkeet, 1992), one might expect a larger gap effect in the amblyopic eye.

Measuring gap effect differences between the eyes in amblyopic patients allows us to account for how changes in the fixation stimulus may be affecting reaction times to targets in the periphery.

1.9 Aim 2 Overview

In Aim 1 we measured saccadic latency to the abrupt appearance of peripheral targets to isolate the role of target visibility on reaction time. In Aim 2, we will measure the saccadic latency to highly visible stimuli while modulating a gap duration between the offset of the fixation spot and the onset of the gabor target. By comparing reaction times with various gap durations we can assess the influence of fixational processes in the delayed response to peripheral targets in the amblyopic eye. We believe the gap effect will be reduced in the amblyopic viewing condition, given that they have less oculomotor control with this eye as measured by larger BCEAs at fixation.

As we know that amblyopes, particularly strabismic amblyopes, have more microsaccades when fixating with the amblyopic eye, the gap could influence reaction time in two ways: (1) If reaction time is greatly reduced in gap conditions that will inform us that a visual stimulus on the fovea is an important part of the delay in responding with this eye. (2) Alternatively, if the gap does not make much of an impact on reaction time when viewing with the amblyopic eye, it provides evidence that there is a smaller capacity for attentional enhancement when viewing with this eye or that there are other factors (e.g. delayed neural transmission) that delay reaction times.

1.10 Improving amblyopic treatment

Taking a step back, furthering our understanding of the neural mechanisms affected by amblyopia is of minimal use unless it can make way for better amblyopic treatment. Current amblyopic treatment involves patching the non-amblyopic eye. Not only is this therapy outdated (it's been the main course of treatment for about 300 years), compliance issues make following the clinician's directions difficult for the child and family, and the monocular viewing that results from patching does not treat the visual system as a whole. This treatment has been consistently used for the past century because it is inexpensive and readily available. Additionally, it does slowly improve acuity in the amblyopic eye at a rate of about 0.1 log unit improvement in logMAR acuity (≈ 1 line on the chart) per 120 hours of patching (Stewart et al., 2004). However, just because a treatment has shown some efficacy does not preclude the possibility that a more efficient and comprehensive treatment is possible.

There has been a rapid increase in the power and portability of consumer grade electronics in the past decade, along with a trajectory of decreasing price and increasing availability. As a result, many research groups have begun to investigate health applications that leverage these new devices. A technology-based intervention for the treatment of amblyopia has many potential advantages to historical therapies. First, it may improve compliance, particularly in the target population of young children, by creating a treatment that is fun and easy to use. Built-in measures could quantify training time and relate this to improvements in visual tasks. These benefits could target a wide variety of measures, from improvements in visual sensitivity and acuity, to improving eye movements, reading, and hand-eye coordination. Finally, and perhaps most importantly, using today's technology we can also split and individually manipulate the display for each eye, allowing *binocular* treatment. This also allows for proper eye alignment and perceptual balancing that creates a more holistic therapy.

Technology-based treatments for amblyopia are founded in the principles of perceptual learning. Two decades ago, Levi and Polat (1996) showed visual improvements in adult amblyopes who practiced the same Vernier acuity task many times in a simple computer program. Their results were especially exciting given the fact that up until this point, most clinicians believed that after the end of the critical period, usually cited as age 7, amblyopes would not have gains in visual function, and treatment was discontinued.

Subsequent studies measured perceptual learning in a variety of different tasks (see Levi & Li 2009; Levi, 2012 for reviews). While the mechanisms of perceptual learning are beyond the scope of the current aims, it's important to note that the improvements that result from this repeated practice go above and beyond simply becoming more accustomed to the task. For example, the observed improvements are often closely related to the visual task, and can be measured in highly experienced observers such as those that designed and programed the experiment. Further, these gains are also linked to neural changes that can be measured with electrophysiological and fMRI methodology.

In patients with amblyopia, perceptual learning has shown to improve contrast sensitivity, logMAR acuity, and even stereoacuity (e.g. Levi et al., 1997; Polat et al., 2004; Zhou et al., 2006; Huang et al., 2008). However, there are several drawbacks with perceptual learning that limit it's use as a clinically prescribed treatment. In the studies mentioned above, patients practiced the same Vernier acuity task for thousands of trials – it quickly became tedious and boring. Additionally, for it to be an effective treatment the benefits should transfer to non-trained parts of the visual field and generalize to other visual functions. Many times this was not the case.

About a decade later after Levi and Polat's groundbreaking study, a promising new tool was shown to lead to visual improvements – action video games. Shawn Green, an undergraduate student working in a visual processing lab consistently out-performed his peers on psychophysical measures. He hypothesized this was due to his hobby of playing action video games many hours a week. Formalized testing comparing gamers to non-gamers, and later, training non-gamers on action video games, lead to a variety of improvements from sensitivity to visual attention (Green and Bavelier, 2003; Dye et al., 2009) and visual short-term memory (Wilms et al., 2013; Blacker and Kirby, 2013).

Video game training was then adapted to help improve vision in amblyopic subjects. Patients played action video games viewing with their amblyopic eye only, the non-amblyopic eye was patched. The video game training had many advantages – it was fast paced, engaging, and improvements in vision both generalized across the entire visual field, and transferred to many visual functions, such as visual acuity, stereoacuity (Li et al., 2011), and temporal processing in a task known as the attentional blink (Li, Ngo & Levi. 2015).

Most recently, the effectiveness of dichoptic training has been tested in a population of adult amblyopes (Vedamurthy et al., 2015). A custom action video-game was developed (Bayliss et al., 2012; 2013) that allowed experimenters to adjust the input to each eye, making them perceptually equivalent. The game was also played with a stereoscope that used mirrors to align the two images onto the fovea of each eye. After playing the game for 40 hours, participants had an average visual acuity improvement of 0.14 logMAR, equivalent to a gain of approximately 27%.

1.11 Aim 3 Overview

In Aim 3 we will apply knowledge from the first two studies to improve our understanding of amblyopic treatment. Specifically, we will test the hypothesis that treatment with two eyes is better than treatment with one by testing the same training paradigm under monocular vs. dichoptic conditions that allow for bifoveal viewing. Bifoveal viewing has been shown to improve fixation stability in strabismic amblyopes (Raveendran et al., 2014). Fixation stability is further improved through adjusting the luminance of the non-amblyopic eye such that it is perceptually equivalent to that in the amblyopic eye.

We will test the luminance-balanced, dichoptic training versus monocular training with an eye patch over the non-amblyopic eye. Unlike previous video game studies that report similar results with whether the training is monocular or dichoptic, we will directly compare these two conditions using the same game. We will test the feasibility of both training methodologies in a younger population of amblyopes, aged 7 to 17. We hypothesize this population will have greater neural plasticity and will show greater and/or faster improvements that previous video game training with adult populations.

1.12 Summary

The following experiments will explore the nature of the reaction time delay in patients with amblyopia, and its potential link to fixation instability and/or and impairment in focusing spatial attention when viewing with the amblyopic eye. We believe irreducible response delays are likely to be more pronounced in strabismic amblyopes, who overall have less fixation stability in the amblyopic eye. We will test this hypothesis by comparing interocular reaction times to perceptually equivalent stimuli in strabismic and anisometropic amblyopes and measuring how the release of attention at fixation effects suprathreshold reaction time. Finally, we believe that perceptually equivalent input presented to both eyes may help stabilize fixation and that binocular treatments that support this feature may lead to faster visual improvements than monocular treatments such as patching. This will be tested directly via dichoptic and monocular video game training in a sample of young amblyopes.

Chapter 2

Reaction Time in Amblyopia as a Function of Equivalent Contrast

2.1 Introduction

When light enters our eye and falls onto our retinas, this information is not processed in a uniform state. Each eye has only a very small area of densely packed cones, called the fovea, which does the majority of our detailed information processing. To compensate, our oculomotor system makes binocular eye movements called saccades, to bring these highly specialized parts of our retinas to the area of visual interest.

It is estimated that we produce approximately three saccades every second that we are awake (Cassavaugh, Kramer, & Peterson, 2004). These eye movements are essential, whether we are reading text on a screen, looking for our keys, or trying to cross a busy street. Saccades are fast – in fact, they have the lowest reaction times and highest velocities of any overt action (Pratt et al., 2006). Despite this, the planning and execution of where we point our foveae in visual space is a rather complex phenomenon.

2.1.1 Overview of the saccadic neural circuitry

Saccadic eye movements are directed by a surprisingly large number of cortical and subcortical brain areas, including frontal and parietal cortices, the basal ganglia and thalamus, the superior colliculus, cerebellum, and brainstem reticular formation. These interact to drive both goal-directed and stimulus-driven saccades. Importantly, they also include sites of convergence of multiple sensory and motor maps that can translate visual space to motor movement, and send information back about precisely where the foveae have landed. This process allows us to make small correctional saccades or plan the next eye movement, all while perceiving a stable world. For the sake of brevity, we will only briefly describe the most well known and influential contributors to this process, but see (Sparks & Barton, 1993; Munoz, 2002; Sparks, 2002, Schor, 2011) for a more detailed review.

Brainstem Saccadic Burst Generator

The eyes are held in place by three orthogonal pairs of extraocular muscles (lateral and medial rectus; inferior rectus and superior oblique; superior rectus and inferior oblique) (Schor, 2011). These muscles are controlled by a series of pulses and steps from neurons in the paramedian pontine reticular formation (PPRF) of the brainstem (Sparks, 2002). To initiate a saccade, pulses of neuronal activity from excitatory burst neurons (EBNs) in the brainstem quickly move the eyes to the desired position, while inhibitory burst neurons (IBNs) suppress activity in the antagonist muscles during movement and also aid in braking the eye movement. The pulses translate directly to the physical movement of the eyes; the frequency of the burst is correlated to the eye velocity and the number of bursts scales with the amplitude of the eye movement. These burst neurons are counteracted by omnipause neurons which serve to keep the eyes steady for a brief time. An imbalance of these neuronal activities leads to drifting of the eyes during fixation.

The Superior Colliculus integrates input

The superior colliculus (SC) is a fascinating structure in the midbrain that can be thought of as the air-traffic control agent, constantly taking in input from the retinae and other brain areas, integrating various sensory and cognitive information, and translating it into directions for motor movement that get sent to the brainstem (Sparks, Roher, and Zhang, 2000). The SC contains several layers that each play a different role in this operation. The superficial layers of the SC take in direct retinal input, organized in a contralateral map of the contralateral visual field. The intermediate layers of the SC represent a motor map, specifying saccades intro the contralateral visual field. At the rostrolateral portion of the SC, neurons correspond specifically to foveal input. Here, neuronal activity translates to fixation and other small eye movements associated with this, such as drift and microsaccades.

Much of the work done to investigate the SC has relied on stimulating different areas of this structure and measuring the resulting eye movements in awake, behaving animals. These neurophysiological studies have shown that along with direction, the amplitude of the eye movement is also spatially mapped in the SC. When microstimulation is moved slowly from the anterior to the posterior end of the SC, eye movements gradually progress from small amplitude, nasally-directed saccades, through to larger, temporal eye movements (Fecteau & Munoz, 2006).

A recent study has also shown that visual input during development is necessary for the fine-tuning of these topographic areas. Wang et al. (2015) reared mice in a dark environment and compared their eye-movement maps to mice raised in a normal light environment. The researchers showed that evoked saccades were less consistent, and that naturally occurring eye movements were larger in the dark reared mice. These findings could have implications for our understanding of eye movements in amblyopic patients, who also receive degraded input from one of the eyes during development. Potential outcomes of this will be discussed later.

Higher brain areas help process and plan

Along with direct retinal input, the SC also receives input from several areas in the cortex (Gaymard et al, 1998; Pierrot-deseilligny et al., 2004; Hutton, 2008). One of the best studied is the frontal eye field (FEF), which is involved with preparing goal-driven saccades. When intending to look at an object or target, whether it is already present, a predictive movement, or memory-guided movement, or a saccade in the opposite direction, it is generally triggered by this brain area.

The FEF is also networked to the parietal eye field (PEF), which generates reflexive saccades in response to the sudden appearance of objects in the periphery. The parietal cortex plays a major role in the deployment of attention, and the PEF may be an important link between attention and eye movements. In the monkey, light stimulation of this area results in a shift of visual attention (without eye movements). Further, stimulating the same area, but to a stronger degree results in a saccade. These findings highlight the close relationship between eye movements and attention.

Several other eye fields also belong to this cortical eye movement network including the supplementary eye field (SEF) and dorsolateral prefrontal cortex (DLPFC). The SEF is involved in motor programs, for example, when a saccade is combined with a body movement, or several successive saccades are made.

The DLPFC is involved in higher level processing, such as short-term spatial memory and decisional processes, but is also thought to play a role in saccade inhibition. This is important, because saccades are a limited resource, occurring only about three times a second. There are often many areas of visual interest, and the DLPFC may help decide which areas are important to inspect, while stopping unnecessary eye movements to lower-payoff locations. Pierrot-Deseilligny and colleagues (2004) write, "*the inhibition of reflexive saccades originating in the DLPFC is probably exerted directly on the superior colliculus*."

2.1.2 Saccadic timing and execution

Several properties are often measured when studying saccades, including the *amplitude, duration, peak velocity, and latency*. The amplitude is the linear size of the saccade, usually measured in degrees or minutes of arc. The duration is defined as the time it takes to complete a saccade, measured from when they eye begins to move and ending when they eye lands at the end point. Saccadic durations are quite short (30-100ms) (Wong, 2014). During this time, the velocity of the eye movement quickly ramps up, reaching a maximum or peak velocity of 30-700 degrees/sec, and the ramps down in a symmetric shape. Because a saccade happens so quickly, they cannot be modified by visual information "in-flight", however, visual information can be updated continuously to modulate the parameters of following eye movements.

The latency of a saccade is measured as the difference from the onset of a saccade target (or signal to initiate a saccade), and the beginning of the eye movement. Saccadic latency will be described in greater detail in a following section, but it's important to note that this property is one of the most variable. While latencies for a medium amplitude saccade (5-10 degrees) are generally about 200ms, they can be as low as 100ms or high as 350ms. The distribution of these latencies can yield insight into how visual information is processed and translated into oculomotor movements.

Once initiated, saccades follow a fairly stereotypical pattern called the *main sequence* (Bahill et al, 1975), in which the velocity increases linearly with the amplitude of the eye movement. At amplitudes of greater than 20 degrees, the velocity begins to plateau, reaching a maximum of about 500°/sec (Wong, 2014). For saccades above 4 degrees, the duration also increases linearly with amplitude, up until about 50 degrees. Although it is possible to voluntarily direct when and where we point our eyes in space, the speed at which they move is not under our direct control. In fact, any change to these stereotypical patterns is generally thought to signify an underlying neuropathology (Ramat, et al., 2007).

2.1.3 Saccadic timing in depth

The case for and against express saccades.

When saccadic latencies from a simple saccade task are plotted on a frequency distribution, there are on some occasions two different maxima: the normal peak that falls at about 200ms, and an earlier peak that falls around 100-135ms (Fisher and Boch, 1983). The eye movements in this earlier peak are referred to as express saccades (Fischer and Boch, 1983; Fischer and Ramsperger, 1984).

Express saccades have been the subject of much debate as not all studies, even with similar methodologies, have been able to produce them (Reuter-Lorenz et al., 1991; Kingstone & Klein, 1993; Wenban-Smith & Findlay, 1991). Some groups believe they are manifested via separate pathways that bypass cortical processing, while others believe that they are correct guesses, initiated just before the stimulus actually appears (A.J. Anderson & Carpenter, 2008). When they do occur, it is often in cases where urgency is encouraged over accuracy (e.g. Reddi and Carpenter, 2000), or in artificial situations with observers who are highly practiced (Carpenter, 2001; Dickov & Morrison, 2006; Pare & Munoz, 1996). They can also be produced when the fixation point is removed before the appearance of the saccade target. This phenomenon, known as the gap effect, will be described in greater detail in a following study.

Are there benefits to delaying a saccade response?

Express saccades aside, a quick saccade to the sudden appearance of a bright target generally has a latency of approximately 120-180ms (Saslow, 1967). Although faster than other overt actions, these latencies are still surprisingly slow, given the temporal properties of the neural synapses involved in these processes. Electrophysiology studies have shown that retinal information takes about 40ms to reach the SC and when the deeper layers of the SC are directly stimulated saccades occur about 20ms later (Carpenter, 1981). Together, these findings point to the capability of a much faster system. With each saccade taking only 60ms, one could make upwards of 16 eye movements per second. Why then, hasn't our visual system evolved to a much higher saccadic tempo? Is there a benefit to limiting the briefness of a saccade?

By increasing the reaction time, or even pausing before initiating a motor movement, the oculomotor system allows time for more sophisticated judgments, using feedback from cortical areas to take other factors, such as distractors, into consideration and even inhibit the movement if necessary (Carpenter, 1981). This sluggishness could be a tradeoff for a more efficient saccade system. Not only can the brain decide where to move the foveae and map out the motor plan to that space, but also evaluate if it is worthwhile before the action is initiated (Hutton, 2008).

What leads to increased saccadic latency?

The determinants of saccade latency can be grouped into two main classes: 1) external or stimulus properties that can change the mean latency, and 2) observer factors that result in a wide latency distribution even when conditions are apparently constant.

The first group contains stimulus properties and includes attributes such as the contrast, spatial frequency, size and eccentricity of the target. Using psychophysical methodology, clear relationships between these attributed and reaction time have been described. One of the most well studied is the effect of contrast (as well as luminance), on reaction time in both manual and saccadic response modalities. These relationships can be modeled quite clearly by Piéron's Law (Piéron, 1914; 1920; 1952; see also Hughes & Kesley, 1984; Pins & Bonnet, 1996; Jaskowski & Sobieralska 2004). A modified version of Piéron's Law (Burr et al., 1998) will be fit to the reaction times measured in this study. The manipulations and curve fitting will be described in greater depth in the following methods section.

Other stimulus properties tangentially of interest to the proposed experiment include spatial frequency and target eccentricity. Modulation of these both result in a u-shaped function. For eccentricity, the fastest reaction times are from about 2 to 10 degrees in eccentricity, at closer and farther distances, reaction times increase

(Sumner, 2011). Similarly, for spatial frequency, reaction times are fastest from approximately 1 to 4 degrees, and rise sharply to the grating cut-off (Levi, Harwerth, & Manny, 1979).

Studies have also shown that as the target size increases, the latency of the saccadic reaction time also increases (Ploner et al., 2004). This change is roughly 20ms when comparing mean SRTs to 1 degree and 10 degree targets, however, what is most interesting is that the overall distribution of responses changes drastically. While the 1 degree stimulus has a sharp peak at approximately 180ms, and an earlier express saccade peak at 120ms, the distribution for the larger stimuli is much more diffuse, with a wider peak at about 250ms. These results highlight the importance of comparing the entire range of observed reaction times, not just the means and standard deviations.

Stimulus property interactions

Plainis and Murray (2000) also showed that stimulus properties of contrast, spatial frequency, and luminance can interact and that reaction times vary as one would expect based upon one's contrast sensitivity function. By measuring reaction time to a series of sinusoidal gratings encompassing a wide range of suprathreshold contrasts, spatial frequencies, and luminances, they determined that all of the resulting RTs could be explained with a simple equation linking contrast, spatial frequency and luminance.

Their first equation is $RT=RT0 +k (1/C)$ where RT is the reaction time, RT0 is the asymptote, k is the steepness of the curve, and C is the contrast. Further, they found that k varied greatly according to spatial frequency in a systematic manner. K was low at low spatial frequencies, where a unit change in contrast has only a small effect on RT. Conversely, at high spatial frequencies a unit change in contrast has a high effect on RT, and the value of k is high. At these higher spatial frequencies, the threshold is higher and the dynamic range over which the target can be detected is narrow.

Plainis and Murray (2000) note that their equation predicts that the value of k will reflect the behavior of RTs for all spatial frequencies and luminances as a function of contrast. They therefore call k the RT-contrast factor with units of ms x contrast. Further, for each spatial frequency, they find that the value of k increases with reductions in luminance, and that the functions are nearly parallel to one another. They use this family of curves to extend their original equation to account for all stimulus characteristics: contrast, luminance, and spatial frequency, as well as a constant, which is specific to the subject. These findings highlight the fact that stimulus properties can well account for most RT differences in a mathematical model that relates to visibility. However, as we'll see in the next section, despite these clear mathematical models, it is impossible to account for reaction time differences at the level of the observer.

Higher-level effects on reaction time

Observer effects, such as alertness or attentional state, have been less studied, in part because they are harder to model. Some of these attributes can be modulated by experimental design, however it's difficult to quantify the associated changes in the internal state of the observer, as these vary millisecond to millisecond as well as across trials. For example, when the fixation dot changes color or luminance before a saccade target appears, latency can decrease by about 20ms (Pratt et al., 1999; Jin & Reeves, 2009). This is called the warning effect and is thought to alter the observer's overall readiness or attentional state. Reaction times are reduced by a similar amount if the observer knows in advance whether the target will appear on the left or right of fixation (Megaw & Armstrong, 1973).

Even the instructions can influence reaction time to the abrupt appearance to a peripheral target. A participant that is allocating attention and holding steady fixation may be slower than one that is 'merely looking at the fixation point' (Becker, 1991). There are also speed accuracy tradeoffs, for example, Kowler and Blaser (1995) reported changes in saccade responses when observers were instructed to "*be as accurate as possible, even if this requires a longer latency*".

Finally, an interesting study by Kowler and Blaser (1995) reflects the ability of one's cognitive state to influence saccadic latency. They used a saccadic go/no-go task, where participants had to make a saccade if the peripheral target was green, but hold fixation if the peripheral target was red. While doing this task, participants had one of three different audio conditions: no sound, bursts of pink noise, and a soundtrack of pre-recorded words. They found both an increase in short-latency saccades, as well as an increase in error rate when participants were under higher levels of cognitive distraction. Subjects performing the task while listening to a string of words had a diminished ability to inhibit reflexive saccades, highlighting the importance of cortical control in eye movements.

2.1.4 Modeling reaction time to visual stimuli

The relationship between stimulus intensity and reaction time can be described by Piéron's law, $RT = \alpha C-\beta + \gamma$; where α and β describe the shape of the slop and γ sets asymptotic floor. According to this model, reaction time is slowest at threshold, and falls with β, a fractional exponent that controls RT decay, until reaching an asymptote, γ, at which point further increases in stimulus intensity no longer effect the RT response. Alpha is the scaling parameter, describing the differences between the maximum RT at threshold and minimum RT at asymptote, while C drives the change in stimulus intensity. Piéron's law holds for a wide variety of sensory phenomenon, including not only the visual domain, but also auditory stimuli (reviewed in Luce, 1986) and gustatory reaction times (Bonnet et al., 1999), as well as different times of reaction time paradigms (e.g. simple and choice tasks, Pins & Bonnet, 1996).

Piéron's law does not just act at the level of behavioral response, but can be traced back to faster response times at the neural level, that is, when the intensity of a visual stimulus is increased the onset of neural activity is decreased. Bell et al. (2006) measured neural activity in the intermediate layers of the SC in monkeys generating saccades to high and low intensity visual stimuli. They found that the single unit responses to the high intensity stimuli were an average of 27ms faster in a gap saccade task, which coincided with 35ms faster saccadic latencies. Their findings provide a direct link between stimulus intensity, neural activity, and reductions in saccadic latency.

Piéron's law models

Various types of models have been proposed for describing the mechanisms of Piéron's law (Link, 1992; Baird, 1997; Stafford and Gurney, 2004; Hsu, 2005; Palmer et al., 2005; Stafford et al., 2011; Servant et al., 2014; Verdonck and Tuerlinck, 2014), but we will mainly focus this discussion on diffusion models. This model is a continuous version of a random walk and is a successor to earlier random walk models of two choice decisions. In a random walk model, an observer's decision is based on a sequence of noisy observations that can have positive or negative values. These successive samples are summed until a boundary is reached.

Diffusion models are defined by two parameters: the drift or rate at which the information is accumulated and the diffusion coefficient which describes the noise within the observer. An important part of this model is that it relates internal noise, signal strength of the stimulus, and reaction or decision time. It can also account for errors, and offers an explanation for why they might happen in a nosier system, or when a fast response is prioritized over accuracy. In relation to the current study, one might hypothesize that there might be more internal noise when viewing with the amblyopic eye, or that the rate of information accrual might be slower for the same physical stimulus in this eye.

Modifications to Piéron's Law

While Piéron's function appears to fit a wide variety of phenomenon with relative robustness, closer inspection does lead to some concern. Luce first noted in his 1986 textbook on reaction time that fitting Piéron's law to data "*provides an estimation problem of some delicacy*". As such, several groups have modified Piéron's formula to improve the fits in cases where the original function does not accurately describe the empirical results. For example, Pins and Bonnet (1996) simplified the function by removing the third parameter (the asymptotic value) when they used a smaller number of luminance levels (five), and the highest intensity was still far from the beginning of the asymptotic RT level. Similarly, Smith et al. (2004), who were studying reaction time to targets near the contrast threshold, also used the two-parameter version of Piéron's law because the asymptotic parameter did not yield an improvement of the fit and showed poorer stability. A third group, Gould et al. (2007), also removed the asymptotic parameter, noting that in cases where the reaction time is slow and stimuli are near threshold, this parameter can be difficult to estimate reliably. However, removing this parameter in our case would not be ideal since a primary goal of the first aim was to determine if the asymptotic values were the same in the amblyopic vs. non-amblyopic eye.

Further, Pianta and Kalloniatis (1998) noted that the two shape parameters of the function, α and β, are not independent. Pianta and Kalloniatis (1998) calculated the dependence of these parameters using the following equation: dependence = (variance of the parameters, other parameters constant)/ (variance of the parameter, other parameters changing). They found with their reaction time data that the parameters α and β have dependencies near one, suggesting that they are strong dependent on one another. This is particularly the case when the data are variable or collected over a small range of intensities, yet can exist even when the data are collected from an experienced observer over a wide range of intensities. They conclude that α and β alone should not be used to describe the shape of these functions, as had been done in the previously mentioned set of studies.

Another group, Burr and colleagues (1998) have modified the Piéron function in a way that improves the estimation of the asymptotic values. In their assessment of the Piéron function, they note, like Pianta and Kalloniatis (1998) that the alpha and beta parameters trade off with one another to give the best fit, and that this trade-off depended on whether the measurements were made near the threshold. They proposed an alternative formula that normalized for contrast threshold and only has one parameter that determines the slope of the equation.

$$R = \alpha /(\log(\chi/\tau)) + R\infty$$

Where R is the reaction time, x is contrast, theta is the threshold, Rinfinity is the reaction time asymptote, and alpha is the contrast determining the steepness of the curve. In this equation, the asymptote reaches infinity and has a single estimate of contrast dependency (alpha). In the Burr study, the fits with the modified equation had lower chi-square values than those reported by the Piéron function. We will use this modified formula in our estimation of asymptotic reaction times, which allow for the characterization of suprathreshold performances while controlling for contrast dependency.

2.1.5 Saccadic latency is increased in patients with amblyopia

Increased saccadic latency in amblyopia was first reported over 50 years ago by Mackensen (1958), who found an approximately 25ms difference in saccadic latency to the appearance of a peripheral stimulus. However, as reported by Ciuffreda, Kenyon & Stark (1978ab), this latency difference varied considerably from patient to patient. By averaging across subjects Mackensen reduced the overall effect, as some patients did not exhibit an interocular RT difference.

Ciuffreda and colleagues (1978a,b) also provided evidence that despite these delays, patients with amblyopia had normal motor movement. That is, while the initiation of the eye movement was slower in the amblyopic eye, saccadic durations in this eye were normal and appropriate for their amplitude, and when both eyes viewed the target eye movements, including the latency, were also normal. They conclude, "*Our results are interpreted in terms of a processing delay in the sensory pathways leading from the central region of the amblyopic eye to the centers involved in amblyopic initiation*".

Hamasaki & Flynn (1981) found a potential explanation for the high degree of observer variability reported in earlier studies. By measuring manual reaction time in normally-sighted observers, strabismic patients without amblyopia, and strabismic patients with amblyopia, they found a simple relationship: as the interocular difference in vision increased, so did the change in reaction time. They found a mean difference of about 45ms, that was related to the presence of amblyopia, not strabismus. A related finding was reported by Gerin et al in 1973, using visually evoked responses (VER). These researchers confirmed the presence of increased saccadic latency in amblyopic eyes, in both eye movements and neural measures, however, and the 100ms average in increased saccadic latency for amblyopic eyes far exceeded the VER delay. These findings show that amblyopia is related to widespread temporal delays and that these can be traced back to the neural level.

In the past 30 years, many groups have supported and expanded upon these findings (Pianta & Kalloniatis, 1998; Niechwiej-Szwedo, Goltz, Chandrakumar, & Wong 2010; Niechwiej-Szwedo, Chandrakumar, Goltz & Wong, 2012; Perdziak et al., 2014), yet little has been done to assess what sites in the network may be causing this temporal disruption. One potential site that could be driving much of this delay is the superior colliculus. Schiller and Stryker (1972) write, "*It has been advanced that the superior colliculus may be intimately involved in coding information regarding the location of objects in space relative to the fovea, the 'foveation hypothesis' and in the initiation of saccadic eye movements.*" This hypothesis is similar to the one proposed by McKee (and colleagues, 2016) who writes, *"...the longer saccadic latencies found in amblyopes may be due to the slow shift in attention prior to saccadic initiation*".

Several other groups have also posited that cells in the superior colliculus may be involved in early information processing for saccadic initiation. Wurtz and Goldberg found increased saccadic latencies following focal lesions in the SC in monkey. While the accuracy did not change and the speed of movement was only minimally affected, saccadic latencies were approximately 150-300ms greater than normal. Converging evidence more directly linking the SC site to increased saccadic latencies in amblyopia was reported by Gordon & Presson in 1977. Kittens reared with unilateral strabismus had decreased responses in collicular cells when viewing with the strabismic eye compared to the dominant eye.

Finally, these delays are primarily observed when needing to attend and respond to a visual signal. Fukushima and Tsutsui (1984) measured saccadic latencies for both visual and auditory evoked saccades and found increased latencies for the visual but not auditory target when initiating the eye movement with the amblyopic eye. This provides further evidence that the motor response is largely unaffected, and that the delay is caused by a sensory impairment. This also aligns with the work by Mackensen showing increased response times to visual targets when making a manual response. It appears that any time an amblyopic patient is responding to visual stimuli regardless of the response type, there is potential for a delay. This widely reported finding suggests that the slowing occurs in the sensory processing of visual information, and support the SC as a likely contributor in the delay.

Given that we now have good models for how saccades are generated in people with normal vision, and have been able to apply this to other clinical groups, amblyopia presents itself as an excellent case to explore how visibility can change reaction times. We know that the root cause of amblyopia is a disruption in visual input during development, but we are far from understanding the implications this has on parts of the brain outside the primary visual cortex. By conducting a systematic measurement of saccadic and manual reaction time across contrasts that vary as a function of effective stimulus intensity we can begin to rule out stimulus properties. This will also allow us to set the groundwork for looking at observer-level differences, such as attentiveness, in a follow-up experiment.

2.16 Hypotheses for Aim 1

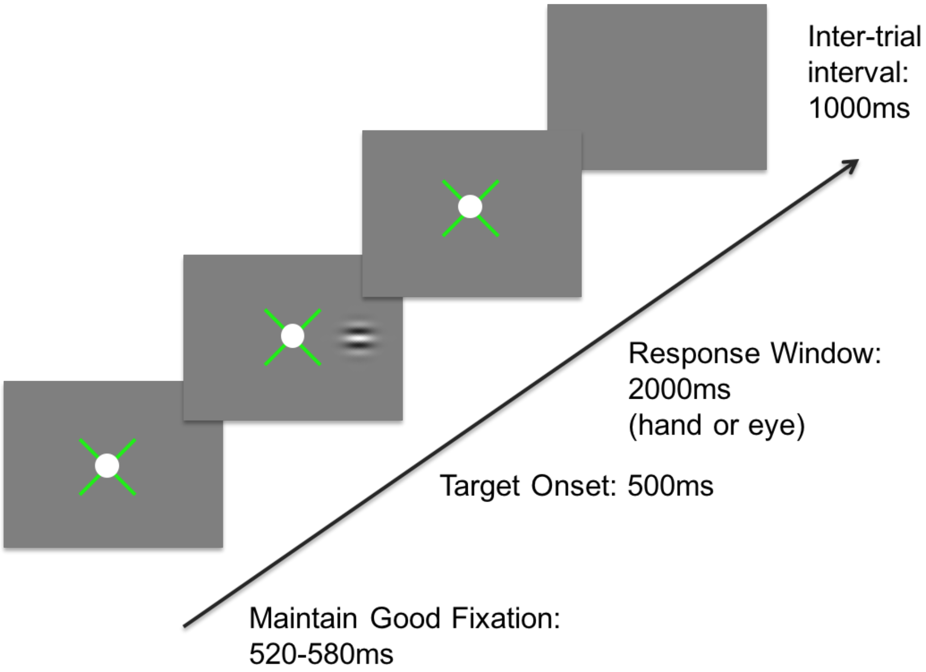
In Aim 1, we will measure the reaction time to peripheral targets as a function of *effective* stimulus intensity, or contrast that is proportional to that eye's contrast sensitivity. We will fit the 'Burr' function to the reaction time data for each eye to compare interocular RT differences along the entire function, but most importantly at the asymptote. If sensory differences in visibility are the exclusive factor in the amblyopic delay, then this scaling should completely account for the reaction time differences. We hypothesize that this may be the case for most anisometropic amblyopes, but not strabismic amblyopes.

2.2 Methods

2.2.1 Procedure

Reaction time functions for saccadic latency and manual response time were calculated for each eye of amblyopic and control participants following the sequence of events illustrated in Figure 2.1. Participants were instructed to fixate a white dot, while gabor targets were presented at 5 degrees to the right or left of this dot. The participants were asked to respond by looking at the target (saccadic latency measurement) or pressing the left or right arrow key specifying the side of target appearance (manual reaction time measurement) *as quickly and accurately as possible*.

Figure 2.1: Procedure for Experiment 1.



An x-shaped fixation guide appeared at the center of the monitor, with a 1 degree white fixation target placed on top. The fixation guide provided feedback, with green representing fixation within a 1 degree window, and red noting that the fixation error was greater than this boundary. A red fixation guide paused the trials and reset the fixation timer. After ~550ms (+/- 30 ms) of proper fixation, a gabor patch briefly appeared at 5 degrees to the left or right, and participants had up to 2 seconds to respond, followed by auditory feedback and a 1-second inter-trial interval.

Stimulus Parameters

The gabor targets were adjusted for each participant to allow reliable detection at mid to low contrast levels in the amblyopic eye. To find the appropriate stimulus properties, a cutoff frequency was first determined in a yes/no procedure, where the mean of three reversals was calculated (mean for all observers was 4 cpd, range 1 to 7 cpd); gabor patches were generated with a standard deviation of ¾ of a cycle). Contrast thresholds, described below, were then measured for targets with half of this cutoff value.

Gabor patches were randomly presented to the left or right of fixation and threshold values for the nasal and temporal side of the amblyopic eye and non-amblyopic eye were measured independently. If one of the threshold values for this spatial frequency was greater than 0.2 absolute contrast, the spatial frequency was shifted lower, and the threshold values were re-measured. This was performed to ensure a sufficient range of stimulus intensities for each participant. Contrast thresholds were measured with the fellow eye using the same spatial frequency as the one used for the amblyopic eye.

For each eye, contrast thresholds were measured with two adaptive staircases, measuring the threshold for the left and right targets independently. Participants pressed the left or right arrow key, indicating the side at which the gabor target appeared, and the contrast of the target increased or decreased logarithmically in a three-up, one-down manner. Each threshold block had 100 trials, with targets randomly appearing on the left or right side. To determine the final threshold for each side, the first reversal was discarded and the mean of the remaining reversals was calculated. If there were less than six reversals for one side, the contrast threshold measurement was rerun for that eye.

RT measurements

After contrast thresholds were determined, participants completed one practice block of 10 trials with an easily detectable contrast level (contrast = 0.6). Subsequent blocks started with 5 practice trials at this level, then a green fixation dot was presented signifying the beginning of reaction time measurements. For the remainder of that block, there were 100 trials with gabors at three of nine possible CTUs. These contrast threshold units were multiplied by the left and right threshold value to determine the raw contrast values measured in that block. There were at least 2 blocks per eye (for 6 total CTUs), per response type. Response modality was kept consistent across a block, but switched every 2-4 blocks.

Set-up and Calibration

Participants were seated 100 cm from a 21” ViewSonic G225f monitor in a dimly lit room, and positioned in a brow bar and chin rest such that the viewing eye was parallel to and gazing at the center of the screen (min luminance = 6 cd/m^2, max luminance = 120m^2). The non-viewing eye was occluded by an eye patch. Eye movements were recorded with an Eyelink 1000, SR research (2000 Hz monocular sampling rate and 0.25°-0.5° average accuracy when properly calibrated), mounted above the brow bar and aligned with the surface of the eye using a beam splitter.

Prior to each block, eye position was calibrated using a 5-point calibration display (center and the four corners). After calibration, participants were instructed to maintain fixation on a bright white dot (1 degree, 120 cd/m^2) that was displayed on a grey background (30 cd/m^2). A red or green crosshair surrounding the fixation dot provided feedback regarding fixation status. Participants maintained good fixation for 550ms (+/- 30 ms) prior to target onset.

2.2.2 Analysis

At least 10 reaction time measurements (mean 35, range 11-76) used to calculate each contrast threshold unit (CTU) data point on the reaction time curve. The contrasts were distributed from 1 CTU (threshold value) to 9. A minimum of 6 contrast levels (maximum 9) were used to fit the reaction time function described below.

For each contrast level, correct responses were averaged to find the mean and standard error of the reaction time. For saccadic responses, response times below 120ms and above 800ms were eliminated to reduce the effect of express saccades, guesses, and exploring the screen to find a target.

Burr function fittings

Mean response times and standard errors were plotted as a function of contrast sensitivity threshold. The data were fit with the Burr function:

$$R = \alpha /(\log(\chi/\tau)) + R\infty$$

Where R is the reaction time, α is the constant determining the steepness of the curve, χ the contrast, τ is the threshold, and R∞ the reaction time asymptote. The Burr equation asymptotes to infinity at threshold and has a single estimate of contrast dependency (α), measured in ms x log-contrast.

2.2.3 Study participants

The Research Subjects Review Boards at Smith Kettlewell Eye Research Institute approved the study protocol. Informed consent was obtained from each participant and the study was conducted according to the tenets of the Declaration of Helsinki.

Eight (n = 8) adults (mean age: 54 ± 15, range 31–68 years) with unilateral amblyopia and 3 normally-sighted control subjects completed at least one part of the study (see Table 1).

Table 2.1: Study Participants

SID	Age	Classification	AE Contrast Threshold	NAE Contrast Threshold	Spatial Frequency Tested (cpd)	AE Acuity	NAE Acuity
S1	68	aniso/strab	0.07	0.1	5.5	20/63+1	20/20-1
S2	31	strab	0.07	0.03	4	20/100	20/25+2
S3	34	aniso/strab	0.05	0.02	1	20/300	20/16-2
S4	68	strab	0.12	0.05	4	20/80+2	20/20
S5	56	strab	0.15	0.07	3	20/125+2	20/20+2
S6	64	aniso/strab	0.14	0.08	3.5	20/63+1	20/16+2
A1	53	Aniso	0.11	0.03	4.5	20/63+2	20/20+2
A2	51	Aniso	0.14	0.07	7	20/50	20/12.5-2
			Non-Dominant Eye Threshold	Dominant Eye Threshold	Spatial Frequency Tested (cpd)	Non-Dominant Eye Acuity	Dominant Eye Acuity
C1		Alternating Strab	0.10	0.09	3.5	20/20	20/20
C2		Control	0.6	0.6	5	20/20	20/20
C3		Control	0.05	0.05	4.5	20/20	20/20

* All control participants, including C1 had acuities of 20/20 or better.

Participants were recruited through advertisements and local ophthalmologists. An experienced ophthalmologist provided an eye exam for each participant prior to enrolling. The inclusion criteria were: (1) age 18 years or older; (2) anisometropic amblyopia, strabismic amblyopia, or mixed type (i.e., anisometropic and strabismic); (3) interocular visual acuity difference of at least 0.2 logMAR; and (4) no history of eye surgery except those to correct strabismus. Participants were excluded in they had any ocular pathological conditions (e.g., macular abnormalities) or nystagmus. All of our participants had visual acuities of 20/12– 20/25+2 in the non-amblyopic eye. The retinal health of all participants was assessed as normal and cover tests were used to assess ocular alignment at both distance and near. The study took place at Smith Kettlewell Eye Research Institute located in San Francisco, CA.

Study participants were classified as either anisometropic ('Aniso') or strabismic ('Strab') amblyopes. Anisometropia was defined as 0.50D difference in spherical equivalent refraction or 1.5D difference in astigmatism in any meridian, between the two eyes (Wallace et al., 2011). Amblyopic subjects with anisometropia and an absence of manifest ocular deviation were classified as anisometropic amblyopes. Those with an ocular deviation (strabismus), as indicated by the cover test, were classified as strabismic amblyopes, irrespective of their refractive state, meaning that participants with both strabismus and anisometropia were classified as ‘strabismic’.

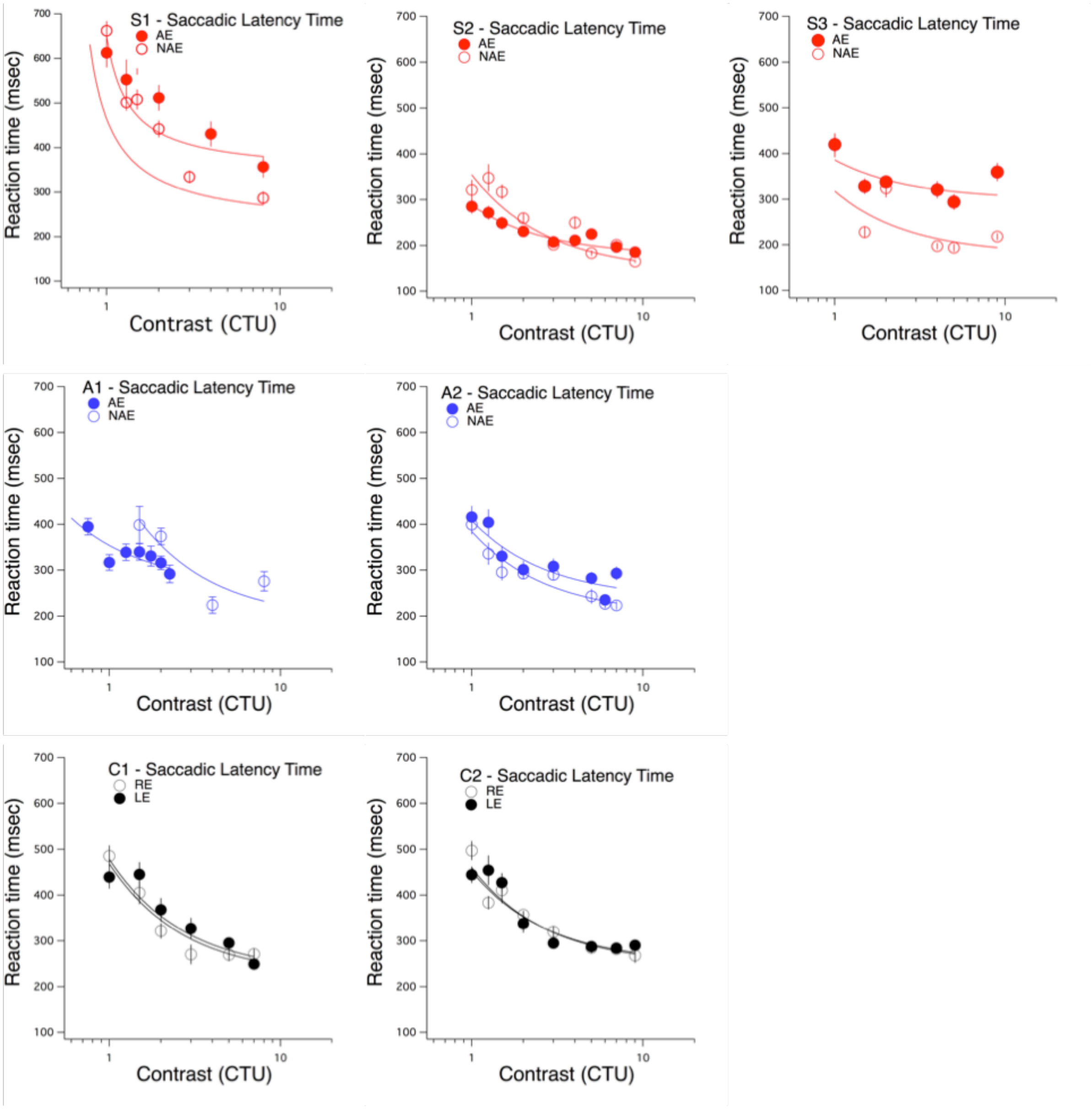
Control subjects were age-matched to match the approximate mean of the patient group. Control subject 1, however, was an interesting case, with alternating strabismus. Her acuity and threshold were impaired with both eye, but given the fact that each eye drove eye movements and perception for some period, and neither eye was under constant suppression, her reaction times may be more similar to normally-sighted controls.

2.3 Results

2.3.1 Saccadic reaction times

Saccadic latencies as a function of contrast threshold units were plotted in Figure 2.2 for each eye of each participant (3 strabismic in red, 2 anisometropic in blue, and 2 age-matched controls in black) and fit with a Burr function.

Figure 2.2. Saccadic latency as function of contrast threshold unit.



The solid symbol is the amblyopic (or non-dominant) eye; the open symbol is the non-amblyopic (or dominant) eye.

The coefficient values for the slope and asymptote from the fits are reported in Table 1. For each patient, amblyopic and non-amblyopic eyes are reported, along with the chi-square value and number of stimulus intensities sampled. The same values were calculated for the control participants, and the dominant (DE) and non-dominant (NDE) eye reported in the table below.

Table 2.3: Burr Fit Coefficients: Saccadic Reaction Time

	SID	Slope (α)	Asymptote (R∞)	Chisq	Num pts
AE	S1*	307.39 ± 40.4	342.08 ± 22.6*	5.46	6
NAE		412.04 ± 25.1	219.55 ± 14*	10.43	6
AE	S2*	109.92 ± 11.2*	177.75 ± 3.31*	14.13	9
NAE		210.84 ± 14.4*	143.86 ± 3.66*	39.55	9
AE	S3*	86.654 ± 25.6	299.5 ± 13.5*	13.85	6
NAE		139.16 ± 20.6	178.68 ± 6.82*	57.98	6
AE	A1	91.45 ± 19.4*	264.88 ± 16.5	8.04	8
NAE		325.7 ± 60.3*	191.82 ± 22.6	12.98	4
AE	A2	170.95 ± 23.9	237.51 ± 10.2	14.82	8
NAE		182.52 ± 19.4	202.31 ± 7.83	9.46	8
DE	C1	246.21 ± 25.9	222.24 ± 11.2	6.92	6
NDE		248.43 ± 27.1	230.04 ± 10.1	10.90	6
DE	C2	212.08 ± 18.1	247.83 ± 8.44	9.74	8
NDE		199.96 ± 18.8	252.46 ± 7.27	13.51	8

For the strabismic amblyopes (in red), asymptotic response times made with the AE were significantly slower than with the NAE, as the difference in R∞ values is greater than the 95% confidence interval (CI) for the two fitted points. Comparatively, the differences between the two eyes in anisometropic amblyopes (blue) and controls (black) were not significant.

Further, two of the patients (one strabismic and one ansiometropic) had the first parameter, which described the slope of the AE, fall outside of this 95% CI of the NAE, with a shallower slope in the amblyopic eye. This shows that not only are the asymptotic reaction times different between the two eyes, but that these RT differences are apparent across the entire shape of the function.

Both coefficients were remarkably similar between the two eyes in the control subjects, showing that the Burr fits can accurately describe the data and that in normal observers there is no difference in slope or asymptote between the two eyes. Further evidence of the goodness of fit can be seen in the Chi square values, with only two of the 14 functions having a Chi square value of greater than 15.

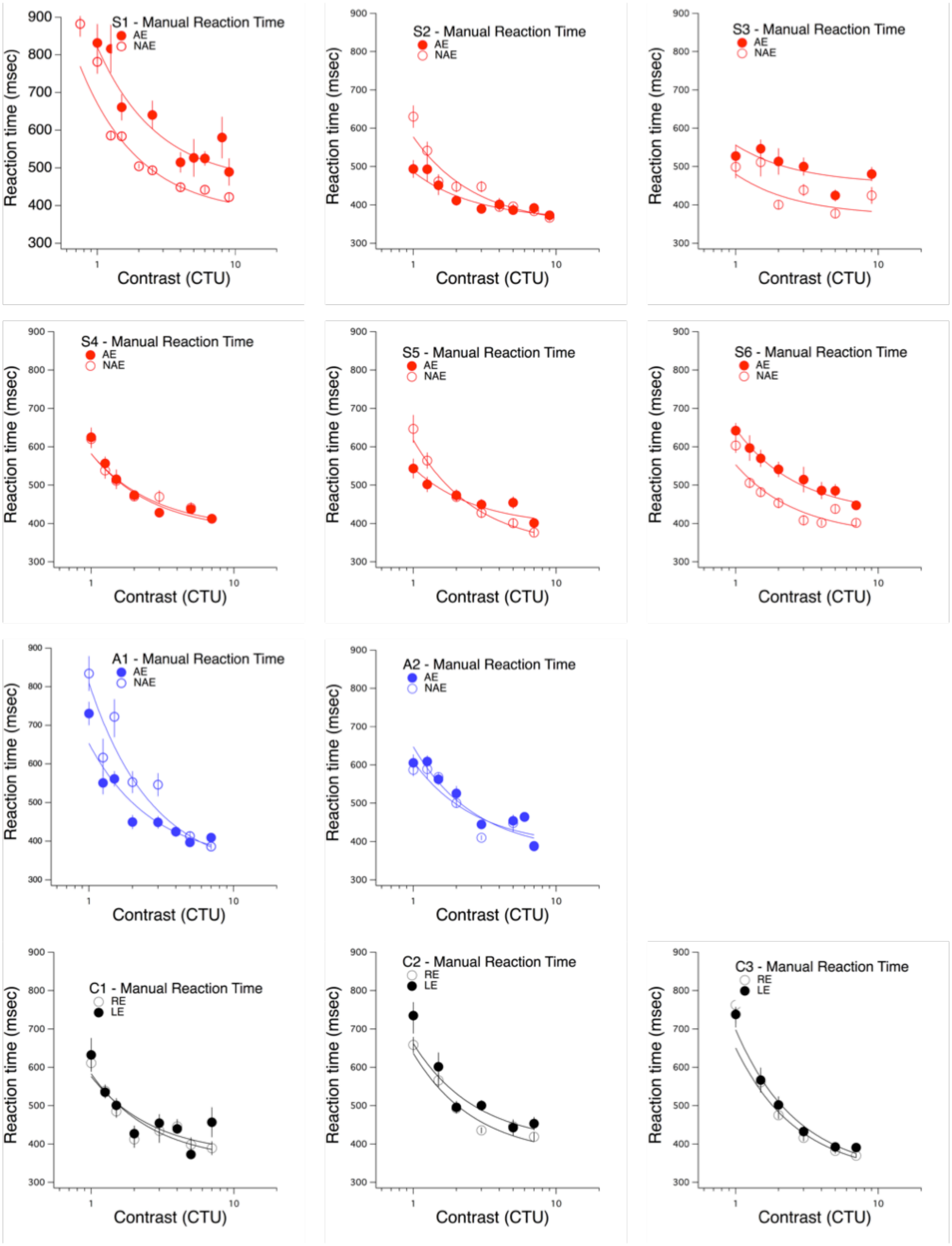
Accuracy

In general, targets near threshold were detectable above chance and quickly plateaued to ceiling by the middle of the curve. Unfortunately, when measuring the saccadic reaction times the eye tracker periodically lost the eye during the block, causing the trial to be recorded as incorrect when the subject did indeed make the correct eye movement. In these instances, the participants usually reported seeing the targets and this was noted. Still, this left some error in the accuracy measurements for these subjects, and this error varied greatly from trial to trial, block to block, and eye to eye. Accuracy values for the same contrast values were measured more robustly for manual reaction times and are reported in the next section. It is unlikely that accuracy would be much different with the eye movement response, and in the a few cases where the eye tracker consistently tracked the eye for an entire 100-trial block, participants had 80-100% accuracy.

2.3.2 Manual reaction times

Manual reaction times as a function of contrast threshold units were plotted for each eye of each participant (6 strabismic in red, 2 anisometropic in blue, and 3 age-matched controls in black) and also fit with a Burr function. The manual reaction time section included a larger number of subjects as these measures could be completed for subjects with which the eye tracker could not be calibrated consistently or with precise accuracy.

Figure 2.3. Manual reaction times as function of contrast threshold unit.



The solid symbol is the amblyopic (or non-dominant) eye; the open symbol is the non-amblyopic (or dominant) eye.

Table 4 provides the manual reaction time coefficients for the 8 patients and 3 controls that completed the second part of the experiment.

Table 2.4: Burr Fit Coefficients: Manual Reaction Time

	SID	Slope (α)	Asymptote (R∞)	Chisq	Num pts	Threshold Accuracy	Ceiling CTU
AE	S1*	361.36 ± 46.2	458.98 ± 18.3*	7.14	9	69.32%	4
NAE		294.94 ± 15.3	376.04 ± 7.53*	41.81	9	88.54%	2.5
AE	S2	125.73 ± 21.6*	360.64 ± 8.09	4.71	9	91.12%	4
NAE		231.59 ± 20.2*	345.68 ± 6.4	13.51	9	81.87%	4
AE	S3	102.25 ± 25.6	453.85 ± 13.5	14.70	6	87.55%	1.5
NAE		116.74 ± 32.9	439.78 ± 14.6	11.76	6	85.62%	-
AE	S4	204.24 ± 18.5	378.13 ± 6.99	15.47	7	90.45%	2
NAE		195.76 ± 16.6	386.28 ± 7.71	6.72	7	89.29%	1.5
AE	S5*	152.11 ± 23.4*	392.3 ± 9.85*	6.40	6	87.34%	2
NAE		281 ± 25.3*	336.64 ± 10*	1.63	6	92.58%	2
AE	S6*	222.42 ± 22	423.6 ± 10.1*	2.21	8	95.83%	1.5
NAE		186.23 ± 15.9	367.09 ± 7.38*	21.82	8	100%	1
AE	A1	306.55 ± 25.2*	346.29 ± 9.37	20.04	8	80.60%	1.25
NAE		495.59 ± 37.2*	314.21 ± 10	12.36	8	71.49%	4
AE	A2	278 ± 178	369.37 ± 6.83	41.13	8	71.26%	1.25
NAE		223.09 ± 19.4	385.62 ± 7.83	54.37	8	86.56%	2
DE	C1	230.15 ± 22.4	353.14 ± 13.4	13.93	8	90.29%	1.25
NDE		205.75 ± 23.9	370.59 ± 9.79	27.35	8	88.46%	3
DE	C2	268.95 ± 22.4	367.68 ± 9.38	16.79	6	89.29%	1.5
NDE		260.05 ± 42.8	401.88 ± 16.9	9.52	6	85.71%	3
DE	C3	333.12 ± 24.1	317.04 ± 7.04	10.00	6	93.33%	5
NDE		376.29 ± 31.6	321.55 ± 9.1	5.80	6	90.48%	2

When responding by hand to a visual target, half of the strabismic amblyopes took significantly longer when viewing with their amblyopic eye, compared to their nonamblyopic eye, as defined by the second coefficient for AE falling out of the 95% CI for the NAE. Two of the strabismic and one of the anisometropic amblyopes also had shallower slopes (α coefficient) for the fits with their AE.

2.3.3 Variability in response times

It is possible that the mean reaction times were slower in the AE than the NAE because it was more likely the amblyopic eye was in a refractory state when the saccadic target appeared. Refectory periods occur in between a saccade or microsaccade and the following saccade or microsaccade and last for about 200ms (Otero-Millan et al., 2008). This means that depending upon where that eye was in this period when the gabor appeared, some amount between 0 and 200ms would be added to the saccadic latency. Therefore, having more frequent microsaccades when fixating with the AE, would cause a broader distribution of reaction times for that eye, and in particular, a longer tail on the right side of the mean.

To test this theory, we compared the variance in response times between the two eyes at the highest CTU where targets were easily perceivable using Levene's variance test. The results from these tests are in Table 5 (for saccadic latency measures) and Table 6 (for manual response times). Patients with the largest between eye RT differences were indeed more likely to have greater variance in the RT data for their amblyopic eye, as compared to their non-amblyopic eyes. For example, looking at the asymptotic values in Table 2.3, the observers with the largest SL differences were S1 and S3. The Levene test was highly significant for S3 and trending for S1. Conversely, S2 had a small, but significant difference in saccadic latency RTs between the two eyes had almost no difference in variance between the two eyes.

A similar pattern was observed with manual RT variances. Patients that had the greatest between eye RT differences were also more likely to have near-significant differences in variance (S1 and S5). Interestingly, while the asymptotic values from the Burr fits were similar between the two eyes, the mean of C1's LE MRT was much slower than her RE's mean, and more variability can be seen on the asymptotic section of this curve in Figure 2.3. This participant and potential explanations for this will be considered in the discussion section.

Table 2.5. Saccadic Latency Variability

SID		Num Trials	Mean	Standard Deviation	Levene's Statistic (p value)
AE NAE	S1	37 43	357.40 286.74	120.34 106.58	3.48 (p=0.07)
AE NAE	S2	48 66	185.34 164.49	22.92 24.53	0.04 (p=0.837)
AE NAE	S3	51 64	359.79 218.09	143.58 60.11	14.16 **(p=0.00)****
AE NAE	A1	26 24	293.06 258.16	56.20 54.06	0.07 (p=0.79)
AE NAE	A2	37 43	357.40 286.73	120.34 106.58	3.47 (p=0.07)
LE RE	C1	76 70	271.13 250.29	101.00 88.80	1.19 (p=0.28)
RE LE	C2	38 21	268.32 290.44	95.16 52.31	1.44 (p=0.23)

Table 2.6. Manual Reaction Time Variability

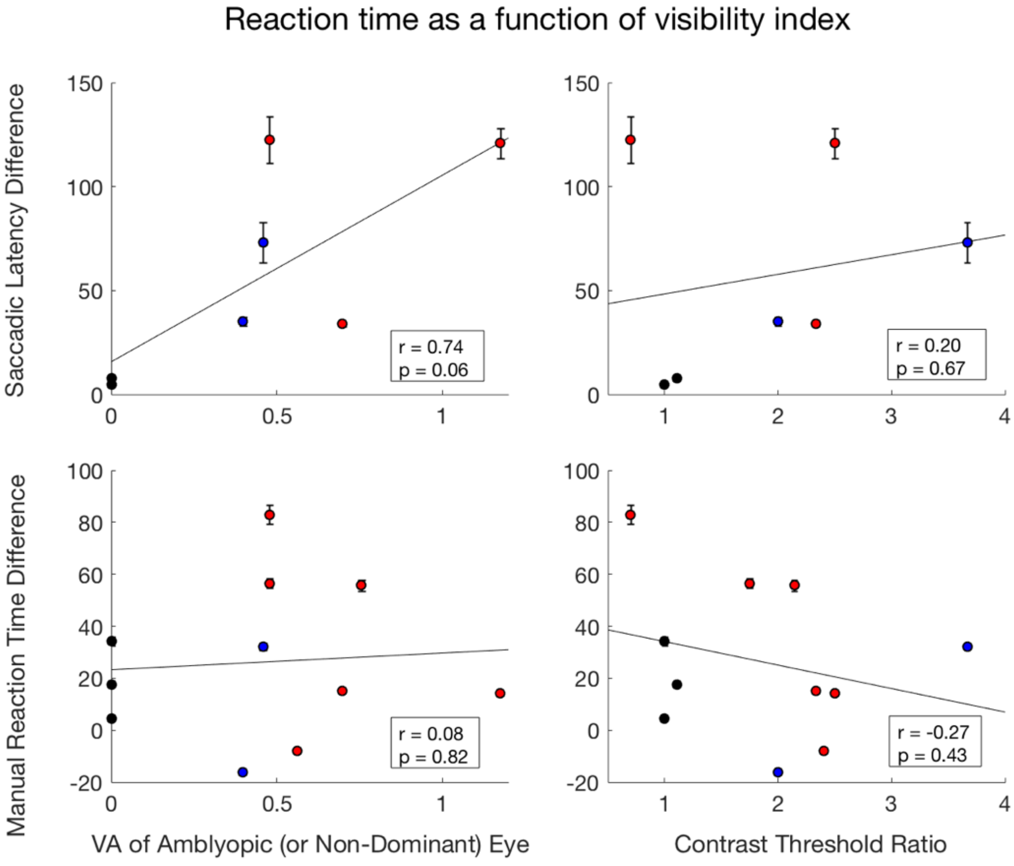
SID		Num Trials	Mean	Standard Deviation	Levene's Statistic (p value)
AE NAE	S1	102 60	477.22 433.65	119.25 88.89	3.25 (p=0.07)
AE NAE	S2	42 26	377.37 366.77	58.54 47.30	0.17 (p=0.68)
AE NAE	S3	33 26	480.53 424.80	104.38 110.44	0.06 (p=0.81)
AE NAE	S4	52 53	412.94 412.71	47.26 49.16	0.00 (p=0.95)
AE NAE	S5	62 30	401.57 376.44	72.72 52.68	3.87 **(p=0.05)***
AE NAE	S6	36 39	447.29 401.74	58.67 59.23	0.63 (p=0.42)
AE NAE	A1	30 32	403.03 386.19	62.89 46.56	0.38 (p=0.54)
AE NAE	A2	33 37	387.25 388.92	38.85 32.50	0.04 (p=0.85)
LE RE	C1	29 29	456.90 389.16	210.64 96.88	8.39 **(p=0.01)****
LE RE	C2	31 31	453.44 419.12	95.85 64.22	1.69 (p=0.20)
LE RE	C3	26 29	390.85 369.92	48.96 29.85	1.95 (p=0.17)

2.3.4 Relationship between reaction time difference and sensitivity

There was a trending relationship between the visual acuity of the amblyopic eye and the magnitude of the saccadic latency difference between eyes (r=0.74, p=0.06). This finding is remarkably similar to the robust relationship reported by McKee et al., (2016) where they found a correlation of r=0.75 with 393 observers (p≤0.001). Unlike Hamasaki and Flynn's 1981 finding, we find no relationship between manual reaction time and the visual acuity of the amblyopic eye, but their study only looked at manual RT in strabismic amblyopes to the appearance of a single, bright target.

Interestingly, we also did not find relationships between the threshold ratio and reaction time difference for both saccadic latencies and manual reaction time measures. This finding is important in that it shows that there is no residual effect of contrast sensitivity in this study. By normalizing for CT threshold, we can effectively remove this factor from the RT differences that were found in earlier studies.

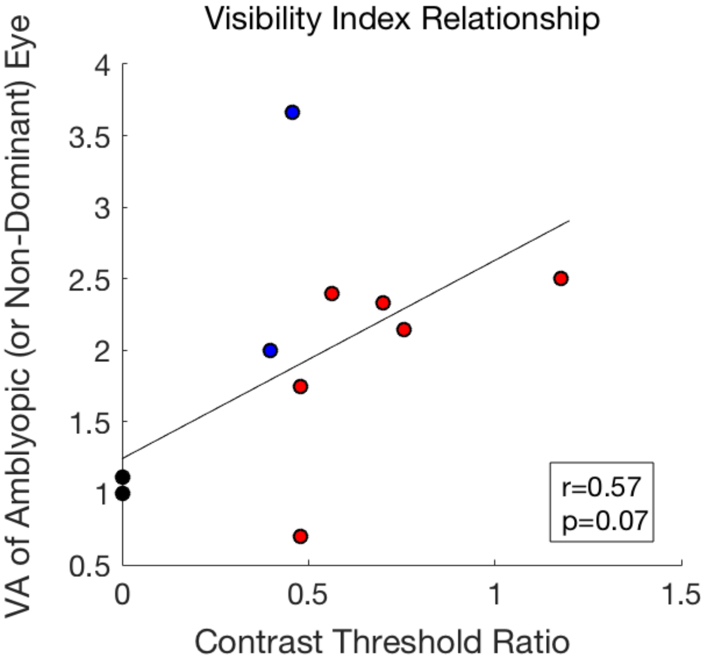
Figure 2.4. Reaction time differences as a function of visibility index.



The left hand columns show the between eye saccadic latency (top) and manual reaction time (bottom) differences as they relate to the acuity of the amblyopic or non-dominant eye. The right column shows the same plots, but with the contrast threshold ratio instead of the visual acuity. For all plots, the strabismic or strabismic aniso patients are red, anisometropic amblyopes are blue, and controls are black.

Further investigation showed that the logMAR acuities and contrast threshold ratios were correlated in our observers, with the strabismic group having poorer contrast thresholds than the two anisometropic observers (see Figure 2.5).

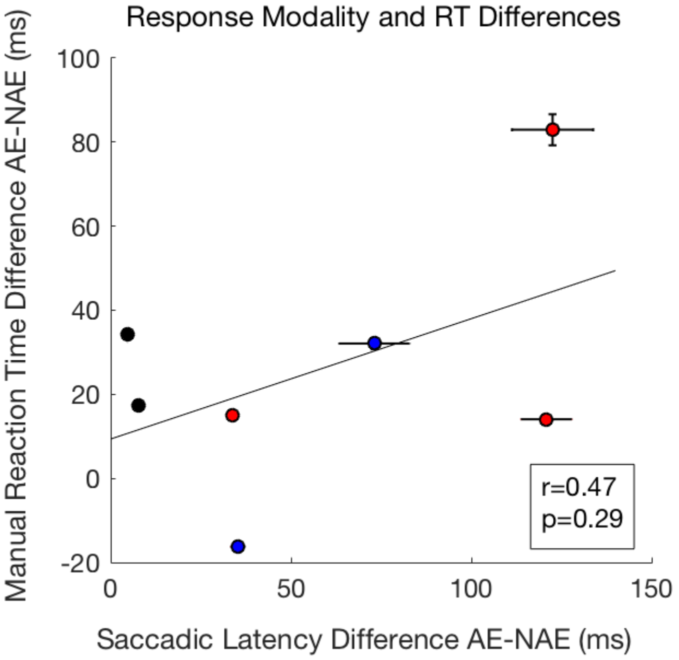
Figure 2.5. The relationship between visual acuity and contrast threshold in the six strabismic (red) and two anisometropic amblyopes (blue), and three age-matched normally-sighted controls (black) that participated in the study.



2.3.5 Relationship between saccadic latency and manual reaction time

In general, patients with larger differences between saccadic latencies in the two eyes also tended to have larger differences between manual reaction times. This overall effect was not significant, but this is likely due to our small sample size (n=7) that completed both the saccadic latency and manual reaction time measures.

Figure 2.6. Relationship between saccadic latency and manual reaction time differences in all participants that completed both measures.

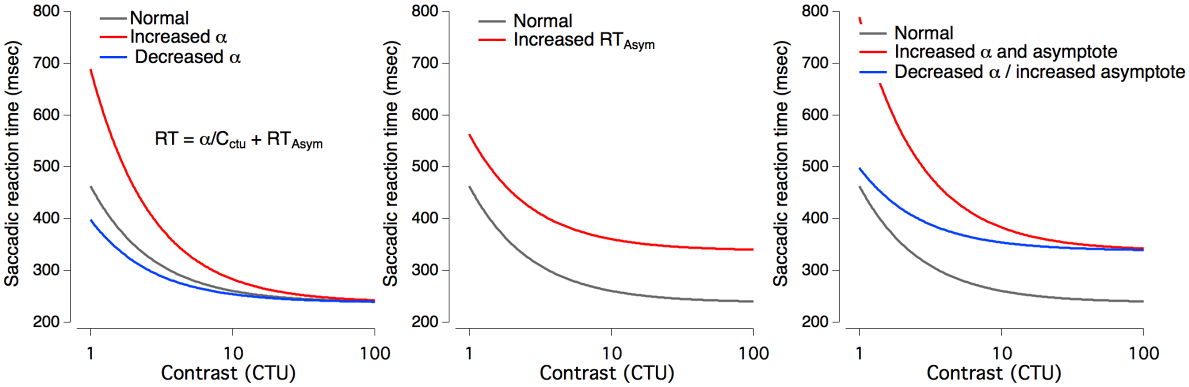


2.3.6 Parallel vs. intersecting burr functions

While the Burr functions for control observers are almost completely superimposed, showing that the reaction-time difference between their two eyes is null for all contrasts, we find a different pattern in the amblyopic patients. In some cases, such as S1, S3, and S6, the two functions are parallel, suggesting an additive effect. Alternatively, other patients have intersecting functions, such S2 and S5. In these cases, the amblyopic eye is faster at the threshold level, but also reaches a plateau earlier. While it’s possible that these patients had overestimated thresholds for their amblyopic eyes, and/or underestimated thresholds for their non-amblyopic eyes, it’s also possible that more than one factor is causing the reaction time delay, and that this factor acts differently at different contrast levels.

While we are unable to answer this question with the current data set, the asympototic value with the Burr fits would be unaffected by an over or underestimated contrast threshold. This can be seen in Figure 2.7, which plots hypothetical Burr fits, manipulating both coefficients. The left figure shows that if the threshold were over (red curve) or under (blue curve) estimated in the AE, alpha, which determines the slope, would be increased or decreased, but the two curves would still converge at the asymptote. Alternatively, in the middle plot, the two curves are parallel over the entire range. In this case, alpha is identical and the parameter determining the asymptote is greater in the AE. This is exactly the pattern observed in several of our strabismic observers. Finally, in the plot on the right both parameters are different from normal. In this case, while the slopes are very different in the three conditions, it's clear that the red and blue lines do not converge with the curve from the normal eye, showing an irreducible delay.

Figure 2.7. Hypothetical Burr fits.



2.4 Discussion

The goal of the first aim was to determine if sensory differences could account for the delayed response time in amblyopic patients. In other words, if the effective stimulus strength were normalized, would amblyopes still be slower when responding with their amblyopic eye? Would the results be different between the strabismic and anisometropic amblyopes?

To determine this, we calculated the contrast threshold of the stimuli for each eye, measured the saccadic and manual reaction time at several intensities that were multiples of this threshold, and fit these data with a Burr function. From our results we can confirm that many strabismic amblyopic patients have an irreducible delay at the asymptote. That is, even when the sensory differences of the stimulus are accounted for, several amblyopes still had large interocular difference (average of 77ms) in saccadic latency, and a smaller interocular difference (average of 25ms) in manual reaction time.

These findings replicate and extend the previous findings of reaction time in amblyopia. Levi and colleagues (1979) measured manual response time as a function of contrast and found a significant difference between the eyes of strabismic patients. However, it is difficult to make strong conclusions regarding interocular differences from their data, given the fact that the target contrast levels were the same for both eyes. This design resulted in an effective stimulus strength for the AE that was weaker than the NAE at each contrast level. When McKee et al. (2016) replotted the original data as a function of contrast threshold units, this RT difference was much smaller.

Pianta and Kalloniatis (1998), also measured manual reaction time differences in amblyopic observers and normalized the contrast between the two eyes to account for sensitivity differences. They report no RT differences in all four of their anisometropic amblyopes. While our two anisometric amblyopes also follow this pattern, our results highlight why it it's important not to generalize the previous findings to the entire patient group, as we find a much different pattern in strabismic amblyopes.

As was described in several other studies (e.g. Ciuffreda, Kenyon & Stark,1978ab; McKee et al., 2016) which measured reaction time in patients with amblyopia, there was considerable variability between the patients. This highlights the need for reporting individual results. In studies (e.g. Mackensen, 1958) where only the mean results are reported, the effect can be blurred, as some patients can have large differences between the two eyes (upwards of 100ms), while others have no significant difference.

We also see considerable variability in response times in several of the patients in the AE compared to the NAE. Further, this between eye variability measure was related to the size of the saccadic latency difference between the two eyes, at least in the case of the strabismic amblyopes. This pattern may have also been present in the manual reaction time data, but to a lesser extent. We hypothesize that microsaccade frequency is a major contributor to the latency differences between the two eyes. The outcome of this would be temporal jitter in SL, which could be causing the increased SL in the AE. The increased microsaccadic rate could also be affecting the manual response times through small spatial shifts in foveal attention.

While many other studies have explored saccadic (Ciuffreda et al., 1978ab; Mackensen, 1958; Niechwiej-Szwedo et al., 2010; Niechwiej-Szwedo et al., 2012; McKee et al., 2016) and manual reaction time (Mackensen, 1958; von Noorden, 1961) in patients with amblyopia, this is the first time that both are measured as a function of effective stimulus. We believe the extent of the two delays may be related, however more subjects would be needed to draw any firm conclusions on this.

Participant C1 was an interesting case in that she was an alternating strabismic patient. If the strabismus itself was the cause of the RT delay, both eyes should be slower than that of normal controls. Instead, her RTs were within the normal range, for both response modalities. This points to a potential explanation of the delay being related to the lack of experience with fixing with the AE and the attentional mechanisms that provide accurate oculomotor control when doing so. This participant did experience more variability in her LE than her RE while performing the manual response task, particularly when targets were highly visible. It is possible that there is some remaining higher level deficit due to a lack of an intact binocular system that could be explore further in future studies.

While this study helps eliminate a main confound of earlier work, that reaction time differences between the amblyopic and non-amblyopic eye may be due to sensory differences alone, it is worth noting several weaknesses with the current design. First, many of the amblyopic patients had difficulty with the contrast threshold measurement in the amblyopic eye. Patients often fatigued toward the end of this procedure, which was also when the contrasts of the targets were closest to threshold. As the subsequent trials were targets based in contrast threshold units, any error in this original measurement would also appear in the subsequent RT measures. However, Figure 2.7 shows that while threshold error may affect the slope coefficient, it should not remove the irreducible delay, should one exist.

Unfortunately, we were unable to investigate express saccade frequency, due to noise when tracking both eyes of the amblyopes, but particularly the AE. One reason for this was the fact that several of our amblyopes had large refractive errors, including high astigmatism. Spurious reflections off of the corrective lenses worn by these patients created false responses to be recorded. To reduce potential errors, we filtered the data to assess RT at the range of greatest signal, between 120 and 800ms. A negative outcome of this was the elimination any express saccades, which usually occur between 80 and 120ms.

There was also more positional variability when viewing with the amblyopic eye. This led to difficulties in eye tracker calibration for several of our patients. Achieving a calibration file with errors of 1 degree or less for all 5 points could take many attempts. If the patient moved slightly during data collection, the calibration file would no longer be valid and the entire procedure would need to be restarted. In some cases, finding an appropriate threshold for the amblyopic eye could take as long as an hour. For these reasons, we were unfortunately unable to measure fixation data, and instead only recorded saccades that were greater than 1 degree as a response. Smaller eye movements during fixation may cause a refractory period and delay RT, but these events were not recorded. We will attempt to measure the effect of fixational saccades indirectly in our next aim, which removes the fixation spot prior to target onset.

One interesting confound that exists in all studies that measure reaction time as a function of stimulus intensity is the issue of different attentional networks at play. While highly salient stimuli may trigger an automatic response through the exogenous network, this is not the case for stimuli near the threshold. When these targets appear, participants must direct endogenous attention to the left and right while maintaining fixation at the center of the screen. This process may be more challenging when viewing with the amblyopic eye, and could also account for some of the difficulties in measuring the contrast threshold with that eye.

Exactly where this transition from exogenous to endogenous attention occurs and how many of the intensity levels are overlapping with input from both networks is unknown. This could, however, account for some of the observed differences in slope for the amblyopic vs. nonamblyopic eye. As many of the amblyopic eye functions were more shallow than the non-amblyopic eye, it could mean that there is more overlap between the networks, or lower involvement of the top-down network overall.

This study adds to the knowledge of reaction time in amblyopia by carefully controlling for sensory differences between the two eyes. By measuring manual and saccadic reaction times as a function of contrast sensitivity, we show that in many patients there is still an irreducible delay, particularly in saccadic latency. This delay is also related to the variability in response time while viewing with the amblyopic eye. In the next experiment we will investigate the role of active fixation in this irreducible delay. As previously mentioned, when the eye is trying to maintain fixation small eye movements may delay RTs, but these movements were out of the range of our measurements. It's possible that the AE requires greater effort to maintain fixation and that this is resulting in the slower RT in our strabismic amblyopes. By removing the fixation spot prior to target onset this should eliminate or reduce fixational effort, and potentially eliminate the irreducible delay.

Chapter 3

Modulating Fixation Offset: The Gap Effect in Amblyopia

3.1 Introduction

In the previous study we found that strabismic amblyopes had an irreducible reaction time delay that was not dependent upon low-level factors, such as a difference in the visibility of the target. As discussed in the previous section, aside from stimulus differences, there are many other observer-level factors that could contribute to this delay. In the case of amblyopia, we can eliminate some of the most basic observer-level contributors, such as age and cognitive state. It's possible, though, that even with balancing the perceptual input to the two eyes, the neural processing of the visual information is still different, depending upon which eye is viewing the target. In the second study we will use the gap paradigm to explore the role of fixation and foveal attention on reaction time, to see if this is a potential contributor to the delay in the amblyopic eye.

3.1.1 Gap effect

What is it?

In the 1960s, George Saslow was working on a series of saccadic latency experiments in which he was trying to determine if the set size of potential target locations influenced the overall saccadic reaction time to a particular area. In these experiments, a neon bulb at the center of a display served as the fixation target. It was extinguished and at the same time one of up to eight eccentric target lights was turned on. While his main effect was null, that is the set size did not alter the saccadic latency to a particular location, he did find a peculiar effect. When the fixation light bulb was extinguished *prior* to the onset of the target light, the saccadic latency was much faster. These unexpected findings were later termed the *gap effect* and have sparked many veins of fruitful behavioral and neurophysiological work in the subsequent 50 years.

Potential Mechanisms

While the mechanisms that cause this reduction in reaction time are unknown, there are several competing theories, which were reviewed in great depth by Jin and Reeves in 2009. The first is the *oculomotor readiness effect*, suggested by Saslow in his original paper (1967). According to this theory, during fixation the eye must continually work, using small saccades to return the gaze to the fixation point after small drifts. This active process could delay subsequent saccades, including those to the target. Removing the fixation spot reduces these fixational saccades, allowing for a faster response to the target. As previously discussed, this hypothesis was ruled out prematurely by a study with Kingstone et al. (2005), involving only a handful of subjects and a now outdated method of quantifying fixational saccades.

Earlier work by Kingstone and Klein (1993a, 1993b) supported a different set of theories. They believed there are actually two separable mechanisms responsible for the gap effect, a general *warning effect*, and a *fixation offset effect*. The general warning effect proposes that any salient cue, such as a diming of the fixation dot, or a preceding tone could alert the motor system that a change is imminent. Several other groups have confirmed this finding, all with similar effect sizes of about 15-20ms (Pratt et al, 1999; Jin & Reeves, 2009).

In the second part of Kingstone and Klein's gap theory, the reduction in saccadic latency is specifically linked to the fixation process. According the fixation offset hypothesis, active fixation, potentially driven by fixation-related neurons in the rostral pole of the superior colliculus, must be disengaged before a saccade can be initiated. Neurophysiological work that supports this theory will be discussed later. It is important to note that this fixation offset effect is different from the oculomotor readiness effect because it does not involve the production of fixational saccades during active fixation. In Kingstone and Klein's 2005 study they report that these microsaccades do not alter reaction time, so it must be some other active process at fixation that is reduced to speed up saccadic latency in the gap paradigm.

The reduction in saccadic latency could also be in part due to the observer's expectations, heightened by the artificial set-up of laboratory and simple dots of light in predetermined locations. In fact, the gap effect can be reduced when more targets are added to the paradigm (Rolfs & Vitu, 2007), or alternatively can be sped up if only one target location is used (Fischer & Ramsperger, 1984).

The salience or strength of the fixation target may also influence the gap effect. This was tested by Jin and Reeves (2009) and they found a reduced, but still present gap effect when the fixation target was dimmed, as apposed to one which remained bright prior to target onset.

Finally, it is unlikely that the gap effect is due to a speed-accuracy-trade-off. This was investigated by Pratt and Nghiem in 2000, and replicated by Jin and Reeves (2009). Both groups found that saccades to targets without a simultaneous fixation spot were no less accurate than those that did have a fixation target.

Foveal processing vs. attention: can the two be separated?

Overall, there are two main categories of potential explanations for the gap effect: foveal and attentional. Several additional experiments by Jin and Reeves as well as other scientists have been conducted to try and untangle these related sources. Several important clues can help us narrow in on the origins of Saslow's unexpected finding. We now know that the timing of the fixation gap is crucial to it's overall effect. The latency reduction is greatest, reaching 80–100 ms, when the fixation point is removed 200 ms before the appearance of the target (Pratt, Bekkering, Abrams, & Adam, 1999). It's interesting to note that this time frame is consistent with that of a covert shift of attention (Reeves & Sperling,1986).

According to Posner's theories of spatial attention (Posner et al., 1984), a covert shift of attention involves three stages: disengagement from the fixation point, shifting to a new location, and re-engaging on the new target. Under this theoretical framework, the gap effect could initiate the speed up the first stage, such that when a new target appears attention is already disengaged.

One of the first studies to explore the roles of foveal processing vs. attentional engagement was by Braun & Breitmeyer (1988). In their study, subjects attended to a peripheral point that extinguished at various offsets, while viewing a continuously visible fixation target. They found similar effects to the offset of a central fixation point published in previous studies, although they did not test this condition directly. Instead, in their control condition, subjects attended to the central fixation dot, while an unattended peripheral point turned off. They found a small gap effect in this case – on par to what was observed in the 0-gap condition.

Perhaps one of the more important results from these studies are the findings related to the timing of this release of attention. While many other studies only compare overlap and 200ms gap conditions (sometimes including an additional 0ms, simultaneous offset condition), Braun & Breitmeyer conducted 9 different temporal offsets, ranging from -500ms to 500ms, with each subject completing 100 trials per offset. From these careful measurements, they were able to establish a U-shaped curve, with subjects requiring at least 100ms to disengage attention, and that this disengagement can only persist for about 300ms.

In Jin and Reeves's study (2009), they assessed the warning effect by adding in conditions with a color changing fixation target and a dimming target. The warning effect could not account for the entirety of the reduction in saccadic latency. The mean SL in the dimmed condition was about 17ms slower than a blank gap, and the mean SL in the condition where the fixation cross changed color was marginally (6ms) slower than the dimmed condition. They also tested the role of foveal vs. parafoveal targets. The latencies with the parafoveal targets that remained on were almost identical to the latencies with a foveal fixation target that remained on. When the parafoveal targets were extinguished 200ms prior to target appearance SLs were reduced, although to a lesser extent than in the standard gap condition (31ms vs. 62ms in the standard gap). These effects, including reduced latencies without removing the fixation target, or even in cases where fixation is guided by parafoveal targets suggest that the gap effect in influenced by factors other than the release fixational activity.

Positive evidence that covert attention plays a role in the gap effect was contributed by Pratt and Nghiem (2000). When a cue was used to signal the location of the target, they found a larger gap effect with a valid vs. an invalid cue. Pratt, Lajonchere, and Abrams (2006) also reported shorter SLs (by 19 ms) when an attended, rather than unattended, line segment in the fixation cross was removed in the gap condition. This result can be explained by covert attention, but not by general warning, although covert attention would be underestimated if the 'unattended' line segment was partially attended.

3.1.2 Neurophysiology of the gap effect

As mentioned previously, the superior colliculus (SC) is a mid-brain structure that plays a vital role in the planning and execution of eye movements. The structure has a highly organized layout, with fixation neurons located in the rostral pole and saccade neurons that are associated with increasing amplitude along the caudal sections. The balance of activation of these neurons is thought to play an important part in maintaining fixation and suppressing the initiation of unwanted saccades (Munoz and Wurtz, 1992). The SC is often implicated in the gap effect, in particular when discussing the fixation offset hypothesis. Studies by Munoz and Wurtz (1992a,b; 1993a,b) have shown that when the fixation stimulus is removed in the gap paradigm, the activity of the fixation neurons decreases, while the activity of saccade neurons increases, and that this change in activity is related to the reduction in reaction time.

Neurophysiological experiments involving behavior and single-cell recordings have also provided strong evidence that the superior colliculus is important to the engagement and disengagement of attention. Studying its functions may lead to important insights into the gap effect and the role of attention in oculomotor processes. Below a few of the most relevant experiments are discussed, but see Krauzlis et al., (2013) for a more detailed review.

Enhancement and Suppression

Microstimulation of the SC can enhance spatial processing, even without eye movements, in a manner that appears very similar to covert attention. In an experiment by Muller et al. (2005), animals judged the motion in a random-dot motion patch, while irrelevant flickering dots appeared elsewhere in the display. When microstimulation was applied to the intermediate layers of the SC, performance improved and the psychometric function shifted leftward, meaning that less visual motion was needed to achieve a particular level of performance. Importantly, this change was only seen if the site of SC stimulation matched the area representing the patch.

More recently a study by Chen et al. (2015) provided groundbreaking neurophysiological work linking eye movements and attention. Monkeys were trained to fixate a spot, while a grating was peripherally presented in the receptive field of a recorded neuron, located either in the SC or FEF. Responses to the gratings were measured and separated into time frames depending upon whether or not a microsaccade occurred. Neuronal response gains for the grating occurred when microsaccades proceeded target appearance, and conversely were suppressed if the target appeared after a microsaccade. It's also of importance to note that these neuronal changes were measured in both purely visual as well as visual-motor neurons. These findings support the notion that microsaccades can enhance or suppress signals, similarly to the more classically studied effects of attention.

Engagement and Disengagement

Several studies have shown that inactivation of the SC can disrupt attentional processes. In a study by Lovejoy & Krauzlis (2010), monkeys were trained to attend to a particular location in space, cued by a red ring. A pulse of motion occurred at this location and monkeys had to indicate the direction of motion with either a saccade or a button press. At the same time, monkeys needed to ignore a distractor pulse of motion in the opposite location. After inactivation of the SC, performance for the affected hemisphere dropped, and the animal often responded with the direction of the non-cued side. These findings suggest that the SC is involved in selecting and maintaining covert attention. Further, as performance was impaired in both the saccade and manual response task, these results suggest that the SC contributes to a generalized allocation of attention, not limited to the oculomotor domain.

Another study showed that inactivation of the SC can impair attentional disengagement in rats. de Araujo and colleagues (2015) trained rats in a two-alternative light-guided spatial choice task. In 75% of the trials, the rats needed to attend to a light on one of two tubes that signaled a large reward. This cued-location was randomly assigned in the beginning, but was consistent for each animal. In 25% of the trials, however, both lights turned on. In these trials, the rat needed to disengage attention to the light which previously signaled a reward and move to the opposite tube, which now had the large reward. The process required all three stages of Posner's attentional processing framework. To be successful, the rats needed to disengage the previously rewarded cue, switch to the opposite cue, and re-engage attention. At baseline, the animals could perform this task very well (approximately 100% and 90% accuracies for the frequent and infrequent trial types). However, after muscimol injection into the SC, the rats could still perform the standard task at nearly 100%, but the trials that required disengagement and reengagement fell to nearly 0% accuracy. The rats which previously could appropriately disengage, shift, and re-engage their attention lost this ability. These studies highlight the importance of the SC in modulating spatial attention.

Human fMRI studies elicit of retinotopic SC activation with covert attention

Evidence linking attentional modulation and superior colliculus activity has also been achieved through functional magnetic resonance imaging in people. A series of experiments by Katyal et al. (2010, 2014) provides interesting insights into the mechanisms of spatial attention and how they might be disrupted in patients with amblyopia. Using high-resolution imaging, they established that the SC in people responds in a retinotopically selective manner and that there are distinctions between the responses in the various layers (Katyal et al., 2010). Responses evoked by a discrimination task with high-contrast stimuli were seen both in visual and visuo-motor neurons in the SC.

In a follow-up study, they measured the attentional baseline response in the SC during a threshold-contrast detection task in which subjects attended to the left or right hemisphere (Katyal et al., 2014). They found retinotopically selective responses for covert attention, even in the absence of significant visual stimulation. Further, the SC responses to the different tasks had different depth profiles, with the threshold-level stimuli responses appearing deeper than the high-contrast discrimination stimuli. They proposed that the more superficial responses for discrimination corresponded to superficial and intermediate neurons, while the deeper neurons activated in the detection task corresponded to cue-evoked baseline enhancement of the visuomotor neurons. These levels could connect to different parts of the attentional network, such as the FEF.

Visual Experience Is Required for the Development of Eye Movement Maps in the Mouse Superior Colliculus

One more key piece to this puzzle involves the development of the SC. As mentioned in the previous series of studies, the SC responds retinotopically to drive eye movements as well as covert attention. But what happens in the case of amblyopia, when the SC doesn’t receive proper input during development? Evidence suggests, at least for mice reared in dark environments, that these eye movement maps can be disrupted (Wang et al., 2015). Compared to mice reared in normally-lighted conditions, the dark-reared mice experienced more spontaneous saccadic eye movements and larger evoked saccades. This was particularly the case for saccades evoked from anterior locations, resulting in a shallower slope along the anterior/posterior axis.

It is possible that these degraded visual maps in the SC also exist in patients with amblyopia, albeit to a lesser extent. These degraded maps could result in impairments in the enhancement and suppression of visual information, as well as disengaging attention and shifting it to a new location. Extra noise in the SC could also result in difficulties with maintaining fixation. In the next section we’ll discuss some results that suggest that this might be the case. We will focus on the potential functions of fixational saccades and how disruptions to these could impair spatial vision in patients with amblyopia.

3.1.3 Temporal encoding hypothesis: large implications for perception

Based on a series of new theoretical and experimental results (Kuang et al., 2012; Rucci & Victor, 2015), a new vision for the role of fixational eye movements in perception is beginning to take form. Instead of being viewed as something that does not occur in free viewing, or an unfortunate outcome of neural noise that smears photons across the retina, a growing consensus now believes that small movements during fixation may actually be an efficient strategy for acquiring information. As the eyes move, they don't just refresh the retinal image. Instead these movements could restructure the spatial information into a spatiotemporal format that emphasizes high spatial frequencies. This amplification occurs up to a cutoff frequency that is close to the spatial resolution of the photoreceptor array. This net effect creates output with approximately equal amplitudes over a wide range of spatial frequencies, a process known as spectral whitening (Kuang et al., 2012).

This restructuring is known as temporal encoding and potentially results in a highly efficient strategy for acquiring information from natural scenes. As the power spectrum of natural images declines with spatial frequency in a way that is approximately proportional to the square of the of the spatial frequency (Field, 1987), high spatial frequency information is perceived as fainter than lower spatial frequency information. The motion on the retina from small fixational eye movements can enhance fine spatial detail, effectively equalizing the power present at different spatial frequencies in the real world. This process also removes correlations in space, and could potentially be a mechanism for feature extraction in early visual processing.

Taking this theory one step further, if the visual system has tight control over the regions being sampled with these small fixational eye movements, it would allow us to exploit these input changes in situations when detailed information is needed. In a sense, by controlling the temporal and spatial properties of fixational eye movements, one could actively control the scale of visual processing for that part of the scene. Short fixation durations would allow one to quickly assess the structure of the scene from low spatial frequency information. Longer fixations would give time for fine spatial information to be extracted from fixational eye movements.

Laubrock et al. (2013) provided evidence that this might be the case. They created four types of gaze-contingent natural scenes: foveal low-pass, foveal high-pass, peripheral low-pass, and peripheral high pass. Participants were told to inspect each scene, visible for 15 seconds, and then answer a question about scene content after each trial. In the foveal high-pass filtering condition, they found that the number of short fixations was significantly reduced, while the number of longer fixations increased. This shows subjects can use adaptive strategies to control the temporal parameters of eye movements depending upon the task and information present.

In sum, these studies highlight the importance of flexibility and control for fixational eye movements. They also point to a potential source of visual impairment that has not been explored. If high levels of noise or a lack of precision exists, that could impair an individual's ability to perform high acuity tasks, and even difficulties in directly spatial attention. These symptoms align with those observed in amblyopia. In order to fully understand how perception is impaired in amblyopia, we must consider not just the stimulus, but also the eye movement activity of the observer. Along with the impaired ability to precisely move the preferred locus of fixation to the desired location in space, fixational velocities that are too slow or too fast could disrupt information processing by blurring the image or reducing the range of whitening to the retinal input.

Additionally, there may be one or more feedback loops linking fixation to cortical selection. These could be disrupted during development in a child with amblyopia. In the next section we will discuss what is currently known about the differences in fixational eye movements in patients with amblyopia, both in comparison to their non-amblyopic eye, as well as in comparison to normally sighted children and adults with intact binocular vision.

3.1.4 Measuring fixation stability in normal and patient populations

Fixational variability in the normal population

Older methods of measuring microscopic eye movements, such as the optical lever technique or the use of eye coils in magnetic fields, were time consuming and invasive to the individual. As a result, many early studies measuring fixation stability in normally-sighted subjects used a small number of highly-trained observers. A recent study by Cherici et al. (2012) investigated fixation using a video-based eye tracker in a sample of 14 observers, 11 of which had never participated in a vision research experiment before. They aimed to not only measure the variability of fixational precision in this large population, but to also to link individual differences in the characteristics of ocular drift and microsaccades to the precision in these observers.

When a 4' fixational spot was used, the span of fixation—the area containing the line of sight with 0.75 probability—had a mean of 367 arcmin^2 and a standard deviation of 251 arcmin^2. More interestingly, the average span of the untrained group was more than three times larger than the span of the experienced observers. Additionally, the range of precision across subjects varied by a factor of 14: 71 arcmin^2 for a highly trained observer to 994 arcmin^2 for the noisiest naive subject. The overall distribution of fixation points also varied greatly across subjects, including radial symmetry and elliptical distributions in both the horizontal and vertical dimensions.

Cherici et al. (2012) also measured fixation stability in these subjects in the absence of a fixational spot. Under these conditions, fixation stability deteriorated drastically. Precision decreased by a factor of 1.45x to 9x, and the gaze distribution also changed shape in several observers. These changes were complex and idiosyncratic, yet the precision in subjects between the two conditions was highly correlated ($r=0.6$, $p<0.03$).

Saccade rate also varied greatly across subjects, from approximately 0.44 saccades/s to 2.29 saccades/s when the fixation dot was present. While the experienced observers made significantly fewer saccades, there was little to no difference in saccadic amplitude between the two groups (17' vs. 20'). Without the fixation dot the rate of saccades decreased significantly, mainly due to a drop in saccadic rate for the naïve observers (1.47 saccades/s decreased to 1.12 saccades/s). The average amplitude also increased in all observers from a mean of 20' to a mean of 40'.

As mentioned previously, one of the main aims of this experiment was to link individual differences in the characteristics of ocular drift and microsaccades to fixational precision. The average measured drift was 52'/s, but this varied by a factor of about 3 across observers, from 30' in a highly experienced subject to 89'/s for the noisiest individual. Several subjects exhibited a bias in the direction of drift, such as a downward left direction, bidirectional, or circular movement. The authors also found that the instantaneous drift speed was significantly correlated with saccade rate ($r=0.62$, $p<0.02$), showing that overall subjects that had faster drift were also more likely to make frequent saccades. Drift slightly increased in the condition without a fixation spot (from a mean of 52'/s to 57'/s), and a preferred drift direction was now present in all observers.

The analysis of drift provides evidence that saccades and drifts counteract each other during fixation. Saccades were significantly more likely to move the eye in the direction opposite to that of the preceding period of drift than in a similar direction. This was particularly the case in subjects with a pronounced bias in the direction of drift. This shows that saccades may act to compensate for drift, a hypothesis proposed by Rolfs in 2009.

Finally, using a multiple linear regression model with four variables, saccade rate, saccade amplitude, drift speed, and drift curvature, they were able to predict the fixational instability across trials. Saccadic variables were greater contributors in 8 of the 14 observers when a fixation dot was present, and all by two observers in the blank screen condition. Thus, saccades contributed more to the dispersion of gaze than drift, and this was particularly the case in the absence of the fixational dot. The presence or absence of the dot modulated both rate and amplitude of saccades, and these variables were highly correlated between the two conditions.

This shows that even in subjects with different saccade characteristics, oculomotor behavior was affected in a similar manner when a fixational stimulus is removed.

In sum, this study produced several highly important findings. First, observers were less accurate in maintaining prolonged fixation than is commonly believed and there was considerable variability across individuals. The two observers with the best fixational precision in this study were also highly trained experts. Only these subjects are in the range of previously reported ranges of fixation, providing evidence that practice and/or motivation can change fixational performance. Second, the findings provide evidence that both drift and saccades contribute to the maintenance of fixation, and that these two eye movements tended to counteract each other during fixation. Finally, drift speed was also approximately one order of magnitude larger than previous values reported in the literature (typically 4'/s; Ditchburn, 1973), and was related to the overall precision of the observer's fixation. Although this study significantly increases our understanding of fixational abilities in the normal population, there is still a large gap in the literature regarding how these differences relate to one's perception. It would have been interesting if the authors had included a measure of letter and/or hyperacuity to see if there was a relationship between fixational accuracy and acuity in this large, heterogeneous population. Hopefully future studies that explore fixational variability will relate this to behavioral performance.

Fixation stability development during childhood

While information on how fixational precision changes during development is sparse, a recent study by Tiadi et al. (2016) helped shed some light on this by measuring fixation in a population of 55 children aged 7-14. After calibration, the children fixated the center of a white dot of 0.5 degrees for 15 seconds, while saccades greater than 2 degrees were measured. The authors found that the number of saccades decreased significantly with the age of the children, suggesting that the quality of the fixation improves with age. This finding is in line with previous studies exploring fixation in younger populations (Munoz et al., 1998; Ajrezo et al., 2013) and provides evidence that the quality of visual fixation in younger children is poor and improves through adolescence. The authors speculate that this could be due to the fact that the frontal and prefrontal cortices involved in fixation and saccadic suppression are still developing in young children, and also that this could be tied to the development of visual attention throughout adolescence.

Fixation is impaired in amblyopia

Fixational eye movements in amblyopic patients have been of moderate interest for almost 40 years, yet the field is still far from reaching a consensus. This is most likely due to the fact that eye tracking technology has changed significantly over this time and there is still not a standard method to classify the various types of amblyopia.

The first discovery was that the speed of slow drifts is higher and the amplitude is larger in amblyopic than in normal eyes (Ciuffreda, Kenyon, & Stark, 1979; Schor & Hallmark, 1978). Ciuffreda and colleagues (1979) also reported the occurrence of “saccadic intrusions” at a rate of about 1 per second second (range: 0.3–2 per second). The frequency and amplitude of these intrusions increased when the amblyopic eye monocularly fixated the target.

More recently, as our eye tracking technologies became more robust and more accessible, several scientists have returned to this research area to deepen our understanding of fixation in amblyopic patients. Shi et al. (2012) used a high-speed eye tracker to track monocular fixational eye movements in the AE and NAE of 28 anisometropic amblyopes as well as 28 age-matched controls. They noted several differences in the fixational saccades (FSs) when viewing with the amblyopic eye, including fewer FSs compared to the NAE, but those that did occur had increased amplitudes, increased peak velocities, and longer inter-saccadic intervals. They performed a new simulation model analysis to suggest a potential cause for these altered FSs: an excitatory-inhibitory activity imbalance in the SC. They speculated that the altered FSs *“may be an effect of amblyopia, an attempt of the visual system to try to capture more information from a broader spatial domain to enhance contrast sensitivity, or it may be the cause or a contributing factor to the original deficit, which may open up a new avenue of research of the neural mechanisms of amblyopia that will be as different form the traditional research route as the pioneering work in visual cortex by Hubel and Wiesel*.”

BCEA as a summary measurement of amblyopic fixation

As the importance of normal fixation on visual function has recently received much attention, scientists are returning to reassess fixation in clinical populations such as amblyopia to better understand how these patients differ from the normal population. Subramanian et al. (2013) measured fixation instability in a large population (n=89) of amblyopic children aged 5-17 y.o. with a Nidek MP-1 microperimeter and a 1 degree ring target. Children viewed the target for 30 seconds, during which the instrument calculated the shift between the reference image and the real-time fundus image at 25 Hz. The resulting fixation points were used to calculate the BCEA for each child.

Their main finding was that the BCEA was significantly larger for the amblyopic group (amblyopic eye = 0.56, nonamblyopic eye = 0.2) compared to a group of non-amblyopic children with strabismus and/or anisometropia (right eye = 0.28), as well as a group of control children with normal vision (right eye = 0.12). Fixation stability was significantly poorer in the amblyopic eye than all other conditions, including the fellow eye, the non-amblyopic group with strabismus and/or anisometropia, and control subjects. The mean BCEA was also larger for the right eye of children in the non-amblyopic group compared to the normally-sighted control group.

BCEAs can also be described by their vertical and horizontal axes. The authors found that the amblyopic eyes had significantly greater major/minor axes BCEA ratios (1.8 vs. 1.5), meaning that the distribution of fixation points was more elliptical in the BCEAs of amblyopic eyes than in right eyes of normal controls. The length of the horizontal axis was also greater in amblyopic group (AE = 3.53 degrees, NAE = 1.98 degrees) compared to the right eye of the control group (1.62 degrees). There was less variability along the vertical axis, but there was still a significant difference between the amblyopic eye (1.99 degrees) compared to the fellow eye (1.28 degrees) and right eye of the controls (1.12 degrees).

The authors also looked to see if the increase in fixation stability was related to clinical variables. They found significant correlations between BCEA and visual acuity ($r=0.6$, $p<0.001$) and stereoacuity ($r=0.25$, $p=0.05$). The visual acuity relationship held for strabismic and mixed type amblyopes, but not the anisometropic group. This is not surprising given that other groups have found that strabismic amblyopes on the whole have greater fixation instability. They did not find relationships with the age of onset, and treatment history (both surgery and length of treatment). This is surprising, given that one would expect fixation stability to improve with changes in acuity, but this was not measured directly. It also hints that specific training of fixation may be important in the recovery of visual function in this population, as well as a potential for top level effects such as visual attention and cortical feedback of eye movements.

Unfortunately, the instrument used by Subramanian et al. (2013) is relatively slow at recording fixation points (25 Hz). This does not allow for the characterization of eye movements and the authors could not differentiate from drift and microsaccades. This study was helpful in that it was one of the first to quantify the overall stability of fixation in amblyopic children, non-amblyopic children with similar visual factors, and normally-sighted control children, however it does little to elucidate the underlying cause of this instability.

A more recent study by Chung et al. (2015) used a scanning laser ophthalmoscope and a cross-correlational technique to model the top contributors of fixation instability and impaired visual acuity based on a population of 28 adult amblyopes. To identify microsaccades, they smoothed the raw traces with a 5-sample moving average filter and applied a velocity cutoff of 8 degrees/second. From the saccadic measurements several parameters were quantified, including the speed, amplitude, rate, and error, which was defined as the distance between the landing position of a microsaccade and the mean retinal location upon which the fixation cross fell. After isolating the microsaccades, eye movements between these events were considered drift, and the drift speed and amplitude were also measured. They also quantified fixation stability for each condition with a BCEA measurement.

In all cases aside from the speed of drifts, the strabismic AE was significantly different from the control eye, anisometropic AE, and the fellow of the strabismic group. The anisometropic AE was often different than the control eye and fellow eye, but to a lesser degree than the strabismic group. BCEAs were larger in strabismic AEs than control eyes, anisometropic AEs, and strabismic fellow eyes. They also found a significant difference between the two eyes of anisometropic amblyopes.

The authors then used these measures to determine the relative importance of the fixational eye movement components, along with visual acuity and type of amblyopia, to the variance of log BCEA with a multiple regression model. Overall, their model was able to account for 87.7% of the variance of the BCEA, with the greatest predictors being the error magnitude of microsaccades, measured as the distance between the landing position and preferred retinal locus (37.62%), acuity (14.9%), amplitude of microsaccades (13.36%), and frequency of microsaccades (13.02%).

Similar modeling was done to assess the greatest contributors to variability in visual acuity. For this trait the greatest predictors were the error magnitude of microsaccades (30.94%), fixation stability (20.81%), amplitude of slow drifts (14.19%), and amplitude of microsaccades (12.40%). For visual acuity the model was able to predict 67.1% of the variance.

Fixation stability and Visual Acuity

Given the fact that fixational eye movements have been linked to the processing of high spatial frequencies, it seems plausible that disruptions in the control of fixation would impair visual acuity. Gonzalez et al. (2012) found no significant correlation between fixation stability and visual acuity, however, they only had 13 subjects. Subramanian et al. (2013) used a much larger population of amblyopic children (n=89) and found significant correlations for the strabismic and mixed groups, but not for the anisometropic group. Chung et al. (2015) also found a significant correlation between fixation stability and acuity in the amblyopic eye. This relationship held for the strabismic group alone (r=0.627) and to a lesser extent, the anisometropic group alone (r=0.555).

Further, Chung's group used mediation analysis to help tease apart the direction of causality for fixation stability and visual acuity. Mediation analysis (described in greater detail in Calabrese et al., 2014) uses multiple linear regression modeling to estimate the coefficients between the independent and dependent variables. The results of this analysis is 1.062, and this implies that the observed findinga are completely due to the effects of the amplitude of microsaccades on fixation stability and fixation stability on acuity.

The mediation analysis also shows that the amplitude of slow drifts is an important factor limiting acuity in the amblyopic eye. This makes sense given the theoretical function of drifts to enhance high spatial frequencies as described in Section 3.1.3. Chung and colleagues speculate that the larger drift amplitudes in the AE would "*smear the retinal stimulus across a greater retinal area, causing a degradation in acuity*". In the theory proposed by Kuang et al. (2012), the slow drifts, which are linked to the enhancement of high spatial frequencies, behave similar to Brownian motion, where there is no preferred direction for the random motion. In the case of strabismic amblyopes, however, their drifts often exhibit a predominant direction along the horizontal direction (Subramanian et al., 2013). Thus, this disruption to the normal drift behavior could play a part in the reduced acuity in the amblyopic eye.

Intact binocularity may be important for fixation stability

Recent evidence also points toward the importance of binocularity for accurate fixational control. González et al. (2012) measured the fixation stability (as defined by the BCEA) of both eyes under three conditions: binocular, and monocularly with each eye. In normally-sighted controls, there was no differece between the two eyes, however, when both eyes viewed the target, the fixation stability was significantly better.

Conversely, in patients with amblyopia, fixation stability of the the fellow eye was similar to that of controls when viewing binocularly or monocularly with the fellow eye, but not when viewing with the amblyopic eye. Fixation stability with the amblyopic eye was poorer in all conditions. Similar results were found for the microsaccade rate. In normally-sighted controls, binocular viewing produced a significantly lower rate of microsaccades than monocular viewing with either eye. In the amblyopic group, there was no significant effect for viewing condition (monocular with either eye and binocular).

In another study, Shaikh et al. (2016) used an infrared permissive filter to measure binocular eye movements while observers fixed monocularly. The authors used an unsupervised clustering method previously described by Otero-Millan et al. (2014) to isolate fixational saccades from drift periods. Not surprisingly, they found that the median amplitude of fixational saccades and BCEA measurements were greater when the subjects viewed with the amblyopic eye. Further, they also reported that these measures increased in relationship to the degree of severity of amblyopia. For example, the population median for severe amblyopes was 1.28 degrees, which is beyond the cutoff used in the conventional definition of microsaccades (1 degree or less). There was also a significantly reduced frequency of microsaccades measured when viewing with the amblyopic eye. Finally, in subjects without stereopsis, there was an increase in the fixational saccade amplitude of the non-amblyopic eye when the amblyopic eye was viewing which was not significant in subjects with gross stereopsis.

Together, these studies of fixational eye movements in patients with amblyopia show that increased fixational instability and vision loss go hand in hand. As we saw in the first study, reduced acuity is also related to the delay in the saccadic and manual reaction when viewing with the amblyopic eye. The goal of this study was to attempt to relate reduced foveal attention, which should also be related to increased fixational instability, to the reaction time delay, via the gap paradigm.

3.1.5 Hypotheses for Aim 2

In sum, behavioral and neurophysiological work related to SC has demonstrated that this brain area is of great importance to the control of fixational eye movements, and in turn, to the modulating of foveal processing of visual information. It is thought that this structure may code the location of objects in space relative to the fovea (Schiller and Stryker 1972) and this information may be used to direct future saccadic eye movements. When focal lesions are made to the SC, saccadic latencies can be increased by 150 to 300ms (Wurtz and Goldberg, 1972).

Additionally, we know that amblyopia could potentially impact the functioning of the SC. Gordon and Presson (1977) showed that in kittens reared with unilateral strabismus, and presumably amblyopia, the ability of the affected eye to drive cells located in the superior colliculus was impaired. We also know that the SC contains eye movement maps, with sensory and motor information that can be disrupted by visual deprivation in rodents (Wang et al., 2015). It is conceivable, then, that amblyopia could affect the fine-tuning of these maps just as is seen in other parts of the visual cortex, and that this noise could result in the increased fixation instability observed in these patients. Further, as both large and small saccades are linked to changes in perception, this would provide a mechanism by which spatial attention is impaired in amblyopia.

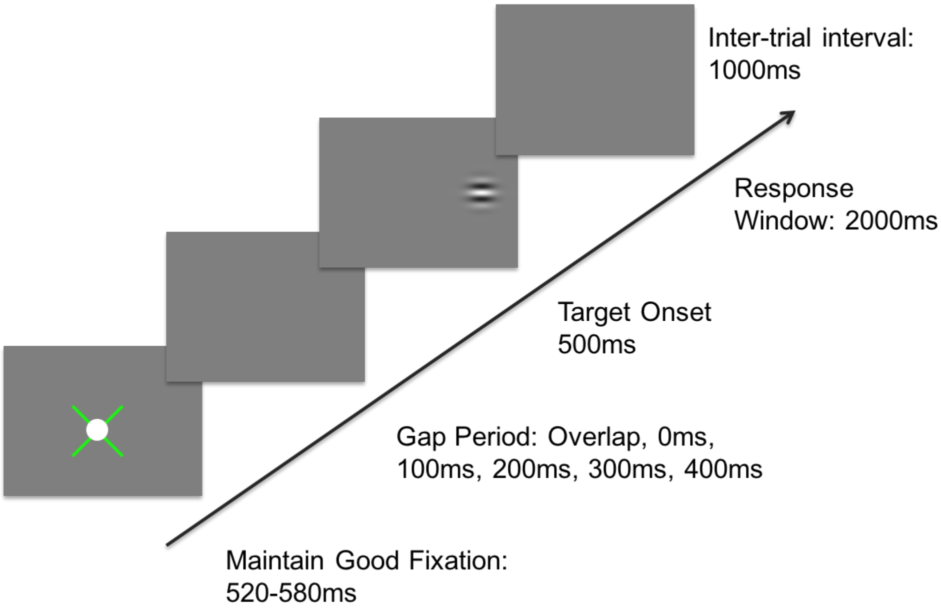
The second experiment will test the effect of fixation on saccadic latency in patients with amblyopia. By comparing reaction time with and without a foveal target, we can isolate the role of active fixation on saccadic latency. Further, by comparing interocular differences in patients with amblyopia, as well as both the AE and NAE to the performance of normally-sighted controls, we can assess how foveal attention may be impacted by amblyopia. We hypothesize that the gap could effect reaction time in one of two ways: (1) If reaction time is greatly in reduced in gap conditions that will inform us that a visual stimulus on the fovea is an important part of the delay in responding with this eye. (2) Alternatively, if the gap does not make much of an impact on reaction time when viewing with the amblyopic eye, it provides evidence that there is a smaller capacity for attentional enhancement when viewing with this eye.

3.2 Methods

3.2.1 Procedure

The procedure for Aim 2 was nearly identical to that of Aim 1, however, instead of modulating the contrast of the gabor on each trial, the temporal gap between the offset of the fixation spot and the onset of the gabor target was randomly chosen from one of six possibilities: an overlap where the fixation target persisted throughout the trial, a 0ms condition where the target appeared simultaneously with the extinguishing of the fixation target, and 100ms, 200ms, 300ms, and 400ms gap conditions. Additionally, unlike Aim 1, where the spatial frequency of the gabor patch was adjusted for each participant, everyone in Aim 2 had a gabor patch of 4 cycles per degree presented at full contrast.

Figure 3.1: Procedure for Experiment 2.



An x-shaped fixation guide appeared at the center of the monitor, with a 1 degree white fixation target placed on top. The fixation guide provided feedback, with green representing fixation within a 1 degree window, and red noting that the fixation error was greater than this boundary. A red fixation guide paused the trials and reset the fixation timer. After ~550ms (+/- 30 ms) of proper fixation, one of six potential gap durations occurred. After the gap period, a gabor patch briefly appeared at 5 degrees to the left or right, and participants had up to 2 seconds to respond, followed by auditory feedback and a 1-second inter-trial interval.

3.2.2 Analysis

At least nine reaction time measurements (mean: 22, max: 51) were averaged for the baseline value; min, mean, and max trial numbers for each gap duration were similar to those in the baseline condition. Response times below 120ms and above 800ms were eliminated to reduce the effect of express saccades and random guesses. Correct responses were averaged to find the mean and standard error of the reaction time.

3.2.3 Study participants

The Research Subjects Review Boards at Smith Kettlewell Eye Research Institute approved the study protocol. Informed consent was obtained from each participant and the study was conducted according to the tenets of the Declaration of Helsinki.

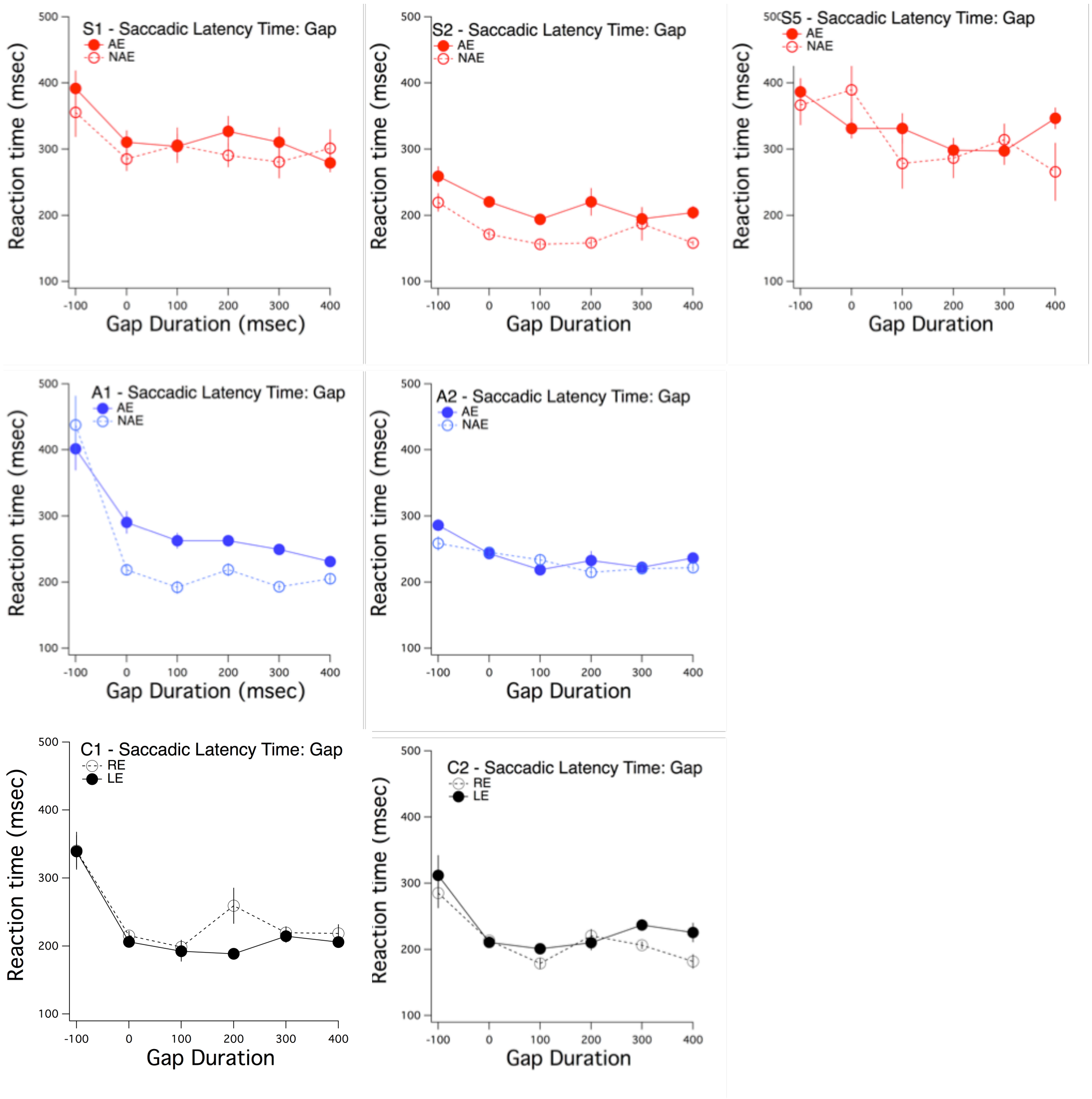
A subset of five (n = 5) adults (mean age: 54 ± 15, range 31–68 years) with unilateral amblyopia and 2 of the normally-sighted control subjects from Aim 1 also completed Aim 2. Subject identification codes have been maintained from the previous study. For more information regarding each participant, see table 1 in Aim 1.

3.3 Results

3.3.1 Saccadic reaction times with a gap

Saccadic latencies as a function of gap duration are plotted in Figure 3.2 for each eye of each participant (3 strabismic in red, 2 anisometropic in blue, and 2 age-matched controls in black). The x-axis shows the the duration between the offset of the fixation target and the onset of the gabor. The -100ms is the overlap condition.

Figure 3.2: Saccadic latency as function of gap duration.



The solid symbol is the amblyopic eye or non-dominant eye; the open symbol is the non-amblyopic eye or dominant eye.

The gap size was calculated as the difference between the baseline (overlap) condition, and the mean reaction time for each gap duration. To assess if there were significant interocular differences in gap size, paired t-tests were run to compare the gap effect for the five durations in the AE vs. NAE. The results of the t-tests are presented in Table 2.2.

Table 2.2: Interocular gap size t-test values (AE vs. NAE)

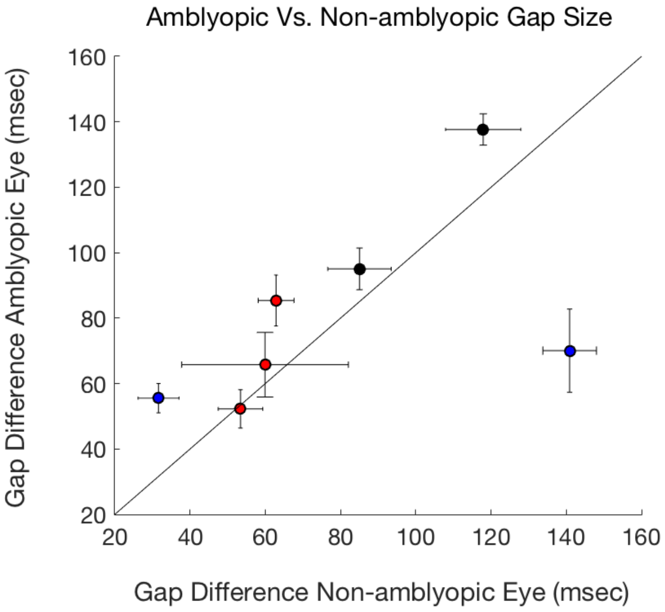
SID	Reject Null hypothesis?	P Value
S1	No	p= 0.11
S2	No	p= 0.91
S5	No	p= 0.82
A1	Yes	**P< 0.01****
A2	Yes	**p= 0.02***
C1	No	p= 0.19
C2	No	p= 0.38

For strabismic amblyopes, removing fixation does speed reaction time in the amblyopic eye, but not anymore so than the non-amblyopic eye. For example, the saccadic latency of S2 is slower in the AE than the NAE, but the gap size in both eyes is more or less the same. A similar pattern was observed, for the controls, although there was little to no difference in the raw reaction times.

The two anisometropic amblyopes did have significant differences in the gap size between the two eyes, however, intriguingly, they were in different directions. A2 had a shallow gap size for both eyes, but because the amblyopic eye was slower in the overlap condition, the gap was slightly, but significantly larger in the AE than the NAE. Interestingly, in this one patient, by removing the fixation target prior to target onset, the RT difference between the two eyes was eliminated. In A1 however, the gap size for the AE was significantly smaller than in the NAE. It's unclear what might drive these differences in anisometropic amblyopes given our small sample size.

To further quantify the gap effect size in our subjects, we calculated the average the gap effect for the five durations to determine one mean gap value for each eye. These results are plotted in Figure 3.3, with the mean gap size in the NAE or dominant eye on the x-axis and the mean gap size for the AE or non-dominant eye on the y-axis. All but patient A1 had a slightly larger gap in the AE or non-dominant eye than the NAE or dominant eye.

Figure 3.3. Mean gap size in the amblyopic and non-amblyopic eyes.



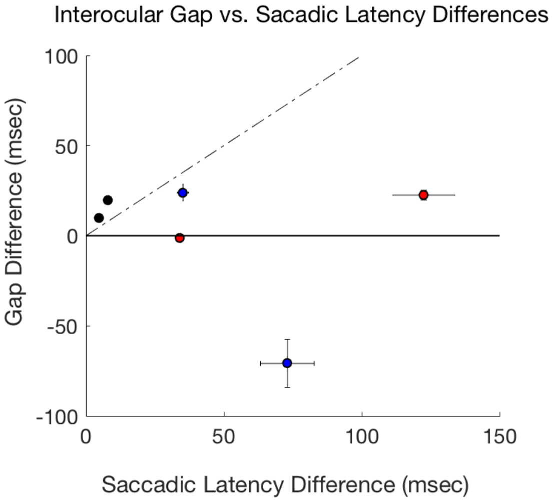
A repeated measures ANOVA was run to compare the within-subject means to the between-subject means. These findings suggest that overall there was much more variation between subjects (F=11.68,p<0.01) than between the two eyes of the observers (F=0.94, p=0.37).

3.3.2 The gap effect does not eliminate the irreducible delay

If the gap effect were to account for the irreducible delay observed in the previous study, it would need to approximately equal the size of the saccadic latency difference between the two eyes. Here we plot the interocular saccadic latency difference on the x-axis and the mean interocular gap effect difference on the y-axis (AE-NAE for both x and y axes). Points above the horizontal line have a larger gap in the AE, and importantly, points that have the same interocular gap size and SL difference would fall on the dashed unity line. With the exception of A2, who did indeed have small saccadic latency and gap differences (that were approximately equal (35ms and 24ms), all other subjects do not have similar gap and SL differences. This is driven mainly by the fact that for most subjects the mean interocular gap difference is very small (< 30ms) while the SL difference is quite large (>30ms).

This plot also shows that for controls, the interocular difference is slightly larger in the gap effect than the saccadic latency measure. For all amblyopic patients, the points fall below the dashed line, illustrating a larger AE-NAE difference for SL compared to SL with a gap. Figure 3.4 also clearly illustrates the extent of the outlier, A1, who only had a moderate difference in SL between eyes, but a large gap effect difference. For this subject, removing the fixation target increases the irreducibly delay almost two-fold.

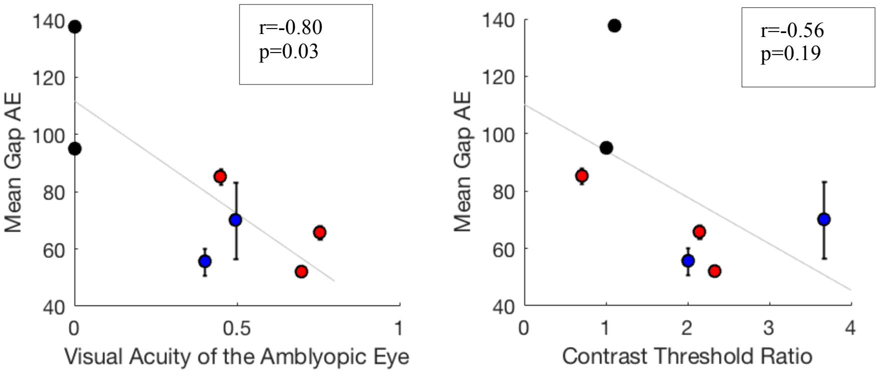
Figure 3.4. Saccadic latency asymptotes differences, and gap size



3.3.3 Gap effect and visibility indices

We previously reported that the saccadic latency difference between the two eyes was nearly significantly correlated with the VA of the AE, but not the contrast threshold ratio. We were curious if the size of the gap effect showed similar relationships (or lack thereof). Here we plot the mean gap size in the AE with the VA of the AE (left) and contrast threshold ratio (right).

Figure 3.5. Gap size and visibility indices



We found a similar pattern of results as with the saccadic latency differences. The gap size in the AE was related to the acuity in the AE, indirectly supporting the notion that VA, gap size, and therefore fixation stability are all related. There may also be a relationship with gap size and contrast threshold ratio, but this was weaker and did not reach significance.

3.4 Discussion

The aim of the second experiment was to isolate the role of fixation on saccadic latency in the amblyopic population by comparing reaction times in conditions with and without a fixation target. We hypothesized that the gap could effect reaction time in one of two ways: (1) If reaction time is greatly in reduced in gap conditions that will inform us that a visual stimulus on the fovea is an important part of the delay in responding with this eye. (2) Or, if the gap does not make much of an impact on reaction time when viewing with the amblyopic eye, it provides evidence that there is a smaller capacity for attentional enhancement when viewing with this eye.

We find preliminary evidence that while removing the fixation target does indeed speed up reaction time in the amblyopic eye, the gap size is about the same in both eyes. This means that the gap effect cannot eliminate the irreducible delay in the amblyopic eye, and we find similar interocular SL differences as were reported in the previous experiment. Additionally, when comparing the gap size in the NAE, we find that it is smaller than in either eye of the normally-sighted control subjects. Finally, we also observe that the size of the gap in the AE is related to the acuity in that eye. Our hypothesis for these findings is that fixational instability is driving both the increased response time and some of the acuity loss. Interestingly, the increased RT appears to be mainly monocular in nature for the saccadic latency task that was used in the previous experiment, but there is also a binocular component of fixation that is impaired, as we see in the current study.

According to the attentional explanation of the gap effect, attention must disengage from the fixation target in the overlap condition before moving and re-engaging at the peripheral target. For gap trials, this attention is already disengaged, allowing for a faster response to the saccadic target. In the case of amblyopic patients, perhaps there is a binocular impairment affecting the disengagement process of spatial attention, but only a monocular impairment (AE only) in the shifting and reengagement functions. This would account for the difference in saccadic latencies observed in the first experiment.

This ties back in to our earlier hypothesis that the delay in response time may be driven by an impairment of the superior colliculus. As mentioned previously, neurophysiological work has shown that the SC is a site where input from the retinae and other brain areas are integrated and translated into directions for motor movement that gets sent to the brainstem (Sparks, Roher, and Zhang, 2000). Most relevant to our findings is a study by Araujo and colleagues (2015) of attention engagement and disengagement in rats. While the animals could previously could perform a task that required disengaging attention from one cue and shifting it to another, their performance fell to 0% after their SCs were injected with muscimol. Importantly, however, the same rats could still perform at 100% on a task that did not require disengagement, but only shifting and engagement of attention. Perhaps, then, our amblyopic patients have a binocular deficit at the SC due to improper visual input during development which specifically impairs the disengagement process.

It should be noted that while previous studies that have modulated the gap duration report a U-shaped pattern in the gap effect, we did not find this, even in our two control subjects. While many experiments have only conducted a 200ms gap condition, citing that this point is when the gap effect is greatest, this may not always be the case. In the study by Braun & Breitmeyer's study (1988), which originally reported this finding, they measured the gap effect at 9 different temporal offsets, ranging from -500ms to 500ms, compared to the 6 offsets (-100ms to 400ms) in the current study. So, it is possible that we are limited by the number of temporal offsets measured.

Alternatively, it's possible that we may have needed to conduct many more trials per offset to observe this pattern, as the participants in Braun and Breitmeyer's study performed 100 trials per duration, compared to the average of 22 in the current study. Still, as our control subjects had mean gap effect sizes at the 200ms offset that were very similar to those reported by Braun and Breitmeyer (1998) as well as many other studies, we do not believe our smaller trial number per gap period is reason to doubt our results. Given these inconclusive findings on the temporal patterns of the gap effect, we are unable to determine whether the amblyopic patients are slower at disengaging attention, or just weaker overall.

As mentioned in the previous study, precise eye tracking in this population is challenging due to the patients' high-power refractive lenses and difficulty obtaining a clean calibration file. As a results we focused our measures on large saccadic eye movements, which were less susceptible to precision errors. However, in order to further untangle the relationship between foveal processing and attention in this population, we should aim to have more careful measurements of fixational eye movements in amblyopic patients, as well as online recordings of eye movements while they are doing a task. In the case of the current experiment, as well as the one in the previous chapter, quantifying microsaccadic rate and amplitude during fixation could have provided a more direct link between fixational stability and reaction time. Unfortunately, our set-up did not allow for such precise measurements.

We hypothesize that fixational instability is driving both the increased response time and some of the acuity loss in the AE. While the current studies focus on reaction time, future work should look at how these abnormal fixational eye movements could be impacting the perception of high spatial frequencies. As discussed earlier in this chapter, there is evidence that fixational eye movements in normal observers may be acting to restructure visual information in natural scenes to enhance fine spatial details (Kuang et al., 2012). In the case of amblyopic patients, drift velocities may be too slow or too fast, which could disrupt information processing by blurring the image or reducing the range of whitening to the retinal input. This lack of efficiency could lead to amblyopic patients making fewer and longer fixations while inspecting a scene, and this may be further exacerbated by an increased microsaccadic rate which is less tightly controlled and acts to smear the locus of attention.

While the above speculations are beyond the scope of the current experiment, we show here that by modulating the period between the fixation spot offset and gabor target onset and the comparing the reduction in saccadic latency between eyes (1) the gap effect cannot eliminate the irreducible RT delay seen in the previous experiment, (2) the gap effect is reduced in both eyes of amblyopic patients, and (3) that the gap size in the AE was related to the acuity in the AE. From this, we can infer that there is a monocular component of the gap effect that is related to fixation stability, and a binocular component that is related to the disengagement of spatial attention.

It would be interesting to see if the gap effect increases with visual training that has been shown to improve visual function in amblyopia. For example, a study by Li and colleagues (2015) provided evidence that patients with amblyopia that played video games improved on an attentional blink measure, a similar task in that it involves both temporal processing and foveal attention. In the next section we will look at treatment in amblyopia and how our findings from the first two studies can be informative to the development of more efficient therapies.

Chapter 4

Dichoptic Video Games as a Treatment for Children with Amblyopia

4.1 Introduction

While the consequences of abnormal visual development have been known for more than a century, millions of children go undiagnosed and therefore untreated ever year. Current reports put the prevalence of amblyopia at about 2.4% of the population, affecting approximately 15 million children worldwide (Wu & Hunter, 2006). As a result, these patients face the possibility of permanent monocular vision loss and a greater likelihood of complete impairment if vision to the good eye is disturbed through injury or disease (Harrad and Williams, 2003). Amblyopia can also negatively impact one's quality of life, resulting in reduced reading and fine motor skills, and may even negatively effect an individual's self image (Choong et al., 2004; Chua & Mitchell, 2004; Horwood et al., 2005; O'Connor et al., 2010b; O'Connor et al., 2009; Packwood et al., 1999; Rahi et al., 2006; Webber et al., 2008a,b). In the next section we will briefly describe the deficits commonly associated with amblyopia.

Clinical profile of patients with amblyopia

Contrast Sensitivity

Contrast sensitivity (CS), particularly at high spatial frequencies, is one of the main impairments reported in amblyopia. It's important to note, however, that the degree of perceptual loss in this domain can vary significantly depending on the factors that contributed to the development of amblyopia. For example, in patients with anisometropic amblyopia, the extent of CS loss may be greater than what occurs in strabismic amblyopes with an equivalent loss in letter acuity (McKee et al., 2003). Patients can also have reduced CS in the non-amblyopic eye (NAE) (Koskela, 1986; Leguire et al., 1990; Wali et al., 1991), and this deficit does not appear to be related to patching the fellow eye. Several theories have been proposed as explanations for these patterns of loss, including neural undersampling, particularly at higher spatial frequencies (Levi & Klein, 1985; McKee et al., 2003), and disruptions in the excitation/inhibition balance (Mansouri et al., 2008).

Spatial Localization

Localization of items in space can also be affected by amblyopia. This is often measured with a Vernier acuity task, where an observer is asked to detect or discriminate the offset between two lines. Thresholds for these tasks are smaller than the diameter of a photoreceptor, and thought to reflect cortical processing (Asper et al., 2000). Even when contrast is adjusted such that it is at the threshold for each eye, Vernier acuity is worse in the amblyopic eye (AE) than the NAE (Barbeito et al., 1988) of strabismic (but not anisometropic) amblyopes. As with CS, depending upon the type of amblyopia, Vernier acuity in the NAE can also be impaired. Levi and Klein (1895) measured the Vernier acuity of the NAE and found that it was worse in strabismic amblyopes when compared to normally-sighted controls, but this deficit was not evident in the NAE of anisometropic amblyopes.

Pattern Perception

Spatial localization difficulties also extend to higher-level perceptual mechanisms. These deficits are often categorized into two groups: tasks that require binding elements across space and tasks that require isolating features from noise. Examples of the first category include averaging the mean orientation over a set of individual items and detecting deviations from circularity (Hess et al., 1999). In the second grouping of deficits, amblyopes have shown poorer performance on tasks such as detecting illusionary contours and resolving glass patterns (Levi et al., 2007; Rislove et al., 2010).

Crowding

Crowding is the phenomenon in which visual acuity measured with several letters on a line is worse than when the same size letters are presented singularly, and can be particularly apparent in amblyopic observers – especially in strabismic amblyopes. While many researchers have sought to understand the mechanisms that cause this enigmatic phenomenon (see Levi, 2008; Whitney & Levi, 2011 for reviews), it is still unknown which of the many potential factors, such as contour interaction, fixational eye movements, and attention, have the greatest contribution to this effect. The ability to read crowded letters improves as a child is developing, along with fixation and performance on attentional tasks, so while there appears to be a relationship amongst these functions, their interactions are not well understood. Since fixation and spatial attention are both impaired in patients with amblyopia, it is not surprising that these individuals also show further deficits in letter acuity when flanking letters are included.

Spatial attention is impaired in amblyopia

Several studies have looked more closely at the relationship between amblyopia and impaired spatial attention. For example, Sharma et al. (2000) had strabismic amblyopes count highly visible Gabor patches that were briefly presented to each eye, and measured a threshold function relating accuracy to the number of patches presented. They noticed that amblyopic observes consistently overestimated the number of patches presented to their AE when the number was less than five and underestimated the number when it was greater than five. For large numbers of items, this underestimation was huge – observers guessed approximately half the number of objects as were actually presented. Several control experiments were run, such as lowering the contrast in the NAE, removing the mask, and increasing the stimulus duration. None of these significantly altered the observed effect, providing evidence that these counting errors were not due to low visibility or impoverished temporal integration. Further evidence of a higher-level deficit comes from the fact that amblyopes also underestimated the number of patches missing on a reverse task. Together, these experiments provide evidence that amblyopes have a more difficult time using attentional mechanisms to individuate the items on a display.

The attentional deficit extends into the temporal domain. When a series of letters are quickly presented and observers are asked to detect two targets in the stream, detection (as well as discrimination), of the second target is reduced when it falls approximately 200-500ms after the first. This dip in performance is referred to as the attentional blink (Raymond et al., 1992). Not only is this performance dip shallower in patients with amblyopia, but the error distributions were also much different than in the NAE. When Popple and Levi (2008) explored this phenomenon in a group of amblyopes, they found that the attentional blink ratio, or the difference between performance measured at 200ms and 700ms after the first target, was negatively related to the degree of amblyopia. That is, the greater the depth of amblyopia, the smaller the attentional blink.

Finally, patients with amblyopia are also poorer at attending to objects moving in space. When asked to track multiple objects moving at once, performance decreases as the number of objects increases. Ho et al. (2006) measured multiple object tracking (MOT) in 18 amblyopic children, as well as 30 age-matched controls. They discovered that spatial perception was relatively intact for low-level tasks, such as a low-level global motion task and a high-level 2-dot apparent motion task. However, performance on single and multiple-object attention tracking tasks was significantly impaired. Children were asked to track one to four of eight moving items and performed significantly worse than the age-matched controls with both the AE and the NAE. Further, the difference between the two groups grew as the attentional load, or the number of target items, increased. A follow-up fMRI study with 7 amblyopes, aged 9-36 years old, provided neural evidence of this impairment. Behavioral results were similar to the previous study, and activity in areas associated with motion processing and spatial attention showed less attentional modulation in the AE compared to the NAE.

Further neural correlates of impaired spatial attention were also reported by Ho et al. (2016). Not only did they find that modulatory effects of spatial attention were diminished when viewing with the AE, but that the degree of degradation was linked to the amount of suppression. The NAE showed normal modulation of attention in early visual cortex, but extrastriate areas had reduced modulation in both eyes compared to normally-sighted controls. This is consistent with behavioral findings that amblyopes have deficits in some spatial tasks in both eyes.

Together, these findings describe a widespread processing deficit, not one solely explained by a reduction in primary visual cortex. Despite this, the standard treatment for amblyopia focuses on improving early visual functions, such as letter acuity. The next section explores the benefits from standard treatment and compares them to what has been observed in recent experimental interventions.

4.1.1 Amblyopia treatment

Spectacles alone can improve vision

The first step to treating amblyopia is often prescribing the optimal refraction to both eyes. This can boost the degraded image to the amblyopic eye that, at least in the case of anisometropic amblyopia, has contributed to amblyopic development. This phase, termed refractive adaptation, often takes place simultaneously with occlusion therapy, however studies on refraction alone have shown that this can improve vision gradually over a period of weeks by 2 lines on an acuity chart (Stewart et al., 2004), and that in some cases can completely resolve the amblyopia (approximately 25% of patients that comply).

Monitored occlusion studies provide evidence that patching is slow

Because amblyopia affects visual acuity (VA) in one eye, it has been historically considered and treated as a monocular disease. For over 250 years, the standard treatment for amblyopia is to cover the dominant eye and have the child to go about life while viewing through the weaker eye. While this does lead to improvements in VA and CS, it wasn't until recently that we had a good sense of the "best practices" when prescribing occlusion therapy.

The first step to understanding the benefit of this treatment was the development of a patch that was lightweight and easy to wear, yet provided an objective measure of the time the patch is worn. Stewart and colleagues (2002) developed a smart occlusion monitoring device (OMD), which measured the temperature difference between the surface of the eye and the surroundings. This allowed the researchers to directly measure the time each child was wearing the patch rather than relying on parental reported data. This was vital because although practitioners knew there was great variability in compliance, the objective measures showed that on average children wore the patch for less than half (48%) of the time that was prescribed. Directly monitoring occlusion also allowed Stewart and colleagues to calculate a dose response, or the degree of acuity change related to the hours/day of occlusion (Stewart et al., 2004, 2005).

These studies helped shape our understanding of the effects of patching in several important ways. First, they found that the occlusion dose required to achieve the observed gains in VA could be described by a monotonic function, with approximately 0.1 log unit improvement in logMAR acuity per 120 hours of patching. Most (82%) of the improvement occurred during the first 6 weeks, but further improvement was seen up to 12 weeks. Second, the studies provided evidence that dose rates between 2 and 6 hours/day resulted in equivalent outcomes, although the longer daily duration did result in faster improvement. Finally, the authors found a large effect of age on the outcome of visual improvement, with children younger than 4 years old showing much better gains than those over 6 years old. A linear regression model including age, starting acuity, total occlusion, and type of amblyopia fit the data well ($R^2 = 0.87$), but further analysis shows that the subtype of amblyopia was not an influential factor.

More recently, another group (Fronius et al., 2014) used ODMs to monitor occlusion treatment in 27 young amblyopic patients. While the studies described above targeted the traditional age group for treatment (3-8 years old), the study by Fronius and colleagues included patients up to 16 years of age. Their results show that while patching does indeed improve VA throughout the age span, patching provides significantly greater benefit in patients younger than 7 years old. For example, Stewart et al. (2007a) report that it takes approximately 170 hours of patching for two lines of improvement in VA for a 4 year old, and 236 hours for a similar effect in a 6 year old. This jumps to over 400 hours for children greater than 7 in Fronius's (2014) study.

Failures of occlusion treatment

While it's clear that occlusion therapy is better than nothing, it's also evident that there are problems with this treatment. As noted in the previous section, patching is slow. Covering one eye is conspicuous, and requires the child to accept reduced visual perception while the fellow eye is covered. For these reasons, compliance can be very challenging. Further, the visual function of many children does not improve all the way. In fact, as many as 50% of amblyopes fail to achieve normal acuity even after extended periods of treatment (Birch and Stager, 2006; Birch et al., 2004; Repka et al., 2003; Repka et al., 2004; Repka et al., 2005; Rutstein et al., 2010; Stewart et al., 2004b; Wallace et al., 2006; Woodruff et al., 1994a). Even when treatment fully resolves the amblyopia, as many as 25% of patients experience a recurrence within the first year of treatment (PEDIG, 2004).

Groups have investigated alternative treatments that are less conspicuous, including administering atropine drops to the NAE (PEDIG, 2003, 2008). The treatment is thought to act by inhibiting accommodation, and preventing this eye from being used during near tasks. While studies have shown that it is effective in improving vision in the AE, it is no more so than occlusion therapy.

The goal of these treatments is to trigger plasticity in the visual system by covering the stronger eye and requiring the weaker eye to do the visual "work". This work can vary greatly depending upon the activities performed while wearing the patch. Given what we know from other areas of basic vision research, there are attributes which could make this visual work more efficient, such as performing a challenging task and receiving feedback. Levi and colleagues combined perceptual learning (PL) methodology with monocular occlusion to more specifically target this plasticity and carefully measured improvements on psychophysical tasks.

Perceptual learning improves visual function in children and adults

Practicing a sensory task will lead to improvements in this task over time. An adaptive staircase can be used to find the observer's threshold, and most stimuli can be presented near this point. This practice leads to small incremental improvements over time. Many scientists believe perceptual learning (PL) can be divided into two different phases: an early phase which is relatively fast and can generalize to a larger set of stimuli, and a later phase which is slower and more specific to the practiced task (Li et al., 2008; Li et al., 2007; Astle et al., 2011).

These later improvements in behavioral sensitivity are associated with neuroplastic changes. For example, in a study by Schwartz et al. (2002), training on a texture discrimination task was directly associated with changes in V1 as measured by fMRI. While an extensive body of PL studies exists which discusses potential mechanisms for these neuroplastic changes, (for examples see Gilbert et al., 2001; Dosher & Lu, 1999) it is beyond the scope of the current discussion. Briefly, benefits from PL may stem from reduced lateral inhibition at the early stages of visual processes (Polat et al., 2004), reduced internal noise and template retuning (Li et al., 2008; Li & Levi, 2004), or a high-level rule based learning process (Zhang et al., 2014).

Given these benefits in V1, and potentially beyond, PL could lead to improvements in amblyopic vision. Initial studies (Levi and Polat, 1996; Levi et al., 1997) provided evidence that when amblyopic patients practiced a Vernier acuity task for thousands of trials their performance improved. Consistent with PL studies on normally-sighted observers, learning was strongest at the trained orientation, but did partially transfer to other orientations. Further, in two anisometropic observers, the learning transferred to the NAE that was patched during the course of the training. These patients also showed improvement in Snellen acuity. The findings of these studies are particularly noteworthy, given that all observers were adults far outside of the age window normally treated in the clinic. They established that not only does PL lead to improved visual function in amblyopic observers, but also that there may be some benefit in treating older amblyopic individuals.

Since these original studies, dozens of others have followed that allow us to begin to fill in our understanding of perceptual learning in amblyopia. These studies include tasks such as letter identification (Levi, 2005; Chung et al., 2008), position discrimination (Li and Levi, 2004; Li et al., 2005, 2007, 2008), grating acuity, motion discrimination, and CS (Polat et al., 2004; Zhou et al., 2006). Meta-analyses of these studies (Levi, 2005; Levi, 2012; Levi and Li, 2009) tell us that the largest improvements in performance come with contrast-based tasks, and that broadband and/or crowded stimuli result in greater improvements compared to narrowband, uncrowded stimuli.

We also see more global transfer in amblyopic observers than the small, specific PL gains measured in normally-sighted individuals. For instance, amblyopes that practiced Vernier acuity also improved in Snellen acuity, and even stereoacuity (Polat et al., 2004; Li et al., 2007; Huang et al., 2008) and binocular combination (Chen et al., 2016). A potential explanation for this is that the training strengthens higher cortical areas instead of or in addition to primary visual cortex, and these areas may be used to process global information and make decisions. It also supports the notion that amblyopic patients retain some of their original visual networks for binocular vision, and that these can be strengthened through targeted exercise.

For PL to be an effective treatment option for amblyopia, improvements must persist to at least the same degree or better, as those resulting from patching. Evidence suggests that PL gains do indeed remain for several months or even years after the experimental procedure was conducted (Li et al., 2004; Polat et al., 2004; Chen et al., 2008; Zhou et al., 2006; Astle et al., 2011).

Though PL is tedious, visual function improves at a faster rate than patching (Chen et al., 2008). A potential explanation is that while the visual input is more specific than every day viewing with a patch, patients are learning to focus their attention on a particular stimulus close to their threshold, process and make decisions about this stimulus, and receive immediate feedback about their decision. Importantly, PL has shown to improve vision in a wide variety of patients, including those of different ages, and clinical profiles (Levi & Li., 2009). These finding provide initial evidence that PL can be an effective treatment for amblyopia.

The experiments mentioned above are all performed monocularly and thus fail to treat the base cause of amblyopia, a disruption in binocular vision. While patching the NAE does eliminate the need to suppress the AE, it does so under situations that would not normally occur in everyday life. An ideal training regime would encourage the use of the AE under binocular conditions. Several groups have looked at adapting PL tasks so that stimuli can be presented to each eye independently. This design, called dichoptic presentation, allows greater control in how the task is perceived by the observer, and the possibility of coordinating the features of the task between the two displays.

Dichoptic perceptual learning studies aim to balance the input to the two eyes

An alternative treatment approach, dichoptic PL, focuses on improving the binocular balance between the two eyes. Studies use similar methods to those described in the previous section, however now high contrast stimuli can be presented to the AE, while lower contrast stimuli can be presented to the NAE, allowing the observer to receive input to both eyes at the same time. Additionally, a mirrored stereoscope or similar device can be used to help align the two foveas to assist binocular fusion, which is particularly important for the strabismic amblyopes.

One of the first approaches in this area used random dot kinomatograms, where randomly moving noise dots were presented to one eye and signal dots moving in the same direction were presented to the other (Hess, Mansouri, & Thompson, 2010a, 2010b). The researchers adjusted the contrast of the noise dots in the NAE, and compared the ratio of the AE to the NAE to determine the suppression threshold. With several weeks of training on the task, the threshold decreased, along with the dichoptic motion threshold, or percentage of signal dots needed to determine the direction of motion. At the same time, VA and stereo acuity measures improved in several of the patients. One problem with this design, however, was that it made it possible for amblyopic patients to simply suppress or disregard the noise dots, thus binocular integration was not required to perform the task.

A different approach was performed by Ding and Levi, 2011. In their study, patients first practiced a fusion and alignment task where patients became proficient in aligning their foveas with a stereoscope, followed by thousands of trials where monocular information were correlated with disparity cues. The authors' reasoning for this was that through repeated practice with stimuli that contained correlated 2 and 3D input, stereoblind observers might learn to perceive depth from binocular disparity. Overtime, several of the patients that were initially stereoblind or stereo deficient were able to gain or improve their three dimensional depth processing.

A third group developed an approach they termed 'push-pull training' (Ooi et al., 2013). During this training, an attentional cue was presented to the AE, followed by a competitive dichoptic target to the NAE. Ooi's hypothesis was that the cue stimulates the weaker eye (a 'push') and causes interocular inhibition to the NAE ('a pull'), and that practicing this training leads to reduced sensory eye dominance (SED). Alternatively, one could view this as training the AE to attend to the cue, and ignore the information to the stronger eye. Ooi et al. (2013) found this training also transferred to other areas, including improved CS of the AE and stereoacuity.

While dichoptic training provides several advantages over monocular PL, these training regimes are still tedious, requiring thousands of trials of near identical tasks for small amounts of improvement. The stimuli are often simple, and while this makes them easier to control and quantify, the lack of complexity can create a barrier to transferability. On the opposite side of the spectrum, several groups have moved from these classical stimuli to the visually compelling worlds generated by modern video games.

Video game training leads to faster and broader improvement

After it was initially documented that video game playing can induce changes in perception (Green & Bavelier, 2003) a surge of related work followed (see Green & Bavelier, 2008; Bavellier et al., 2011 for reviews). Many of these studies piggybacked their methods on earlier work in perceptual learning. At first glance PL and video game playing (VGP) seem to have little in common, however, when video games are broken down into individual components, it becomes clear that the two share many commonalities.

For example, let's consider the case of action video games, a genre of games that has received the most attention in recent scientific investigations. These games have targets and enemies that move into and across the visual field. To succeed, players must be able to shift focus to the most relevant areas of the screen, and make fast-paced, spatial decisions by aligning a cross hair or viewing scope to the area of interest. Once a decision has been made, the players receive immediate feedback in the form of points or negative consequences. Like perceptual learning, the level of game difficulty also increases as the players improve.

Unlike perceptual learning studies however, the gaming industry is a multi-billion dollar segment of the entertainment media, and designers face intense competition to create rich, immersive environments. The result is a more compelling experience that is more enjoyable and overcomes much of the tediousness experienced in perceptual learning regimes. Action video games also trigger arousal and provide nuanced feedback on performance, which may be critical for efficient learning. Most importantly, however, might be the fact that video games have a variety of important content over the entire screen, leading to behavioral enhancements that are broader than the retinotopic and task specific changes observed in PL.

Methodology and Criticisms

There are two main methods for conducting video game studies: *cross-sectional* studies, where task performance of action video game players (VGPs) is compared to non-video game players (NVGPs), and *training* studies, where task performance is compared before and after an intervention, which is either video-game playing or a control condition. Training studies can include anywhere from 8 to 50 hours of video game exposure, with a fixed or variable schedule.

Several groups, perhaps most notably Boots and Simons (2011), have criticized early video game studies for design weaknesses. For example, they hypothesize that targeted recruitment of video game players could lead to increased motivation and performance bias on computer tasks. Additionally, they point to the fact that it is not possible to prove directionality in cross-sectional video game studies. Instead, it could be argued that individuals with superior visual skills enjoy playing video games because they are inherently better at them. Further, even after research groups moved to intervention studies, where gaming is controlled in the lab and dosage can be carefully monitored, it is challenging to identify the appropriate control condition. Groups have used different strategies, ranging from a non-action video game, to a no-contact/no intervention group. These differences, as well as the variability in game type, training regime, etc.

make it difficult to directly compare the results from one study to another. As a result, it’s unclear if one game is better at inducing neuroplasticity than the next, or what form of training leads to the greatest outcomes.

Areas of Improvement

Primary Visual Functions

Early video game studies focused on lower-level visual functions, such as contrast sensitivity and visual acuity. Li et al. (2009) measured CS at several spatial frequencies in participants with either extensive practice playing action-based video games or with no video game experience. They found that VGPs had increased CS at all but the lowest spatial frequencies when compared with age-matched NVGPs. They followed this with a training study where a group of NVGPs played a video game for 50 hours over a nine-week period. Half of these participants played an action-based video game, while the other half played a non-action video game. Contrast sensitivity was measured for these two groups both before and after the training, and however substantial improvements were only observed in the action-based video game group.

Another study focused on spatial visual processing in video game players. Green and Bavelier (2007) measured crowding in cross-sectional and training studies. As mentioned previously, crowding is primarily measured as the critical spacing between a target and its surrounding flankers needed to accurately determine the identity of the target. The effect of flankers can be much greater in the amblyopic population, particularly in strabismic amblyopes (Bonneh, 2007; Levi et al., 2007; Chung, 2008). Green and Bevelier’s study compared VGPs to NVGPs and showed that critical spacing thresholds in peripheral vision were smaller in the VGP group, and this effect was also evident when NVGPs played action video games for 30 hours.

Although action video game study results are often framed in terms of benefits to the primary visual cortex, without the ability to isolate the specific mechanism(s) leading to these improvements it’s also possible that these changes could be explained by enhanced attentional and/or oculomotor abilities. For example, a variety of studies show a close link between contrast thresholds and attention. Carrasco et al. (2000) provided initial evidence that directing attention to a stimulus can lower contrast thresholds. Here we will briefly review three areas of video game research that are the most relevant to amblyopia and the results of the previous chapters: *attention*, *reaction time*, and *oculomotor processing*.

Attentional Enhancements

Attentional processes can be divided into three separate categories: space, time, and objects. When spatial attention is engaged, a particular region of the visual field is prioritized, such that when a target is presented in this region, it is detected faster than if it were presented in an unexpected location. Video game players (VGPs) have superior abilities in detecting targets in space, whether it be in an insolated environment, such as a Goldmann perimeter (Buckley et al., 2010), or in situations where the participant must identify a target in a complex scene with distractors, such as in the Useful Field of View (UFOV) task (Green and Bavelier, 2003), or a visual search task (Wu & Spence, 2013).

VGPs also perform better at tasks requiring temporal attention. As previously mentioned, when a stream of letters is rapidly presented with two targets interleaved with distractors, observers have a much harder time identifying the second target if it is presented 180-450ms after the first. This ‘attentional blink’ is thought to reflect a bottleneck in the temporal processing of items. VGPs, and those that have received video game training are better at detecting this second target, demonstrating a shallower blink than non-VGPs and control participants (Green and Bavelier, 2003).

Finally, VGPs are also better at directing attention to objects of interest. For example, they are able to track more targets in a MOT task (Boot et al., 2008, Green & Bavelier, 2006b, Trick et al., 2005. Here, unlike the previous scenarios, the objects move independently and continuously across the entire screen. Therefore, the attentional processing needed to excel at this task is different from the spatial attention measures described above.

Oculomotor Changes

Several groups have investigated how video game playing may lead to changes in the oculomotor system. In a paradigm by West et al. (2011), VGPs and NVGPs made saccades toward a target, while trying to ignore a concurrently presented distractor. In this situation, depending upon when the distractor was presented, saccades can curve toward or away from the distractor, reflecting enhancement and suppression of the miscellaneous information.

The authors report two notable findings: NVGPs show a rapid transition to curving away from the distractor, but this diminishes at longer latencies. VGPs, however, are slower to change from curvature toward to curvature away, but this shift away is sustained at the longer latencies. West et al., discuss several hypotheses for these findings, such as differences in FEF functioning and more residual attentional resources to encode distractor stimuli. Regardless of the specific mechanism causing these differences in oculomotor behavior, it is clear that the processing of visual information differs between these two groups.

Second, VGPs improved over the course of the study, while NVGPs did not. While performance was similar between the two groups in the first half of the study, in the second half the VGPs were significantly better at ignoring the distractor than the NVGPs. This findings points to a trend other groups have also noted – that action video game players learn faster than non-action video game players. Others (for example see Green et al., 2012) note this could reflect more flexibility in the executive system.

Top-down control of eye movements in VGPs was also investigated by Chisholm and Kingstone (2012). In their study, participants performed a visual search task, during which an additional non-target item abruptly appeared on half of the trials. Individuals will often make a reflexive saccade to this target, even though it is known that is irrelevant to the task. VGPs, however, made significantly fewer saccades to the distractor, thus demonstrating superior suppression and top-down oculomotor control.

VGPs have faster reaction times

What do these studies have in common? A meta-analysis of more than 80 different video game studies showed that VGPs were on average 12% faster than NVGPs, yet were just as accurate (Dye et al., 2009). These reaction time differences existed over a wide range of durations, from extremely short simple decision tasks (250ms), to longer, more complex visual search paradigms (1.5s). This RT difference is not due to a change in response criterion, as the VGPs were as or more accurate than the NVGPs. Instead, the fact that this robust effect persists across a wide variety of tasks and is multiplicative, not additive, suggests that there are differences in how video game players process information.

Potential Mechanisms

Along with the behavioral changes described above, there is also building evidence that video game playing can lead to appreciable neural changes. Mishra et al. (2011) was one of the first research groups to link behavioral advantages to neural differences in VGPs compared to NVGPs. While a previous study described how video game players were better at suppressing eye movements to a known distractor, Mishra et al. takes this one step further by providing neural evidence of this suppression. In their study, participants were cued to attend to a peripheral or central stimulus and detect occasional targets. The VGPs had faster reaction times and higher accuracy, and this was correlated with an increased suppression of steady state evoked potential (SSVEP) amplitudes to unattended peripheral sequences. This is a potential neural signature of the superior ability for VGPs to suppress distractors on attentional tasks.

Mishra et al. (2011) also found group differences in event-related potentials to the targets, with larger P300 components in VGPs than in NVGPs. While still under debate, this component is often linked to sensory decision making processes and is particularly evident in cases when a target is not expected, but occurs (for a review, see Dinteren et al., 2014). This finding is a potential explanation for why VGPs have faster reaction times than NVGPs.

Similar findings to these were reported by Bavelier et al. (2012) using functional magnetic resonance imaging (fMRI). In their study, VGPs and NVGPs were asked to decide if the presented target was a square or diamond. The task was complicated by the presence of static distractors and moving dots, in high and low perceptual load condition. They found that VGPs were faster at the task than NVGPs, and had greater suppression of both the distractors and irrelevant motion. They also hypothesized that the VGPs had more flexible cognitive control because there was less of an increase in the recruitment of the fronto-parietal attention network when switching from the low demand task to the high demand task.

Several other groups (Voss et al., 2012; Wu et al., 2012; Krishnan et al., 2013; Kühn et al., 2014) have also investigated cross-sectional differences or training-induced plasticity in the brain. These studies demonstrate that the neural effects of video game play are far reaching, which may not be surprising, given the large number of studies reporting behavioral benefits associated with video game playing. These findings have led scientists to investigate the use of these games to boost brain function and perception in patient groups with deficits in these areas.

Video games as a treatment therapy

Researchers have used video games as a potential treatment intervention for a wide variety of clinical groups, including those suffering from PTSD (Goncalves et al., 2012), multiple sclerosis (De Giglio et al, 2016), and brain damage due to a stroke (Li et al., 2016). One particular study that has much in common with the goals of amblyopia treatment research was conducted by Franceschini et al. (2014), who studied the effect of action video game playing in children with dyslexia.

The research group had dyslexic children play an action or non-action game on a Nintendo Wii for 12 hours over 9 days. Like the adult action game studies, the wii action video games encouraged visuo-motor control, and the engagement of focused attentional resources. Meanwhile, the control games emphasized fast motor execution without any prioritization on when or where to focus attention (e.g. press a button as fast as possible). In the action video game group, they found large reading improvements, including increases in reading speed without decrease in reading accuracy. These changes were not small. In fact, the children showed approximately the same improvement as occurs in one year of spontaneous development. The researchers also found concurrent gains in attentional skills, measured on focused and distributed attention tasks. These results show that video games can lead to improvements in quality of life in children with developmental disorders.

Video games as a treatment for amblyopia

One of the most popular applications of video game therapies is aimed at improving visual function in patients with amblyopia. In the past six years, at least 16 studies have been published on the subject. The reasoning is clear: while the neural changes in normally-sighted video game players might not make a noticeable difference in quality of life, the potential for this is much greater in patients with amblyopia.

In a groundbreaking series of pilot studies Li et al. (2011) enrolled 18 adult amblyopic patients to receive one of several treatments, including playing an off-the-shelf action video game with their NAE patched. Other conditions included playing a non-action video game with their NAE patched, or simply patching while performing other visually demanding tasks such as watching TV, reading books, knitting, using the computer, et cetera. Every 10 hours, the patients had their VA and several other measures of visual function assessed. Amblyopes in the action video game group improved by about 0.16 logMAR in their AE, and showed boosted performance on several of the other visual tasks. Three subjects who played a non-action game (Sim City) also showed VA improvements of about 0.15 logMAR. Two of these amblyopes further improved by 0.1 logMAR after switching from the non-action to the action video game and training for 40 more hours (the third had already reached normal vision at the end of the first training round). Finally, the 7 amblyopes that patched for 20 hours showed no significant VA improvement, however, 5 of these amblyopes crossed over to play action video games and improved by 0.17 logMAR.

While it was not a large scale, randomized study, this investigation showed for the first time that playing video games could induce visual changes in patients with amblyopia, and that these gains were just as, if not more beneficial than those measured in studies with PL. This pioneering study initiated many fruitful avenues of video game research that will be discussed in the next few sections.

Dichoptic games control the content to each eye

A follow-up to this original study was recently conducted (Vedamurthy et al., 2015). While action video games were still used as the active treatment, there were several important methodological modifications to the study. Instead of using off the shelf action games, a special game was designed, specifically for the treatment of amblyopia. The content for each eye was split so that it was possible to adjust the luminance to each eye. Dichoptic presentation also made it possible to align the images with a stereoscope, allowing patients to see the content on both foveas, even if they did not normally have good binocular alignment. Gabor patches were added to the view of the AE to monitor suppression of this eye during game play. The spatial frequency of the patches could be adjusted with a staircase to measure changes in the resolution of the amblyopic eye (AE) during game play. This design provided the benefit of stimulus control from the PL studies, with the engaging content from video games. A control group watched action televisions shows with the NAE patched. This control was chosen to directly compare the effects an active dichoptic treatment to traditional treatment, and to assess if supervised patching in adult amblyopes is beneficial. Finally, a larger patient group was recruited (n=38), which allowed the researchers to investigate factors such as age, amblyopic type, etc.

There were several interesting findings that resulted from this study. First, both anisometropic and strabismic amblyopes improved by about 0.14 logMAR, similar to the findings in the previous, monocular action video game study. Second, the anisometropic amblyopes in both the video game group and the control group improved by a similar amount, while the strabismic amblyopes only improved in the game group. There were additional improvements in the visual tasks in the dichoptic game group, compared to the control group, such as stereoacuity, reading speed, and CS. When the amblyopic participants returned after a 2-month, no-contact period, these improvements were largely maintained. Together, this and the earlier pilot study by Li et al (2011) provided clear evidence that playing video games can improve visual function in adult amblyopes, although it does not fully answer what types of games, what display methods, or participant factors lead to the best outcome.

Around this same time, another group began researching the effect of video game training on amblyopic vision, but instead of using an action video game, they had their amblyopic subjects play Tetris (To et al., 2011). In some ways these game-based treatments were similar – in both participants scanned and processed moving objects on a screen, made perceptual decisions, and received feedback. However, in other ways, they were quite different. Unlike the first person shooter games, the Tetris game had no crosshair to help localize the point of focus, and the blocks moved across the screen in predictable vertical and horizontal orientations. One large benefit, however, was the ease of use of the platform developed by To and colleagues. Study participants were given an iPod touch for the duration of the study and could play at home. The mobile device was also fit with a lenticular film to provide a different (albeit degraded) image to each eye. The downside of this method, however, was that it is difficult to properly control the dichoptic input, which can change based on head position and viewing angle. In an ideal situation, some content is sent to the AE at high contrast, a second portion is sent to the good eye at reduced contrast and a third set is sent to both eyes. To help alleviate the cross-talk due to improper viewing, the group added a chin rest and remote control so that the angle of the screen was kept constant, but this limited the portability of the game treatment. Another important problem with this design is that it is possible to play the game (badly) with one eye.

In a pilot test of 9 amblyopic patients (game play ranged from 6.25-25.25 hours), they found the contrast ratio between the two eyes was reduced over the course of the training, and upon completion of the training, a majority of the patients could tolerate the same contrast in both eyes. To et al. also found improvements in VA (mean 0.18 logMAR) and stereoacuity, including 3 patients that went from no measurable stereo to clinically significant depth perception.

The Montreal-based group followed-up this up with two more recent studies (Hess et al., 2011; Hess et al., 2014). The findings were consistent with the original study, despite small changes to the design of the device, such as wearing red/green anaglyph glasses, and playing the game at home, where viewing angle and training schedule were less controlled.

Video game training for children with amblyopia

The anaglyph version of the Tetris game has also been used to test the feasibility of video game-based treatments for children with amblyopia. In a study by Li et al. (2014), 50 children underwent dichoptic treatment and 25 underwent a sham treatment where the colors of the anaglyph glasses were flipped such that the contrast-reduced content was viewed by the AE instead of the NAE. After 4 weeks of training (children were instructed to train for 4 hours per week, but actual training time varied due to compliance), patients returned to the lab for a vision screening. Visual acuity improved in the dichoptic group by about 0.08±0.01 logMAR, while no change was observed in the sham group. A handful (11%) of the children in the dichoptic group also showed some improvement in stereoacuity.

Twenty-seven of the 45 children that finished the first 4 weeks of testing continued for a second set of 4 weeks, however, there was no additional improvement. While some of the children involved in the study continued to patch for some period of the time while not playing the games, this patching did not result in a significant difference in VA improvement compared to the non-patching individuals. Follow-up measures in 21 of the participants showed that the acuity gains were maintained for at least three months after finishing the study.

The authors ran a follow-up study (Birch et al., 2015) on a slightly younger population (here the median was 5.7 y.o.; individual data not reported in the previous study but the range was reported as 4.5–12.7 y.o). Mean improvement was 0.09 logMAR in the dichoptic group, compared to a mean improvement of 0.02 logMAR in the sham group, and three children in the dichoptic group improved in stereoacuity. These results were near identical to those in the previous study. Together, these studies point to the robustness of the benefit of video game training for amblyopia, and age not being an import exclusion criterion for treatment.

To fully assess the impact of the dichoptic iPad treatment as it directly compares to current clinical treatment, researchers should include a patching control group. A recent study by Kelly et al. (2016) ran 28 children (aged 4-10) in a randomized clinical study, where half of the children received 10 hours (1 hour/day, 5 days/week, 2 weeks) of the experimental treatment, a dichoptic adventure game played with anaglyph glasses, and the other half patched for 28 hours (2 hours/2, 7 days/week, 2 weeks). After the two-week treatment period, vision was assessed and children in the dichoptic game group improved an average of 2x more than the patching group (0.15 logMAR vs. 0.07 logMAR). The dichoptic treatment group continued for an additional two weeks, while the patching group crossed over. The authors found the children that had crossed over caught up to the dichoptic game group in terms of VA improvements from baseline (0.17 logMAR for the 4 week dichoptic game group, vs. 0.16 logMAR for the cross over group). They also report near 100% compliance with the dichoptic game, which may have contributed to this robust effect.

A larger, multi-site study was recently completed that had similar methodology (Holmes et al., 2016), however it lasted much longer (16 weeks), and children played the dichoptic Tetris game instead of the adventure game. In this study, 182 children played the game at home for an hour a day, while 188 children patched for 2 hours each day. The results of this study conflicted with those reported above, with the dichoptic group improving by an average of 0.12 logMAR and the patching group improving 0.14 logMAR. One significant difference, however, from the Kelly et al. (2016) study was a low rate of overall compliance. While the Kelly group had near 100% compliance with the dichoptic game, only 22.2% of the children in the current study played the game for at least 75% of the prescribed time. The authors note that the children often lost interest in the Tetris game in a number of days or weeks, which would actually support a shorter training period, such as in the previous study. It also points to the importance of game design, particularly for this younger population, and that games such as Tetris may be too tedious for younger children. Another important finding was that the magnitude of improvement was much higher for younger children that had not received previous treatment (0.25 logMAR in the binocular group and 0.28 logMAR in the patching group). This result is often discussed in relation to the mixed results of training studies, including the two mentioned below.

A different type of dichoptic treatment platform, the interactive binocular treatment system (I-Bit), was developed several years ago by Herbison and colleagues (2013). This system is similar to the anaglyph approach used above, however, instead of color filters shutter lenses are used to view a computer monitor with alternating frames and the image to each eye is separated temporally. Additionally, unlike the dichoptic iPad game, the content strength to each eye with the I-Bit system was the same. The authors initially conducted a small pilot study with, ten young (mean age of 5.4 years) children. Treatments, which consisted of 20 minutes of dichoptic video watching and 10 minutes of dichoptic video game playing, were completed once a week for 6 weeks (for a total of 3 hours). Nine of the children completed the study, and showing a mean VA improvement of 0.18 logMAR (range 0.025 to 0.45 logMAR). This change was much greater than was seen in the dichoptic iPad study that had a longer training duration, however, conclusions about this are limited due to the small sample size, lack of control, and large variability in effect size.

A follow-up study with a larger sample of 75 children (aged 4-8) and a control condition, where both eyes received the same content, was recently published (Herbison et al., 2016). After 6 weeks of treatment, small improvements (average of 0.07 logMAR) were seen in the experimental condition (I-BiT movies and I-Bit games), as well as in the control condition (average of 0.05 logMAR).

These results are similar to those reported in the dichoptic iPad study (Li et al., 2014), and the authors note that this may be due to the fact that a majority of the children were residual amblyopes (67 of 75 had received previous treatment compared to a 1/3 in the pilot study). Another potential explanation is that a majority of the patients were strabismic (70 of 75) and the angle of deviation was not corrected in the viewing setup.

Video game treatments: alternative assessments

Best corrected visual acuity (BCVA, or more simply VA) is almost always the main assessment used to track improvement in amblyopic vision with training. This is chosen as it has historically been used to monitor amblyopia treatment in the clinic and studies on patching or atropine use VA as the primary outcome measure. There are however, several other visual assessments, such as reading speed, CS, and stereoacuity, that have been investigated in some training studies. One could argue that these might provide better insight into improvement in quality of life due to the fact that they are more similar to the skills needed for everyday visual function.

Li and colleagues (2011, 2015) have investigated how the attentional blink in amblyopia is lessened through video game playing. As described earlier, amblyopia can affect the temporal processing of rapidly presented letters, but counter-intuitively, the blink is actually weaker in the AE. In Li's study, the attentional blink was significantly reduced in both eyes after 40 hours of video game playing with the NAE patched. This "relieved blink" was particularly evident at a lag of 200ms. In comparison, amblyopes that patched for 10 hours and participated in visually demanding activities had no significant blink reduction in either eye.

This series of studies also demonstrated that while perceptual learning can improve VA, the acuity improvement is separable from the attentional enhancements obtained from video game playing. One patient practiced a Vernier acuity task before playing the video games and improved in acuity, but not blink. Afterward, this patient played action video games for 40 hours and had their vision reassessed. At this point, the researchers found no evidence of further VA improvements, but they did find a significant reduction in attentional blink. Given that video game playing without Vernier acuity training can lead to improvements in both functions, this finding supports the broader benefit of video game playing.

Another group looked at how dichoptic game playing can improve fine motor skills in children with amblyopia (Webber et al., 2016). Amblyopic children performed worse than normally sighted children on a series of fine motor skills, such as placing beads on a string and drawing vertical lines, however, after training for 5 weeks on the dichoptic Tetris iPad game (To et al., 2011), they showed significant improvements on these fine motor tasks. The age-matched controls did not receive training, and their measures at 5 weeks were not significantly different from baseline. The fine-motor improvements in the amblyopic children persisted at least 12 weeks after the training. One important note is that all amblyopic children in the study had received conventional treatment prior to enrollment, yet had some residual deficits. In previous studies, these children may have been viewed as not having a successful outcome because VA did not improve significantly with the training. This study is important in that it demonstrates video game playing can help relieve deficits that traditional treatment does not target, and that these additional benefits can make an impact on quality of life.

Challenges and Design Considerations

One facet of these studies that is often underappreciated is the importance in the design of both the video game and the study. One group that did discuss these considerations in relation to their findings was Hussain et al., (2014). These researchers worked with a software development company (Ilixa, game available here: http://www.pangame.eu/beta/), to create a contrast-based video game that could be played at home on a variety of displays. Viewing distance was adjusted for study participants such that all stimuli were supra-threshold, and the participants' home monitors were calibrated using an observer-based procedure developed for LCD displays. To reduce technical issues that could arise and provide a set-up that could be replicated in all participant's homes, the game was played monocularly, with a patch over the NAE.

Hussain et al. enrolled 10 children and 10 adults with amblyopia to play the game. After an on-site visual screening and test run where the game was explained, the participants were asked to play the game for 25 minutes everyday and return after at least 2 weeks for a follow-up vision screening. Game play data for each participant was saved remotely on a secure server, allowing the researchers to track the progress of game play of the study participants. They found that the game provided reliable estimates of contrast thresholds for the adults and that these thresholds decreased with game play. For the children, however, contrast thresholds were much more variable and did not improve significantly with the training.

On the other hand, LogMAR acuity did improve for both groups by about 1.3 lines (range 0–3.6 lines across both group), after an average of 11 hours of training distributed over multiple sessions. For four of the mild amblyopic participants (2 adults and 2 children, starting VA between 0.14 and 0.10) the acuity difference between the two eyes at the conclusion of the study was less than 2 lines, making them no longer amblyopic according to the study criterion. Half of the participants (6 children and 4 adults) continued to train past the initial period and later returned for a third visual screening. There were slight improvements in the children's logMAR acuity (mean 0.05 logMAR), but no further acuity changes for the adults, and no improvements in contrast threshold for either group.

Why is it that little to no benefit was observed after the initial training period, and why did one of the primary outcome measures of this study, CS threshold, fail to provide an accurate metric for half of the patient population? Thanks to the large body of knowledge attained from patching studies, we know that there are several key factors related to the rate and degree of visual improvement with this treatment. Meanwhile, we know relatively little about which of these factors are most important to a successful outcome with the behavioral training paradigms described above. Below we summarize the main findings from previous training studies and focus the remaining discussion on several open questions in this field that will be addressed in the current study.

Behavioral Training Summary and Key Factors

Most behavioral training studies for amblyopia report improvements of 1-2 lines of logMAR acuity with 5-40 hours of training, with a mean improvement of 0.17 logMAR (see Levi and Li, 2009; Levi, 2012; Tsirlin et al., 2015 for reviews). This effect size is fairly stable, despite a large variety of training tasks, study durations, participant types, et cetera. It's also important to note that while several studies do report resolved cases of amblyopia (that is, after training patients have less than two lines difference in VA between the two eyes), this only happens in mild amblyopia where an improvement of 1-2 lines is all that is needed to reach this criterion. If long-duration patching can yield greater improvements, perhaps these behavioral studies are operating on different neural mechanisms than patching. Alternatively, perhaps the effects of game playing has two phases: a fast, exponential boost in VA where attentional and cognitive effects are occurring, and a slower, linear gain where primary visual cortex continues to receive benefit. The duration of the second phase could be upwards of the 100-400 hours, the reported saturation point in patching studies, however no training studies exist for this length.

This leads to an important question: given what we know from previous training studies, how does the training schedule factor into the study outcome? Hussain et al.,(2014) state that almost all of the training benefit is realized in the first training period of about two weeks, and it is "not clear that further training would have produced further improvement". Meta analyses (Levi & Li, 2009; Tsirlin et al., 2015) report that across training methods, training duration was not a significant factor in VA or stereo improvement. These large scale analyses, however, only assess the total training time, and fail to take into account gaps in training, session duration, and perhaps even how training duration is related to skill improvement. More work is needed to extend the duration of training and understand the temporal dynamics of VA and stereoacuity recovery.

Training schedule is related to another important in these studies: motivation. Hussain et al. (2014) hypothesize that a contributing factor to the differences in the reliability of CS measure for the adults vs. children may be motivation. The adults in the study were externally motivated to follow the training directions (play for 25 minutes every day), because they would like to improve their vision. As a result, overall compliance is high, with a mean play duration of 24 minutes, and the range was very low (20-25 min). Compliance was much lower and more variable for the children enrolled in the study, with a mean play duration for the children of 12 min, and a range of 5-25 min. The authors report that keeping the children motivated was challenging, particularly after the first week, and that "as with patching, even video games carry issues of compliance when prescribed for children".

Motivation may also be linked to the game design and difficulty. Good game design is needed to keep the player engaged, both within a single session, and across multiple sessions. In the study by Hussain et al. (2014), each level timed out at 90 seconds, and participants were encouraged to obtain as many points as possible during this time. While this helped keep the training time consistent across participants, time-based stopping rules may not be as effective as a performance-based stopping rule, where game flow can be longer and more engaging. Additionally, Hussain et al. (2014) did not order the levels by difficulty and participants were free to choose which levels to play. This may have reduced the overall motivation, particularly across sessions, since the achievement on one level was not related to unlocking other levels or an overall score. If the game had moved the participants on a guided progression of difficulty across the levels, there may have been a greater sense of overall achievement. More work from game experts should be included in the study design to keep all ages of participants engaged and motivated throughout the entire course of treatment.

Also worthy of discussion is whether dichoptic games lead to greater benefits than monocular games. As dichoptic games are logistically more challenging, and potentially increase the risk of developing diplopia, it is important to understand if there is a significant benefit from adding dichoptic presentation to the study design. Several new studies have investigated training with dichoptic games, but only one has directly compared the same game with dichoptic and monocular conditions. Instead, others have commonly used a sham condition as a control. In this condition, the contrast-reduced cues are sent to the NAE instead of the AE. An unfortunately outcome of this design is that it eliminates any insight into whether the dichoptic presentation can result in greater vision recovery than a simpler treatment, such as playing a game with a patch.

Li et al. (2013), did compare the effect of a monocular vs. dichoptic condition of the Tetris game. After 10 hours of training, the dichoptic group had improved by 0.17 logMAR, while the monocular group had little to no change in VA (0.04 logMAR). The dichoptic group also had significant improvements in stereoacuity and a measure of suppression, while the monocular group did not. The monocular group then crossed over for 10 hours of dichoptic training, and showed similar improvements to the original dichoptic group (0.18 logMAR). These results suggest that dichoptic training is faster, and perhaps more effective than monocular training, but the group size for each condition was small (n=9) and more work is needed to support this claim. This will be one of the primary goals of the current study.

Finally, along with these design-based factors, there are participant-based factors that can influence the study outcome including: age, amblyopia type, and initial acuity. Given that young children have more flexible sensory systems and show faster improvements with patching one might expect that they would also have greater improvements with behavioral training for a given dosage. Hussain et al. (2014) did not find this result in their study where both children and adults were tested with the same game, yet this is confounded by the fact that adults demonstrated better compliance and overall played the game for longer at each session. Children and adults that played the dichoptic Tetris game in different studies received similar amounts of benefit, or if any difference existed, the adults received slightly larger benefits from the training. A meta-analysis of 23 studies by Tsirlin et al. (2015) also showed there was no effect of age, but none of the studies in this analysis included participants under 18 years old.

However, there may be some important holes in the conclusion that age does not effect training outcome. When Levi (2012) compared all amblyopic PL studies, they found the largest gains were in a sample of 5 children (mean age 7.3 years) that had undergone patching treatment but had either failed to improve, or were non-compliant (Polat et al., 2009). Their naiveté may be important in this outcome, as another study where children that had already patched for 2 years and improved by a 2-3 factor of in acuity, later failed to show improvement with subsequent PL (Liu et al., 2011). Previous treatment exposure may help explain some of these mixed results.

This notion may be coupled to the finding that initial visual acuity is negatively correlated with VA improvement (patients that had poorer AE vision at the beginning of training showed more capacity for change). Tsirlin et al. (2015) showed that this relationship held across studies and treatment types, and importantly was not related to a ceiling effect. Unfortunately, it is not possible to disassociate the effect of previous treatment from this finding (and the fact that these patients are more likely to start with a greater deficit), as this information is not reported in most of the studies.

Although most studies do not analyze effect size by amblyopia type, or only include one type of amblyopic patients, the few that have enough power to do so, also show mixed effects. This is further obfuscated by the fact that not all studies classify amblyopia type by the same criteria. Vedamurthy et al. (2015) report an interesting result where anisometropic amblyopes improved by approximately the same amount in the experimental (dichoptic game group) and control condition (watching action television series with an eye patch). Strabismic and mixed amblyopes, on the other hand, only improved in the active treatment condition. A meta-analysis by Levi et al. (2015) expanded on this, showing that in the stereoacuity domain, strabismic amblyopes are much more likely to improve in dichoptic conditions, compared to monocular conditions.   Tsirlin et al. (2015) did not show an effect of amblyopia type on VA in their meta-analysis of behavioral training, however, they group patients with microstropias into the anisometropia group, while other groups (e.g. Levi et al., 2015) classify these as mixed-type amblyopia.

While the research community has made great progress in measuring visual improvement with behavioral training, and particularly video games, in the past few years, there are still several important unknowns that will be addressed in the present study.

4.1.2 Study aims

The aim of this chapter is to test the feasibility of action video games with a younger population of amblyopes (age 7-17). While several groups have recently conducted studies with similar goals, they all cite motivation and compliance as a challenging factor that may be limiting their results. Instead of sending the children home to play games, the present study will conduct supervised training in the lab. While logistically more challenging to the patients and their families, this design offers several key advantages. First, we can ensure that all patients receive the same training dosage with the proper optical correction. Second, we can have participants use a mirror-stereoscope to view the game content dichoptically, which may be important for proper binocular alignment in some of the patients. For example, several of the strabismic amblyopes initially experienced difficulty fusing the dichoptic content. By starting with a very low contrast image in the NAE and adjusting the mirrors on the stereoscope until bifoveal alignment was achieved, patients learned to fuse the two images. These patients became more proficient with maintaining fusion as the training progressed. While several of the studies used dichoptic content, it was presented with anaglyph or shutter glasses, which do not allow the same control over binocular alignment.

The dichoptic condition will be compared to a monocular treatment, where patients play the same action video game with their NAE patched. This control condition is more similar to traditional treatment than a sham condition where the content to the two eyes is flipped, and will help provide further insight into whether a dichoptic setup yields better improvement than the monocular treatment.

Finally, we aim to assess outcome differences according to amblyopic type. As previously noted, research that shows that dichoptic game playing is more beneficial for strabismic amblyopes than the more passive condition of patching and watching action TV shows (Vedamurthy et al., 2015). It is unknown if the dichoptic nature or active treatment was more influential in this result. The present study will add to this knowledge by investigating whether there are differences in effect size for strabismic vs. anisometropic amblyopes in the two action video game conditions. This will provide valuable insight in understanding the mechanisms affected by strabismic and anisometropic amblyopia, and will be essential if training studies move from the lab to the clinic.

4.2 Methods

4.2.1 Study participants and ethics statement

The study took place at two research laboratories, one at University of Rochester in New York and the other at University of California, Berkeley. The Research Subjects Review Boards at both institutions approved the study protocol, and did not ask for the study to be registered as a clinical trial. The study was conducted according to the tenets of the Declaration of Helsinki and informed consent was obtained from each participant and their parent. Twenty (n=20) children (mean age: 9.95 ± 3.14, range 7-17 years) with unilateral amblyopia completed twenty hours of video game training (see Figure 4.1. Consort Diagram, for numbers of participants screened, qualified and dropped out). Participants were recruited through the eye clinic at UC Berkeley and through print advertisements at both locations. Three experienced optometrists provided complete eye exams for all participants prior to enrolling.

The inclusion criteria included: (1) age 7-17; (2) anisometropic amblyopia, strabismic amblyopia, or mixed (i.e., anisometropic and strabismic); (3) interocular VA difference of at least 0.2 LogMAR; and (4) no history of eye surgery except those to correct strabismus. Exclusion criteria included: (1) non-comitant or large angle constant strabismus (>30 prism diopters); and (2) any ocular pathological conditions (e.g., macular abnormalities) or nystagmus. All of our participants had normal or near normal VA in the NAE ($20/12^{-1}$– 20/25). The retinal health of all participants was assessed as normal, and they all had clear ocular media as assessed by ophthalmoscopy. Cover tests were used to assess ocular alignment at both distance and near. Clinical data of all study participants is summarized in Table 4.1.

Table 4.1. Study Participants

SID	Age	Classification	Training Type	Starting VA: AE (logMAR)	EOS VA: AE (logMAR)
A1	9	Aniso	Mono	0.20	0.32
A2	11	Aniso	Mono	0.56	0.56
A3	9	Aniso	Mono	0.40	0.22
A4	11	Aniso	Mono	0.48	0.42
A5	7	Aniso	Mono	0.40	0.10
A6	15	Aniso	Mono	0.58	0.52
A7*	10	Aniso	Dichoptic	0.28	0.32
A8	7	Aniso	Dichoptic	0.46	0.10
A9	17	Aniso	Dichoptic	0.32	0.18
A10	10	Aniso	Dichoptic	0.34	0.18
A11	7	Aniso	Dichoptic	0.96	0.94
A12	11	Aniso	Dichoptic	0.44	0.32
A13	13	Aniso	Dichoptic	0.44	0.34
S1	8	aniso/strab	Mono	0.72	0.48
S2	9	aniso/strab	Mono	0.34	1.02
S3	11	aniso/strab	Mono	0.70	0.20
S4	8	aniso/strab	Mono	0.66	0.56
S5	9	aniso/strab	Mono	0.48	0.84
S6	7	aniso/strab	Dichoptic	0.54	0.18
S7	17	aniso/strab	Dichoptic	1.06	0.66
S8	7	strab	Dichoptic	0.36	0.62
S9	7	strab	Dichoptic	0.74	0.44

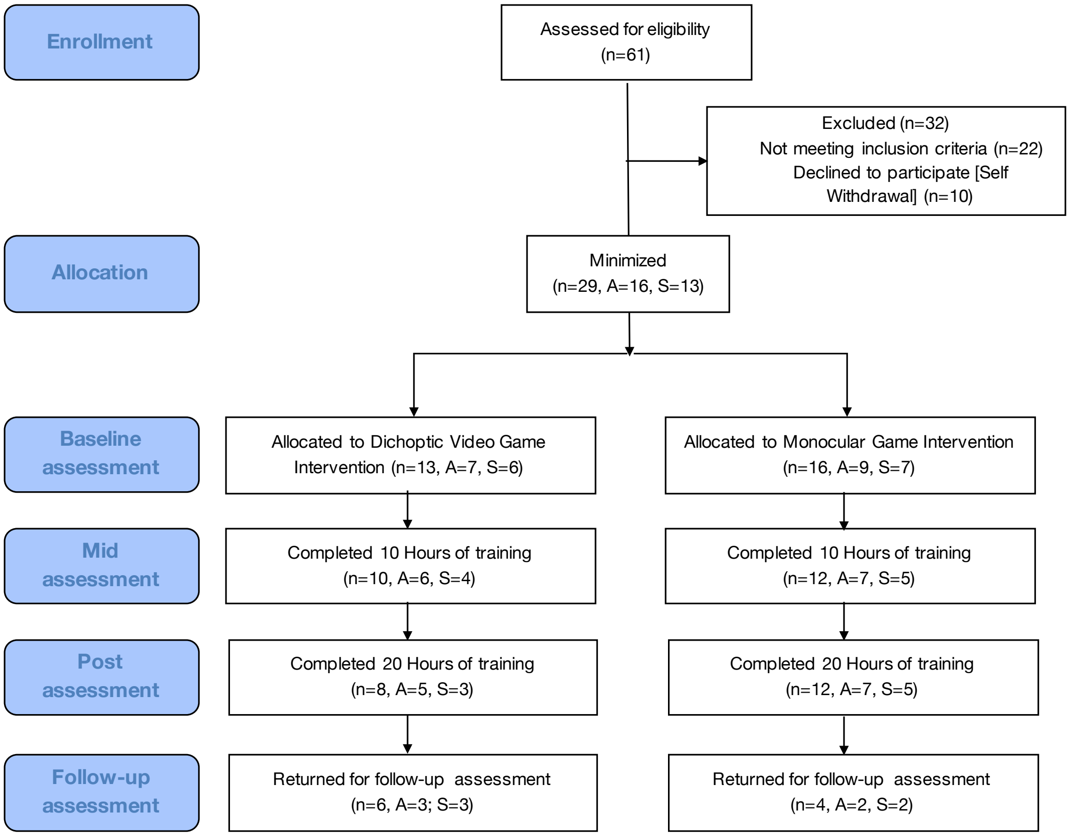
*Dropped from analysis. Participant wore different Rx corrections for pre/post assessments.

Subject Classification. Study participants were categorized as either anisometropic ('aniso') or strabismic ('strab') amblyopes. Anisometropia was defined as ≥ 0.50D difference in spherical equivalent refraction or ≥ 1.5D difference in astigmatism in any meridian, between the two eyes (Wallace et al., 2011). Amblyopic subjects with anisometropia and an absence of manifest ocular deviation were classified as anisometropic amblyopes. Those with an ocular deviation (strabismus), as indicated by the cover test, were classified as strabismic amblyopes, irrespective of their refractive state, meaning that participants with both strabismus and anisometropia were defined as ‘strabismic’.

4.2.2 Study design overview

The complete experimental design is detailed in Figure 4.1. Following consent and screening, participants were assigned into one of two intervention groups: (1) dichoptic game group (n=13): playing the custom-made dichoptic videogame using a mirror stereoscope and balanced input (see description below); (2) monocular game group (n=16): playing the same game with the non-amblyopic (NAE) view turned off and that eye occluded with a black eye patch. Groups were assigned via minimization, i.e. the first several participants were randomly assigned to a treatment and later participants were assigned to reduce the imbalance between the groups. This was particularly important to approximately balance the amblyopic subtypes (anisometropic and strabismic) for each training method.

Figure 4.1. Study Design



Total number included in each portion of the study (n), further divided into anisometropic amblyopes (A), and strabismic amblyopes (S).

Drop-out rate for both groups was moderate because of the substantial time commitment required for training in the lab. We note that the two groups were similar in age (10.2± 4.0 and 9.72.1± 2.2 years in the dichoptic and monocular groups, respectively), and in distribution of amblyopia type (≈ 40 % strabismic and 60% anisometropic in each group), but differed slightly, although not significantly, in their baseline VA (0.58±0.28 vs. 0.50±0.16 logMAR in the dichoptic and monocular game groups, respectively).

Before starting the 20-hour intervention, participants completed a battery to assess vision and related functions ('baseline assessments'). Participants repeated the battery after the completion of 10 hours ('mid-assessment'), and 20 hours ('post-assessment'). Additionally, half (n = 10) of the participants that completed the study returned for one last assessment following a six-week no-contact period ('follow-up assessment'). One child (anisometropic, monocular treatment) was removed from analysis because he wore different refractive corrections for each of the assessments.

4.2.3 Study interventions

Participants from both groups were required to complete a total of 20 hours of experimental treatment, in sessions lasting approximately 1 hour, 1-3 times/week. Participants were instructed to wear their most recent optical prescription at all times, but were given trial frames with their refractive correction for training in the lab if they arrived without spectacles or contacts. Prior to enrollment, we assessed whether participants AE acuity could be improved with further refraction alone. A few children met this criterion and they were given 6 to 8 weeks of refractive adaptation with an updated prescription. They then returned to the lab for a new baseline assessment before starting the study.

Participants in both groups played a child-friendly version of the Unreal Tournament video game (Epic Games, 2004), which we have developed from a previous version of the adult game (see Figure 4.2, for full details see Bayliss et al., 2012, 2013 and Vedamurthy et al., 2015). In the child-friendly version of the action game, we removed the violent elements of the original action game while maintaining the motivating high-speed nature of a commercial game.

Figure 4.2 Game Design

Children in the dichoptic group aligned the game with a mirrored stereoscope (left). During set-up, the children adjusted the mirrors and alpha level of the content (right) to the NAE such that both the green (AE) and red (NAE) part of the fixation scope were visible. The experimenter monitored responses to the Gabor patch to ensure that the AE was not suppressed during game play. In the control condition, the NAE image was turned off and an eye patch was used to cover this eye.

Easier training levels were also included so that young children with little game experience could master the skills required to play the game. The initial training levels included basic environmental cues and boundaries to keep the children focused while learning to move, pick up objects, and orient their pointer tools. Children were instructed to move throughout the scene, collecting health points and tag one or more robot opponents. To tag the opponents, the fixation scope was aligned onto the center of the robot and the mouse was clicked to activate the pointer tools. This was operationally similar to a first-person-shooter game, but instead of guns the pointer tools included a juice machine, bubble wand, etc.

Once basic proficiency with the game was achieved, the children played the tag game in one of three main worlds, each with a variety of colorful features at a variety of spatial frequencies. To maintain engagement over the course of the entire study, additional robot opponents were added, and the difficulty level of the game was modulated, causing the robots to move faster across the screen.

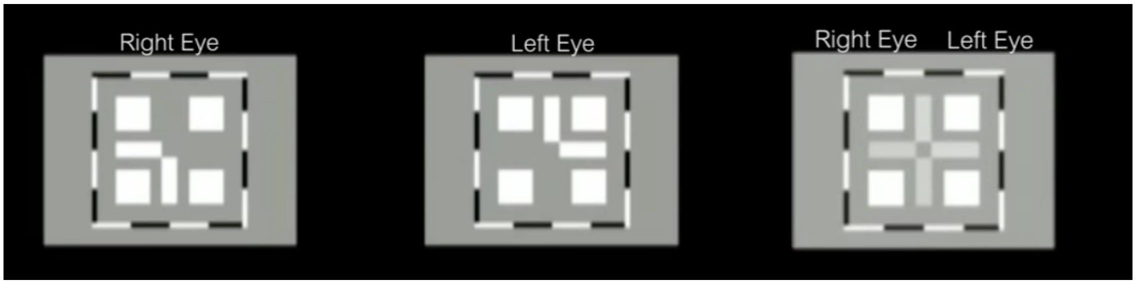
An orientation discrimination perceptual learning task was seamlessly integrated within the game, with a Gabor patch that would randomly appear every few seconds in the view of the AE only. Participants were required to respond to one orientation by tagging the target, and to the other orientation by ignoring the patch or pressing the letter ‘E’. An incorrect response transformed the Gabor patch into a particularly powerful game enemy. The spatial frequency of the Gabor patch was adapted to maintain participant’s performance at 79% correct (Levitt, 1971). The Gabor patch task enabled us to monitor the AE’s resolution limit, while simultaneously serving as a suppression check (particularly important under dichoptic mode, see below), ensuring that the AE was actively engaged during game play.

4.2.4 Dichoptic game mode

In the dichoptic game mode, the game presented a split screen view, allowing independent control of the images presented to the left and right eyes, and in particular their respective alpha level. The split images of the game were viewed with a custom designed stereoscope at a distance of 68 cm. These dichoptic viewing conditions were designed in order to reduce suppression and promote fusion, while challenging the AE with an embedded psychophysical resolution task. Alpha blending was used to balance the perceived image strength of the NAE with that of the AE eye at the start of each play session, in an effort to reduce suppression and facilitate fusion.

At the beginning of each session, children aligned the dichoptic nonius lines (see Figure 4.3) by adjusting the mirrors of the stereoscope. If there was a vertical offset, the images on the screen could also be nudged up or down. The image to the right eye was the bottom and left side of the cross, while the image to the left eye was the top and right side of the cross. With proper alignment, the image was a cross with a square cutout of the center, surrounded by four additional squares and a high contrast border that was visible in both eyes. After launching the game, the experimenter checked in again with the child to ensure that both parts of the fixation scope were visible. The experimenter could also monitor the performance of the gabor task to confirm that the child was not suppression the AE.

Figure 4.3. Dichoptic Alignment



Fusion was achieved by aligning dichoptic horizontal and vertical lines to make a cross. A high contrast border and additional squares to presented to both eyes provided context to aid in this process.

We note that there are important differences between our method of dichoptic presentation and those used by others. Our action video game presented the same image to each eye (except for Gabor patches and part of the fixation scope) with reduced luminance/contrast in the NAE, in an attempt to promote binocular fusion, whereas other dichoptic video game studies have presented different game elements to each eye so that binocular combination is required to play the game (see Hess et al., 2014a for a review). Both approaches have been shown to reduce binocular suppression as well as to improve VA and stereopsis (Vedamurthy et al., 2015).

4.2.5 Monocular game mode

Participants in the monocular game group played the custom videogame described above, but with the NAE display turned off, and a patch over this eye. Other features of the game, such as the perceptual learning task presented to the AE, remained identical to the dichoptic group. Training parameters, such as game difficulty and duration of sessions were also kept the same in both groups.

For all participants, the videogame was displayed on a gamma corrected monitor (Mitsubishi Diamond Pro 2070 SB), with resolution 1024 x 768 pixels and refresh rate of 60 Hz.

4.2.6 Visual function assessments

Participants were required to wear their best optical correction (if any) for all visual assessments. Our assessments included two primary measures, VA and stereoacuity, and two secondary measures, CS and reading speed. Assessments were administered at baseline and following 10 and 20 hours of training. Follow-up assessments 6-10 weeks post training were conducted in half of the participants that completed the study.

Visual acuity (VA)

Clinical visual acuity (VA) at distance was measured using either Bailey-Lovie logMAR letter charts (UCB site), or the high-contrast ETDRS format chart with Sloan optotypes (catalog No. 2104; Precision Vision, La Salle, Illinois; U of R site). Monocular acuities for both the AE and NAE, as well as binocular acuity were all measured with the same conditions.

Stereoacuity

Stereopsis was measured using the Randot Stereotest (Stereo Optical Co., Inc.; See description in Simons, 1981). Analyses were performed on the logarithm (base 10) of the stereoacuity values. Participants that ‘failed’ the stereo test were assigned a value of 600 arcsec (similar to Vedamurthy et al., 2015 and Wallace et al., 2011). Analyses were repeated on a subset of the study populations to only include the patients that had measurable stereoacuity. We found similar results in these analyses to those that included all study participants. There were no patients that went from no measureable stereoacuity to some consistently measurable stereoacuity.

Contrast Sensitivity (CS)

We used the Pelli-Robson Contrast Sensitivity Chart to measure CS (Pelli et al., 1988). Measurements were made for each eye separately, as well as binocularly, at a distance of 1 meter.

Reading Speed

Reading speed for reading out-loud was evaluated using the standardized MN Read Acuity Chart (Legge et al., 1989). This chart includes 16 lines, with a full sentence on each line, and each successive line is reduced in letter size by 0.1 Log units. The words chosen for the sentences are ones which commonly occur in second or third grade reading material. All but one child in the study could comfortable read the sentences with suprathrshold print size. This child was removed from the reading analysis. The test was run for each eye separately and then binocularly. One of two charts, each with the same parameters, was chosen for each viewing condition.

The time it takes to read each line, and the number of errors on that line are used to assess reading metrics. Basic reading speeds were calculated in words per minute (WPM) after accounting for reading errors. We then calculated a difference reading speed score for each participant. This was derived by first calculating the reading speed difference (post minus pre or follow-up minus pre) for each print size value, and averaging across the number of print sizes read by that participant. This difference between WPM was used for data analysis.

We also investicated the critical print size (CPS), which is the smallest sentence line that the participant can read without a significant reduction in reading speed or increase in the number of errors. To calculate this value, we found the mean reading speed of the first 5 lines, and the threshold speed, which was this mean multiplied by 1.96 times the standard deviation. The CPS was the smallest print size that was less than this threshold.

4.2.7 Data analysis

Our primary hypothesis concerns the efficiency of the dichoptic game versus monocular game intervention on visual, stereoacuity, contrast sensitivity, and reading speed. Because the number of subjects within each subgroup (amblyopia type: anisometropic and strabismic; treatment type: monocular and dichoptic) was small, we performed repeated-measures ANOVAs (without within-subjects factors) to assess the overall change, or main effect, for each dependent measure.

To assess sub-group pre/post differences with limited sample sizes, we performed dependent t-tests with Bonferroni correction. As there were 4 tests run (dichoptic, monocular, aniso, strab), the criterion required for significance on these tests was p=0.01 or below. In all cases, the data reported are the mean ± the standard error.

Missing data. Ten participants dropped out or returned to their primary amblyopia treatment following post-training and before the follow-up assessments, hence their data is missing from follow-up. In addition, CS data is missing for nine participants, as this assessment was added midway through the study. MN Read data was missing for one or more assessments for 5 of the participants, in all cases due to data not being recorded correctly. In cases of missing data, these participants were omitted from the analysis.

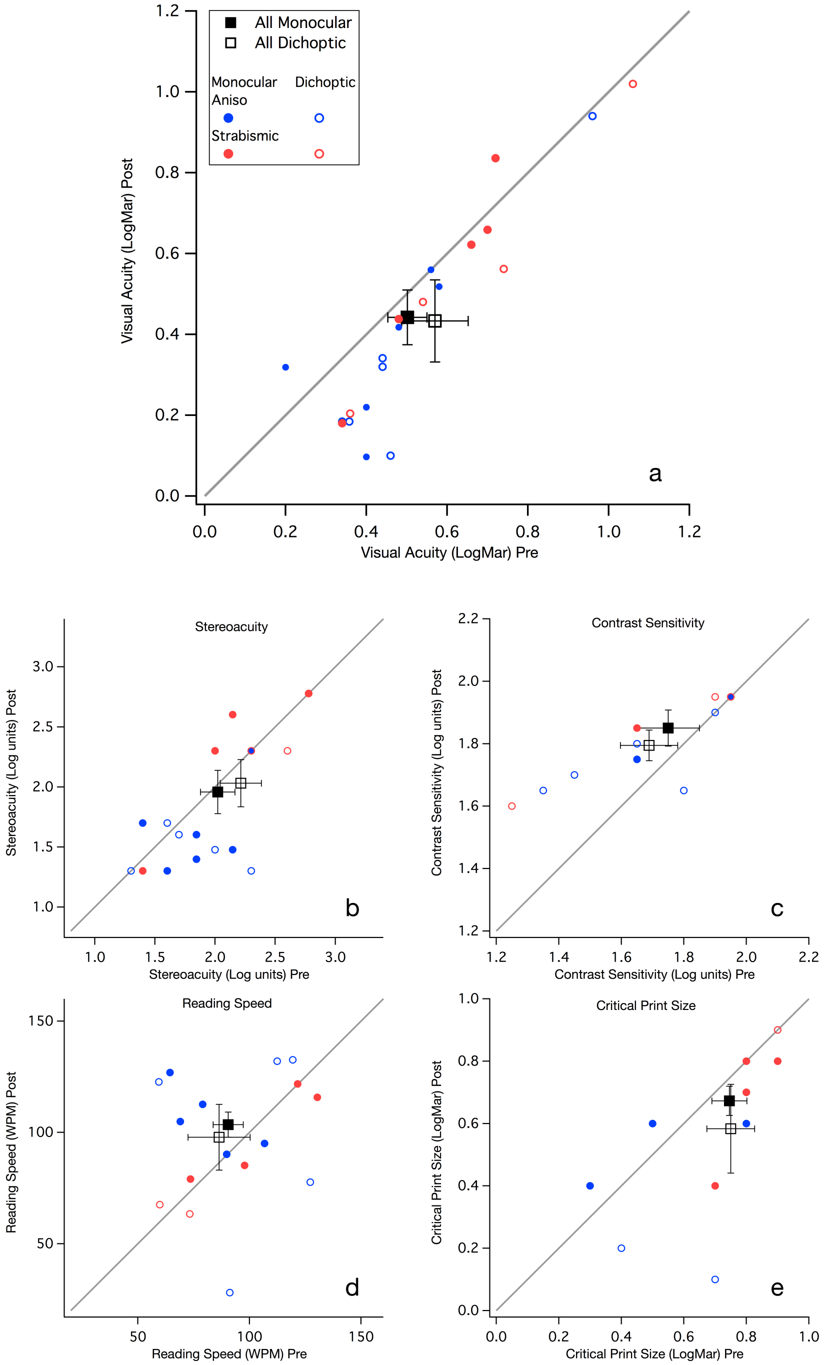
4.3 Results

4.3.1 Changes in clinical visual acuity (VA)

Playing action video games can lead to significant VA improvements in children with amblyopia. The mean change for all subjects (n = 21) was 0.1 ± 0.025 logMAR units, or the equivalent of one line on a letter chart. This result was significant on a repeated measures ANOVA (**F=13.60, p=0.001**).

In Figure 4.4a data of individual participants are plotted, showing improvement in pre vs. post logMAR acuity as displacement below the diagonal line. This figure shows that the mean acuity improvement varied somewhat amongst the four subgroups. To further assess the significance and effect size for the training and amblyopic-type subgroups, paired t-tests (pre/post) were run on the following groups: anisos, strabs, dichoptic, monocular. From this analysis, we can see that VA improvement in the dichoptic group was significant (mean change = 0.14 ± 0.02 logMAR, **t=4.46, p<0.01**) while the monocular group was not (mean change = 0.06 ± 0.04 logMAR, t=1.57, P=0.15). We can also see that the improvement in the aniso group was significant (mean change = 0.12 ± 0.04 logMAR, **t=3.12, p<0.01**), while those in the strab group were trending, but ultimately not significant (mean change = 0.07 ± 0.03 logMAR, t=2.16, p=0.06).

Figure 4.4. Pre/post changes in visual function.



Pre/Post plots of dependent variables: a. visual acuity, b. stereoacuity, c. contrast sensitivity, d. reading speed, e. critical print size. Open symbols are the dichoptic participants (open square is the dichoptic mean). Filled symbols are monocular participants (filled square is the monocular mean). Blue symbols are anisometropic amblyopes, red symbols are strabismic or mixed amblyopes.

4.3.2 Changes in stereoacuity

We were also curious if the children would improve in stereoacuity, and if these gains would be greater in the dichoptic group than the monocular group. The mean change in stereoacuity was 0.12 ± 0.07 log units, which did not have a significant main effect (F=0.12, p=0.74). Not surprisingly, there was a between-subjects effect, with the anisometropic group improving significantly more than the strabismics group (**F=5.96, p=0.03**). There was no a significant difference between the two training groups (F=0.32, p=0.58) and no significant interaction between patient type and training group (F=0.01, p=0.94).

Log-transformed stereoacuity data are plotted in Figure 4.4b, again with improvement visualized as displacement below the diagonal line. Paired t-tests (pre/post) were also run on the following groups: dichoptic (mean change = 0.18 ± 0.11, t=1.77, p=0.13), monocular (mean change = 0.06 ±0.1, t=0.62, p=0.55), anisos (mean change = 0.24 ± 0.11 log units, **t=2.30 p=0.04**), and strabs (mean change = -0.04 ± 0.07, t=0.53, p=0.63). While the dichoptic group did improve more than the monocular group, this was not significant. The anisometropic group showed the greatest amount of change and this was trending, however it was not significant due to the Bonferroni correction. None of the strabismic children improved in stereoacuity. Further, the change in stereoacuity was not related to starting stereoacuity (R^2=0.00, p=0.87).

4.3.3 Changes in contrast sensitivity (CS)

Children training with the action video games also improved significantly in contrast sensitivity CS, by approximately 0.1± 0.04 log units (**F=6.16, p=0.03**). Figure 4.4c shows the pre/post contrast sensitivity data, however this time improvement is demonstrated by a higher CS score, and therefore displacement above the diagonal line.

Paired t-tests (pre/post) were also run on the following groups: anisos (mean change = 0.09 ± 0.06 log units, t=1.57, p=0.17), strabs (mean change = 0.12 ± 0.07 log units, t=1.76, p=0.15), dichoptic (mean change = 0.11± 0.06 log units, t=0.11, p=0.09), monocular (mean change = 0.1 ± 0.06 log units, group too small for t-test), however none of these were significant. This is likely due to the smaller number of participants that completed this assessment (n=12, with A=7, S=5, D=9, P=3).

Unlike the VA and stereoacuity changes, however, there was a significant relationship between the starting CS and the amount of change, with children that started with poorer CS being more likely to have a larger gain in this measure (**R^2=0.79, p=0.0001**). This is likely due to a ceiling effect, as half of the group initially had good CS (>1.75 log units) and showed little to no improvement, while those that had room to improve, did. When the 6 individuals with good CS were removed from the analysis, the remaining 6 showed an average improvement of 0.23 ± 0.04 log units. Three of these children were in the aniso dichoptic group, while the other 3 were evenly distributed amongst the aniso monocular, strab dichoptic, and strab monocular groups.

4.3.4 Changes in reading ability

These changes in visual function also transferred to improvements in reading, an important visual-motor skill that can have a lasting impact on a child's education. Overall, children were able to read significantly more words per minute in their AE after training (mean change = 15.47 ± 4.97 wpm, **F=5.47, p = 0.03**, see Figure 4.4d. Like contrast sensitivity, improvement was measured in an increase in words per minute, and children that improved in this measure are plotted above the diagonal line.

Again, we assess the significance of the change, this time in reading speed, using paired t-tests (pre/post) with Bonferroni correction. There was a trending improvement in reading speed for the aniso group (mean change =21.99 ± 5.99 wpm, **t=2.78, p=0.02**), but not for the strabismic group (mean change = 5.69 ± 7.77 wpm, t=1.72, p=0.15). There was no significant change for the dichoptic group (mean change = 20.12 ± 6.72 wpm, t=1.56, p=0.19), or the monocular group (mean change = 11.66 ± 4.97 wpm, t=2.19, p=0.06).

We also assessed the change in critical print size for the participants in the study (Figure 4.4e). While we expect that some children might show slight improvements in reading speed as a result of natural development, this should not be related to the print size at which they can read comfortably. There was a trending reduction in critical print size after 20 hours of training (0.11 ± 0.04 logMAR**, F=6.16, p=0.03**). Interestingly, this effect was the reverse of the WPM results, with a trending difference for the strabismus group (0.1 +/- 0.03 logMAR, **t=3.06, p=0.02**), but not for the aniso group (0.11 +/- 0.07 logMAR, t=1.55, p=0.16). This could be due to the larger degree of variability in the aniso group for this measure. There was also no significant pre/post difference for the the dichoptic group (0.16 +/- 0.09 logMAR, t=1.81, p=0.13) or the monocular group (0.07 +/- 0.04 logMAR, t=0.69, p=0.51).

4.3.5 Follow-up Assessments

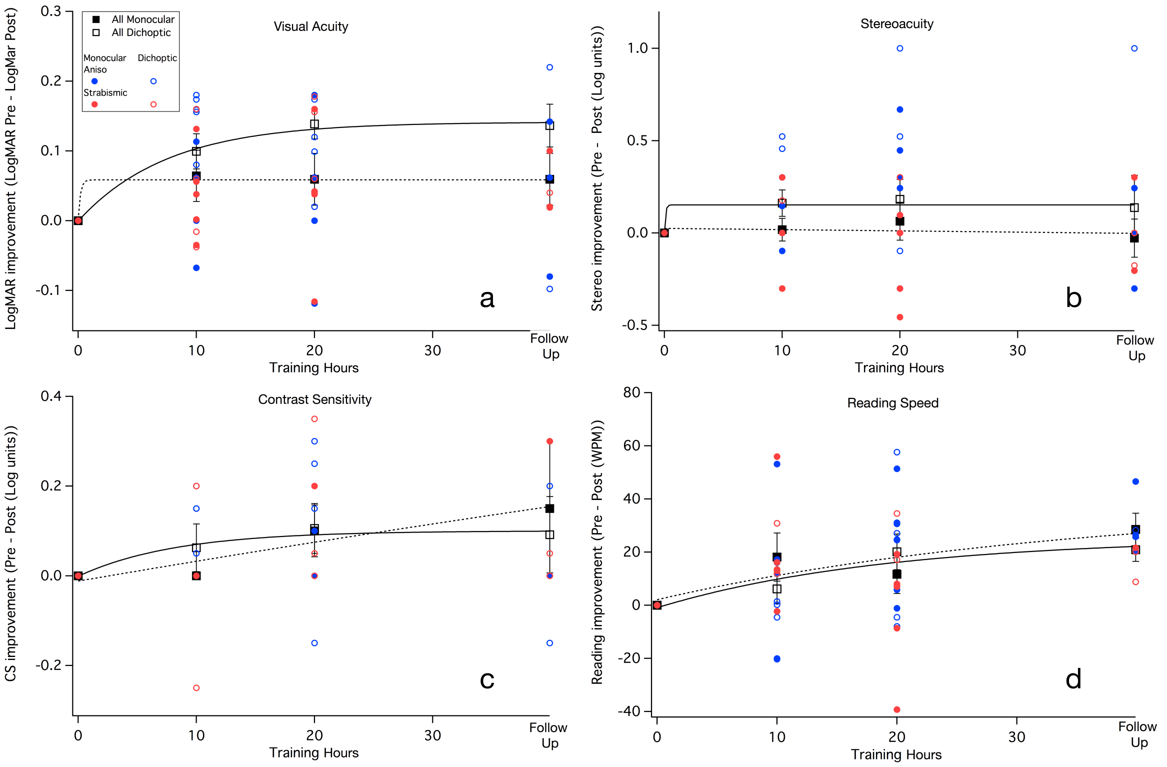
Visual improvements were maintained for at least 6 – 10 weeks after training. For VA, when only the children that returned for a follow-up were included (n=13) there was a 0.11 ± 0.04 logMAR gain from baseline to the end of study, an identical gain (0.11 ± 0.04 logMAR) from baseline to to follow-up. This was different from the observed stereoacuity pattern. In this case, when only the 12 children that returned for a follow-up were included in the total measure, the mean change at the end of the study was 0.11± 0.11 log units, compared to 0.05 ± 0.10 log units at follow up. This could be because there was more variability in the stereoacuity measurements that in the other assessments.

Eight children also completed all CS measures, with an overall change of 0.12 ± 0.06 log units from the baseline to the end of training, and 0.11 ± 0.07 log units from baseline to follow-up. Four children completed all MN read assessments. At the end of the study reading speed in their AE had increased by 14.22 ± 3.72 words per minute. This gain was maintained beyond the training period, with an overall increase in reading speed from the baseline assessment of 24.71 ± 3.77 wpm. These results clearly show that the visual improvements for video game training can persist even after the treatment has stopped.

4.3.6 Dynamics of visual function change

Understanding the time course of treatment efficacy allows one to maximize the treatment benefits and minimize training fatigue. We measured the change in visual function at several time points (baseline, 10HR, 20HR, follow-up) over the course of treatment. These findings are summarized in Figure 4.5.

Figure 4.5 Dynamics of visual functional improvement



Change in dependent variables over training hours, in a. visual acuity, b. stereoacuity, c. contrast sensitivity, and d. reading speed.

For visual acuity (Figure 4.5a) almost all of the improvement occurred during the first 10 hours of training (mean change 0-10HR = 0.08 ± 0.02, **t= 3.65, p=0.002** 10-20HR 0.02 ± 0.02, t=1.05, p=0.31). Looking for more closely, we can see a very different pattern for the two treatment groups. Playing the game monocularly may yield a small (non-significant) improvement initially, but it slows or stabilizes after 10 hours of training (0-10HR mean change = 0.06 ± 0.04 logMAR, t=1.72, p=0.12; 10-20HR: mean change = 0.00 ± 0.03 logMAR, t=0.02, p=0.99). For dichoptic training, most of the change also occurred within the first 10 hours (0-10HR mean change = 0.10 ± 0.03 logMAR**, t=3.96, p=0.003**, but acuity continued to improve slightly (and non-significantly) with more training (10-20HR mean change = 0.05 ± 0.03 logMAR, t=1.49, p=0.18).

Comparing the two patient subgroups in the dichoptic training group, we can see further differences in the dynamics of VA change. The anisometropic patients had greater improvements in the first 10 hours of training (0-10HR mean change = 0.12 ± 0.03 logMAR, **t=3.41, p=0.006**), but then stabilized with little to no improvement after this point (10-20HR mean change = 0.01 ± 0.02 logMAR, t=0.59, p=0.57). In contrast, the strabismic group had slow and consistent (non-significant) gains across all training periods (0-10HR: mean change = 0.04 ± 0.02 logMAR, t=1.68, p=0.13; 10-20HR: mean change = 0.03 ± 0.04 logMAR, t=0.84, p=0.43).

Similar patterns were seen in the time course of stereoacuity improvement (Figure 4.5b). While the overall change was not significant across any of the time points it was slightly more in the first period than the second (0-10HR mean change = 0.08 ± 0.05, t=1.71, p=0.10; 10-20HR mean change = 0.01 ± 0.07, t=0.23, p=0.82). The dichoptic group had the largest gains and approached significance in the first 10 hours, but then leveled out and was not significant in the second (0-10HR mean change = 0.16 ± 0.07**, t= 2.26, p=0.05**; 10-20HR mean change -0.03 ± 0.09, t=0.30, p=0.78). Similarly, the anisometric group approached significance in the first 10 hours (mean change = .15 ± 0.06, **t= 2.53, p=0.03**). Although some individuals continued to improve, others did not and the mean change for the second 10 hours of training was moderate and not significant (0.08 ± 0.08 t=1.10, p=0.30). Finally, the strabismic group did not show improvements during either time period (0-10HR mean = -0.02 ± 0.07, t=0.21, p=0.84; 10-20HR mean = -0.09 ±0.11, t=0.78, p=0.46).

Different patterns were observed for CS (Figure 4.5c) and reading speed (figure 4.5d). For the contrast sensitivity measures, all groups showed more or less similar, nonsignificant changes (total group change mean = 0-10HR = 0.05 ±0.04, t=1.66, p=0.27; 10-20HR mean = 0.06 ± 0.04, t=1.56, p=0.15). This pattern was the same in the dichoptic group (0-10HR mean change = .06 ± 0.07, t= 1.17, p=0.28;

10-20HR mean = 0.04 ±0.06, t=0.8, t=0.45). The increase was more varied in the monocular group, but this was based off of only 3 participants (0-10HR mean = 0 ± 0; 10-20HR mean = 0.1 ± 0.06 (sample size too small for testing, but all participants increased from 10-20hrs). The aniso group had the largest change in the first 10 hours of training that approached significance (0-10HR mean = 0.11± 0.04, **t= 2.99, p=0.04**), but this flattened out with no additional gains in the second 10 hours of training (10-20HR mean = 0.01 ± 0.02, t=0.42, p=0.70). Finally, the strabismic group showed little change in the first 10 hours of training (0-10HR mean = 0.01 ±0.07, t=0.14, p=0.90), and a larger change in the second 10 hours of training (10-20HR mean =0.13 ± 0.08), however, the sample size for the second period was too small for testing.

For reading, there were little to no gains across all groups for both training periods (0-10HR mean =5.23 ± 6.58, t=0.79, p=0.44; 10-20HR mean = -0.01 ± 8.74, t=0.92, p=0.38). Similarly, there was little to no mean difference in the dichoptic group for the first 10 hours, (0-10HR mean = -0.23 ± 4.63 wpm, t=0.91, p=0.40) and a small, but insignificant increase for the second 10 hours (10-20HR mean = 17.09 ± 10.76 wpm, t=1.59, p=0.19). On the other hand, for the monocular group, there was a small (insignificant) increase in the first 10 hours (0-10HR mean = 16.33 ± 8.6 wpm, t= 1.01, p=0.35), but not in the second (10-20HR mean = -0.65 ± 8.10 wpm, t=0.53, p=0.61). The aniso group had a little to no change in the first 10 hours (0-10HR mean = 4.54 ± 7.55 wpm, t=0.60, p=0.57), and a moderate (trending) increase in the second 10 hours (10-20HR mean = 19.11 ± 6.61 wpm, **t=2.89, p=0.03**). Finally, the strabismic group had moderate (not significant) improvement in the first 10 hours (0-10HR mean = 15.48 ± 8.42 wpm, t=0.51, p=0.63) and little to no improvement in the second 10 hours (10-20HR mean = -8.93 ± 9.23 wpm, t=0.96, p=0.38).

4.3.6 Change in dependent variables not correlated

Interestingly the changes in the dependent variables were not related, so a participant who improved significantly in VA was not more likely than someone who showed no VA improvement to see gains in stereo, contrast sensitivity or reading, (VA/CS: R^2=0.05, p=0.47; VA/Stereo: R^2=0.02, p=0.59; CS/Stereo: R^2=0.12, p=0.26; VA/WPM: R^2=0.07, p=0.26; VA:CPS: R^2=0.02, p=0.59; CS/WPM: R^2=0.05, p=0.51; CS/CPS: R^2=0.29, p=0.27; Stereo/WPM: R^2=0.02, p=0.52; Stereo/CPS: R^2=0.03, p=0.51). This finding provides initial evidence that there are most likely multiple mechanisms being activated through the video game training and that they show different levels of plasticity in the different participants. One factor that may be contributing to the different patterns of visual function recovery may be previous treatment, which will be discussed in a section below.

4.3.7 Game related measurements

All children started on the introductory game level and advanced in difficulty level once they scored approximately 10-15 points per 20 minute session. All children reached a minimum of level 2 and a maximum of level 5 over the course of the 20 hours of training. We hypothesized that game proficiency may have served as a proxy for overall engagement with the game. While older children were more likely to reach higher game levels (**R^2=0.39, p=0.002**), skill level was not related to the degree of improvement in visual acuity (R^2=0.01, p=0.68), stereoacuity (R^2=0.00, p=0.97), contrast sensitivity (R^2=0.11, p=0.32), or reading measures (WPM: R^2=0.05, p=0.34; CPS: R^2=0.07, p=0.30).

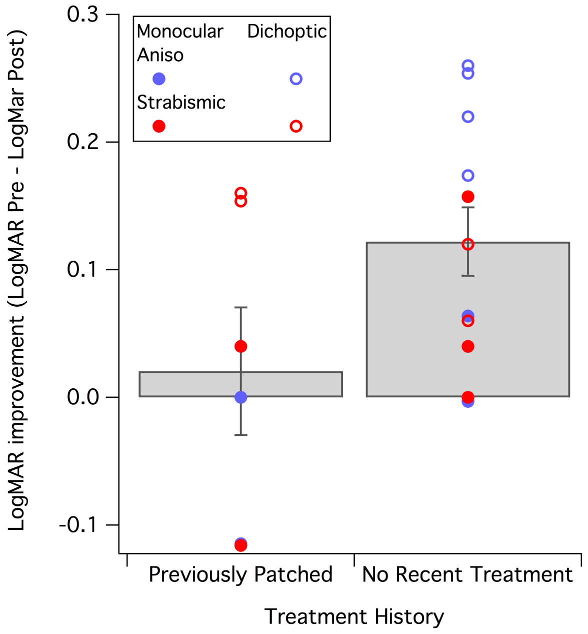
4.3.8 Participant factors

Given the mixed results of previous studies, we were curious if participant factors such as the child's age and start acuity could be related to improvement on visual assessments. We found no relationship between age and VA improvement (R^2=0.01, p=0.62), as well as no relationship between the starting VA in the AE and VA improvement (R^2=0.01, p=0.70). We also found a lack of correlation for both participant factors in Stereo change (Age/Stereo change: R^2=0.04, p=0.39; Initial VA/stereo change: R^2=0.20, p=0.09) and contrast sensitivity (Age/CS change: R^2=0.02, p=0.57; Initial VA/CS change: R^2=0.00, n=12, p=0.997). Finally, we also see no relationship between improvement in reading metrics and age (WPM: R^2=0.02, p=0.55; CPS: R^2=0.12, p=0.17) or starting acuity (WPM: R^2=0.01, p=0.67; CPS: R^2=0.01, p=0.70).

However, one participant factor did have significant relationships with the degree of improvement in all 4 visual functions: *treatment history*. We separated our patients into two groups: those that had previously patched in the previous 6 months (n=6), and those that had not had recent treatment (n=12). The treatment history of four patients was unknown and they were excluded from this analysis. The two groups were significantly different in age, with the patching group being an average of about 3 years younger (mean age recent treatment = 8.33 ± 0.61 years old vs. 11.66 ± 1.06 years old, **t=1.80, p=0.05**). However, both groups had similar starting VAs in their amblyopic eye (0.53 ± 0.17 logMAR in the patching group, 0.57 ± 0.22 logMAR in the non-patching group, t=0.41, p=0.34). While the recently treated group had little to no improvement in VA (0.02 ± 0.05 logMAR), the group that had not patched in the last six months had significantly more improvement (0.12 ± 0.03 logMAR, **t=1.97, p=0.03**) (See Figure 4.6). It should be noted, however, that all of the anisometropic children that received dichoptic training were in the group that did not have a history of recent treatment. As this group had the greatest overall gains, this may have contributed to the findings on treatment history.

Similar patterns were seen in the stereo (0.04 ± 0.09 log units vs. 0.16 ± 0.11 log units), CS (0 logMAR vs. 0.11 ± 0.06 logMAR gain), and reading CPS (0.08 ± 0.04 logMAR vs. 0.3 ± 0.08 logMAR) variables, but not reading WPM (15.08 ± 19.49 words vs. 16.50 ± 6.98 words). Sample size for several of these additional measures were too small to test for significance.

Figure 4.6 Change in logMAR acuity by treatment history



Total change in logMAR acuity in children that had recently patched (left column) and had not recently patched (right column). Grey bars are the group means ± the SE. Individual data points follow the standard convention from earlier figures.

4.3.9 Training dosage

While an independent t-test shows the dichoptic group trained significantly more minutes per week than the monocular group, the mean training time per week did not significantly correlate with change in VA (R^2=0.07, p=0.26), stereo (R^2=0.15, p=0.08), CS (R^2=0.09, p=0.39), or reading metrics (WPM: R^2=0.18, p=0.06; CPS: R^2=0.00, p=0.95). Other training measures, such as the variability in training schedule (standard deviation of training duration per day, stdev) or the number of missed sessions (ms) while enrolled in the study also did not significantly correlate with change in any of the visual assessments, including: VA (stdev: R^2=0.01, p=0.68; ms: R^2=0.00, p=0.87), stereo (stdev: R^2=0.00, p=0.84; ms: R^2=0.02, p=0.50), CS (stdev: R^2=0.02, p=0.72; ms: R^2=0.01, p=0.91), or reading (WPM stdev: R^2=0.00, p=0.91; WPM ms: R^2=0.04, p=0.46; CPS stdev: R^2=0.13, p=0.15; CPS ms: R^2=0.00, p=0.94).

4.4. Discussion

4.4.1 Summary

Our goal for this study was to provide further evidence that action video game playing is a beneficial treatment for children with amblyopia, and to determine if dichoptic game playing is more effective than playing with the AE only. Our results show that VA improvement in the dichoptic game was significant (0.14 ± 0.02 logMAR, **t=4.46, p<0.01**), while VA improvement in the monocular group was not (0.06 ± 0.04 logMAR, t=1.57, P=0.15). We were also curious if there would be outcome differences between the two subgroups of amblyopia. While both groups improved by about the same amount in CS (0.09 ± 0.06 log units for the anisos, vs. 0.12 ± 0.07 for the strabs), the anisometropic group had significantly larger gains in both VA (0.12 ± 0.04 vs. 0.07 ± 0.03 logMAR, **t=3.12, p<0.01**) and stereoacuity (0.24 ± 0.11 vs. -0.04 ± 0.07 log units, **t=2.30 p=0.04**). Children also improved significantly on a reading task, reading more words per minute and smaller print sizes at a comfortable reading speed, after video game training.

4.4.2 Action video game training in children vs. adults

The children in our dichoptic group had similar VA improvements as the adults that played a dichoptic action game in a previous study (Vedamurthy et al., 2015), yet they reached this with half the training time of the adults (20 vs. 40 hours). We looked to see if the rate of improvement in the children was faster, and found inconclusive results. This was because the blocks of training hours at which vision was assessed were slightly longer for the adult group, and improvement slows over the course of the training, making it difficult to directly compare the two groups. However, we did look at the overall gain and rates of improvement at each time point and found that VA appeared to improve slightly more quickly for the children (0.10 ± 0.03 logMAR with 10 hours of training for the dichoptic kids for a dosage rate of .01 logMAR /hour vs. 0.08 ± 0.02 logMAR with 13 hours of training for the dichoptic adults for a dosage rate of 0.006 logMAR /hour). With 20 hours of training the children had an improvement of 0.14 ± 0.02 logMAR for a dosage rate of 0.007 logMAR /hour vs. 0.13 ± 0.01 with 26 hours of training for the adults, for a dosage rate of 0.005 logMAR /hour.

Our results are consistent with previous studies testing video game playing as a treatment for kids with amblyopia, which have reported a benefit of 0.08 – 0.18 logMAR. There are several hypotheses for this moderately large range of results. First, one major factor in our study was treatment history, where children that had recently undergone occlusion therapy were much less likely to show improvements in visual function. This has also been noted in several other children's studies. For example, Holmes et al. (2016) report an improvement of 0.12 logMAR for all of the children that played a dichoptic game in their study, however, when they only included children that had not recently patched, the mean improvement more than doubled to 0.25 logMAR.

Second, there may also be an effect of compliance, as this varied widely among the studies, from near 100% (Kelly et al., 2016) to 22% (Holmes et al., 2016). Many research groups send the children home with an iPad, or have the children complete the training on a computer at home. This design results in a wide variation in compliance, both within and between studies, causing large differences in dosage, despite approximately the same time period between assessments. On the other hand, our study had the children come into the lab to train, which allowed us to make sure each child had the same dosage between visual assessments, but the time to complete this training varied widely (from 3 weeks to 20 weeks). In both study designs, there does not appear to be a direct relationship between overall compliance and visual improvement, however, as studies that report better compliance also have slightly better outcomes, more work is needed to understand this complex factor.

4.4.3 Dichoptic vs. monocular training

As previously mentioned, it is important to understand if dichoptic training provides an additional benefit beyond monocular training, as it is logistically more challenging. Additionally, there is a potential for the development of diplopia with the dichoptic training, although few if any cases of this have been reported. Thus, along with our main effects, we also investigated the pre/post effects in the two treatment groups. We found that the 10 patients in the dichoptic group improved significantly in visual acuity, while the 12 in the monocular group did not. There were no significant differences in training type for the other visual assessments, however these measures had smaller sample sizes. For example, when just comparing stereo improvement in the aniso group (as this was the only group to show a significant change), the mean gain was near identical for the two training groups (monocular mean = 0.23 ± 0.14 log units, vs. 0.25 ± 0.17 log units for the dichoptic group, both n = 6). While we hypothesized that the dichoptic group would show greater stereoacuity improvements, previous studies have shown improvement in both training types, so perhaps this is not altogether surprising.

The study most similar to this is by Li et al. (2013), who measured visual acuity and stereoacuity improvement in adults that played either a dichoptic or monocular version of the Tetris game. After 10 hours of training, their dichoptic group improved by 0.17 logMAR, which is identical to the improvement of VA improvement in our study for the children that played dichoptic games and did not recently patch. Their monocular group also showed similar (non-significant) gains as our children that had not previously patched (0.04 logMAR in the Li study, compared to 0.05 logMAR in the current study). Unlike our study, however, they did find a significant difference in stereoacuity improvement between the two groups.

Together, these two studies show that there does appear to be an advantage for dichoptic training, however, more work is needed, both with larger patient populations to better clarify the training outcomes, as well as in understanding if there are differences in the neural mechanisms that lead to these results. For example, we hypothesize that fixation stability and bifoveal alignment may be key. As discussed in earlier chapters, fixation stability and oculomotor control is linked to visual acuity (Chung et al., 2015) and intact binocularity may be important for fixation stability (Shaikh et al., 2016). Therefore, we believe that the dichoptic presentation, which provided content to both foveas, may have allowed for better fixation stability than when viewing the content with the amblyopic eye only. Over the course of the training, the luminance to the non-amblyopic eye was increased, training better binocular integration. This, along with the ability to fine tune the foveal positions using the mirrored stereoscope, may be what is providing the additional benefit in the dichoptic treatment group.

Anecdotally, we saw clues that support this claim. At first, our young participants, particularly the strabismic amblyopes, had a difficult time setting up the stereoscope. The concept of “making the lines (presented independently to each eye) look like a cross” was confusing, because they were not accustomed to paying attention to both eyes at the same time. Setting up the display could take a long time, although this was aided by turning the luminance down on the non-amblyopic eye to almost zero. Over the course of the study, all children in the dichoptic condition learned to set up the stereoscope themselves, achieving near immediate fusion with brighter and brighter content presented to their non-amblyopic eye. Meanwhile, this skill was not practiced in children playing the game with a patch over the NAE. We believe this bifoveal fixation, even if it is transient, could boost the attentional mechanisms thought to be important in the cognitive and perceptual benefits from playing video games.

4.4.4 Training effects in anisometropic vs. strabismic amblyopes

The authors that conducted the adult action video game study noticed very different outcomes for their anisometropic and strabismic groups. In sum, their anisometropic group improved in both the control condition (watching action television shows while wearing an eye patch), and the experimental condition (playing the dichoptic action video game), while the strabismic group only improved in the experimental condition. As strabismics have more difficulty with oculomotor control, this fits in with our bifoveal fixation stability hypothesis of why the dichoptic games are more effective.

We were curious to see if these etiological differences were related to treatment in our study with children. Unfortunately, our sample sizes (n=4-6) in each of these subgroups was too small for detailed analyses. However, we do see preliminary evidence that this may be the case. For our anisometropic group, there was marginal (not significant) VA improvement in the monocular group significant (0.08 ± 0.06 logMAR, t=1.35, p=0.23), but trending improvement in the dichoptic group (0.15 ± 0.05 logMAR, **t=3.33, p = 0.02**). For our strabismic group, there was little to no VA improvement in the monocular group (0.03 ± 0.04 logMAR, t=0.71, p=0.52), and moderate improvement in the dichoptic group (0.11 ± 0.03 logMAR, n=4, too small for testing).

We see a similar pattern in our reading results. The anisometropic children improved in reading by similar amounts with both treatment types (23.74 ± 7.75 wpm for monocular training; 20.24 ± 9.81 wpm for dichoptic training). Meanwhile, for the strabismic children, participants in the dichoptic group showed about the same improvement as the anisometropic children (19.89 ± 7.76 wpm), and participants in the monocular group showed no improvement (-2.83 ± 10.13 wpm). As reading is an active task that requires good oculomotor control, these findings also support our hypothesis that binocular training may be needed for improvement in strabismic amblyopes, but that this is less important in anisometropic amblyopes that on the whole have better fixation stability.

While most training studies do not separately compare anisometropic and strabismic amblyopes, these findings show it is important to consider etiology. More work is needed in this area, as these findings have great potential significance for treatment of amblyopia in the clinic.

4.4.5 Feasibility and other limitations

Due to the challenging nature of visiting the lab 2 to 3 times a week, we had moderate dropout in both training groups. Of the 29 children that completed the baseline assessment and were allocated to one training group, only 22 completed the first 10 hours of training. Two more children dropped out prior to completing the second phase of training. More work is needed to simplify the experimental treatment so that the training is portable and engaging. While several groups have looked into this, such as Hess's group which developed a dichoptic Tetris game that can be played on an iPad with anaglyph glasses, and Hussain's group, who developed a game that can be played on a computer at home with a patch over the non-amblyopic eye, there are still problems with these designs. In both of these cases, compliance can still be an issue, as the games can be played without the glasses, or without the patch. As Stewart et al. (2004) found with their occlusion monitoring device, self or parental reports of wear time frequently do not match up with actual usage. Although our design was challenging in that it required kids to travel to the lab after school or on weekends, we were able to directly monitor game play to make sure the children were following the rules and difficulty levels could be adjusted to maintain engagement.

As noted previously, children are more challenging to motivate in training studies, even when games rather than PL regimes are used. As such, several studies have noted more variability in results, and a lack of significance in measurement tools that are informative for adult participants. Therefore, although several of our assessments did not find significant differences between training types, or patient group, it does not rule out the possibility that these differences did exist.

4.4.6 Changes in quality of life

While not all children received a significant improvement in visual acuity, we looked to see if there was a perceivable difference in at least 1 of the 4 visual functions assessed. Our criteria for this was the following, an AE improvement of: 0.1 log unit or more in VA, CS, stereo, or for reading, an increase in 20 wpm *or* 0.1 log unit for critical print size. Twenty of our 21 children met this criterion. Further, 15 of these children reached at least two of these criteria by the end of training. Interestingly, the group of children that met this more stringent set of improvement criteria includes all of the patients in the dichoptic group (n=10). Anisometropic children are about as likely to reach reach these criteria with either training type (5/6 for monocular training vs 6/6 for dichoptic training), however, children in the strabismic group had very different outcomes depending on the type of training they received. All four strabismic children that received the dichoptic training met at least 2 of the improvement criteria, while none of the children in the monocular group improved in more than one area. This is further evidence that dichoptic training yields a better outcome than monocular training, particularly in strabismic patients, and additionally shows that studies should consider more than one visual outcome when determining treatment success.

Finally, we were curious if the children that had previously patched would meet any of the functional improvement criteria described above. We found that all of the children in the recently-patched category improved in reading, and several of the recently-patched anisometropes also improved in stereoacuity. Like the study by Li et al (2015), this shows that there may be additional benefits beyond visual acuity improvement achieved by playing action video games. Thus, a child that has received occlusion therapy and reached a plateau or achieved “normal” vision (defined as 20/20 acuity), can still benefit from action video game treatment. This is likely due to improvements in fixation stability and attentional mechanisms, and may provide further benefits than those measured in this study, such as an improvement in fine motor skills (Webber et al., 2016).

4.4.7 Future directions and clinical applications

While we have made remarkable progress in understanding how video games can be used as a treatment for patients with amblyopia in the 6 years since Li et al. (2011) first reported on this topic, there are still many important questions that remain unanswered. In particular, we need to understand how binocular treatment affects oculomotor control. Eye movements skills are important for proper development of spatial attention and learning activities such as reading. As work has shown that children with amblyopia have reading impairments, even when using both eyes (Kelly et al., 2015), understanding how to improve these functions is important to the clinical outcome of these patients. Future studies should assess eye movements online (while doing the training) and at assessments, including saccadic measurements and fixation stability.

4.5 Conclusion

This study adds to the growing body of work which shows that using action video games to treat amblyopia can be as effective (if not more) as traditional occlusion therapy. Our results also strongly suggest that dichoptic video game playing results in larger improvements than playing a game with a patch over the non-amblyopic eye. Thus, there must be something unique about this viewing condition. We propose that fixation stability and bifoveal alignment may be key to unlocking a holistic treatment for this developmental condition. Additionally, incorporating stereo cues, as was done in Vedamurthy et al. (2016) may lead to the greatest overall improvement, as this provides an additional cue to aid in sustained binocular fusion. Future work should investigate the mechanisms behind these improvements, to differentiate what is special about the dichoptic viewing condition. Finally, easier set-ups, that include an assortment of engaging action video games in a portable unit, and the ability of researchers and clinicians to track the data regarding a patient’s compliance and progression, will be essential to future iterations of this work.

Chapter 5

Conclusion

5.1 Summary

This series of experiments explored the nature of the amblyopic deficit in several important ways. First, we sought to understand the why amblyopic patients are slower at responding when viewing with the amblyopic eye. In the first experiment, we measured reaction time for each eye separately, using stimuli of equivalent stimulus strength. As reaction times are faster to more intense stimuli, this procedure allowed us to rule out a sensory basis for the delay. Our results show that while our two anisometropic patients had little to no RT difference between the two eyes once the stimulus intensity was accounted for, our strabismic patients were significantly slower at responding when viewing with their amblyopic eye. Several of these strabismic patients also had significantly more variability in response times in their AE compared to their NAE, which could be due to greater fixational instability when viewing with that eye.

To further explore the nature of this delay and its potential link to fixation instability and/or impairment in focusing spatial attention, we next measured reaction time with a temporal gap between the disappearance of the fixation spot and the appearance of the saccadic target. This gap is thought to release attention at the fovea and significantly reduces RT in normal observers. This study yielded two important results: (1) the gap effect cannot eliminate the irreducible RT delay in the AE that was measured in the previous experiment and (2) the gap effect is reduced in both eyes of amblyopic patients compared to normal observers. This provides evidence that there is a binocular deficit in amblyopic patients, specifically related to the disengagement of spatial attention at fixation.

Training patients to use both eyes to fixate and detect objects randomly appearing at different parts of the visual field may help alleviate this binocular deficit. In our last study, patients that played action video games with both eyes showed significant visual acuity improvement after 20 hours of training, while those that played the game monocularly did not. In sum, these studies add to the growing body on literature that suggests that amblyopia is a binocular impairment, and should be treated as such.

5.2 Vision is an active process with a binocular advantage

Previous work has shown that normally sighted observers have better fixation stability when viewing with two eyes, compared to one, however, this advantage is not observed in amblyopic patients (Gonzalez et al., 2012). Moreover, there is increasing evidence that fixational eye movements are not merely random, but an important contributor to an active process that allows us to perceive high spatial frequencies.

Natural scenes are highly redundant, dominated by relatively uninformative low frequencies and extensive spatial correlations. In a recent study by Kuang et al. (2012), the authors recorded the eye movements of normal observer and analyzed the resultant ‘natural movie’ inputs to the retina using spectral analysis. They found that the the statistics of normal fixational eye movements matched the statistics of natural images, equalizing the power over a wide range of spatial frequencies, and accenting the perception of informative regions such as edges. Poorer control over these fixational eye movements is one potential explanation for why amblyopes are worse at tasks that contain these types of stimuli.

Further, additional eye movements, such as microsaccades, can enhance spatial vision by directing the locus of attention to areas of interest. In a study by Ko et al. (2010), eye movements were measured while observers performed a very high precision task: threading a virtual needle by placing a small “thread” into a tight gap between two lines. The pattern of microsaccades while performing the task was quite striking – microsaccades became progressively smaller and more frequent as the thread approached the needle. They also precisely shifted the gaze from the thread to the eye of the needed, and rarely landed on other parts of the scene. The eye movements also correlated to behavior. Observers were more likely to adjust the thread position immediately after a microsaccade from one object to the other, compared to when fixation was held in steady in one place. Accuracy was also linked to the eye movements while performing the task. On trials where microsaccades were less precise, subjects were less likely to thread the needle. These findings suggest that the fine control over microsaccades is an important part of active vision and that impairment of this precision could lead to difficulties in tasks requiring high-spatial frequency processing.

These studies highlight the importance of oculomotor control in optimal visual function. Amblyopia may impair performance on such a wide variety of spatial vision tasks because these patients lack the ability to precisely control their foveas. Fortunately, evidence exists that this skill can be improved upon through practice.

5.3 Improving fixation stability with training

First person shooter games require accurate fixation at the crosshair, while scanning the periphery to identify and respond to targets and opponents. It should not be surprising, then, that people that practice this skill in everyday life also have superior fixation stability. Russo et al. (2003) measured fixation stability and saccadic latency in a group of elite shooters as well as normal observers.

Participants in the elite shooters group had trained for at least 16 hours a week for 4 years in a minimum of two of the three Olympic shooting disciplines (trap, double trap, and skeet). Participants first performed a stability task, where they were asked to keep their fixation as still as possible for 1 minute in a sparse condition with just the fixation target, and a complex condition where 48 stimuli were flashed near the fixation point. They also performed a saccadic latency task where they were asked to respond as fast as possible to the appearance of a peripheral target with and without distractors. The elite group had similar stability performance in both fixation conditions, while the control group had more variability in eye position in the distractor condition. The elite group also had faster saccadic latencies, both in the simple RT task, as well as in the presence of distractors.

Of great relevance to the current work, one of the control participants participated in a training exercise where he practiced the saccadic response task in one of the four tested directions. After approximately 1500 trials, this control participant had significantly reduced RTs (40ms in the simple task and nearly 100ms in the distractor task) that were comparable to the elite shooter group, but only in the trained direction. The authors hypothesize that part of this speeding up effect may be the result of faster disengagement of attention at fixation, similar to the processes associated with the gap effect.

This training effect is likely similar to what is occurring in action video game players, but at a more general level, as the location of targets and opponents cannot be predicted in the action video games. Thus, this study provides a link between fixation stability and saccadic latency, and shows that they can be improved through training, both with perceptual learning or a real world experience.

We believe this fixational training can improve visual acuity in patients with amblyopia, and can also boost performance on a variety of other visual tasks where precise oculomotor control is needed, such as reading. Further, we believe additional modifications to the action video games can enhance the fixational training. A study by Raveendran and colleagues (2014) measured fixation stability in strabismic amblyopes and normally-sighted observers in four conditions: unaligned/high contrast, unaligned/contrast balanced, aligned/high contrast, and aligned/contrast balanced. Alignment was achieved with a haploscope, similar to what was used in Ch 5, and the contrast balance point was measured with a dichoptic global motion task. The authors found that the fixation stability of the AE was significantly improved in the aligned/high contrast condition, and further improvements were observed in the aligned/contrast balanced condition. In fact, in the later condition, fixation stability was not statistically different from control observers.

These modifications may be why the children in our dichoptic group improved more than those in the monocular group, particularly for the strabismic amblyopes. In the next section we will consider what further changes can be made that could maximize the potential for visual improvement in behavioral training paradigms.

5.4 Stereo training may provide the greatest benefit

Acquiring stereoacuity may be an important component of amblyopia treatment. Not only does this function improve everyday binocular viewing by adding the ability to perceive an extra dimension, evidence suggests that it may also help lock in other visual benefits achieved through training. Birch (2013) reported that children with no measureable stereopsis have more than twice the risk for persistent amblyopia. Ciuffreda et al. (1979a) tracked the progression of a deep amblyope with eccentric fixation that underwent intense orthoptic training that lasted more than a year. The training included hand-eye coordination exercises, accommodation exercises, and later on fusion training. Along with remarkable acuity recovery from 20/230 to 20/20, the patient's stereoacuity also improved from less than 800 seconds of arc to 60 seconds. Ciuffreda et al. also carefully tracked the performance on several eye movement metrics, many of which normalized with training. These changes included decreases in drift amplitude and velocity, and an increase in frequency and duration of steady fixation. In their conclusions, the authors note that "*not all visual functions in the AE improve concurrently*", and that "*perhaps orthoptic training should continue until visual acuity, fixation, and oculomotor control (as well as other vision functions) are normalized and/or remain stable for a suitable period of time*".

While just a case study of one patient undergoing intensive treatment, these findings demonstrate that active binocular treatment can lead to the improvement of VA, stereoacuity, and oculomotor control. Further, these functions may be related so that improvement in one makes it easier for others to develop and persist. To optimize treatment for amblyopia, then, we should also consider adding stereo cues to the dichoptic images. There have only been a handful of stereo training studies to date, however, a recent meta-analysis by Levi et al. (2015) provided initial evidence that stereo training can provide additional benefits beyond dichoptic training. The authors compared the number of anisometropic and strabismic amblyopes that improved in stereoacuity with monocular training, dichoptic training, and stereo training. They found that while stereo training may only provide a slight advantage in the anisometropic group (24/39 of the monocular group improved in stereoacuity, compared to 5/11 in the dichoptic group, and 12/15 in the stereo group), the stereo cues provided a massive benefit in the strabismic group (only 2/36 of the monocular group improved, compared to 17/53 in the dichoptic group, and 8/13 in the stereo group).

Therefore, this type of training could be greatly advantageous to patients that do not have stereo perception, which includes many strabismic amblyopes. As mentioned previously, we still do not understand the differences in mechanisms for monocular vs. dichoptic training, and the same holds true for stereo training. It's possible that the benefit from stereo training may actually come from practicing fusion with bifoveal alignment, as was done in our dichoptic game condition. Levi et al. (2015) write, that the fusion training "*may provide a useful scaffold for integrating information from the two eyes, and may therefore present a more efficient way to restore stereovision in amblyopic patients, while simultaneously fostering improved visual acuity*."

In conclusion, decorrelated visual experiences during development can harm the networking of the binocular vision system, leading to fixation instability and poor stereoacuity. As the visual attention networks require the ability to hold the foveas steady at a particular location and make goal-directed saccades to areas of interest, these too can be disrupted as a consequence. We show that strabismic amblyopes have an irreducible delay in their AE, and that the attentional mechanisms which help mediate the process of disengagement of attention at fixation the subsequent initiation of saccades are affected in both eyes. We believe fusion training, and potentially stereo cues, may help foster stable fixation, which in turn can enhance visual attention. Therefore, bifoveal fixation, even if it is transient at first, could boost the attentional mechanisms thought to be important in the cognitive and perceptual benefits from playing video games. This holistic treatment should lead to quality of life improvements by boosting a variety of visual functions, such as reading and fine-motor skills, and these should persist long past the end of training.

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