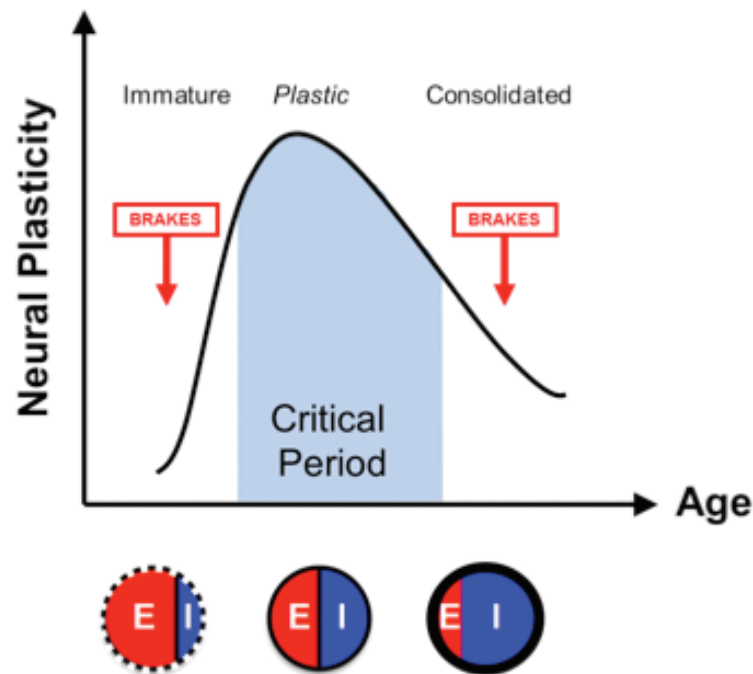




Amblyopia: Challenges and Opportunities

**The
Lasker/IRRF
Initiative for Innovation
in Vision Science**





Critical period plasticity as a function of age. Initially, immature brain circuits are dominated by excitatory inputs and fail to express plasticity. As inhibitory circuits mature, a highly plastic critical period is induced. Plasticity then declines with age as inhibitory circuits and brake-like factors dominate, harboring the potential for plasticity throughout life. Dynamic changes in the excitatory/inhibitory balance across age are shown below the graph. See Chapter 3. Figure courtesy of Takao Hensch (Harvard University)

Cover:

Top left: A schematic representation of the columns or stripes that extend across area V1 of the normal visual cortex in a monkey. The cells in one stripe, either dark or light, receive their input from one eye, whereas input from the other eye is in the other stripe and the stripes alternate. Thus, each eye has equal representation in the visual cortex in the normal animal.

Bottom right: In a monkey in which form vision has been deprived in one eye during the critical period, the amount of cortex receiving input from the deprived eye (dark stripes) has been much reduced. The stripes are thin and discontinuous. Thus, input from the non-deprived eye has taken over much of the territory belonging to the deprived eye. It does this by the input neurons extending their axon terminals into the deprived eye's territory and presumably forming new synapses there.

The drawings derive from micrographs in the paper, "Plasticity of ocular dominance columns in monkey striate cortex" by DH Hubel, T. N. Wiesel and S. LeVay (*Phil. Trans. R. Soc. Lond. Biol. Sci.* 1977; 278 (961): 377-409. Drawings from *Creating Mind: How the Brain Works* by John E. Dowling (WW Norton, New York, 1998).

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The Lasker/IRRF Initiative for Innovation in Vision Science

Amblyopia: Challenges and Opportunities

Background and Acknowledgements

The Lasker/IRRF Initiative for Innovation in Vision Science is a ten-year collaboration, launched in July 2008, between the Albert and Mary Lasker Foundation (Lasker) and the International Retinal Research Foundation (IRRF). The Initiative was designed to identify knowledge gaps in vision research and propose innovative strategies to accelerate the discovery of sight-saving treatments and methods to prevent diseases of the eye, especially retinal degenerative diseases, using novel scientific, engineering and technological approaches.

The Initiative has conducted three studies to date: *Astrocytes and Glaucomatous Neurodegeneration* (2010), *Diabetic Retinopathy: Where We Are and A Path to Progress* (2012) and *Restoring Vision to the Blind*. (2014). Each of these reports may be viewed and downloaded at <http://www.laskerfoundation.org/programs/lasker-irrf-initiative-innovation-vision-science/> or <http://www.irrfonline.org/laskerirrf.html>.

In addition, in 2015 the Initiative conducted a follow-up of the first Initiative to assess outcomes from its examination of glaucoma, i.e., where the field is now, whether and how the report stimulated innovative lines of research, what scientific hurdles have been overcome and what problems now confront the field. This report is being published as a special edition of *Experimental Eye Research (EER)*, 2017.

In late 2014, the Initiative's Joint Advisory Board (Appendix 1) decided that recent scientific advances provided a compelling opportunity to examine the scientific challenges in the field of amblyopia and to propose new approaches and novel treatments for this condition. Amblyopia is a disorder that results in varying degrees of monocular, or occasionally binocular, vision impairment, mainly in children; if not successfully treated, it can lead to permanent vision impairment for life. Its etiology and a precise definition have long defied science's best efforts, but we do know that risk factors for amblyopia include strabismus (crossed eyes), anisometropia (asymmetric eye focus), and deprivation (lens or lid defects). Amblyopia affects 1 - 3% of the population, though estimates vary widely because diagnostic and screening tools may not always be sufficiently comprehensive or available to all populations. How and when to treat amblyopia remains an ongoing challenge, and even whether innovative technologies can restore improved visual function in patients who were not treated when young remains an open question.

John E. Dowling, Chairman of the Initiative, appointed a Steering Committee (Appendix 2) to define goals and key issues for exploration by invited participants. During the summer of 2015, two workshops were convened at the NAS J. Erik Jonsson Center in Woods Hole, MA. Each workshop consisted of approximately 30 scientists and clinicians with different backgrounds and expertise who identified core questions and key issues that impeded progress in the field, leading to a more formal meeting in March 2016 at the Howard Hughes Medical Institute's Janelia Research Campus in Ashburn VA. The report

chapters are the result of Targeted Breakout Sessions held at the Janelia meeting, but all participants had the opportunity to comment on the entire report. We have tried to achieve a consensus document, but not all participants may agree with everything in the report.

The Initiative thanks the Boards of Directors of the Albert and Mary Lasker Foundation and the International Retinal Research Foundation for their support; the Initiative's Joint Advisory Board and Steering Committee, for their counsel; the Discussion Leaders who guided the development of the key issues discussed in this report and the scribes who recorded the discussions and, in most cases, drafted chapter texts; and all participants, for their energy, expertise, and lively discourse. Special thanks go to Karen M. Wright, Program Administrator, for her vital and seasoned administrative direction; to Kate Guthrie, Project Manager, for her adept logistical support; and to Sandra Blackwood, Executive Director of the IRRF, and Claire Pomeroy, President of Lasker, for their constancy and contributions to this endeavor.

The Initiative is most appreciative to both the Howard Hughes Medical Institute for its very generous in-kind contribution by making available the facilities at its Janelia Research Campus in Ashburn, Virginia, for the Initiative's plenary session, and the staff of the National Academy of Sciences' J. Erik Jonsson Center in Woods Hole, Massachusetts, for their gracious hospitality during the two summer workshops.

The Initiative gratefully acknowledges *Visual Neuroscience* (VNS) for publishing this report as a special edition of its journal, which will be available at <https://www.cambridge.org/core/journals/visual-neuroscience>.

For further information about the Initiative, please contact: Karen M. Wright, Program Administrator at kwright@laskerfoundation.org. For additional copies of this report, please make your request to Kate Guthrie, Project Manager at kguthrie@laskerfoundation.org.

Introduction

During the summer of 2015, the Lasker/IRRF Initiative on Amblyopia convened two groups of scientists and clinicians with diverse backgrounds and expertise. The objective was to focus on a problem that seriously affects vision, and to see if new thinking and ideas might be helpful in the understanding of amblyopia and how these ideas can be applied to advance the field. At these sessions, David Hunter described what is currently known about this complex condition and identified the research and clinical challenges that persist. A summary of his presentation serves as an introduction to this report.

Amblyopia: The Clinician's View **David Hunter**

As a practicing pediatric ophthalmologist, I continue to be frustrated by how frequently I encounter children with amblyopia when it is too late to restore normal or near-normal function. In this introduction to the Lasker/IRRF Initiative's report *Amblyopia: Challenges and Opportunities*, I will provide the basics of amblyopia from a clinician's view to provide common ground that facilitate open-ended discussions among clinicians and researchers interested in improving our understanding of this disease. I will present the current clinical definition of amblyopia and how we make the diagnosis, review what is understood about the cause and the nature of the deficit, provide an overview of current approaches to screening for the disease as well as its treatment, and place amblyopia in the context of its burden to society.

The current clinical definition of amblyopia is best provided by the American Academy of Ophthalmology's Preferred Practice Pattern on Amblyopia (AAO Pediatric Ophthalmology/Strabismus Panel 2012).

"Amblyopia is a unilateral or, less commonly, bilateral reduction of best-corrected visual acuity that occurs in the setting of an otherwise normal eye, or a structural abnormality involving the eye or visual pathway, with reduction in visual acuity that cannot be attributed only to the effect of the structural abnormality. Amblyopic eyes may also have deficits in contrast sensitivity and accommodation. Often the fellow eye is not normal but has subtle deficits. Amblyopia is caused by an abnormal visual experience early in life."

A similar definition is provided by the American Optometric Association (Care of the Patient with Amblyopia):

"Amblyopia, also referred to by the public as "lazy eye", is a unilateral or infrequently bilateral condition in which the best corrected visual acuity is poorer than 20/20 in the absence of any obvious structural anomalies or ocular disease. Amblyopia represents a syndrome of compromising deficits, rather than simply reduced visual acuity, including increased sensitivity to contour interaction effects, abnormal spatial distortions and uncertainty, unsteady and inaccurate monocular fixation, poor eye tracking ability, reduced contrast sensitivity, and inaccurate accommodative response."

Practically speaking, this means that visual acuity is reduced despite a normal structural eye examination due to the presence of at least one amblyopia risk factor early in life. These risk factors include deprivation (induced by congenital cataract or ptosis, for example), manifest strabismus of any type (esotropia, exotropia, hypertropia), or anisometropia (asymmetric refractive error) of as little as 0.50 D. Each of these risk factors leads to a different type of amblyopia: deprivation, strabismic, and anisometropic amblyopia, respectively, with each subtype demonstrating distinct features. Deprivation amblyopia is most refractory to treatment, while anisometropic amblyopia has the best prognosis, probably due to the preservation of some degree of binocularity in these patients.

Given that visual acuity is the primary measure of amblyopia, the method used to determine visual acuity is key to obtaining the proper diagnosis. Older children will read an eye chart as ably as a literate adult, but in preschool children commonly used alternatives include the HOTV test (in which only four letters are offered, and a child is allowed to match the letters with a card held in the lap) and picture optotypes (such as LEA symbols). When testing vision in young children who might get lost viewing an entire line of letters, it is tempting to provide just one letter or picture at a time, but single letter optotypes will overestimate visual acuity in amblyopic eyes. If it is not possible to present a full line of letters, it is acceptable to instead surround an individual letter with “crowding bars” to provide the most accurate estimate of visual acuity. For non-verbal children, a preferential looking test may be performed. In this test, a pattern is presented on one side of a card and the child’s fixation behavior is observed to decide whether the pattern was seen. Preferential looking tests tend to underestimate the vision deficit in amblyopia (Kushner, Lucchese and Morton, 1995).

Once visual acuity is known, unilateral amblyopia is diagnosed clinically when there is a 2-line difference between eyes using the logMAR (log of minimum angle of resolution) scale; that is, a difference of 0.2 log units between eyes. Bilateral amblyopia is diagnosed when best-corrected visual acuity is worse than 20/40 in the better-seeing eye at age 4 or older (or worse than 20/50 in children under age 4.)

The prevalence of amblyopia ranges from 1 to 3% depending on the population studied and the exact definition used (Hendler, Mehravaran, Lu *et al.*, 2016). While this may seem uncommon, amblyopia has been described as the number one cause of monocular visual impairment in children and young adults (NIH Facts About Amblyopia). The risk of amblyopia increases with prematurity, developmental delay, and maternal smoking, drugs, or alcohol. There is also a genetic predisposition, with the likelihood of amblyopia higher when a first-degree relative is affected.

What exactly is the nature of the deficit in amblyopia? Our understanding of this continues to advance, and many of the chapters in this document will address these questions in different ways. Briefly, we have known for decades that amblyopia is a disease of the brain, with documented structural abnormalities in visual cortex (Hubel and Wiesel, 1962). Some of the deficits that will be discussed in the chapters ahead include reduction in contrast sensitivity, errors in accommodation, reduced binocular vision, and neural deficits in higher-order visual and motor areas. There is growing evidence that although all amblyopia patients have a measured reduction in vision in at least one eye, most unilateral amblyopia patients have deficits even when performing under binocular conditions, including deficits in real-world visuomotor tasks (Grant and Moseley, 2011) and reading (Kelly, Jose, De La Cruz *et al.*, 2015). Most amblyopia patients also have more subtle deficits such as microstrabismus, eccentric fixation, and fixation instability (González, Wong, Niechwiej-Szwedo *et al.*, 2012).

In patients with deprivation amblyopia, treatment starts with correction of the underlying structural anomaly that has created the problem in the first place, such as cataract or ptosis. For all forms of amblyopia, if refractive error is a contributing factor, the first step in treatment is appropriate refractive correction. (There is some debate as to whether correction of strabismus alone can have a positive impact on strabismic amblyopia.) Once the underlying anomaly is corrected, the current standard of care for amblyopia treatment revolves around impairing or occluding the fellow eye to interfere with its dominance of visual cortex and allow development of visual pathways serving the amblyopic eye. For more than two centuries, occlusion of the sound eye with an adhesive eye patch has been the primary method of achieving this goal. Occlusion may also be achieved by placing blurring filters over the sound eye (Bangerter foils). In more recent decades, a series of randomized, controlled trials have shown that atropine eyedrops are equally effective to patching for amblyopia (PEDIG, 2002). Atropine is a cycloplegic agent that blurs vision for more than 24 hours after instillation, making it considerably easier to implement atropine penalization than occlusive patching in a non-cooperative child. (While a child may resist the atropine as much as the patching, in practical terms just a moment of conflict is required to administer an atropine drop, whereas with a patch parents must exert constant vigilance to assure that it has not been removed by the child.) Systemic therapies for amblyopia have been explored, but their efficacy has not been demonstrated in clinical trials, except that one controversial prospective study suggested that, surprisingly, acupuncture improved visual acuity as effectively as occlusion therapy (Zhao, Lam, Chen *et al.*, 2010). Amblyopia may recur even after successful treatment, with one study showing recurrence in 25% of children within a year of discontinuing therapy. Slow tapering of treatment can prevent this recurrence.

Regardless of the method used, earlier treatment of amblyopia yields better outcomes presumably due to the loss of plasticity in the visual cortex that occurs with advancing age. There is no exact age cut-off for when amblyopia treatment will no longer be effective, but initiation of treatment in the preschool years is most effective, and treatment after this age is unlikely to yield normal visual acuity in most patients except perhaps a subset of patients with anisometropic amblyopia. With that said, some gains in visual acuity can be seen with treatment in older children and even adults; however, these gains do not seem to correlate with any improvement in binocular function. For example, if the sound eye has 20/20 vision and treatment of an amblyopic eye in an adult brings vision from 20/80 to 20/60, there will be little or no immediate clinical impact for that patient. Treatment of amblyopia in older children and adults is not likely to carry a clinical imperative until such treatment is shown either to improve vision to nearly normal or to improve binocular function.

Other recent studies of amblyopia have yielded some surprises. Occlusion of as little as 2 hours per day is an effective treatment of moderate amblyopia (Repka, Beck, Holmes *et al.*, 2003), and prescribed occlusion of 6 hours per day is as effective as full-time patching (Holmes, Kraker, Beck *et al.*, 2003). Spectacles alone (without patching or atropine penalization) may improve vision by 2 or more lines, not just in anisometropic amblyopia but even in strabismic amblyopia (Cotter, Foster, Holmes *et al.*, 2012).

Newer approaches to therapy will be discussed in the chapters ahead. There is renewed excitement about the potential for binocular therapy (Kelly, Just, Dao *et al.*, 2016), which, rather than profoundly occluding or blurring the sound eye, provides just enough blur or contrast reduction to engage both the sound eye and the amblyopic eye simultaneously. These treatments can be implemented on handheld

devices while the patient wears red-green or polarized glasses to provide different inputs to the two eyes. Various forms of LCD (liquid crystal display) shutter glasses have been introduced as another means of implementing binocular therapy. Perceptual learning, the process of improving perception by performing repetitive discriminative tasks, has also shown some promise, particularly for treatment of adults (Levi and Li, 2009).

Given the loss of plasticity of the brain in childhood years, the importance of initiating therapy early in life, and the binocular and functional deficits in amblyopic adults, one would think that healthcare systems and insurers worldwide would focus intensive resources on early detection and treatment. Unfortunately, this is not the case, and efforts to screen for amblyopia in preschool children, when they are implemented at all, have been plagued by low sensitivity and specificity with a few notable (if costly) exceptions in Scandinavia (Sloot, Hoeve, de Kroon *et al.*, 2015). In an effort to reduce the cost of vision screening, automated photoscreeners have been introduced to test preschool children for amblyopia risk factors. Implementation of these devices is increasing but remains limited, in part due to over-referral, with one study showing that only 13% of children referred by a photoscreener had amblyopia (Bregman and Donahue, 2016). The topic of early detection of amblyopia is detailed in one of the chapters in this document.

The cost of amblyopia to society is enormous, with poor screening and delayed treatment leading to poor outcomes in many patients. In addition to the obvious monocular deficits and the binocular deficits noted above, patients with amblyopia have double the lifetime risk of total blindness, with limits on career opportunities and even an increased risk for anxiety and depression (Packwood, Cruz, Rychwalski *et al.*, 1999). The annual cost of screening, unnecessary referrals, and missed cases has been estimated at between \$1 and \$7 billion. Amblyopia treatment is thus highly cost-effective when compared with other interventions in health care (Membreno, Brown, Brown *et al.*, 2002).

In conclusion, amblyopia is a common and important clinical problem. The diagnosis is based on visual acuity testing, with current treatment focusing on glasses, patching, and atropine penalization. Many children are not diagnosed in a timely manner, leading to a lifetime visual deficit that can affect far more than monocular visual acuity. The burden of amblyopia on society is for the most part hidden, and yet it is enormous. We hope that with this Initiative, the interaction of basic and clinical amblyopia investigators will bring about new insights into the condition while also helping to focus our priorities on the most promising areas of investigation in the science of amblyopia.

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Chapter 1

Classification and Diversity of Amblyopia

Discussion Leaders: Daphne Maurer and Suzanne McKee

Scribe: Michael Richards

Session Participants: Rowan Candy, Alistair Fielder, Irene Gottlob, Creig Hoyt, Paul Harris, Jonathan Holmes, Zhong-Lin Lu, John Sloper, Ben Thompson

Introduction

Amblyopia is a developmental disorder that affects the spatial vision of one or both eyes in the absence of an obvious organic cause; it is associated with a history of abnormal visual experience during childhood. Estimates of the prevalence of unilateral amblyopia in preschool children range between 1 and 3%, with the proportion depending somewhat on the ethnic composition of the study sample (MEPEDS, 2008; Friedman, Repka, Katz *et al.*, 2009; Ying, Maguire, Cyert *et al.*, 2014). It is the most common cause of monocular visual impairment in children (Webber and Wood, 2005; Gunton, 2013). A similar proportion of adults suffer from unilateral amblyopia (Attebo, Mitchell, Cumming *et al.*, 1998). Bilateral amblyopia is much less common (0.1 – 0.45%; Robaei, Rose, Ojaimi *et al.*, 2005; MEPEDS, 2008) and is generally associated with a history of visual deprivation produced by cataracts, ptosis, or high refractive error in both eyes.

The generally accepted definition of amblyopia is reduced visual acuity, despite best optical correction, when measured with an optotype chart, such the LogMAR chart. Typically, a two-line difference between the eyes is taken as evidence of unilateral amblyopia. Special pediatric charts with single letters or pictures are used to test preschool children. Amblyopia has been sub-divided traditionally based on the condition thought to be its cause, primarily strabismus (misaligned eyes) or anisometropia (difference in refractive error between the two eyes) or some combination of both, yielding the common labels of strabismic amblyopia, anisometropic amblyopia, and strabismic-anisometropia or mixed amblyopia. Amblyopia in patients with the rare history of deprivation is labeled deprivation amblyopia.

Does this classification, based on purported etiology, produce different functional types of amblyopia, or are there different functional types that would arise from some other classification scheme, or is amblyopia, however caused, essentially the same abnormality? This answer to these questions is especially important in determining treatment because the etiology may not be fully apparent at the time that amblyopia is diagnosed, and different forms of amblyopia may require different management.

Classification Based on Visual Function

Numerous behavioral studies, made almost exclusively on adults with amblyopia, have suggested strongly that strabismus and anisometropia do indeed lead to different patterns of visual loss (Levi and Klein, 1982; 1985; Hess and Pointer, 1985). In the largest study to date, McKee, Levi and Movshon (2003) measured the acuity and contrast sensitivity of 427 individuals either with amblyopia or with risk factors for amblyopia, e.g., strabismus or anisometropia, plus 68 normal control observers. Participants were assigned to one of eleven groups, based on their history and a detailed clinical examination of their oculomotor and refractive characteristics. Participants were not classified on the basis of their optotype (LogMAR) visual acuity; instead the study explored the relationships between optotype acuity and four other measures (grating acuity, vernier acuity, edge contrast sensitivity and contrast sensitivity measured with Pelli-Robson chart). Statistical analysis of this large data set revealed that two factors accounted for about 80% of the variance separating the 11 clinical groups: an acuity factor and a sensitivity factor. In Figure 1.1, the average sensitivity factor of the non-preferred eyes (the eyes with the poorer acuity) is plotted versus the average acuity factor of the non-preferred eyes for each of these clinically defined groups. Note that these groups include both amblyopic and non-amblyopic observers, e.g., all pure patients with strabismus whether amblyopic or not.

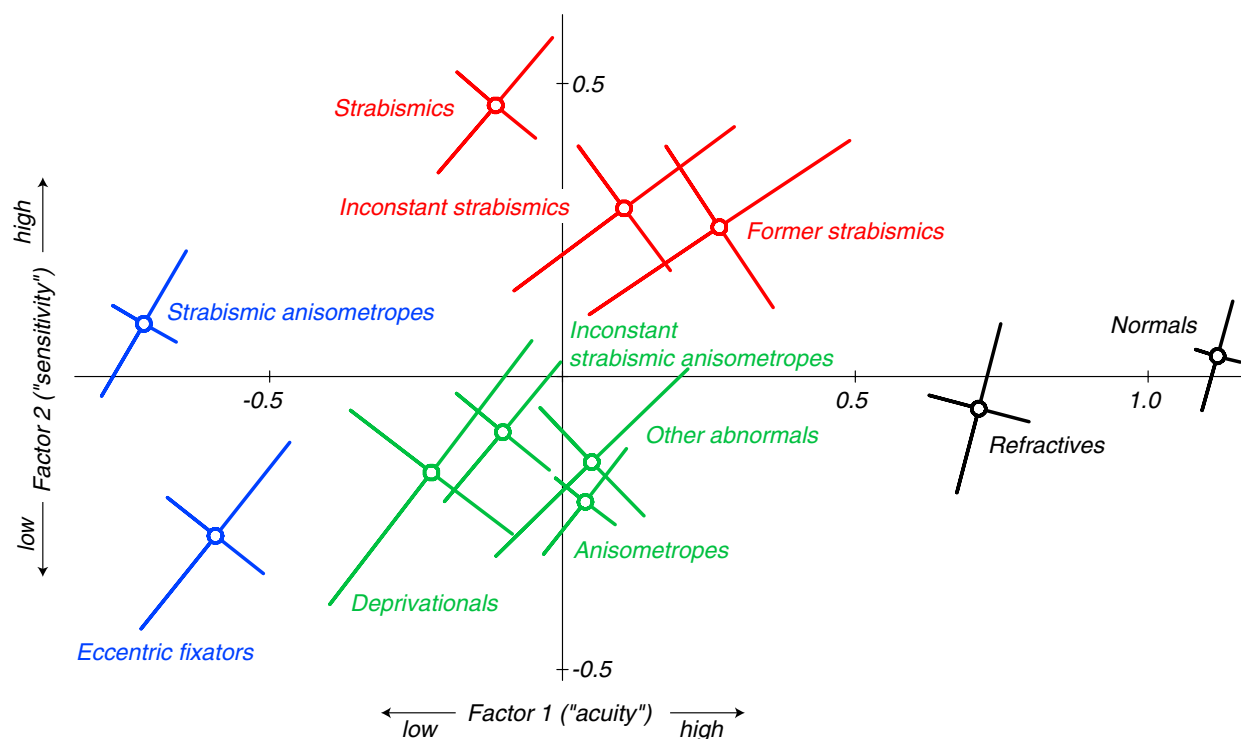


Figure 1.1. McKee SP, Levi DM, Movshon JA. The pattern of visual deficits in amblyopia *J Vis.* 2003 3:380-405 (used with permission)

Figure 1.1 represents a kind of ‘map’ of amblyogenic conditions with the normal control group (black symbol) on the right showing the best acuity and the strabismic-anisometropic group (blue symbol) on the left showing the poorest acuity. Clearly, the strabismic groups (red symbols) and anisometropic group (green symbol) fall in different sectors of this map, suggesting that different patterns of visual loss are associated with these different conditions. The difference between the red and green groups is largely dependent on a significant difference in contrast sensitivity. The strabismic groups are more sensitive than the anisometropes; indeed, they are significantly more sensitive than the normal controls, a curious hypersensitivity that may arise from the loss of binocularity. McKee *et al.*, (2003) speculated that the reorganization produced by the loss of binocularly-driven neurons would lead to greater visual input to monocularly driven neurons, which would thus enhance their thresholds at lower contrast. Thus, they predicted that monocular contrast sensitivity in both eyes of the strabismic groups would be slightly better than normal, which is indeed what their results showed. Another item of interest in this map is that the deprivation group falls near the anisometropic groups, which may arise from the degraded retinal image produced by both anisometropia and deprivation.

What is missing from this map is an important functional difference between the strabismic groups and the anisometropic group – namely the quality of their binocular performance. In addition to their measures of contrast and acuity, McKee *et al.*, (2003) made two measurements of binocular function. The first was a widely used clinical test of stereopsis – the Randot “Circles” test. Observers viewed the test circles with best optical correction, but without prisms. The second test, binocular motion integration (BMI), was an experimental measure of the cortical capacity to integrate or fuse diverse images into coherent motion. This test used the dichoptic quadrature motion stimulus devised by Shadlen and Carney (1986). Briefly, each eye viewed a horizontal sinusoidal grating whose contrast was modulated sinusoidally at 2Hz. The stimuli presented to the two eyes were spatially and temporally 90 deg out of phase; the direction of the phase shift determined whether the gratings appeared to move up or down. Observers were asked to judge the direction of motion for 30 trials. Performance on each of these two tests was graded as ‘pass-fail’. If an observer had any measureable stereopsis based on the Circles test, he or she was given a pass. In the BMI test, an observer was given a pass if he or she correctly judged the direction of motion on more than 70% of trials. About 80% of participants either passed both tests or failed both; all normal observers passed both. Most members of the strabismic groups (~90%; red and blue symbols) failed both measures, but a significant proportion of anisometropes (~64%) passed both. In short, based on these tests, most of the strabismic participants had difficulty combining information binocularly, while the majority of anisometropic participants retained this capability.

Although the classification scheme shown by the ‘map’ is based on rigorous psychophysical measurements made on a large sample of participants, it can be criticized on many grounds. First, the psychophysical measurements tested only acuity, contrast sensitivity and binocular functioning. A different configuration might emerge if more functions had been tested. Some types of motion judgments (Simmers, Ledgeway, Hess *et al.*, 2003; Ho and Giaschi, 2006; Thompson, Richard, Churan *et al.*, 2011), subitizing (counting number of items seen during a brief presentation; Sharma, Levi and Klein; 2000), and attentional control (Poppo and Levi, 2009; Farzin and Norcia, 2011; Kiorpes, Pham and Carrasco, 2012) have been shown to be abnormal in amblyopia. The inclusion of oculomotor measurements might also alter the classification scheme since they appear to vary with amblyogenic conditions. Many studies have found that strabismic patients with amblyopia show poorer

oculomotor control than anisometropic patients with amblyopia; their fixation is much more unsteady and the latency of their saccades is also longer (Schor and Hallmark, 1978; Cuiffreda, Kenyon and Stark, 1979; Zhang, Stevenson, Cheng *et al.*, 2008; Niechwiej-Szwedo, Goltz, Chandrakumar *et al.*, 2010; Niechwiej-Szwedo, Chandrakumar, Goltz *et al.*, 2012; Gonzalez, Wong, Niechwiej-Szwedo *et al.*, 2012; Chung, Kumar, Li *et al.*, 2015; McKee, Levi, Schor *et al.*, 2016). It is also possible to break down the clinical groups shown in the map into further subgroups. For example, there is considerable evidence that early onset (infantile) strabismus produces different functional deficits than late onset (refractive) strabismus (Schor, Fusaro, Wilson *et al.*, 1997; Brosnahan, Norcia, Schor *et al.*, 1998; Sloper, 2016).

Another problem with the ‘map’ is the categorical designation of the clinical groups. These designations were based on measurements made by ophthalmologists and optometrists who had been trained on an extensive clinical protocol. Strabismus and anisometropia were defined by the conventional criteria at the time of the study: a difference between the eyes of 1 diopter in refractive error at the maximum anisometropic meridian for anisometropia; eye movement seen under unilateral and alternating cover tests at near (0.3 m) and distance (6 m) for constant strabismus. The dichotomy between these two conditions might have been less obvious if more precise measurements of ocular alignment had been used (Hunter, Patel and Guyton, 1999; Gramatikov, Zalloum Wu *et al.*, 2007). Were the two eyes of amblyopic members of the anisometropic group really aligned within the normal range? About half of patients with anisometropic amblyopia passed both of our binocular tests, so we do know that their binocular functioning was better than the amblyopic members of the designated strabismic groups. However, it is intriguing that other functional characteristics (vernier acuity, saccadic latency) of the patients with anisometropia who failed both of our binocular tests were somewhat similar to strabismic patients (McKee, 1998; McKee, Levi, Schor *et al.*, 2016). Generally, the non-binocular anisometropic patients were those with the poorest acuity in their non-preferred eye – individuals suffering from severe unilateral amblyopia. If they had no capacity for binocular integration, what mechanism was keeping their eyes aligned? A more sensitive measure of alignment might have revealed that these non-binocular anisometropic patients were micro-strabismic.

The fluidity of these categories probably reflects the complex interaction between refractive error (spherical, astigmatic and anisometropic), spatial visual performance and eye alignment during development. Apparently similar infants can develop along quite different paths (Babinsky and Candy, 2013; Barrett, Bradley and Candy, 2013). Thus a patient with strabismic amblyopia may present with quite different forms (ranging from intermittent exotropia, or microtropia to constant alternating or unilateral esotropia) and different forms of neural adaptation or consequence (from suppression to diplopia). We are yet to fully understand the factors that predict the developmental path different infants will take and that define the range of untreated visual function that presents for an examination in a clinic.

In conclusion, it may be more useful to describe prototypes that differ but have unclear boundaries between them, rather than to assign amblyopic individuals or individuals at risk for amblyopia to distinct clinical categories.

Does Classification Matter?

Despite the vast amount of basic research demonstrating that different patterns of functional loss are associated with different amblyogenic conditions, the presenting condition does not predict treatment outcome. Following patching or penalization, which force use of the amblyopic eye by depriving the fellow eye of vision, patients classified as suffering from strabismic and anisometropic amblyopia, treated before age 7, both show the same amount of improvement in acuity (PEDIG, 2003abcd). One could argue either that the patients were misclassified due to insensitive measures of misalignment, or that this analysis was too narrowly focused on acuity, and that more extensive testing including measures of contrast sensitivity and binocularity (Birch, 2013; Bosworth and Birch, 2003) would show that the associated condition does matter to outcome. Using a battery of tests to classify patients might lead to a better prediction of outcome, although obtaining reliable behavioral measures in preschoolers, the age at which amblyopia or an amblyogenic condition is diagnosed, is challenging. However, a test battery might be prognostic in older patients with amblyopia in whom a later intervention is being contemplated and provide insight on treatment of young patients with amblyopia if more reliable behavioral measures in preschoolers become available.

Future Directions

- As has been proposed in Chapter 4, it would be useful to agree on a common set of sensitive tests that can be used clinically and in research to classify patients with amblyopia. That list should include measures of crowded acuity (acuity measured with symbols spaced as in words), contrast sensitivity, and binocular function (stereopsis and binocular interactions). Models of binocular interactions are necessary to understand patterns of empirically observed binocular suppression in different behavioral paradigms (Huang, Zhou J, Zhou Y *et al.*, 2011). Normative data for agreed-upon measures are needed at different ages. Although some of the measures may not be possible with very young children, the patterns found in older children can inform understanding of etiology and possibly effective treatments. There is an urgent need for both more efficient and precise behavioral measurements of functional vision (e.g., Lesmes, Lu, Back *et al.*, 2010) as well as theoretical models that link core visual deficits in amblyopia to observed poor visual performance of patients with amblyopia.
- One of the major problems in amblyopia is regression – a return to amblyopic acuity levels following successful treatment. Clinical classification, based on amblyogenic condition, may be useful in determining who is likely to regress, and thus, who may benefit from additional treatment or alternative treatments during elementary school years.
- Currently, there are a variety of new experimental treatments, involving perceptual learning, video game play, and dichoptic presentation (Zhou, Huang, Xu *et al.*, 2006; Li, Ngo, Nguyen *et al.*, 2011; Hess, Thompson, Black *et al.*, 2012). These new treatments might be especially useful for improving acuity in older children and young

adults who have residual amblyopia following treatment as children. Here again, a new classification system may predict who will benefit from these new treatments.

- As part of a new classification system, it would be worthwhile to pursue research on genetic markers, and epigenetic factors, which may predict which patients with amblyopia are likely to resist treatment or to regress to poor acuity following treatment. Special treatment protocols could be developed for those individuals whose risk profiles, based on a new classification, will make them more likely to suffer from persistent amblyopia.

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Chapter 2

Early Diagnosis of Amblyopia

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Introduction

Amblyopia can be improved or eliminated more easily when treated early in life. Because amblyopia in older children is generally less responsive to treatment (Holmes, Lazar, Melia *et al.*, 2011), there is a premium on the early identification of amblyopia and its risk factors and the subsequent treatment thereof. Clinical preference is to institute treatment in children before 7 years of age when an optimal visual outcome is typically easier to obtain.

Considering that most amblyopia is preventable and reversible, this common cause of visual impairment could be greatly reduced with early detection of the 1 to 3% of affected preschool children in the United States (Friedman, Repka, Katz *et al.*, 2009; McKean-Cowdin, Cotter, Tarczy-Hornoch *et al.*, 2013; MEPEDS, 2008). Unfortunately, young children often appear to function well despite subnormal vision in their amblyopic eye; consequently, monocular visual impairment from amblyopia often goes unrecognized until later in life when treatment is more difficult and less effective. Diagnosing amblyopia is limited by our ability to assess vision in infants and preverbal children. At present, eradication of amblyopia would require either early intervention when amblyopia is deemed likely to occur in the presence of specific risk factors or prompt initiation of treatment when amblyopia first develops. The goal of the discussion in this targeted session was to develop consensus on approaches toward optimizing the early diagnosis of amblyopia.

Early Diagnosis

While it seems intuitive that the earlier the diagnosis of amblyopia is made, the better chance for optimal visual function, the existing literature does not conclusively address the optimal age for initiating treatment. A population-based study in the UK did report data supporting screening and treatment before age 3 (Williams, Northstone, Harrad, *et al.*, 2002). The definitive answer may depend on several factors, including the type of amblyopia (i.e., strabismic, anisometropic, combined strabismic and anisometropic, or deprivation; discussed in a separate section), the time of onset and duration, the depth of amblyopia, and associated visual deficits. A full evaluation of response to early versus late diagnosis and treatment will require more than the assessment of high-contrast visual acuity testing. Defining and determining the treatment response of higher-order visual functions that are disrupted in amblyopia, ranging from contrast sensitivity and binocular vision to functional measures such as reading speed and certain visuomotor tasks, would be helpful. With these unknowns in mind, we reviewed the tools and standards for making a clinical diagnosis

of amblyopia with the goal of reaching a consensus on areas of investigation that might enhance early detection and diagnosis.

What is the youngest age at which a diagnosis of amblyopia can be made? The answer depends on the definition of amblyopia, which is clinically described as a decrease in best-corrected visual acuity, accompanied by one or more known amblyogenic factors (strabismus, anisometropia, high refractive error, or obstruction of the visual media) present in early childhood in an apparently healthy eye (Ciuffreda, Levi and Selenow, 1991). The clinical definition is predicated on monocular measures of distance visual acuity with optimal refractive correction in place. Because the measurement of visual acuity is largely dependent on the ability of the patient to report what he or she can see, this means that amblyopia diagnosis is problematic when assessing pre-verbal and/or uncooperative younger patients. The tools used – and the training of the examiner who is employing those tools – are critical contributors. Today's gold standard visual acuity test for the diagnosis of amblyopia in young children is the use of single optotypes with crowding bars, most often the HOTV letters or LEA symbols. When visual acuity testing is performed by an eye care specialist, reliable results can be obtained with most 3-year-old and nearly all 4-year-old children with otherwise normal neurocognitive development (Cotter, Tarczy-Hornoch, Wang *et al.*, 2007; Vision in Preschoolers Study Group, 2004); however, monocular visual acuity measures in children younger than 3 years of age are often difficult or impossible to obtain.

For infants, Teller acuity cards® (grating acuity with preferential looking) may be used to assess monocular visual acuity; however, measures of grating acuity can be cumbersome to administer. When amblyopia is present, grating acuity often underestimates the magnitude of difference in optotype acuity between the two eyes, particularly in patients with strabismic amblyopia (Sireteanu, Fronius and Katz, 1990; Sokol, Hansen, Moskowitz *et al.*, 1983). Emerging methods using electronic displays and automated tracking of infant gaze to measure resolution acuity could supplant the acuity card procedure in the future (Jones, Kalwarowsky, Atkinson *et al.*, 2014). Experienced eye care providers can make inferences about monocular visual function based on a child's fixation preference; however, this approach has been shown to be a poor surrogate measure of interocular difference in visual acuity (Cotter, Tarczy-Hornoch, Song *et al.*, 2009; Friedman, Katz, Repka *et al.*, 2008), having particularly poor sensitivity for anisometropic amblyopia and poor positive predictive values for both anisometropic and strabismic amblyopia. Therefore, when testing is performed by an experienced eye care provider, amblyopia is most reliably diagnosed when visual acuity obtained using single-surrounded optotypes is considered together with the child's history and measures of ocular alignment, refraction, and ocular structure. For most children, this becomes possible at approximately 3 years of age.

While the diagnosis of amblyopia is predicated on reduced visual acuity (in the absence of structural anomalies and with a history of or in the presence of one or more amblyogenic risk factors), other aspects of visual function might be useful surrogates for the diagnosis of amblyopia. For example, contrast sensitivity is reduced, particularly at high spatial frequencies in amblyopia (Hess, 1979; Levi, 1988; McKee, Levi and Movshon, 2003); however, the clinical assessment of contrast sensitivity using currently available approaches is difficult or impossible in infants and preschool children. Thus, despite the potential value of contrast sensitivity in characterizing amblyopia, it is not routinely assessed in the clinic, nor is it often measured in clinical trials of amblyopia treatment.

Binocular function is impaired in all forms of amblyopia, and stereoacuity is the most sensitive measure of binocular function. When stereoacuity testing is performed by an eye care specialist, reliable results can be obtained with most 3-year-old and nearly all 4-year-old children with otherwise normal neurocognitive development (Birch, Williams, Hunter *et al.*, 1997; Tarczy-Hornoch, Lin, Deneen *et al.*, 2008). Widely instituted modes of measuring stereoacuity in young children typically rely on separate images presented through polarized lenses to produce a stereoscopic image. These tests are easy to implement and thus are used routinely in clinical and research settings. While stereopsis testing used on its own has not proven to be an effective population-based screening tool for detecting amblyopia in preschool children, it is more accurate than visual acuity, autorefraction, or photorefraction in detecting strabismus (which is an amblyogenic condition) (Schmidt, Maguire, Dobson *et al.*, 2004).

Amblyopia Risk Factors

Amblyopia risk factors – that is, ophthalmic conditions that cause amblyopia – include significant refractive error, strabismus, and conditions that interfere with clear retinal image formation. These risk factors can be diagnosed much earlier than amblyopia itself.

Refractive error can be measured in infants and is a known risk factor for both unilateral and bilateral amblyopia. Anisometropia, particularly hyperopic anisometropia, is a strong predictor of amblyopia (Barrett, Bradley and Candy, 2013; Tarczy-Hornoch, Varma, Cotter *et al.*, 2011; Weakley and Birch, 2000). Generally, greater than 1.00 D of hyperopic anisometropia is considered potentially amblyogenic, with increasing risk as the magnitude of anisometropia increases (Tarczy-Hornoch, Varma, Cotter *et al.*, 2011). A more significant interocular difference is required for myopia to cause amblyopia; lower degrees of myopic anisometropia tend not to be associated with amblyopia (Levi, McKee and Movshon, 2011). Thresholds for astigmatic anisometropia that can lead to amblyopia appear to be lower than the 1.50-2.00 D that was previously thought based on clinical samples of non-strabismic children (Weakley, 2001); population-based studies suggest that interocular cylinder differences as small as 0.50 to 1.00D confer increased risk for amblyopia, although the absolute risk is low at this level (Tarczy-Hornoch, Varma, Cotter, *et al.*, 2011).

In regard to bilateral amblyopia, preschool children with bilateral hyperopia of ≥ 4.00 D SE or astigmatism of ≥ 2.00 D are 11 and 17 times more likely, respectively, to have bilateral amblyopia (Tarczy-Hornoch, Varma, Cotter *et al.*, 2011). Bilateral hyperopia also places infants and young children at increased risk for esotropia, which is itself a risk factor for amblyopia. Hyperopia between 2.00 and <3.00 D poses more than a 6-fold increase in esotropia risk, with a marked rise in risk with each diopter of increasing hyperopia (Cotter, Varma, Tarczy-Hornoch *et al.*, 2011). Data from a hyperopia-enriched clinical population have indicated that low amounts of hyperopia (2.00 to <4.00 D) that coexist with anisometropia place children at an increased risk for accommodative esotropia (Weakley and Birch, 2000).

At present, data from two large population-based cross-sectional studies clearly indicate that there is a strong dose-dependent link between refractive error and both amblyopia and esotropia; however, we cannot predict the clinical consequences of a given risk factor in any one child. Not all young children with amblyogenic risk factors are destined for eventual amblyopia – in fact, many of these children never develop amblyopia. Furthermore, the temporal relationship between these risk factors and amblyopia

development remains unclear – an infant or younger child identified as being at risk at one point in time may have not yet developed impending amblyopia. Thus, longitudinal data relating early refractive error at different ages to subsequent eye alignment and vision outcomes at older ages are needed.

The majority of amblyopia is attributable entirely or partly to refractive error (Friedman, Repka, Katz *et al.*, 2009; Groenewoud, Tjiam, Lantau *et al.*, 2010; McKean-Cowdin, Cotter, Tarczy-Hornoch *et al.*, 2013; MEPEDS, 2008). However, there is a lack of consensus on when refractive error should be treated to prevent amblyopia and when it is safe to observe instead. In combined analyses of the Multi-Ethnic Pediatric Eye Study (MEPEDS) and the Baltimore Pediatric Eye Study (BPEDS), ≥ 2.00 D spherical equivalent anisometropia was found to be a major risk factor (odds ratio of 39.8) for unilateral decreased visual acuity in children aged 2.5 to 6 years, yet 40% of the children with this magnitude of anisometropia had <2 lines of interocular difference in visual acuity (Tarczy-Hornoch, Varma, Cotter *et al.*, 2011).

There is similar uncertainty about when uncorrected hyperopia will lead to amblyopia or strabismus. Atkinson and colleagues reported that 7- to 8-month old infants with hyperopia between $+4.00$ D to $+7.00$ D in at least one meridian in one or both eyes who were prescribed a partial spectacle correction were less likely to show measureable visual acuity deficits or strabismus by 4 years of age as compared to a non-treated comparison group (Atkinson, Braddick, Nardini *et al.*, 2007; Atkinson, Braddick, Bobier *et al.*, 1996). In a second similar study, they found a significantly reduced rate of amblyopia but no difference in the rate of strabismus in infants with hyperopia greater than $+4.00$ D who were prescribed a partial refractive correction compared to those who had not been prescribed spectacle lenses (Anker, Atkinson, Braddick *et al.*, 2004). There is presently no single threshold level of hyperopia that is known to be an optimal criterion for referral of children at risk for amblyopia or strabismus or for consideration of prophylactic spectacle prescription (Jones-Jordan, Wang, Scherer, Mutti, 2014).

In addition to concerns about the amblyogenic consequences of hyperopia, poor performance on visual-motor visuocognitive (including attention) (Atkinson, Anker, Nardini *et al.*, 2002; Roch-Levecq, Brody, Thomas *et al.*, 2008), and spatial (Atkinson, Anker, Nardini *et al.*, 2002) measures have been reported for young children with uncorrected moderate hyperopia as compared to emmetropic controls. Recently, the Vision in Preschoolers-Hyperopia in Preschoolers (VIP-HIP) study found significant deficits in preschool literacy among 4- and 5-year-old children with >4.00 D of hyperopia compared to emmetropic controls (Kulp, Ciner, Maguire *et al.*, 2016).

Strabismus is another detectable risk factor for amblyopia; however, angles of strabismus too small to be identified by families or by primary care providers are still large enough to cause amblyopia in many cases, particularly when the strabismus is unilateral and constant. Refractive risk factors alone do not suffice for identification of strabismus because children with strabismus can have refractive errors in the normal range. Furthermore, strabismus is often not present in infancy, but instead develops in the preschool years – thus strabismus screening must be ongoing. While not all patients with strabismus develop amblyopia, the duration of abnormal visual experience may be an important factor in limiting a child's potential for recovery of binocularity (Birch, 2003). There is consensus that prompt management of strabismus is a priority in these cases and most likely to result in improved long-term visual outcomes (American Academy of Ophthalmology Preferred Practice Pattern Guidelines.

Esotropia and Exotropia; 2012; American Optometric Association Clinical Practice Guideline. Care of the Patient with Strabismus; 1995); thus, ideally, screening programs for amblyopia should also include testing for strabismus.

Deprivation amblyopia, which is caused by conditions such as congenital ptosis or cataract, accounts for a small percentage of amblyopia cases (Friedman, Repka, Katz *et al.*, 2009; McKean-Cowdin, Cotter, Tarczy-Hornoch *et al.*, 2013; MEPEDS, 2008). While in more severe cases it is obvious that timely treatment is necessary, when milder ocular defects are present it is not always clear whether or not the defect must be treated to prevent amblyopia. Early assessment of visual function would help determine which children in the borderline range are at risk of developing deprivation amblyopia.

Vision Screening for Amblyopia Detection

As with any public health screening program, the benefits of any one screening method depend on the balance of sensitivity (and the consequences of missing disease) with specificity (the cost and burden of false referrals). Systematic, ongoing cost-benefit analyses of various programs will be critical to answer this question. Key questions to consider include who will perform the screening and at what age or ages should screening be performed. While the goal of most preschool vision screening programs is early detection of amblyopia, the possibility that early treatment of moderate hyperopia may have educational benefits raises the question of whether low or moderate hyperopia should be the target of screening. The cost of casting a wider net for referral in the context of education rather than amblyopia would need to be calculated and considered separately from the cost of a more focused program of screening for amblyopia and strabismus.

Vision screening can potentially be performed by primary care providers (physicians and physician extenders), orthoptists, school nurses, lay persons in the community, and other screening personnel in public health settings. For many screening tests, the validity of the examination is highly dependent on the training of the individual performing the screening test. Primary care providers are optimally positioned to screen for amblyopia because they have access to infants and young children at annual well-child visits. While screening in the primary care clinic keeps this activity in the medical home, it places an additional burden on already-busy providers, who must manage multiple best-practice guidelines and recommendations (and perform a complete physical examination) during a time-limited well-child visit. In addition, inaccurate screening results from non-specialist staff members may limit cost-effectiveness (Williams, Harrad, Harvey *et al.*, 2001). If vision screening is to be conducted effectively in the primary care setting, the screening tools need to be validated by methodologically sound studies performed on the target population, be efficient and easily administered by nonmedical personnel in various environments, require minimal or no subjective interpretation by the screener, and be available at low cost (Cotter, Cyert, Miller *et al.*, 2015). In addition, any screening method should have good sensitivity as well as reasonable specificity to reduce false referrals (Kemper and Clark, 2006).

At what age and how often should a vision screening for amblyopia or amblyogenic risk factors be performed? Screening for amblyogenic refractive errors in infancy can be performed using videorefractive, photorefractive, or other instrument-based screening methods (see forthcoming section “Screening Tools”); however, detection of amblyogenic refractive risk factors is likely to result

in over-referrals of presumed amblyopia because not all children with amblyogenic refractive error have (Cotter, Varma, Tarczy-Hornoch *et al.*, 2011; Tarczy-Hornoch, Varma, Cotter *et al.*, 2011) or will develop (Atkinson, Braddick, Nardini *et al.*, 2007; Atkinson, Braddick, Bobier *et al.*, 1996) amblyopia or strabismus. While vision screening at school age is easier because most school-aged children can read the eye chart and perform other basic testing, amblyopia is generally more difficult to treat in older children age (Holmes, Lazar, Melia *et al.*, 2011). The age of 3 to 4 years represents a time when most amblyopia and/or associated risk factors have become manifest, and allows for detection of amblyopia at an age where there is sufficient cortical plasticity to expect acceptable results in most patients. Thus, if there were only one age at which vision screening for amblyopia and strabismus could be performed using currently available methods, it would probably be age 3 to 4 years. A single screening in childhood would represent the least expensive approach in terms of cost; however, it is unlikely to be sufficiently sensitive because there will be cases of amblyopia and accommodative esotropia not yet manifest that would be missed by early screening. Depending on the sensitivity, specificity, cost, and complexity of the screening tests used, it might be possible to perform screening at more than one age, including annually. For example, the Netherlands has a population-based child health-monitoring program where vision screening is conducted during a series of 7 pre-determined well-child visits between birth and 6 years of age. In a birth cohort study (de Koning, Groenewoud, Lantau *et al.*, 2013; Groenewoud, Tjiam, Lantau *et al.*, 2010), nearly 3,000 children in the city of Rotterdam were examined by orthoptists at the age of 7 years to determine the program's effectiveness in detecting amblyopia. Of the children diagnosed with amblyopia, 73% had screened positive at least once; the majority of them were identified as a result of monocular visual acuity screenings conducted at 3 to 5 years of age, as opposed to the screenings conducted prior to age 3 years. Furthermore, there was a higher amblyopia detection rate among those who had been screened 5 or 6 times versus those screened 4 or fewer times. The authors reported that the Dutch vision screening program had reduced the prevalence of undetected or insufficiently treated amblyopia at age 7 years by more than 50% (de Koning, Groenewoud, Lantau *et al.*, 2013; Groenewoud, Tjiam, Lantau *et al.*, 2010). By contrast, a more conservative approach of a single vision screening at age 4-5 years is the current recommended national policy in the UK (Solebo, Cumberland and Rahi, 2014).

If there were a screening approach that could identify amblyopia directly rather than detect amblyogenic risk factors, then this could allow children with only amblyogenic risk factors but without disease to be followed with annual screening so that referral would occur only if and when amblyopia developed. This is assuming, of course, that children with risk factors known to cause amblyopia at high rates or be problematic in other ways (for example, a young child with high hyperopia deemed significant enough to interfere with visual function) would be referred to an eye care professional for appropriate management regardless of the presence of amblyopia. Whether and under what circumstances early detection and treatment of amblyogenic risk factors can benefit patients in a cost-effective manner requires further prospective study, and the answer will ultimately guide recommendations with respect to the timing of screening and conditions for referral.

Screening Tools

According to a recent report by the National Expert Panel to the National Center for Children's Vision and Eye Health (Cotter, Cyert, Miller *et al.*, 2015), current U.S. best-practice vision screening recommendations for amblyopia and amblyogenic risk factors in preschool children 36 to <72 months

of age are either (1) monocular visual acuity testing using single HOTV letters or LEA Symbols surrounded by crowding bars at a 5-ft (1.5 m) test distance or (2) instrument-based testing using the Suresight Vision Screener (recently discontinued by manufacturer, Welch-Allyn, Inc, Skaneateles Falls, NY) or the Retinomax (Right Mfg Co Ltd, Tokyo, Japan) autorefractor. With specificity set at 90%, sensitivity for lay screeners was 78% and 85% for the identification of amblyopia and amblyogenic risk factors using LEA Symbol visual acuity testing and the Retinomax autorefractor, respectively (Vision in Preschoolers Study Group, 2005). Because Retinomax results are provided in a prescription form, however, this technique is not always readily interpretable by non-eye care professionals and has not been widely adopted for preschool vision screening.

In addition to the aforementioned autorefractor technology, there are a variety of other automated or semi-automated instruments that have been developed in an effort to improve vision screening accuracy while reducing cost in terms of time and trained personnel. Contrary to visual acuity screening, these instruments focus almost exclusively on the assessment of refractive risk factors with an important advantage that minimal cooperation is required from the child being tested.

Many of these new instruments are photoscreeners, which are similar to autorefractors in that they provide an estimate of refractive error (cycloplegic eye drops are not administered). Unlike autorefractors, however, some photoscreeners assess both eyes simultaneously, thereby providing the opportunity to identify grossly visible strabismus. Most published studies have assessed “accuracy” in the context of detecting amblyopia risk factors rather than detecting disease (strabismus and/or amblyopia). This may give misleadingly optimistic results in terms of positive and negative predictive value; ideally, if the goal of screening is to detect treatable conditions at a time when intervention will have maximal impact on outcomes, the gold standard for diagnosis in cost-benefit studies of refractive error screening is the presence or subsequent development of amblyopia and/or strabismus. Furthermore, the ideal refractive error criteria that should be used for vision screening cut-offs are presently debated (Nathan and Donahue, 2011). The most commonly used amblyogenic refractive error criteria are consensus-based rather than evidence-based (Donahue, Arthur, Neely *et al.*, 2013), and are predicated on cycloplegic-determined refractive error, whereas vision screening instruments provide an estimate of refractive error without cycloplegia. In a population-based screening study of children 8, 12, 18, 25, 31, and 37 months of age that did use clinically-confirmed amblyopia and strabismus as the outcomes, non-cycloplegic photorefractive error was only sufficiently specific (88%) and sensitive (97%) for detecting straight-eyed (anisometropic) amblyopia at 37 months of age; unfortunately, it only detected 35% of the strabismic cases at this age (Williams, Harrad, Harvey *et al.*, 2001).

An electrophysiological assessment of visual function using square-wave grating patterns during visual evoked potential (VEP) testing has been considered as a possible vision screening instrument for amblyopia. (Simon, Siegfried, Mills *et al.*, 2004). Because results are analyzed for intraocular differences in the cortical response from each eye in an attempt to identify patients with amblyopia, risk factors such as strabismus and refractive error are not detected by this method. This approach has not been widely adopted, and it is unlikely that it could be a cost-effective tool for large-scale screening.

Recently approved by the FDA for the screening of amblyopia and microstrabismus associated with amblyopia, the Pediatric Vision Scanner (PVS) assesses retinal birefringence to detect foveal fixation in both eyes simultaneously (Loudon, Rook, Nassif *et al.*, 2011). This approach detects binocular

alignment with high accuracy, generating a “binocularity score” that reflects the presence or absence of strabismus and of microstrabismus associated with amblyopia. Testing can be completed in 10 seconds in children as young as age 2 years of age. Thus far, the ability of the PVS to detect amblyopia and strabismus has been promising (Jost, Stager, Dao *et al.*, 2015; Jost, Yanni, Beauchamp *et al.*, 2014; Loudon, Rook, Nassif *et al.*, 2011). While the device was originally designed to assess strabismus, it has been hypothesized that the PVS also detects amblyopia because, similar to strabismic patients, amblyopic patients also have inaccurate and/or unstable fixation (Gonzalez, Wong, Niechwiej-Szwedo *et al.*, 2012; Jost, Stager, Dao *et al.*, 2015; Loudon, Rook, Nassif *et al.*, 2011).

New Avenues of Research

There remains considerable opportunity for innovation and discovery of new critical clinical features of and biomarkers for amblyopia. Potential avenues include identifying genetic markers that predict or contribute to the development of amblyopia. Neurophysiologic correlates such as visual evoked potentials and anatomic or structural correlates of amblyopia are potential future avenues for research in the diagnosis of amblyopia. These markers may also be helpful in predicting response to treatment and priority for or timing of screening in individuals or groups. Discovering novel correlates of amblyopia will require advances in neurophysiologic measures and high-resolution neuro- and retinal imaging. For example, accumulating evidence that long-term chronic interocular suppression may play a key role in the development of amblyopia (Li, Thompson, Lam *et al.*, 2011; Narasimhan, Harrison and Giaschi, 2012) highlights the need for further study. A better understanding of the effects of decorrelated binocular visual experience and the development of amblyopia could lead to the design of a clinical test of suppression to screen for patients with or who are at high risk for developing amblyopia. For any new, sophisticated assessment for amblyopia to have a positive impact on public health, it will need to be translated into a practical implementation that is widely available and cost-effective.

At present, there is no single clinical factor that can be used to predict the presence of or development of amblyopia in very young children. This is also true in other fields of medicine, where no single clinical feature or biomarker is sufficient to predict an adverse event related to disease. Multivariate regression analyses can help to integrate the most important contributing risk factors into a scoring system. In turn, this scoring system can be tweaked to optimize sensitivity and specificity, thereby facilitating effective identification of patients at risk for or with a disease while limiting over-referral of false-positive cases and reducing cost. Such scoring systems have been developed for several systemic diseases as well as for primary open angle glaucoma (Gordon, Torri, Miglior *et al.*, 2007). These systems use readily available clinical or laboratory values to facilitate wide implementation. Such a system for amblyopia could potentially be built from already existing datasets, enhanced by advances in amblyopia science, and deployed in the context of new testing devices. Currently, our knowledge of the risk increase in relation to the magnitude of a given amblyogenic risk factor is still only fragmentary and obtained from large, population-based, cross-sectional studies (Cotter, Varma, Tarczy-Hornoch *et al.*, 2011; Tarczy-Hornoch, Varma, Cotter *et al.*, 2011). Large, prospective clinical trials with proper randomization of study participants to two groups, with and without early treatment of the risk factor, that are heedful of treatment allocation concealment are needed to assess the risk increase in relation to the magnitude of the risk factor more accurately (Jones-Jordan, Wang, Scherer, Mutti, 2014).

Recommendations

- Untreated or insufficiently treated amblyopia may result in life-long impairment in visual function. Early identification of patients with amblyopia is an important step toward effective treatment, but the lack of visual dysfunction evident to laypersons and primary care providers supports the need for more effective screening tools and systems. Refractive risk factors are potential targets for detection, but screening for risk factors must be weighed against the burden of over-referrals, since many infants and young children with risk factors will never develop amblyopia, and a program with a high false referral rate is not likely to be adopted universally. Longitudinal studies of young children with amblyogenic risk factors with and without early intervention would be ideal.
- We can improve the screening and referral process standards through the development of practical and cost-effective vision screening methods that have a strong evidence base. Ideally, vision screening instruments should undergo a robust assessment that includes the following study design characteristics: prospective, large-scale vision screening of children within the targeted age range and with a sufficient number of children with the disease of interest (e.g., amblyopia, strabismus, and refractive error); screening performed by lay screeners in the field; all children who undergo the screening also undergo a comprehensive eye examination that includes a cycloplegic refraction; and both the screeners and eye care providers are masked to the results of other testing. Any given vision screening program also should have a component to ensure that children who need treatment are indeed referred and seen by an eye care professional in a reasonable interval. Ideally an integrated data system for recording vision screening and eye care follow-up and treatment outcomes to “close the loop” should be in place (Hartmann, Block and Wallace, 2015).
- There is considerable opportunity for innovation and development of screening and diagnostic tools for amblyopia. Because moderate levels of hyperopic refractive error place young children at significant risk for future development of amblyopia and strabismus, and because they have been associated with impaired developmental and academic performance, we encourage ongoing work to identify clinical features that predict failure to emmetropize among infants with hyperopia. New technology that can empower lay persons and primary care providers to detect amblyopia and strabismus in preschool children with high sensitivity and specificity could improve the effectiveness of vision screening worldwide. New physiologic, anatomic, and genetic markers could provide valuable insight into the pathophysiology of amblyopia, but should also have practical and wide-spread application if they are to be incorporated into a screening and diagnostic system for amblyopia. Existing and new clinical features could be integrated into a scoring system to predict risk for amblyopia development to guide referrals. Successful implementation of research and clinical programs that embody these principles will lead to earlier detection and treatment of amblyopia, which would have a profound positive impact on the health and well-being of future generations.

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Chapter 3

Critical Periods in Amblyopia

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Introduction

The shift in ocular dominance of binocular neurons induced by monocular deprivation is the canonical model of synaptic plasticity confined to a postnatal critical period. Developmental constraints on this plasticity lend stability to mature visual cortical circuitry, but also impede the ability to recover from amblyopia beyond an early window. Advances with mouse models utilizing the power of molecular, genetic and imaging tools, are beginning to unravel the circuit, cellular, and molecular mechanisms controlling the onset and closure of critical periods of plasticity in primary visual cortex (V1). Emerging evidence suggests that mechanisms enabling plasticity in juveniles are not simply lost with age, but rather that plasticity is actively constrained by the developmental up-regulation of molecular ‘brakes’. Lifting these brakes enhances plasticity in the adult visual cortex, which can be harnessed to promote recovery from amblyopia (see inside of front cover). The reactivation of plasticity by experimental manipulations has revised the idea that robust ocular dominance plasticity is limited to early postnatal development (Bavelier, Levi, Li *et al.*, 2010; Takesian and Hensch, 2013; Sengpiel, 2014). Here, we discuss recent insights into the neurobiology of critical periods, and how our increasingly mechanistic understanding of these pathways can be leveraged toward improved clinical treatment of adult amblyopia.

Differences Between Juvenile and Adult Plasticity

It is well appreciated that defined windows in early life exist when neural circuitry can be robustly restructured in response to experience (reviewed in Hensch, 2004). These time-limited critical periods have been demonstrated for many brain functions across many brain regions, and are thought to allow developing neural circuits to establish an individualized, optimal neural representation of a highly variable environment. The enhanced plasticity corresponds to peak phases of physical growth, and may therefore allow for constant perception during expansion of the body surface. For example, visual receptive fields must repeatedly remap as the distance between the two eyes increases. Indeed, experience-dependent matching of stimulus selectivity of visual input from the two eyes occurs during the critical period (Wang, Sarnaik, and Cang, 2010).

The relative stability in cortical circuitry that follows the critical period may also allow for conservation of energy/resources. However, the enhanced stability with age also inhibits large-scale adaptations to changes in input during adulthood. Importantly, the adult cortex retains the ability to express some

forms of synaptic plasticity, but the mechanisms for induction and expression of plasticity differ from those utilized during the critical period. In this context, it is important to bear in mind that many measures are in current use to study ‘ocular dominance plasticity.’ Originally defined as a change in the eye preference of spiking output of V1 neurons (Wiesel & Hubel, 1963), it has grown to encompass visually-evoked synaptic potentials, intrinsic hemodynamic signals, immediate early gene activation, thalamocortical axon or dendritic spine morphology and motility, and calcium responses in individual cell types. Each of these methods yields different resolution and may be variably sensitive to subthreshold inputs (Morishita and Hensch, 2008), which are important considerations when informing therapies for recovery of visual function in both amblyopic children and adults.

During the critical period, an asymmetry in the quality of visual input across the two eyes leads to reduced visual acuity and visually-evoked spiking response through the affected eye with no obvious pathology in the eye, thalamus or cortex. The severity of amblyopia depends on the age at initiation and the type of asymmetry, which can be caused by unequal alignment (strabismus), unequal refractive error (anisometropia) or form deprivation (e.g., cataract). The critical period for developing amblyopia in children extends to 8 years, and is relatively easy to correct until that age by improving the quality of visual input in the affected eye (reviewed in Daw, 1998; Mitchell and MacKinnon, 2002; Simons, 2005), but becomes increasingly resistant to reversal with age.

In animal models, amblyopia is most often induced by monocular deprivation (MD) – eyelid suture which significantly occludes the patterned visual input to one eye. Across various species, MD unleashes a sequence of functional and structural changes in V1 that shifts the ocular dominance of binocular neurons away from the deprived eye and toward the open eye, resulting in a reduction in deprived-eye acuity (Wiesel and Hubel, 1963; 1970; Olson and Freeman, 1975; Hubel, Wiesel, and Levay, 1977; Movshon and Dürsteler, 1977; Blakemore, Garey, and Vital-Durand, 1978; LeVay, Stryker, and Shatz, 1978; Shatz and Stryker, 1978; Antonini and Stryker, 1993; Fagiolini, Pizzorusso, Berardi *et al.*, 1994; Gordon and Stryker, 1996; Hensch, Fagiolini, Mataga *et al.*, 1998; Taha and Stryker, 2002; Trachtenberg and Stryker, 2001; Mataga, Nagai and Hensch, 2002; Prusky and Douglas, 2003; Frenkel and Bear, 2004; Sato and Stryker, 2008).

While ocular dominance plasticity peaks during the postnatal critical period, it generally persists at some level in many species, including rats, mice and cats, beyond sexual maturity. The age at initiation and duration of MD then strongly impacts the severity of the subsequent amblyopia, as well as the potential for recovery (Liao, Krahe, Prusky *et al.*, 2004; Eaton, Sheehan, and Quinlan, 2016). Accordingly, short durations of MD within the critical period induce a shift in ocular dominance and a reduction in deprived eye visual acuity that are rapidly corrected by restoring normal vision (Schwarzkopf, Vorobyov, Mitchell *et al.* 2007). In contrast, long term MD initiated early and persisting through the end of the critical period induces a shift in ocular dominance and a loss of visual acuity that is highly resistant to reversal.

The initial response to MD during the critical period is a reduction in functional strength and selectivity of deprived eye visual responses (Gordon and Stryker, 1996; Hensch, Fagiolini, Mataga *et al.*, 1998; Trachtenberg, Trepel and Stryker, 2000; Frenkel and Bear, 2004). Depression of deprived-eye responses may occur by synaptic depression at both thalamocortical and intracortical connections. Notably, rapid shifts in the visual response of parvalbumin (PV)-expressing inhibitory interneurons

may enable these first functional changes within V1 (Yazaki-Sugiyama, Kang, Câteau *et al.*, 2009; Kuhlman, Olivas, Tring *et al.*, 2013; Aton, Broussard, Dumoulin *et al.*, 2013). Depression is then followed by a relatively slower, strengthening of open eye responses (Sawtell, Frenkel, Philpot *et al.*, 2003; Frenkel and Bear, 2004; Kaneko *et al.*, 2008).

Robust morphological plasticity is also induced by MD during the critical period. An initial degradation of the extracellular matrix by the upregulation of proteases occurs within the first 2 days after MD in the mouse, and may elevate spine motility (Mataga, Mizuguchi, and Hensch, 2004; Oray, Majewska and Sur, 2004). Studies in cats, monkeys and humans suggest that structural plasticity is facilitated by a reduction in the neurofilament-light protein within V1, which may de-stabilize the cytoskeleton and promote plasticity (Duffy and Livingstone, 2005; Duffy, Murphy, Frosch *et al.*, 2007; Duffy and Mitchell, 2013). Brief MD during the critical period alters spine density on pyramidal neurons (Mataga, Mizuguchi and Hensch, 2004; Tropea, Majeska, Garcia *et al.*, 2010; Yu, Majewska and Sur, 2011; Djurisic, Vidal, Mann *et al.*, 2013), and induces a transient decrease in the density of synapses formed by thalamocortical axons originating from the lateral geniculate nucleus (LGN) (Coleman, Nahmani, Gavornik *et al.*, 2010). Long term MD yields enduring alterations in the length and extent of thalamocortical arbors serving the two eyes (Hubel, Wiesel and LeVay, 1977; Shatz and Stryker, 1978; Antonini, Fagiolini and Stryker, 1999) and a significant reduction in dendritic spine density (Montey and Quinlan 2011).

Studies from humans and non-human primates suggest a protracted decline in visual plasticity that extends into adulthood rather than an abrupt closure of the critical period. However, the residual plasticity that persists in adult visual cortex appears to differ from the plasticity during the critical period in several important ways: 1) the shift in ocular dominance in adults is slower and smaller, and may require a longer duration of deprivation to engage; 2) may not require depression of deprived eye responses for subsequent strengthening of responses to the non-deprived eye; 3) may be restricted to synapses in supragranular and infragranular lamina, as plasticity in layer 4 has been shown to be constrained early in postnatal development; 4) may be restricted by saturated synapses, setting limits on the amount of recovery of visual function that can be accomplished using this pathway. Additionally, MD in adults does not elicit the robust structural alterations that accompany ocular dominance plasticity during the critical period such as increased spine motility and pruning (Mataga, Mizuguchi and Hensch, 2004; Oray, Majewska, Sur, 2004; Lee, Huang, Feng *et al.*, 2006). Indeed, a general decline in structural plasticity is one of the hallmarks of the termination of the critical period. However, residual increases in the rate of formation and stability of dendritic spines may persist in adult layer I after MD (Hofer, Mrcic-Flogel, Bonhoeffer *et al.*, 2009).

Inhibition and Critical Period Induction

Powerful new tools in neuroscience, especially the molecular genetic control available in mice, are beginning to elucidate the cellular and molecular mechanisms that may initiate and terminate critical periods. Ocular dominance plasticity peaks during the 3rd postnatal week in rodents, demonstrating that elevated plasticity is not the initial state of immature circuits. Indeed, the maturation of specific inhibitory circuitry is necessary to initiate the critical period, which can be accelerated by activating inhibitory GABA_A receptors with allosteric modulators such as benzodiazepines (Hensch, Fagiolini, Mataga *et al.*, 1998; Fagiolini & Hensch, 2000; Iwai, Fagiolini, Obata *et al.*, 2003; Fagiolini, Fritschy,

Löw *et al.*, 2004). Promoting early maturation of a specific class of inhibitory interneurons that express the calcium binding protein parvalbumin (PV) by increasing levels of growth factors (Huang, Kirkwood, Pizzorusso *et al.*, 1999; Hanover, Huang, Tonegawa *et al.*, 1999; *Otx2*: Sugiyama, Di Nardo, Aizawa *et al.*, 2008; Spatazza, Lee, Di Nardo, *et al.*, 2013), or removing cell-adhesion (PSA: Di Cristo, Chattopadhyaya, Kuhlman *et al.*, 2007) or DNA binding proteins (MeCP2: Durand, Patrizi, Quast *et al.*, 2012; Krishnan, Wang, Lu *et al.*, 2015) can induce premature initiation of the critical period.

The perisomatic inhibition mediated by these fast-spiking (PV) interneurons exerts powerful control over the excitability and plasticity of downstream pyramidal neurons, potentially sharpening the spike-timing required for synaptic plasticity (Katagiri, Fagiolini, and Hensch, 2007; Toyozumi, Miyamoto, Yazaki-Sugiyama *et al.*, 2013; Kuhlman, Olivas, Tring *et al.*, 2013). Several proteins that regulate synaptic strength and/or number are highly enriched at excitatory synapses onto PV interneurons, and impact the timing of the critical period (NARP: Gu, Huang, Chang *et al.*, 2013; NRG1: Gu, Tran, Murase *et al.*, 2016; Sun, Ikrar, Davis, *et al.*, 2016). Accordingly, NARP-deficient mice fail to initiate a critical period unless rescued by enhancing the strength of inhibitory output or excitatory drive onto PV interneurons (Gu, Huang, Chang *et al.*, 2013; Gu, Tran, Murase *et al.*, 2016).

A further increase in perisomatic inhibition is thought to terminate the critical period. Hence, the critical period can be reopened in adulthood by pharmacological reduction of inhibition (Harazov, Spolidoro, DiCristo *et al.*, 2010) or the knockdown of *Otx2* (Beurdeley, Spatazza, Lee *et al.*, 2012; Spatazza, Lee, Di Nardo *et al.*, 2013). Treatment with an NRG1 peptide induces a precocious termination of the critical period, while inhibition of the activity of the NRG receptor (ErbB) reactivates the critical period in adults (Gu, Tran, Murase *et al.*, 2016). Indeed, a developmental reduction of plasticity at excitatory synapses onto FS interneurons may explain the requirement for longer durations of MD with age (Kameyama, Sohya, Ebina *et al.*, 2010). Together, these studies indicate that PV inhibitory cells exert bidirectional control over ocular dominance plasticity (van Versendaal and Levelt, 2016).

Other classes of inhibitory neurons may influence the expression of plasticity, either independently or through the regulation of PV neurons. Interestingly, inhibitory neurons in layer 1 (L1) of the visual cortex and those expressing vasoactive intestinal peptide (VIP) are strongly activated during certain behavioral states, and exert cortical effects by disinhibition of pyramidal neurons (Letzkus, Wolff, Meyer *et al.*, 2011; Pfeffer, Xue, He *et al.*, 2013; Pi, Hangya, Kvistsiani *et al.*, 2013; Donato, Rompani, and Caroni, 2013; Fu, Kaneko, Tang *et al.*, 2015). Locomotion activates VIP interneurons, which enhances neural activity in V1 (Niell and Stryker, 2010) and promotes adult plasticity by increasing inhibition onto other interneuron subtypes that target pyramidal neurons (Fu, Tucciarone, Espinosa, *et al.*, 2014; Fu, Kaneko, Tang *et al.*, 2015). Similarly, reinforcement signals (reward and punishment) during the performance of an auditory discrimination task activate VIP neurons in auditory cortex, which increase the gain of a functional subpopulation of pyramidal neurons by disinhibition (Pi, Hangya, Kvistsiani *et al.*, 2013). Thus, disinhibitory circuits which transiently suppress other inhibitory interneurons may be a general mechanism for enabling plasticity in the adult cortex.

Molecular Reactivation of Critical Period in Adulthood

Increasing evidence demonstrates that removing molecular ‘brakes’ in adulthood can enhance plasticity and promote recovery from amblyopia. For example, epigenetic mechanisms, such as histone

deacetylase (HDAC) activity, may down-regulate expression of genes that promote plasticity over development. HDAC inhibition then enhances plasticity in adult V1, allowing for recovery from amblyopia (Putignano, Lonetti, Cancedda *et al.*, 2007; Silingardi, Scali, Belluomini *et al.*, 2010). However, the downstream targets of histone acetylation at specific stages of development remain to be identified.

Alternatively, increased expression of specific genes over development can actively limit rewiring. The expression of *Lynx1*, an endogenous inhibitor of nicotinic acetylcholine receptors, emerges in V1 coincident with critical period closure, which would dampen neuromodulatory actions of acetylcholine (Miwa, Ibanez-Tallon, Crabtree *et al.*, 1999; Morishita, Miwa, Heintz *et al.*, 2010). Both genetic deletion of *Lynx1* and administration of acetylcholinesterase inhibitors enhance spine motility and the morphological plasticity induced by MD (Sajo, Ellis-Davies, Morishita *et al.*, 2016) and enables recovery of visual acuity following MD throughout life (Morishita, Miwa, Heintz *et al.*, 2010). The major histocompatibility complex class I (MHCI) receptor, PirB, is another molecular brake. Disruption of PirB signaling enhances ocular dominance plasticity throughout life, and facilitates recovery from amblyopia in adults (Syken, Grandpre, Kanold *et al.*, 2006; Bochner, Sapp, Adelson *et al.*, 2014). Another immune system molecule, Stat1, restricts the increase of open eye responses following monocular deprivation, and its genetic deletion enhances this component of plasticity (Nagakura, Van Wart, Petravic *et al.*, 2014). The identification of specific molecules that actively suppress plasticity in the adult visual cortex may inform strategies for pharmacological interventions to reopen the critical period.

Molecular brakes can also present physical barriers to morphological plasticity. Perineuronal nets are highly enriched around PV neurons, and reach maturity at the end of the critical period. Disrupting the molecular latticework of this extracellular matrix (Pizzorusso, Medini, Berardi *et al.*, 2002; Pizzorusso, Medini, Landi *et al.*, 2006; Carulli, Pizzorusso, Kwok *et al.*, 2010) or the molecules which bind to it (i.e., *Otx2*: Beurdeley, Spatazza, Lee *et al.*, 2012) enables ocular dominance plasticity and recovery from amblyopia in adults. Consistent with this, mice lacking Nogo receptor (*Ngr1*), a bimodal receptor for chondroitin sulfate proteoglycans and myelin-derived inhibitory factors (Dickendesher, Baldwin, Mironova *et al.*, 2012), also retain critical period plasticity into adulthood and spontaneously recover visual acuity following long-term MD (McGee, Yan, Fischer *et al.*, 2005; Stephany, Chan, Parivash *et al.*, 2014). Interestingly, PirB may act in concert with *Ngr1* (Atwal, Pinkston-Gosse, Syken *et al.*, 2008) to dampen morphological plasticity of dendritic spines on layer 5 pyramidal neurons in adults (Bochner, Sapp, Adelson *et al.*, 2014).

Interestingly, one recently discovered molecular brake may lie within the dendritic spine itself. Postsynaptic density protein 95 (PSD-95), an intracellular scaffold highly enriched at excitatory synapses, is thought to accelerate maturation of excitatory synapses. PSD-95 promotes the incorporation of AMPA-type glutamate receptors into synapses containing only NMDA receptors, which are normally functionally “silent” at resting membrane potential. In contrast, the immediate early gene *Arc* promotes removal of AMPA receptors from cortical synapses and precludes visual plasticity when deleted (McCurry, Shepherd, Tropea *et al.*, 2010). Genetic reduction of PSD-95 in adulthood increases the number of silent synapses and reactivates the juvenile form of ocular dominance plasticity, characterized by a rapid and robust deprived-eye depression (Huang, Stodieck, Goetze *et al.*, 2015). Notably, no changes in GABAergic or NMDA receptor currents are observed, suggesting

that the reactivation of plasticity by PSD-95 deletion lies downstream of the regulation of inhibitory circuitry. A conversion of ‘silent’ to functional synapses has been proposed as a general mechanism to constrain plasticity across brain regions (Huang, Stodieck, Goetze *et al.*, 2015; Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014).

Environmental Reactivation of Critical Period in Adulthood

Characteristics of the physical or sensory environment strongly impact the function and plasticity of cortical circuits. Remarkably, adding social, sensory or motor enrichment to the typically impoverished environment of the laboratory rodent influences the expression and time course of ocular dominance plasticity. Robust ocular dominance plasticity persists into adulthood when mice are raised in large complex cages with multi-sensory and motor enrichment (Sale, Maya Vetencourt, Medini *et al.*, 2007; Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2013). In fact, enriched rearing may better reflect the sensorimotor environment of primates including humans. At a molecular level, exposure to enriched environments in adulthood increases H3 acetylation (Baroncelli, Scali, Sansevero *et al.*, 2016), reduces the expression of PV and GAD67 within inhibitory neurons of the visual cortex, weakens GABA signaling and fosters plasticity in both the cortex and hippocampus (Sale, Maya Vetencourt, Medini *et al.*, 2007; Donato, Rompani, and Caroni, 2013; Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014).

In this regard, it is intriguing that total visual deprivation also reactivates robust plasticity in adult V1 and promotes recovery from chronic MD (He, Ray, Dennis *et al.*, 2007; Montey and Quinlan, 2011; Duffy and Mitchell, 2013; Stodieck, Greifzu, Goetze *et al.*, 2014; Eaton, Sheehan, Quinlan *et al.*, 2016; Mitchell, MacNeill, Crowder *et al.*, 2016). The BCM theory on the regulation of a sliding synaptic modification threshold forecasted that dark exposure would enhance plasticity, and indeed several mechanisms are engaged by dark exposure that are predicted to lower the threshold for synaptic plasticity in pyramidal neurons (Cooper and Bear, 2012). For example, the composition of the NMDA type glutamate receptors is reset to a “juvenile” form (containing the NR2B subunit) which exhibits enhanced temporal summation (He, Hodos and Quinlan, 2006; Yashiro, Corlew, Philpot, 2005). In addition, synaptic plasticity typically limited to juveniles is re-expressed (Huang, Gu, Quinlan *et al.*, 2010; Montey and Quinlan, 2011), spines on pyramidal neurons are shifted toward immature structure and dynamics (Tropea, Majewska, Garcia *et al.*, 2010), and immature excitatory synapses on pyramidal neurons are strengthened, thereby increasing excitability and expanding the integration window for spike-timing dependent plasticity (He, Ray, Dennis *et al.*, 2007; Goel and Lee, 2007; Guo, Huang, de Pasquale *et al.*, 2012).

In contrast, dark exposure decreases the excitability of PV interneurons and the reactivated plasticity can be reversed by increasing the strength of excitatory synaptic input onto them (Gu, Tran, Murase *et al.*, 2016). A loss of specific neurofilament protein associated with cytoskeletal stability is observed in the LGN following dark exposure, which may further contribute to the reactivation of structural ocular dominance plasticity beyond the peak of the critical period (O’Leary, Kutcher, Mitchell *et al.*, 2012; Duffy, Lingley, Holman *et al.*, 2016). Importantly, dark exposure restores a period of susceptibility to MD and also promotes the recovery of visual function in adults with amblyopia, as has been demonstrated in rats, mice and cats (He, Hodos and Quinlan, 2006; He, Ray, Dennis *et al.*, 2007; Duffy and Mitchell, 2013; Stodieck, Greifzu, Goetze *et al.*, 2014; Duffy, Lingley, Holman *et al.*, 2016). The reactivation of plasticity by dark exposure has also been shown to strengthen thalamic input

to cortex (Montey and Quinlan, 2011). Thus, the seemingly opposite interventions of environmental enrichment and dark exposure may both enhance cellular plasticity by the removal of functional and structural constraints that normally accumulate over development to stabilize V1 circuitry.

It is important to note that dark exposure alone does not impact visual acuity or neuronal stimulus selectivity, which is regained only after repetitive visual experience (Montey, Eaton, and Quinlan, 2013; Eaton, Sheehan, Quinlan *et al.*, 2016). Likewise, enrichment or locomotion alone does not strengthen visual performance (Greifzu, Kalogeraki and Löwel, 2016; Kaneko and Stryker, 2014). This suggests that the recovery from amblyopia in adulthood is a two-stage process that requires 1) the reactivation of plasticity in the adult amblyopic cortex (permissive step) and 2) focused visual experience to stimulate perceptual learning (instructive step). One of the challenges, therefore, is to identify the optimal visual stimulation to drive recovery of function, which may differ based on age and depth of amblyopia (Montey and Quinlan 2011; Eaton, Sheehan, Quinlan *et al.*, 2016). In addition, prolonged plasticity by environmental enrichment raises the question whether complex environments better mimic those of primates including humans. At a minimum, it provides a valuable condition with which to better understand the biological basis of critical period closure.

Reactivating Plasticity to Enhance Recovery

The reactivation of plasticity in primary visual cortex has revised the idea that critical periods are strictly limited to early postnatal development (Bavelier, Levi, Li *et al.*, 2010; Takesian and Hensch, 2013; Sengpiel, 2014). However, the mechanisms that engage the cortical plasticity necessary to treat amblyopia may be very different from the plasticity that enables the cortex to regain sensitivity to MD. Indeed, it has long been known that the critical period for ocular dominance shifts in response to MD differs from the critical period for recovery of binocularity and orientation selectivity by removing the MD (Liao, Krahe, Prusky *et al.*, 2004). Although initially assumed to overlap with the critical period for susceptibility to amblyopia, it is now clear that the treatment window for reversal of amblyopia in humans may extend beyond early life (reviewed in Daw, 1998). It is therefore important that key molecular effectors be tested in their ability to recover (not induce) amblyopia in adults.

Mechanistic studies (above), performed primarily in mice, have identified novel therapies with translational potential to reverse the developmental constraints on recovery from amblyopia. Several commonly prescribed drugs, such as cholinesterase inhibitors (Morishita, Miwa, Heintz *et al.*, 2010), valproate (Gervain, Vines, Chen *et al.*, 2013; Lennartsson, Arner, Fagiolini *et al.*, 2015), or selective serotonin reuptake inhibitors (SSRIs) (Maya Vetencourt, Sale, Viegi *et al.*, 2008), could be repurposed to reactivate plasticity in adult amblyopic patients. Interestingly, reduced PV interneuron function may be a mechanism common to several of these interventions. The SSRI antidepressant fluoxetine reduces basal levels of extracellular GABA (Maya Vetencourt, Sale, Viegi *et al.*, 2008) and the number of PV interneurons surrounded by dense perineuronal nets (Guirado, Perez-Rando, Sanchez-Matarredona *et al.*, 2014). Similarly, dark exposure may rejuvenate intracortical inhibition by reducing the excitatory drive onto PV neurons (Gu, Tran, Murase *et al.*, 2016).

Future work that explores non-invasive ways to tap into these mechanisms to trigger plasticity may generate novel amblyopia treatments for adults. It will also be important to learn if the success of behavioral manipulations, such as dark rearing, environmental enrichment and exercise, promotes

the recovery from amblyopia in rodents by reversing molecular brakes or engaging residual plasticity mechanisms that are normally expressed in the adult cortex (Sale, Maya Vetencourt, Medini *et al.*, 2007; He, Ray, Dennis *et al.*, 2008; Duffy and Mitchell, 2013; Eaton, Sheehan, Quinlan *et al.*, 2016). Such biological insights gleaned from animal model systems have provided the foundation for a number of promising ongoing clinical trials aimed at improving vision in amblyopic patients (see Chapter 7).

In addition, it is important to keep in mind that while robust ocular dominance plasticity is lost with age, the adult visual cortex does retain the ability to learn. This is reflected in the success of visual training (repetitive visual task performance, visual perceptual learning) to promote enhancement of acuity and recovery of stereoscopic vision in both amblyopic humans and experimental animals (Sengpiel, 2014; Bonaccorsi, Berardi, and Sale, 2014; Kawato, Lu, Sagi *et al.*, 2014; Levi and Li, 2009). Recent approaches to visual training include dichoptic visual stimulation (in humans and cats) to normalize the quality of visual input across the strong and weak eye, and the use of action video games, to recruit neuromodulatory pathways that engage attention and motivation (see Chapter 7; Mitchell and Duffy, 2014; Hess and Thompson, 2015; Murphy, Roumeliotis, Williams *et al.*, 2015; Levi, Knill and Bavelier, 2015). However, the improvements in visual acuity achieved with these methods in humans have been relatively modest to date (Tsirlin, Colpa, Goltz *et al.*, 2015; but see Hess and Thompson, 2015).

Expanding the Focus Beyond Ocular Dominance

The primary aspects of visual system function assessed in animal studies of amblyopia are ocular dominance and spatial acuity. However, amblyopia is associated with a range of visual deficits, including loss of stereoscopic depth perception, crowding, impairments in shape discrimination, deficits in motion and direction perception and object tracking (reviewed in Daw, 2013). Some of these impairments, such as the loss of stereoscopic depth perception and visual crowding, may greatly impact the quality of the life of the amblyopic patient (Levi, Knill, and Bavelier, 2015). Expanding the focus of animal studies of amblyopia beyond the recovery of ocular dominance will broaden the ability of this work to inform clinical strategies.

Furthermore, separable neuronal response properties of individual V1 neurons have distinct, overlapping critical periods (reviewed in Kiorpes, 2015). For example, it has long been known that the critical period for direction selectivity in kittens precedes the critical period for ocular dominance (Daw & Wyatt, 1976). In the primate visual system, critical periods for basic spectral sensitivities end relatively early (6 months), whereas those for complex representations such as contrast sensitivity and binocularity extend much later (25 months; Harwerth, Smith, Duncan *et al.*, 1986). There is also evidence that some manipulations may globally reinstate V1 plasticity across these distinct visual functions. For example, dark exposure in adulthood, which reactivates plasticity for the recovery of normal ocular dominance in amblyopic rats, mice and kittens (He, Ray, Dennis *et al.*, 2007; Montey and Quinlan, 2011; Duffy and Mitchell, 2013; Stodieck, Greifzu, Goetze *et al.*, 2014; Eaton, Sheehan, Quinlan *et al.*, 2016; Mitchell, MacNeill, Crowder *et al.*, 2016) promotes the recovery of stimulus selectivity and visual response strength (Montey, Eaton, and Quinlan, 2013). As critical periods for different visual functions may depend on separate underlying mechanisms, some manipulations may restore only selective features of V1 responses. For example, a genetic deletion of PSD-95 disrupts the development of orientation preference in mouse visual cortex, without impacting the development or plasticity of ocular dominance in juveniles (Fagiolini, Katagiri, Miyamoto *et al.*, 2003).

It is particularly important to ask if the interventions that promote recovery of ocular dominance and/or visual acuity also promote the visual functions that underlie stereopsis, such as retinal disparity tuning and/or binocular matching of stimulus preference. Binocular integration in the primary visual cortex is an important first step in the perception of depth from retinal disparity (Scholl, Burge, Priebe *et al.*, 2013). It has been demonstrated that shortly after eye opening, V1 neurons exhibit orientation tuning and respond to visual stimulation of either eye; however, the orientation preference through each eye, which is initially mismatched, becomes tuned to similar orientations during the critical period (Wang, Sarnaik, and Cang, 2010). Indeed, manipulations that prolong V1 plasticity, such as environmental enrichment, accelerate binocular matching of stimulus selectivity in the developing mouse primary visual cortex (Gu, Tran, Murase *et al.*, 2016). In contrast, manipulations that accelerate plasticity in the cortex, such as heterozygous loss of *Mecp2* prevent the acquisition of matched stimulus selectivity (Krishnan, Wang, Lu *et al.*, 2015). Recovery of stereopsis in rodents can be assessed through behavioral measures such as visual cliff or SLAG performance (Gil-Pagés, Stiles, Parks *et al.*, 2013). Incorporation of physiological and psychophysical assessments that examine contrast sensitivity, direction selectivity and stereoscopy would greatly improve assessment of treatment efficacy across multiple aspects of vision in patients with amblyopia.

Expanding the Focus Beyond Primary Visual Cortex

The majority of animal work on amblyopia has focused on regions early in the visual pathway, as MD induces significant structural re-arrangements in V1, including pruning of thalamocortical inputs that serve the deprived eye (see front cover) (Wiesel and Hubel, 1963; Hubel, Wiesel and LeVay, 1977; Shatz and Stryker, 1978). Long-term MD induces a near complete loss of stimulus selectivity for input coming in through the chronically deprived eye (Montey and Quinlan, 2011). Given these severe structural and functional deficits in V1, it is even more remarkable that full recovery of visual acuity has been demonstrated with some interventions.

However, the magnitude of compromised vision observed in psychophysical experiments is often not mirrored by changes in the function of V1 neurons, suggesting that physiological changes may be propagated and amplified in higher cortical areas (Shooner, Hallum, Kumbhani *et al.*, 2015). Indeed, psychophysical and neural recording data suggest that amblyopia is also associated with abnormalities in extrastriate regions (reviewed in Kiorpes, 2015). For example, deficits in higher order visual functions, such as motion perception have been described in amblyopic monkeys (Kiorpes, Tang, and Movshon *et al.*, 2006) may be partly explained by aberrant development of extrastriate area MT/V5. Here neurons driven by the amblyopic eye exhibit reduced sensitivity to coherent motion and reduced ability to integrate motion information over time (El-Shamayleh, Kiorpes, Kohn *et al.*, 2010).

Higher brain areas and neuromodulatory pathways are also potential targets to facilitate visual responses and plasticity within V1 of amblyopic adults. For example, children with macular degeneration show large regions of V1 that are unresponsive during passive viewing of visual stimuli, which can be activated by engaging the subjects in a stimulus-related task, suggesting a powerful role of top-down influences. Remarkably, the same visual task-related responses are not observed in simulated lesion zones in normal binocular subjects suggesting that macular degeneration may potentiate or unmask feedback signals (Masuda, Dumoulin, Nakadomari *et al.*, 2008).

Regions outside of the primary sensory cortices are thought to express late, prolonged windows of plasticity that extend well beyond that of V1. Thus, devising treatments to target these regions may be an effective strategy for recovery of visual function in adulthood that does not require the reactivation of plasticity in V1. Advanced tools enabling the monitoring, activation or silencing of specific neural circuits in mice or higher species will contribute to our understanding of the top-down influences on plasticity within V1. Future primate studies will also be essential to examine plasticity within higher order visual regions.

Path Forward

Rapidly evolving genetic, imaging and physiological tools have allowed mechanistic insights into how critical period plasticity is regulated, including the identification of molecular ‘triggers’ and ‘brakes’ that control the initiation and termination in V1. Much of this knowledge has been gleaned recently from mouse models, which offer unprecedented experimental control of specific neuronal and synaptic populations, including optogenetic, chemogenetic and magnetogenetic approaches. However, to better inform amblyopia treatment, mechanistic work should be expanded to additional species, especially those with a columnar organization of ocular preference and neurons tuned for small retinal disparities. The increasing availability of molecular genetics techniques such as CRISPR makes this a likely goal.

In addition, examination of the incidence and expression of amblyopia across human populations may elucidate the impact of environmental and genetic factors on individual differences in visual plasticity. An assessment of ocular dominance plasticity across a large number of recombinant inbred mouse strains revealed striking variability in the response to MD. Interestingly, there was no correlation between the weakening of deprived eye responses and the strengthening of non-deprived eye responses, suggesting that these two pathways may be regulated by separate genetic factors (Heimel, Hermans, Sommeijer *et al.*, 2008). In addition, several molecules implicated in regulating the timing of the critical period, including the constraints on adult plasticity, are known risk factors for neurodevelopmental disorders such as schizophrenia. These include HDAC and NRG1 (Penzes, Buonanno, Passafaro *et al.*, 2013; Rico and Marín, 2011). Interestingly, male schizophrenics are two times less likely to have refractive errors (Caspi, Vishne, Reichenberg *et al.*, 2009), raising the possibility that common genetic risk factors contribute generally to the maturation of neuronal circuitry, including the normal development of binocular vision. Further work is necessary to identify human populations that may be at greater or lesser risk for the development of amblyopia.

Capitalizing on these biological insights, one goal is to develop targeted strategies to guide clinical trials by enhancing plasticity in post-critical period visual cortex in humans. In addition, such critical period regulation could also be extended to strabismus, eye movement control disorders, and the restoration of optimal neural function after damage from stroke or other traumatic brain injury.

Recommendations

- During developmental ‘critical periods,’ neural circuitry can be potently shaped by experience. Although the brain retains the capacity to re-wire beyond early life, adult forms of plasticity may utilize distinct underlying mechanisms. Understanding the differences between developmental and adult plasticity, including differences in how they are measured, will provide

key insights into novel therapies for recovery of visual function from amblyopia in both children and adults.

- Evolving tools in neuroscience have shed new light on the ‘triggers’ and ‘brakes’ that determine the onset and offset of critical periods. Strikingly, the brain’s intrinsic potential for plasticity is not lost with age, but instead is actively constrained beyond early critical periods. Indeed, lifting molecular ‘brakes’ unmask potent plasticity in adulthood. Ongoing work to determine how the various ‘brakes’ act within common cellular and circuit networks will lead to targeted therapeutic strategies to promote plasticity – biologically-inspired clinical studies for amblyopia recovery.
- Most animal studies have focused on reinstating a period of susceptibility to monocular deprivation in adulthood. Yet, the mechanisms underlying the plasticity necessary to recover from amblyopia may be distinct. Thus, future work should emphasize animal studies to specifically examine recovery of visual function in amblyopic brains.
- Amblyopia is associated with a range of visual impairments beyond acuity, but the majority of studies in mouse models of amblyopia have exclusively focused on ocular dominance (OD) shifts. Future work should identify other physiological measures and behavioral paradigms to examine widespread visual functions beyond OD, such as contrast sensitivity and stereopsis that can be applied across species.
- Some of the deficits associated with amblyopia may result from abnormalities in regions beyond primary visual cortex (V1). Moreover, signals from higher brain areas may facilitate visual responses and plasticity within V1. Thus, understanding developmental trajectories and critical period mechanisms in regions outside of V1 may identify new treatments for the recovery from amblyopia in adulthood.
- Future work should include the development of better models for amblyopia across animal species and humans. Identifying biochemical correlates of plasticity will allow us to compare developmental trajectories more readily across species. Capitalizing on genetic diversity in mice and humans will provide insight into the individual variability that influences the etiology or recovery from amblyopia.

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Chapter 4

Treatment of Amblyopia as a Function of Age

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Introduction

Although, historically, treatment of amblyopia has been recommended prior to closure of a critical window in visual development, the existence and duration of that critical window is currently unclear. Moreover, there is clear evidence, both from animal and human studies of deprivation amblyopia, that there are different critical windows for different visual functions, and that monocular and binocular deprivation have different neural and behavioral consequences (Lewis and Maurer, 2005). In view of the spectrum of critical windows for different visual functions and for different types of amblyopia, combined with individual variability in these windows, treatment of amblyopia has been increasingly offered to older children and adults, notwithstanding that treatment beyond age 7 years tends to be, on average, less effective than in younger children (Holmes, Lazar, Melia *et al.*, 2011).

Challenge of Individual Variability of Response

There is a high degree of variability in treatment response among patients regardless of age (Figure 4.1) (Holmes, Lazar, Melia *et al.*, 2011), suggesting that age is only one of many factors determining treatment response.

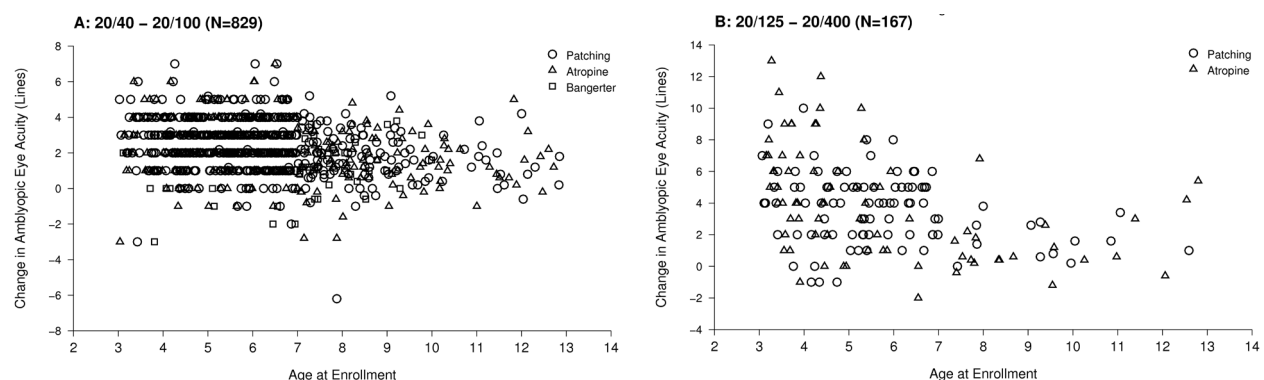


Figure 4.1. Relationship between age and amblyopic eye visual acuity improvement, in children 3 to less than 13 years of age with moderate amblyopia (20/40 to 20/100, n=829, A) or severe amblyopia (20/125 to 20/400, n=167, B) from a meta-analysis of 4 amblyopia treatment trials. (Pediatric Eye Disease Investigator Group, 2008; Pediatric Eye Disease Investigator Group, 2009; Pediatric Eye Disease Investigator Group, 2010; Pediatric Eye Disease Investigator Group, 2008). Regarding improvement, starting at 20/40 it would take 3 lines improvement to reach 20/20, whereas starting at 20/200 it would take 10 lines improvement to reach 20/20. (used with permission)

Poor compliance with prescribed treatment has most often been blamed for a suboptimal treatment response, but when actual patching time is measured (using occlusion dose monitors) (Fielder, Auld, Irwin *et al.*, 1994), it is apparent that only a fraction of the variability can be explained by compliance (Stewart, Moseley, Stephens *et al.*, 2004; Stewart, Stephens, Fielder *et al.*, 2007). Since multiple factors (some known and many unknown) affect amblyopia treatment response, clinicians are poorly equipped to make recommendations for amblyopia treatment (regarding whether to treat and how to treat), and currently have to approach each patient as an ‘average patient.’ Clearly, there is a pressing need to identify the many factors that influence treatment response, of which age is only one.

Age Considerations for Current Treatment Modalities

Overall, there appears to be a reduction in the effect of common forms of treatment (patching, atropine and Bangerter filter) with increasing age, particularly over the age of 7 years (Figure 4.1). Stewart *et al.* (Stewart, Moseley and Fielder, 2003) reported that comparing children <4 years, 4 to 6 years, and ≥6 years-old, the outcome for all was similar, but for older children this required a greater dose of patching compared with those <4 years old.

Nevertheless, in many previous large randomized treatment trials of patching and atropine, ‘age’ has not been found to be an effect modifier, in that there appears to be no greater or lesser effect of patching vs atropine, different doses of patching or different doses of atropine, dependent on age (Pediatric Eye Disease Investigator Group, 2004; Pediatric Eye Disease Investigator Group, 2002; Pediatric Eye Disease Investigator Group, 2003; Pediatric Eye Disease Investigator Group, 2003). Even when patching has been compared with continued optical treatment alone (after maximal improvement with optical treatment alone), age was not found to be an effect modifier (Pediatric Eye Disease Investigator Group 2006). The failure to find an age effect in these earlier PEDIG studies may have been due to the limitations of the study population (age 3 to <7 years), not including children >7 years old where a modest effect of age begins to appear (Holmes, Lazar, Melia *et al.*, 2011).

Despite these findings, there are several practical and theoretical ways in which age may affect the outcome of specific modalities of amblyopia therapy. For example, patient age may influence the ability and/or willingness to comply with a specific treatment. Some treatments, such as patching and atropine, may be easier for infants, who can less effectively resist treatment, whereas they may be resisted more strongly and more effectively by older children.

Recent studies have confirmed the importance of refractive correction (optical treatment) for amblyopia, regardless of whether the cause of the amblyopia is anisometropia, strabismus or both (Pediatric Eye Disease Investigator Group, 2012; Pediatric Eye Disease Investigator Group, 2006). ‘Optical treatment of amblyopia’ can be defined as the long-term effect of putting a focused image on the retina of an amblyopic eye, in contrast to the immediate effect of correcting optical blur. The instantaneous improvement of visual acuity when correcting blur with refractive correction would not be considered treating amblyopia, but the slow improvement of visual acuity over weeks and months while wearing new refractive correction would be considered ‘optical treatment’ of amblyopia. Perhaps paradoxically, when also considering the effect of patching, atropine and Bangerter filters (Figure 4.1), the effectiveness of optical treatment of amblyopia may be independent of age, with marked improvement in some teenagers (Pediatric Eye Disease Investigator Group, 2005) and even adults

(B. Thompson, personal communication). It would be worthwhile to formally study the effect of optical treatment of amblyopia in adults to substantiate these initial observations. From the standpoint of future clinical trial design, patients enrolled in an amblyopia treatment trial should first be provided with optimal refractive correction, and have achieved maximal benefit from that optical treatment prior to the baseline visual acuity assessment and commencement of additional treatment. If optical treatment is not completed prior to starting the additional treatment to be studied, it will be impossible to separate the improvement due to optical treatment from the improvement from the treatment to be evaluated. It is also entirely possible that optical treatment of amblyopia and patching treatment of amblyopia might be mediated by entirely different neural mechanisms and, therefore, it is important to separate these effects when designing future studies.

Correcting refractive errors even before amblyopia develops may be very important for preventing amblyopia. Atkinson's research group (Anker, Atkinson, Braddick *et al.*, 2004; Atkinson, Braddick, Nardini *et al.*, 2007) reported that infants with hyperopia of +3.50 D and greater (identified by photorefractive screening, confirmed on cycloplegic retinoscopy following screening) who wore spectacle correction were much less likely to have reduced visual acuity (including amblyopia) at age 4 years than those who did not. The Pediatric Eye Disease Investigator Group is currently conducting a similar study in 1- to 5 years olds, which may confirm or refute these findings, but further studies involving identification of amblyogenic refractive errors by photoscreening and the prophylactic treatment of amblyogenic factors are needed. Some initial population-based work has been published on the concurrent association of specific refractive errors with amblyopia (Tarczy-Hornoch, Varma, Cotter *et al.*, 2011), but longitudinal studies are needed to determine what levels of refractive error, if untreated, lead to amblyopia and in what proportion of children.

Patching of the non-amblyopic eye has been the cornerstone of amblyopia treatment for many years. Recent randomized clinical trials have substantiated the effectiveness of specific prescribed patching regimens for both moderate and severe anisometropic, strabismic and combined-mechanism amblyopia (Pediatric Eye Disease Investigator Group, 2003; Pediatric Eye Disease Investigator Group, 2003). Lack of compliance remains a major pitfall in patching therapy (Stewart, Moseley, Stephens *et al.*, 2004; Stewart, Stephens, Fielder *et al.*, 2007), and may mediate some of the age effect of generally lesser improvement with increasing age (Holmes, Lazar, Melia *et al.*, 2011). For example, intensive patching regimens (e.g., >2 hours daily) are much easier to implement in preschool children than in school-aged children because of different visual demands and psychosocial concerns (Pediatric Eye Disease Investigator Group, 2003). Nevertheless, patching remains the standard for comparison for alternative and new amblyopia therapies.

Pharmacologic penalization (with atropine drops administered to the fellow eye) is also used to treat amblyopia, and is supported by a series of randomized clinical trials demonstrating similar treatment effectiveness of daily or weekend atropine compared with part-time patching for anisometropic, strabismic and combined amblyopia (Pediatric Eye Disease Investigator Group, 2004; Pediatric Eye Disease Investigator Group, 2002). Although applying an eye drop seems less onerous to some patients and parents compared with patching, the efficacy of penalization regimens may also be limited by poor compliance due to the child resisting the drop. There are some drawbacks associated with penalization. First, since the sound eye is blurred for the full day, school-aged children must rely on their amblyopic

eye to do their schoolwork (unless prescribed a separate pair of glasses for reading). Second, in younger children (<3 years old), reverse amblyopia is difficult to detect because these young children often can rarely complete optotype visual acuity testing and remains a concern with any continuous treatment.

Emerging Treatment Modalities

Several emerging therapies present potential advantages but also potential new drawbacks over patching and atropine in patients of specific ages with amblyopia. Shutter glasses/goggles transiently occlude the fellow eye at various frequencies either as an alternative method of occlusion (BenEzra, Herzog, Cohen *et al.*, 2007) or to occlude alternate eyes in an effort to eliminate suppression (when alternating at high frequency). At low frequency, the shutter glasses might produce problems similar to those of patching: preventing activities such as school work in school-age children; however, in small pilot studies using 45 seconds on, 55 seconds off (BenEzra, Herzog, Cohen *et al.*, 2007) and 30 seconds on, 30 seconds off (Wang *et al.*, 2016 in press), the shutter glasses were well tolerated.

New approaches, including perceptual learning and video game play (both monocular and dichoptic), seem to be promising additions to the amblyopia treatment armamentarium. Nevertheless, these new treatments have not yet been studied in randomized clinical trials (RCTs). Recent reviews, and meta-analyses of case series, suggest that visual acuity improvement with such treatments is modest, on average 0.1 to 0.2 logMAR lines, although other modalities of vision also improve (Levi, 2012; Levi, Knill and Bavelier, 2015). It is important to note that for many of these studies the treatment period was quite limited, typically 2 or 4 weeks. Current binocular (dichoptic) therapy of amblyopia is based on the principle of anti-suppression therapy to promote simultaneous use of both eyes by decreasing the contrast and/or luminance of the fellow eye in order to equalize the perceptual strength of the input to the two eyes. Several game formats have been created, including ones that can be performed on a tablet or PC (Li, Thompson, Deng *et al.*, 2013; Li, Jost, Morale *et al.*, 2014). In children able to play the game and adults, we would expect better compliance with such binocular games than with patching, (Kelly, Jost, Dao *et al.*, 2016) although initial large randomized trials have been disappointing (Holmes, Mahn, Lazar *et al.*, 2016). Through the success and experience of the gaming industry, much is known about how to incentivize game play in children and young adults, and the same principles can be applied to optimize these therapeutic approaches for amblyopia. On the other hand, these games require dedicated time away from schoolwork or other activities that could otherwise be performed with a patch. Importantly, only children old enough to understand and interact with these devices and games can benefit from these therapies. Passive binocular activities, such as watching dichoptic movies (Li, Reynaud, Hess *et al.*, 2015), may be a more practical approach for even younger children or patients with neuro-cognitive or other developmental impairment. A major limitation is providing specific games or movies that are sufficiently engaging. However, it may be possible to merge the passive approach with computer displays used for homework, reading and entertainment, which would provide the variety that could support consistent treatment.

Regarding pharmacological approaches to treating amblyopia, although a recent RCT investigating patching with and without systemic oral levodopa had disappointing results (Pediatric Eye Disease Investigator Group, 2015), there is continued interest in other systemic pharmacologic therapies combined with conventional therapy (such as patching), predicated on the hypothesis that pharmacologic manipulation of the molecular ‘brakes’ that preclude synaptic plasticity will facilitate

a more robust response to treatment. These pharmacologic approaches include selective serotonin reuptake inhibitors (Maya Vetencourt, Sale, Viegi *et al.*, 2008), acetylcholine esterase inhibitors (Morishita, Miwa, Heintz *et al.*, 2010), and histone deacetylase inhibitors (Bavelier, Levi, Li *et al.*, 2010; Silingardi, Scali, Belluomini *et al.*, 2010), and temporary binocular inactivation of retinal ganglion cells with blockade of sodium channels (Fong, Mitchell, Duffy *et al.*, 2016). While these approaches may hold some promise, there are practical issues for consideration. First, some of these medications are psychoactive, having significant adverse effect profiles in adults that have not yet been studied in children and they currently are only FDA-approved for unrelated use in adults. Second, systemic administration is likely to have effects throughout the central nervous system, not just in the visual cortex. Unintended effects on synaptic physiology elsewhere in the brain could have unforeseen effects on neurodevelopment which would need to be carefully evaluated before such medications could be used for treating amblyopia.

Similar considerations may apply to transcranial magnetic or direct current stimulation (Thompson, Mansouri, Koski *et al.*, 2008) complete darkness (Bavelier, Levi, Li *et al.*, 2010; He, Ray, Dennis *et al.*, 2007; Duffy and Mitchell, 2013). In contrast to established, and perhaps more benign, therapies (such as optical treatment, patching and atropine), much less is known about unintended and adverse effects with these proposed new treatments. It would seem reasonable that, initial studies should first be conducted in adults, and perhaps non-human primates before humans, avoiding many of the special ethical and regulatory issues pertaining to children. That said, a negative result in a study of adults with amblyopia should not necessarily be interpreted as a complete failure of the therapy, and studies in children with amblyopia may still be warranted with appropriate attention to safety, consent and ethics.

Role of Motor Activity

Recent animal studies raise the question of whether motor activity could be relevant to the treatment of amblyopia. Is it possible that the treatment of amblyopia could benefit from concurrent motor activity? Experiments in rodents have suggested that motor activity in addition to the visual system stimulation promotes recovery from monocular deprivation. In adult rats reared in an enriched environment where they can run on wheels and have toys to play with in the company of other rats, the loss of visual acuity resulting from monocular deprivation is reversed (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014; Sale, Maya Vetencourt, Medini *et al.*, 2007). When the various components of the environmental enrichment are isolated, enhanced physical exercise, enhanced visual enrichment, and perceptual learning are all shown to contribute to the recovery, but social enrichment does not (Baroncelli, Bonaccorsi, Milanese *et al.*, 2012). Moreover, mice running on a styrofoam ball have rather more plasticity in the visual cortex than mice that are stationary. Evidence suggests that motor-related signals can gain access to the visual cortex via two different ‘neuromodulatory’ routes. One is carried by acetylcholinergic afferents from the basal forebrain to parvalbumin cells, resulting in disinhibition of visual cortical circuits in the visual cortex (Stryker, 2014). The other involves more direct excitatory input from nuclei of the visual thalamus (including the lateral geniculate nucleus) that is probably inherited from head and body movement-related activity in the superior colliculus (Roth, Dahmen, Muir *et al.*, 2016).

Held showed more than 50 years ago that plasticity in the visual system is affected by feedback from the motor system (Held, 1965). There are indications that widespread augmentation of cholinergic activation of human sensory-motor and attention-based cortical systems can enhance perceptual learning of novel, behaviorally-relevant visual tasks (Rokem and Silver, 2010). However, the degree to which the addition of visuomotor feedback and motor activity to treatment may be useful is unresolved, and would benefit from rigorous assessment. Investigation of whether the response to amblyopia therapy is enhanced by motor or visuomotor input could begin to address this question. Appropriately randomized and controlled studies in humans would ultimately be required.

If concurrent motor activity is found to enhance plasticity, it should be explored for integration into the treatment of amblyopia, except perhaps in the very youngest infants where specific motor activity may be difficult to direct and control, or in patients who have other disabilities that preclude a specific type of motor activity. In addition, if general or specific motor activity is found to enhance improvement of amblyopic eye visual acuity, it may be useful when treating adults where, on average, response to other treatments may be somewhat more limited.

Areas of Focus and Ongoing Challenges

As we consider future studies of new treatments for amblyopia and how patient age might influence their effectiveness, there are several challenges that merit further discussion. First and perhaps most importantly, individual responses to all amblyopia treatments appear to be highly variable (Figure 4.1). From the standpoint of clinical trial design, high variability drives up the needed sample size and, in turn, the cost of conducting a treatment trial. In addition, we need outcome measures beyond optotype visual acuity for amblyopia treatment trials. Novel biomarkers and better categorization of amblyopia may help address this problem of variability of outcome. Regarding better categorization of amblyopia, there are practical limitations to increasing the number and complexity of tests used within a multicenter clinical trial design, and therefore additional focused ‘deep phenotyping’ studies are needed to help define a limited set of high yield tests. Such ‘deep phenotyping’ projects will probably involve a small number of interested dedicated sites.

Compliance remains an ongoing challenge. When combined with prescribed patching, educational regimens have been found to improve treatment adherence (Loudon, Fronius, Looman *et al.*, 2006). Measures of actual duration of game play can also now be incorporated into electronic games that are being developed for amblyopia treatment, as a more direct assessment of compliance. Incorporating objective measures of compliance, such as occlusion dose monitors (Fielder, Auld, Irwin *et al.*, 1994) for patching and actual measure of treatment duration for hand held or computer-based treatment, would be beneficial in future amblyopia treatment trials.

The choice of outcome measures for future amblyopia treatment trials also deserves re-evaluation. The current standard outcome measure of optotype visual acuity has proven to have a high degree of test-retest variability (Beck, Moke, Turpin *et al.*, 2003; Holmes, Beck, Repka *et al.*, 2001) and may not be the optimum or only important outcome measure for amblyopia treatment trial. Contrast sensitivity (CS) is also impaired in amblyopia (Levi and Harwerth, 1977; Hess and Howell, 1977) but we need new methods for rapid evaluation of CS thresholds before we could use CS as a primary outcome measure for treatment trials. In addition, ongoing work (Sharma, Levi and Klein, 2000;

Popple and Levi, 2008; Hou, Kim, Lai *et al.*, 2016) has highlighted the importance of higher order visual processing in amblyopia and we need methods to efficiently assess those parameters in a clinical setting. Recent emphasis on treatment using dichoptic tasks suggests that measurement of suppression and binocular fusion may be especially important. There is an opportunity for new visuo-motor tests to be incorporated into the software used on new therapeutic devices (such as hand held tablets) which may lower the burden of testing these parameters in future clinical trials. Such clinical trials should focus not just on children with amblyopia but also adults, who have hitherto been somewhat neglected in RCTs directed at amblyopia treatment.

Lastly, current measurements of visual function in the clinician's office do not necessarily reflect the functional consequences of amblyopia and its treatment and their effects on health-related quality of life, or the economic consequences of the condition and treatment. New patient-derived instruments to assess functional vision in children, and health-related quality of life in children and their parents, are under development (Liebermann, Leske, Castañeda *et al.*, 2016; Hatt, Leske, Wernimont *et al.*, 2017). Appropriately designed, patient derived, questionnaire instruments can be used in both clinical and research environments without placing a significant burden on patient and families. Such questionnaires can easily be incorporated in to amblyopia treatment trials because they can be completed by the patient and/or parents while waiting before and between clinical tests.

Recommendations

While amblyopia is a disorder rooted in the principles of critical periods for synaptic plasticity, the concept of an 'age limit' for plasticity is being challenged, both in its occurrence and through pharmacologic manipulation. This change in how we view age as a factor in the treatment of amblyopia, no longer as a relative contraindication for amblyopia treatment, guides what treatments might be most appropriate and most likely to result in better compliance and outcomes.

- Variability of response may not be so much driven by age and/or compliance but other, as yet unknown, factors that require further studies for elucidation. Such studies will probably involve 'deep phenotyping,' using new and existing clinical tests (beyond optotype visual acuity), and lead to an improved clinical classification scheme for amblyopia based on new and existing biomarkers. Such biomarkers may better predict treatment response to current therapies such as patching and ultimately may guide decision-making and therapeutic approaches. New outcome measures with less variability of treatment response, may also allow more efficient treatment trials.
- New and evolving treatments show promise in therapeutic efficacy, particularly for adults, but we must consider the risks and adverse effects if considering these treatments for children.
- The impact of amblyopia on patients' functional vision and health-related quality of life, including the health-related quality of life of parents and care-givers (in childhood amblyopia), along with economic consequences, should be a focus of future research.

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Chapter 5

Cortical Correlates of Amblyopia

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Introduction

In clinical cases of amblyopia, the common standard for diagnosis and treatment is the presence and severity of monocular acuity loss. Treatment of amblyopia is typically benchmarked by improvements in acuity, where a reduction in the interocular difference in acuity is the treatment goal. However, it is known that amblyopic individuals, even those who have been treated for acuity losses, often suffer a diversity of deficits related specifically to binocular and/or high order visual functions (Daw, 2014). These additional deficits are also present in animal models, particularly nonhuman primates (Kozma and Kiorpes, 2003). Investigation of cortical correlates of amblyopic vision in animal models has been focused mainly on primary rather than higher order visual cortex, although there have been a few studies of neural deficits beyond V1 (Bi, Zhang, Tao *et al.*, 2011; El-Shamayleh, Kiorpes, Kohn *et al.*, 2010; Shooner, Hallum, Kumbhani *et al.*, 2015; Tao, Zhang, Shen *et al.*, 2014). Here, we discuss what is known from both human studies and animal models of amblyopia regarding the cortical correlates of visual deficits found in association with amblyopia, particularly those relevant to binocular vision and high-order vision in striate and extrastriate cortex, and relevant associated visual behaviors. In this chapter we focus mainly on anisometropic and strabismic amblyopia, rather than the more severe deprivation amblyopia, which is comparatively rare in humans.

Binocular Vision

While cortical responses to stimulation of an amblyopic eye are degraded, meaning that the range of spatial stimuli to which amblyopic eye neurons respond is often reduced, the severity of these monocular changes does not fully explain changes in visual behavior (Shooner, Hallum, Kumbhani *et al.*, 2015). Furthermore, binocular cortical responses are strongly reduced both in anisometropic and strabismic patients with amblyopia (Bi, Zhang, Tao *et al.*, 2011; Kiorpes, Kiper, O'Keefe *et al.*, 1998; Smith, Chino, Ni *et al.*, 1997). Understanding the cortical correlates of the binocular combination of visual information is therefore essential to understanding the deficits associated with amblyopia.

Binocular Vision – Suppression

Even during normal binocular viewing, competition between inputs from the two eyes occurs at the cortical level. When the binocular inputs are discordant, there is a need to eliminate or adjust the signals so as to prevent diplopia or confusion. Depending on the degree of discordance, input from one

eye can be suppressed (dichoptic masking) or the two retinal images will alternate (binocular rivalry) (Schor, 1977). It is unclear if these two processes are mechanistically distinct, particularly beyond the level of V1, but both are likely relevant for the range of retinal disparity seen in anisometropic and strabismic patients with amblyopia. In fact, the depth of suppression can vary depending on the type of amblyopia: weaker suppression is seen in anisometropic amblyopia (similar to dichoptic masking) compared to strabismic amblyopia (similar to rivalry suppression) (Harrad, Sengpiel and Blakemore, 1996). Moreover, patients with strabismus lacking suppressive mechanisms frequently experience diplopia - this is particularly true when strabismus occurs later in adult life due to paralytic causes. Neural correlates of binocular suppression induced either by incongruities present in the retinal images in patients with amblyopia or created artificially in normal individuals have been found in LGN, V1, and V2 (Sengpiel and Blakemore, 1996; Sengpiel, Blakemore and Harrad, 1995).

The anatomical substrate for silencing retinal input from one eye in favor of another could be independent of amblyopia; however, in the case of amblyopia, suppression or rivalry may be invoked on a more sustained basis, potentially leading to a less reversible rewiring of normal circuitry. Since suppression is common across normal and amblyopic individuals when confronted with different images in the two eyes, we propose that suppression itself is not a circuit abnormality; rather, the stimulus leading to aberrant suppression should be corrected. This is consistent with treatments to correct either the anisometropia or eye misalignment in patients with amblyopia. An alternative hypothesis is that some pathological adaptation to mismatched visual input must be overcome to fully correct abnormal cortical binocular interactions, in which case the time-course and extensiveness of correction could depend on age and plasticity of the brain. Therefore, the development of appropriate treatments will diverge depending on the cortical correlate of amblyopic suppression when compared to normal vision or across types of amblyopia.

Questions regarding the degree of suppression found across the visual field are particularly relevant to amblyopia. Suppression itself can vary dramatically depending on the stimulus used (Joosse, Simonsz, Spekreijse *et al.*, 1997, 2000), and suppressive mechanisms could vary depending on the degree or type of amblyopia. Because the size of receptive fields and the degree of acuity loss in amblyopia differ across visual space, the mechanisms of suppression and the sensitivity to retinal disparities, and thus the suppressive response, could be very different depending on whether stimuli are foveal or peripheral (Sireteanu and Fronius, 1981, 1989). For example, many corrected patients with strabismus retain a small angle strabismus that could recruit high levels of local suppression, perhaps most dramatically in the fovea. Physiological recordings from animal models are typically biased away from the fovea due to the difficulty of recording foveal responses, and the lack of a fovea in some animal models. Therefore, physiological data relevant to the implications of small foveal disparities are limited. Correlates of foveal suppression should be investigated further.

Many studies on binocular suppression have focused on neural correlates in V1; however, it is unclear the extent to which downstream visual areas contribute to suppression either in normal or amblyopic vision. Strabismic amblyopic macaques are found to have increased binocular suppressive interactions in both V1 and V2; the V2 result may be established as a feedforward consequence or may be qualitatively different, but the degree of change is similar in the two areas (Bi, Zhang, Tao *et al.*, 2011). In general, it is unknown whether amblyopia represents a feedforward dominance of the fellow eye or the feedback selection of the dominant eye's input via a top-down attentional mechanism

that originates in extrastriate cortex. However, the representation of the amblyopic eye feeding forward is clearly compromised (Bi, Zhang, Tao *et al.*, 2011; Shooner, Hallum, Kumbhani *et al.*, 2015) and likely contributes to abnormal binocular interactions. While the contribution of extrastriate areas to amblyopia is likely complex (discussed below), knowing the higher order cortical correlates of rivalry or suppression and their time-courses of development could be illuminating in understanding whether suppression of the amblyopic eye input is driven by low or high level processing.

Binocular Vision – Stereopsis

One of the major benefits of correlated binocular input is stereoscopic depth perception, which is based on disparities between the locations of objects on the two retinae. Both absolute and relative disparities are important for depth perception, but the ability to code for relative disparity is essential for stereoacuity, which may be severely impaired in amblyopic individuals. While neural correlates of absolute disparity have been recorded in V1, relative disparity seems to be encoded elsewhere (Cumming and Parker, 1999; Parker and Cumming, 2001). V2, MT/V5 and V4 have all been shown to exhibit neural correlates of relative disparity and thereby stereoscopic depth perception (Krug and Parker, 2011; Thomas, Cumming and Parker, 2002; Umeda, Tanabe and Fujita, 2007). However, it has been suggested in humans that absolute disparity is encoded by the dorsal stream, while the ventral stream is the source of neural coding for relative disparity (Neri, Bridge and Heeger, 2004). While studies have been done on relative disparity tuning in V1 and V2 of strabismic monkeys (Mori, Matsuura, Zhang *et al.*, 2002; Nakatsuka, Zhang, Watanabe *et al.*, 2007), to date no recordings have been done in amblyopic primates performing relative depth tasks. This type of data could be central to understanding where in visual cortex stereoacuity deficits are most pronounced. Alternatively, because the loss and recovery of stereoacuity in human patients with amblyopia is not fully understood and may be different depending on the disparity range in question (Giaschi, Lo, Narasimhan *et al.*, 2013), locating a brain region of interest, especially in an animal model, could be clouded by insufficiencies in characterization of the deficits themselves. In addition, human anisometropic patients with amblyopia recover stereoacuity more readily than their strabismic counterparts (Astle, McGraw and Webb, 2011; Ding and Levi, 2011; Levi, Knill and Bavelier, 2015; Wallace, Lazar, Melia *et al.*, 2011), so it is important to consider both populations in future studies. It could perhaps be more beneficial to study the recovery of stereoacuity in human patients with amblyopia using techniques such as high density electroencephalography (EEG) (Cottetereau, McKee, Ales *et al.*, 2012) or functional MRI (fMRI) to obtain a more complete picture of the origin of the deficits and changes that take place during recovery.

An underexplored area of research in regard to the disruption of stereoacuity in patients with amblyopia involves the circuitry associated with vergence, which likely has both sensory and motor contributions. Because vergence is the scaffold for stereopsis, a loss in cortical binocular combination could result in a disconnect between sensory and motor circuits that serve fusion. Data from nonhuman primate models suggest that indeed there is a relative independence of sensory and motor fusion, but that stereo and vergence anomalies exist at both coarse and fine levels of disparity (Fredenburg and Harwerth, 2001; Harwerth, Smith, Crawford *et al.*, 1997). However, accommodative vergence is essentially normal in strabismus and amblyopia, despite disrupted disparity vergence, suggesting that some motor aspects of vergence remain functional (Kelly, Feliuss, Ramachandran *et al.*, 2016; Kenyon, Ciuffreda and Stark, 1980, 1981). It is an open question whether the loss of disparity vergence has differential importance for the recovery of fine or coarse stereoacuity, and whether it contributes differentially to

coding disparities across retinotopic space in amblyopic individuals. Considerable work has been done in the past on the circuitry underlying vergence in animal models other than primates (Hughes, 1972; Stryker and Blakemore, 1972; Zuidam and Collewijn, 1979). However, it is unclear how relevant studies in mammals lacking a true fovea will be to understanding the circuitry behind and the deficits in vergence in amblyopic primates; more work is needed to draw a comparison across species. The interplay between motor and visual circuitry in amblyopia emphasizes the importance of studies beyond V1 and perhaps an emphasis on whole brain mapping, which can be best achieved using high resolution EEG and fMRI methods.

Extrastriate Cortex

The majority of studies of neural loss in amblyopia have been directed at striate cortex, V1. Here, in addition to the reduced binocularity discussed above, neurons driven by the amblyopic eye show reduced acuity (Kiorpes, Kiper, O'Keefe *et al.*, 1998) and contrast sensitivity (Movshon, Eggers, Gizzi *et al.*, 1987), but otherwise relatively normal receptive field properties. Studies of extrastriate areas are motivated by the findings that the losses in V1 sensitivity are not sufficient to explain the behaviorally measured deficits (Kiorpes, Kiper, O'Keefe *et al.*, 1998; Shooner, Hallum, Kumbhani *et al.*, 2015) and that patients and animal models show high order functional vision deficits, including deficits in motion perception, which persist in some cases after “successful” treatment with patching (Giaschi, Chapman, Meier *et al.*, 2015; Grant and Moseley, 2011; Lerner, Pianka, Azmon *et al.*, 2003; Levi, Yu, Kuai *et al.*, 2007; Rislove, Hall, Stavros *et al.*, 2010); see Hamm, Black, Dai *et al.*, (2015) for recent review of higher order deficits. The search for the correlates of these losses should avoid the simplistic notion of pairing a visual behavior with an anatomical brain region, and instead focus on pinpointing the location of a breakdown in information transmission along a processing stream that is essential to a visual behavior.

Extrastriate Cortex – Additional Deficits Beyond V1

Neural recording beyond V1 in animal models, especially nonhuman primates, have found an amplification of losses seen in V1, as well as qualitatively different abnormalities. Amblyopic V2 shows abnormalities of receptive field structure and orientation tuning that are not seen in V1 (Bi, Zhang, Tao *et al.*, 2011; Tao, Zhang, Shen *et al.*, 2014). Deficiencies at the level of V2 correlate strongly with those seen behaviorally in the same animals. Ocular dominance imbalance is amplified in V2 and MT/V5 compared with V1 (Bi, Zhang, Tao *et al.*, 2011; El-Shamayleh, Kiorpes, Kohn *et al.*, 2010). Functional losses in motion sensitivity are reflected in population models of MT processing, although not consistently at the single unit level (El-Shamayleh, Kiorpes, Kohn *et al.*, 2010). These and other recent studies (Shooner, Hallum, Kumbhani *et al.*, 2015) highlight the need to understand the neural output at each stage, including interneuronal interactions and correlations, to fully appreciate the quality of the information feeding forward from the amblyopic eye. Functional imaging studies have also made important contributions to understanding at what levels of the visual hierarchy correlates of functional losses might be found. For example, population receptive fields measured by fMRI can be analyzed for both their size and position to address questions regarding amblyopic losses in resolution or topological precision between visual areas (Clavagnier, Dumoulin and Hess, 2015). These methods will contribute substantially to the understanding of neural deficits beyond V1 and how these deficits are fed forward or backward along processing streams.

Extrastriate Cortex – Hierarchical Critical Periods

Across sensory systems, both sensory behaviors and associated neural structures exhibit distinct periods of maturation, suggesting a differential level of plasticity across the brain during certain periods of life (Hensch, 2005). Symptoms of amblyopia are not fully explained by V1 deficits, suggesting that circuit abnormalities could be found outside of V1. One hypothesis is that a cascade of development, where downstream (extrastriate) areas do not mature until upstream input has matured (V1), would leave higher-level visual behaviors differentially vulnerable to amblyopia and to inadvertent treatment effects.

High order deficits may persist in treated patients with amblyopia perhaps because treatment is focused on low-level functions, specifically monocular acuity. If extrastriate areas are actually more plastic than V1, due to longer or later critical periods, treatment focusing on V1 functionality could overly impact high order visual behaviors. For example, patching an eye for long enough to effect a change in V1 could produce novel deprivation amblyopia in a higher order cortical area. On the other hand, since the identified deficits in neuronal acuity at the level of V1 do not account fully for the behavioral losses or predict higher-order losses, treating acuity alone is unlikely to affect the degree of higher order deficits. Again, it is important to understand the differential contribution of feedforward and feedback causality in amblyopia, as well as the nature of the developmental hierarchy, to determine which points in the cortical stream of information should be the focus of treatment for full recovery.

What is the best way to measure sequential cortical maturation? Functional MRI data have been helpful in understanding neural correlates of amblyopia, but these data represent a very coarse scale of analysis and are difficult to obtain in young children. Multiunit physiological recording across brain areas, ages, and behavioral tasks is technically quite challenging and has not been attempted. A useful technique for understanding brain maturation as well as changes related to amblyopia is high density EEG (Cottareau, McKee, Ales *et al.*, 2012), which can be implemented in a non-invasive and spatially broad way to track the development of many brain areas across age. EEG recording can also be made in other species, and in correlation with psychophysics the results can be compared with direct neural measurements.

Open Questions and Recommendations

- The role of oculomotor abnormalities in the assessment of behaviorally measured visual losses remains an open question. Retinal image motion from unsteady fixation does not contribute significantly to poor contrast sensitivity of patients with amblyopia (Higgins, Daugman and Mansfield, 1982), but it does appear that acuity losses can be explained to at least some degree by fixation instability (Chung, Kumar, Li *et al.*, 2015). Furthermore, oculomotor deficits contribute to abnormalities and inaccuracies in visually-guided reaching and other visuomotor behaviors (Grant and Moseley, 2011; Niechwiej-Szwedo, Goltz, Chandrakumar *et al.*, 2011, 2014), and these deficits are not accounted for by the reduced visual acuity of amblyopic eyes (Niechwiej-Szwedo, Kennedy, Colpa *et al.*, 2012; Niechwiej-Szwedo, Chin, Wolfe *et al.*, 2016). It will be important for future studies to determine the role of oculomotor abnormalities in stereoscopic deficits and disparity vergence errors as well as losses in visual sensitivity.

- The disruption of neural mechanisms related to suppression and balanced ocular selection are not well understood. Many psychophysical studies have described these deficits but little is known of the physiological bases. It will be important for future studies to combine awake physiological recordings with behavioral assays of these important binocular functions. In addition, temporal aspects of binocular interaction are understudied. Reports of longer latency for amblyopic eye signals to reach the cortex (McKee, Levi, Schor *et al.*, 2016; Niechwiej Szwed, Goltz, Chandrakumar *et al.*, 2014) suggest that eye selection could favor the earliest arriving signals, triggering suppression of the amblyopic eye. Alternatively, eye selection could be influenced by top-down signals feeding back from downstream extrastriate areas or disrupted attentional mechanisms (Hou, Kim, Lai *et al.*, 2016; Montero, 1999). However, some recent work shows intact attentional resources in patients with amblyopia (Kiorpes, Pham and Carrasco, 2013; Roberts, Cymerman, Smith *et al.*, 2016), although attention problems have been found in children with amblyogenic refractive errors (Atkinson, Anker, Nardini *et al.*, 2002; see Chapter 2). Studies directed at discriminating these alternatives are urgently needed.
- The majority of psychophysical and clinical studies of amblyopia are conducted under free viewing conditions, with the assumption that the retinal area of highest sensitivity – typically the fovea – is directed at the target. In the case of anomalous retinal correspondence (ARC), this could be a locus other than the fovea. Little is known about visual sensitivity at non foveal loci, or whether suppressive mechanisms respond differently to conflicting signals at foveal vs. peripheral loci. Moreover, most neurophysiology to date reflects parafoveal rather than foveal neuronal properties; Shooner, Hallum, Kumbhani *et al.*, (2015) is an exception. Future studies should include evaluation of function at multiple areas of the visual field and neurophysiological investigations should include evaluation of foveal neuronal properties.
- On a related point, much current, as well as past, research on neural mechanisms of amblyopia are conducted in species lacking a fovea, often with deprivation as the model. It is at present unclear what the relationship is to the effects of amblyopia, more typically strabismic or anisometropic amblyopia, in primates. Comparative studies are needed to understand the relevance of circuit anomalies found in afoveate species following deprivation and neural correlates of amblyopia in primates. Nonhuman primates should remain the animal model of choice given the shared evolutionary history with humans, and similarity in cortical organization and nature of amblyopic deficits.
- Amblyopic individuals are now known to have many visual deficits beyond visual acuity, which is the metric assessed clinically and monitored during standard treatment. The additional deficits include binocular as well as monocular losses, high-order perceptual losses as well as threshold elevation, fellow eye deficits, and abnormalities of visuomotor control. Many of these deficits persist despite successful treatment of acuity with patching or other methods. This raises the question of whether the treatment of amblyopia should move beyond patching, or include more “global” therapies with the aim of improving high-level as well as low-level visual function.
- The field would benefit from increased application of assessment tools such as whole-brain fMRI and high-density EEG methods to address open questions regarding the mechanisms of

amblyopia development and recovery during treatment. To understand the progression of development, as a hierarchical or holistic process, and the relationship between the organization of feed-forward and feedback projections and critical periods for amblyopia, as well as critical brain areas involved in recovery during treatment, these coarser-scale tools have the potential to provide valuable information to move the field forward.

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Chapter 6

Animal Models of Amblyopia

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Introduction

Unquestionably, the last six decades of research on various animal models have advanced our understanding of the mechanisms that underlie the many complex characteristics of amblyopia as well as provided promising new avenues for treatment. While animal models in general have served an important purpose, there nonetheless remain questions regarding the efficacy of particular models considering differences across animal species, especially when the goal is to provide the foundations for human interventions. Our discussion of these issues culminated in three recommendations for future research to provide cohesion across animals models as well as a fourth recommendation for acceptance of a protocol for the minimum number of steps necessary for translation of results obtained on particular animal models to human clinical trials. The three recommendations for future research arose from discussions of various issues including the specific results obtained from use of different animal models, the degree of similarity to the human visual system, the ability to generate animal models of the different types of human amblyopia as well as the difficulty of scaling developmental timelines between different species.

In considering animal models of all human diseases, including developmental disorders such as amblyopia, there is a concern as to whether experiential or other manipulations imposed in early postnatal life on animals having a normal genetic background can adequately mimic the human situation where there is a possible genetic contribution to the experiential abnormality. For example, in human amblyopia there is a potential genetic contribution to anisometropia, strabismus and media opacities that are the experiential abnormalities associated with the common subtypes of amblyopia and their presumed cause. Although research performed with animal models cannot at present mimic a possible genetic susceptibility for these amblyogenic factors in certain patients with amblyopia, researchers are aware that experiential manipulations of the early visual input alone in animal models may not precipitate the entire set of molecular, anatomical and physiological events that occur in all human patients with amblyopia.

The four specific recommendations we make emerged from a wide-ranging discussion of the value of the various commonly employed animal models for amblyopia from rodents to non-human primates (NHP). The obvious advantages of NHP (such as monkeys) that possess similarly organized visual pathways and vision to humans (such as a fovea, smooth pursuit eye movements, excellent spatial vision including stereoscopic vision, semi-decussated visual pathways and multiple visual cortical areas) are offset by many considerations that have motivated the choice of alternative models. Among

the barriers to widespread use of NHP have been their long gestation time, small litter size, and the protracted length of key critical periods in visual system development. Added to these barriers are the attendant regulatory requirements and costs associated with establishment and maintenance of a primate breeding colony required to produce infant animals of known ages. Discussion of the value of various animal models and models of strabismic amblyopia in particular, prompted debate among participants on the importance of a fovea or a region of central retinal specialization with respect to the ability of detection of strabismus or eccentric fixation. In passing it was noted that although carnivores such as cats do not possess a rod-free fovea they do have a central region of retinal specialization with a high cone density, the area centralis. The decline in resolution with eccentricity in cats has been documented by both behavioral techniques (Berkeley, Kitterle and Watkins, 1975; Pasternak and Horn, 1991) and from electrophysiological recordings of the spatial resolution of retinal ganglion cells (Clelan, Harding and Tulunay-Keesey, 1979). Both measurements reveal a regular decline from a central peak but with a more gradual slope than observed in humans. The four recommendations are discussed below in turn.

Recommendation 1.

Documentation of the Perceptual Performance Space of Present and Putative Animal Models

Consensus on the behavioral and perceptual repertoire of each species will identify the most appropriate features of amblyopia to address with particular animal models. Furthermore, discovery of similarities in experimental results regarding the performance spaces of two or more species would promote efforts to establish the extent of consensus across results from use of these species as models of various forms of amblyopia.

Because of the complexity and diversity of amblyopia in humans, as well as species differences in the organization of the retina and central visual pathways, it is not surprising that certain animal models do not allow a perfect recapitulation of the clinical symptomology. Primate models exhibit remarkably similar behavioral deficits to those observed in the various forms of human amblyopia so that their cortical correlates are of particular interest for an understanding of their underlying cause. By contrast, the ability to replicate certain forms of amblyopia such as strabismic or anisometropic amblyopia is limited or impossible in other species. Cats and particularly rodents show a declining similarity to humans in their manifestation of deprivation amblyopia. Deprivation amblyopia is the only form of amblyopia that can be modeled in all species, but the reduction in acuity upon monocular deprivation is dramatically different across species. While rodent models exhibit a single octave reduction in grating acuity, macaques remain effectively blind in the deprived eye after this manipulation (Harwerth, Smith, Boltz *et al.*, 1983). Kittens also appear blind immediately after monocular deprivation (MD) but show some recovery afterward (Giffin and Mitchell, 1978; Mitchell, 1988). Participants discussed, but without reaching consensus, whether the discordant magnitude of the effects of MD reflected fundamental anatomical or physiological dissimilarities in the organization of visual pathways across species, or else arose from species differences in the plasticity of neural circuitry.

As an offset to considerations based solely on the inability for adequate modeling of all forms of amblyopia and their poor vision, rodents by far possess the highest throughput and flexibility of experimentation, which has allowed visual circuits to be probed down to the cellular and molecular level in ways that are unfeasible for other species and particularly NHP models. The power of these

two contrasting arguments with respect to the use of rodents versus NHP suggests that it would be imprudent to discard any given species on the basis of an individual shortcoming but to use each animal model based on its strengths.

Discussion of the value of various animal models revealed substantial gaps in our knowledge of the perceptual performance of many species and especially rodents. The lack of knowledge of specific visual thresholds became evident in discussion of the role of particular anatomical or physiological features or the magnitude of the perceptual deficits on different measures of spatial resolution such as grating acuity, Snellen acuity and the various hyperacuties. Another example arose on contemplation of the role of binocular cells in the visual cortex of rats and mice. Although the recent observation (Schol, Burge and Priebe, 2013) that cells in the binocular zone of the visual cortex of mice were tuned to retinal disparity, albeit in a crude fashion with respect to that observed in the cat, discussion of their possible functional role was hampered by the absence of a clear demonstration of stereoscopic vision in mice. A number of studies of depth perception have been made on rats that test either their ability to jump across a gap or their performance on a visual cliff (Howard, 2002). Because these studies did not compare monocular with binocular performance or control for the use of motion cues such as motion parallax, the possible contribution of stereoscopic vision was unclear. Nonetheless, a recent study (Baroncelli, Braschi and Maffei, 2013) that tested rats on a graded series of depth differences between the two sides of a visual cliff apparatus provided evidence of superior performance with use of both eyes suggestive of the presence of stereopsis. However, the possible use of motion parallax could not be ruled out. Innovative new tests of the depth perception of rats and mice that target the specific use of retinal disparity cues are needed to establish whether they possess true stereoscopic vision.

Table 6.1 lists the results of existing measurements of various visual thresholds, the methods of assessment as well as the presence or absence of particular key anatomical and physiological features in the central visual pathways for the various common animal models for amblyopia. As with clinical screening for amblyopia in humans and to assess progress during and following treatment, assessment of visual performance in animal models is most commonly made in terms of the effects on visual acuity. The depth of amblyopia in humans is graded according to the specific acuity measure employed with the severity of the deficit increasing from grating acuity to letter acuity to the various hyperacuties such as vernier acuity. Grating acuity has been measured in all the commonly employed species including mice and spans a range of six octaves from 0.5 cycles per degree in mice (Prusky and Douglas, 2003) to 30 cycles per degree in macaques, or 100-fold with respect to humans. The vast discrepancies of grating acuity across species reflect fundamental differences in retinal anatomy that include the lack of a fovea in species other than primates, or variation in the extent of central retinal specialization with respect to cone density. Vernier acuity, which is likely a more accurate reflection of behavioral amblyopic deficits, has not yet been measured in mice, but has been measured in rats (Seymour and Juraska, 1997).

Table 6. 1.

The perceptual performance space for the different species that are commonly employed as animal models of amblyopia and the assessment methods employed. Also shown are the presence or absence of various anatomical and physiological features in the central visual pathways.

Anatomical and physiological features

	Macaque	Marmoset	Cat	Rat	Mouse
Ocular dominance columns	+	±	+	-	-
Visual processing streams	+	+	+	?	+ [1]
Disparity selectivity	+	+	+	?	+ [2]
Physiological and anatomical MD effects	Severe shrinkage of OD columns, drastic OD shift	Appearance of deprived-eye columns, drastic OD shift [3]	Severe shrinkage of OD columns, drastic OD shift	Moderate OD shift	Moderate OD shift (reduction in OD index by ~0.3)
Cortical Suppression	+ [4, 5]	?	+ [6, 7]	+ [8]	?

Perceptual performance space

	Macaque	Marmoset	Cat	Rat	Mouse
Stereopsis	++	+	+	+? [9]	?
First-order (grating) acuity	30 c/deg	>10 c/deg	6 c/deg	1 c/deg [10]	0.5 c/deg [11]
Second-order (Vernier) acuity	10-12 sec arc [12]	?	1.2 min arc [13]	34 min [14]	?
Contrast sensitivity	~100 at 5 c/deg [15]	?	~100 at 0.3 c/deg [16]	~30 at 0.05 c/deg [17]	C57BL/6 mice 6%, [18]
MD effects on visual function	Fct blindness	?	Fct blindness	1 octave reduction in acuity	1 octave reduction in acuity

Assessment methods

	Macaque	Marmoset	Cat	Rat	Mouse
Single- or multi-unit recording	+	+	+	+	+
VEP / EEG recording *	+	+	+	+	+
Intrinsic signal imaging	+	+	+	+	+
Two-photon imaging	(+)	+	+	+	++
Neuro-anatomical markers (IEG expression)	+	(+)	+	+	+
Genetic modification	-	+ [19]	-	+	++
Behavioural tests of acuity	+	+	+	+	+

* Note that this is the only technique that is also widely used in humans

References for specific data entered in Table 6.1 are as follows: 1. Wang *et al.*, 2012; 2. Scholl *et al.*, 2013; 3. Sengpiel *et al.*, 1996; 4. Smith *et al.*, 1997; 5. Sengpiel and Blakemore, 1996; 6. Chino *et al.*, 1994; 7. Sengpiel *et al.*, 1994; 8. Pietrasanta *et al.*, 2014; 9. Barancelli *et al.*, 2013; 10. Prusky *et al.*, 2000; 11. Prusky *et al.*, 2003; 12. Kiorpes, 1992; 13. Murphy and Mitchell, 1999; 14. Seymoure and Juraska, 1997; 15. De Valois *et al.*, 1974; 16. Bisti and Maffei, 1974; 17. Birch and Jacobs, 1979; 18. Yeritsyan *et al.*, 2012; 19. Sasaki *et al.*, 2009.

Not only is acuity substantially lower in typically reared rodents compared to primates, but the reduction in acuity upon monocular deprivation is dramatically different across species. While rodent models exhibit a single octave reduction in grating acuity, macaques remain effectively blind in the deprived eye after this manipulation (Harwerth, Smith, Boltz *et al.*, 1983). Kittens also appear blind immediately after MD but show some recovery afterward (Giffin and Mitchell, 1978; Mitchell, 1988). Participants discussed but without reaching consensus, whether the discordant magnitude of the effects of MD reflected fundamental anatomical or physiological dissimilarities in the organization of visual pathways across species, or else arose from species differences in the plasticity of neural circuitry.

In connection with the discussion of the effects of MD some participants questioned whether this manipulation was the most appropriate way to model deprivation amblyopia as a way to mimic the development of cataracts. And, as debated in other Targeted Sessions, amblyopia is at its root a binocular condition so that the ability to probe the status of binocular vision across different species including tests of stereopsis and suppression is important. While mice exhibit neural correlates of

disparity selectivity in visual cortex (Scholl, Burge and Priebe, 2013), it is not known whether they possess stereopsis and if so whether it is affected by experiential manipulations such as MD.

Recommendation 2.

Comparative Measurements of Key Perceptual Abilities Across Species by Use of the Same or Very Similar Techniques

While the expansion of technologies in physiology, imaging, and molecular biology has allowed neural circuits to be probed at unprecedented resolution, many of these techniques are not equally applicable across species and especially not to humans. Mice in particular offer high throughput molecular characterization of visual circuits and changes to them during development and in response to experiential manipulation. On the other hand, NHP such as macaques easily outperform rodents on visual tasks that accurately reflect human amblyopic deficits. Comparison of the perceptual abilities on various visual tasks has been made difficult by differences in the techniques employed across species. Rather than an exclusive focus on identification of the techniques best suited for each individual species, participants thought it would be valuable to propose a common technique that could be deployed with ease across species including humans. Discussion focused quickly upon the use of electrophysiological measures and particularly the use of various types of visual evoked potentials (VEP) and, in particular, upon the steady-state VEP, or the SSVEP (Norcia, Applebaum, Ales *et al.*, 2015). A VEP corresponds to electrical changes in large populations of neurons in the cortex, and can be recorded from the surface of the brain non-invasively using electroencephalography (EEG). High-density EEG recordings can be used to improve source localization of this common technique and are applicable across species, including humans. By use of a fan of electrode contacts placed strategically over the scalp it is possible to obtain a high yield analysis of visual responses across different visual areas. A particular form of SSVEP, the sweep VEP that measures the changes in response to a stimulus that is swept (varied) over a range of values is used widely to measure various visual thresholds. For assessment of acuity or contrast sensitivity the SSVEP is measured in response to parametric sweeps of gratings of different spatial frequency or contrast. The method is fast and has been used on human infants and on various species including NHP (Nakayama & Mackeben, 1982) and rodents under light anesthesia (Guire, Lickey and Gordon, 1999; Xu, Tian, Zhang *et al.*, 2015).

On the basis of this discussion, participants proposed that use of the SSVEP would be the most efficient way to assess visual thresholds across species and in the various animal models of amblyopia. In addition to measurement of key visual thresholds across species by use of the SSVEP, the suggestion was made that certain methods of non-invasive imaging could also be used to assess the functional integrity of the visual cortex and possibly other visual areas in the various animal models of amblyopia.

Recommendation 3.

Advocacy for the Use of Marmosets as an Animal Model of Amblyopia

Despite the value of rodents and carnivores such as cats as animal models of amblyopia, there are issues for which there is arguably no alternative to the use of NHPs. In addition to the need for their use to refine the optimum timing and the dosage for projected interventions in human patients with amblyopia, as a species with a highly developed fovea, NHP may provide the only valid choice to model strabismic amblyopia. The presence of a similar organization of higher visual cortical areas to that

observed in humans and which are likewise specialized for processing complex visual stimuli, attracts use of non-human primates for study of the underlying neural basis for the deficits of various higher visual functions observed in amblyopia. Macaque monkeys are easily trained to make very repeatable behavioral observations and the documented perceptual deficits associated with early amblyogenic manipulations are remarkably similar to those demonstrated by human patients with amblyopia.

In addition to the case that can be made for the use of NHP to model human amblyopia, it is very important to recognize their participation in translation of the results from animal studies to clinical trials. These issues are raised in the discussion of Recommendation 4 that follows.

Most of the earliest studies of the functional anatomy and physiology of the visual cortex and its development, including investigations of the consequences of early periods of selected visual deprivation for anatomy, physiology and behavior, were conducted on macaque monkeys. As such, there exists a large body of data documenting the close similarity of spatial vision, oculomotor characteristics as well as organization of higher visual cortical areas between macaques and humans, which makes the choice of the former the ideal primate animal model. Unfortunately, there are considerable practical barriers to their use. Macaques are expensive to purchase and to house. There are regulatory barriers as well as vociferous resistance to their use from the public and media expressing growing ethical concerns. Many of these issues are exacerbated upon consideration of their use as animal models of amblyopia where experimental manipulations must be made in infancy. The long gestation (5.5 months), the unitary litter size, the length of critical period of vulnerability to monocular deprivation (> 1 yr) together with the need for experimental interventions near birth, in most cases requires the existence of an on-site breeding colony. The recent closure of the New England Primate Center and the continual pressures from diverse sources directed against remaining macaque colonies (in North America as well as in Europe) indicate that the ability to initiate a new macaque breeding colony would be close to impossible. As many of the same barriers to use of macaque monkeys apply to familiar New World monkey species, participants at the Targeted Session Group discussed the potential use of marmosets as a primate model of amblyopia. This discussion benefited from the insight provided by Dr. Sam Solomon (University College, London) who has conducted a number of investigations of the central visual pathways of marmosets in recent years in collaboration with Dr. Marcello Rosa and others at Monash University (Melbourne, Australia).

Detailed information relevant to the breeding and housing of marmosets is provided in a multi-center review (Tardiff, Smucny, Abbott *et al.*, 2003). The arguments for their use in visual neuroscience (Solomon and Rosa, 2014; Mitchell and Leopold, 2015) and in particular as a model for amblyopia are worth serious consideration. Marmosets possess a fovea with a peak cone density of 200,000 cones/mm² similar to that observed in macaque monkeys and humans. While the volume of the marmoset brain is approximately 12 times smaller than that of macaques, the cerebral cortex is relatively smooth so that the vast majority of the visual cortex lies exposed and not buried in sulci. Importantly, V2 as well as the third tier visual also lie exposed on the cerebral surface. Important as these features of the marmoset cortex are for investigations of the neural basis for vision, they are more than matched by the short gestation time, large litter size and the comparative ease of housing. The fact that they can be housed socially in groups of 5 or 6 reduces the costs of colonization. In a multi-colony database of 3,714 marmosets, the litter size ranged from 1-4, with twins as the most common but more than a third were triplets or quadruplets (see Table 2 from Tardiff, Smucny, Abbott *et al.*, 2003). The gestation

time of 143 days is shorter than that for macaques (164 days) but longer than that for cats (63 days). Although data as yet are somewhat conflicting, ocular dominance columns in V1 of marmosets, like other New World monkeys, may be transitory or even variable across animals (Roe, Fritsches and Pettigrew, 2005) but have been shown to exhibit experience-dependent change in response to MD (Sengpiel, Troilo, Kind *et al.*, 1996; Fonta, Chappert and Imbert, 2000) or enucleation (Ribic, Flugg, Schlumbohm *et al.*, 2011).

A major additional argument for the use of marmosets beyond the development of a new non-human primate model for amblyopia is the potential for genetic modification (Sasaki, Suemizu, Shimada *et al.*, 2009). As summarized in a recent Nature News item, our Recommendation is reinforced by the launch of a Brain/MINDS project in Japan to study cognition and cognitive disorders in marmoset models (Cyranoski, 2014). Higher visual functions including contrast sensitivity or vernier acuity have not yet been experimentally investigated in the marmoset, but a physiological substrate for stereopsis has been documented (Table 6.1). Our recommendation for the increased use of marmosets as a NHP model for amblyopia should not be taken to mean that they replace completely the well-established macaque model as the latter holds a number of advantages over marmosets for application of particular techniques. For example, the larger body size of macaques allows for both much longer daily behavioral measurements as well as longer awake behavioral recording experiments. Moreover, their longevity makes them invaluable for extended behavioral studies that also serve in part to mitigate against their high initial cost.

Recommendation 4.

A Balanced Suggestion for Replication of a Laboratory Finding Made on One Animal Model to a “Higher” Species as a Necessary Step to a Clinical Trial: a “Two-Species Rule”

For the most part, the use of animal models in the study of amblyopia should have a clear goal of applying the findings of such studies eventually to clinical practice. In some cases, this would mean the development of a therapy, either behavioral or pharmacological, that could be tested in clinical trials. It is therefore prudent to understand how to most efficiently and safely transition between the world of animal models and human patients.

No species matches the efficiency (both cost and time) or the genetic toolbox available to probe the visual system for new approaches to treatments as the mouse. Unfortunately, the relative simplicity of their visual system is the least comparable to humans, although recent studies have found underappreciated complexities in the mouse visual pathway (e.g., Scholl, Burge and Priebe, 2013) as well as sophisticated perceptual abilities such as the perception of motion coherence (Douglas, Neve, Quittenbaum *et al.*, 2006). They also exhibit qualitative differences in visual plasticity compared to other models such as cats and primates. For example, mice living in environmentally enriched conditions display ocular dominance plasticity well beyond the classical critical period (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014), a finding not replicated in other mammals. Mice also exhibit a form of plasticity in adulthood that is not observed in cats or NHP (Satwell, Frenkel, Philpot *et al.*, 2003). In addition, strategies shown to be successful in one species may be less so in another. For example, reverse occlusion but not binocular visual exposure is quite effective in promoting recovery when combined with various approaches to enhance plasticity in rats (He, Ray, Dennis *et al.*, 2007), while cats benefit from binocular visual experience (Mitchell, Cynader and Movshon, 1977; Mitchell, 1988; Mitchell, Gingras and

Kind 2001). Dark exposure is effective in promoting recovery from long-term monocular deprivation in adult rats (He, Ray, Dennis *et al.*, 2007), but is only effective in juvenile cats (Duffy and Mitchell, 2013; Holman, Duffy and Mitchell, 2014; Duffy, Lingley, Holman *et al.*, 2016). Varied responses across species are also observed in response to pharmacological treatments. For example, enzymatic digestion of perineuronal nets with chondroitinase restores ocular dominance plasticity in adult rats (Pizzorusso, Medini, Landi *et al.*, 2006) but has much less of a beneficial effect in cats (Vorobyov, Kwok, Fawcett *et al.*, 2013); Fluoxetine promoted recovery from MD in rats (Maya-Vetencourt, Sale, Viegi *et al.*, 2008) but according to a Press Release (<http://www.evaluategroup.com/Universal/View.aspx?type=Story&id=453937>) failed to show an enhancement of results above that achieved by video training in a recent Phase II human clinical trial.

As researchers we are torn between two extreme viewpoints with respect to the steps to a clinical trial. At one extreme is the view that a result from a single species provides sufficient evidence to mount a Proof of Principle clinical trial. Of course, there are variations of this viewpoint depending on the specific species employed; results obtained from NHP would likely meet with wider acceptance than data from other species. The opposing and more conservative viewpoint is that it is necessary for confirmation of the initial result in at least one other species, with a NHP serving as the second species in the extreme interpretation of such a “two-species” rule.

The strongest argument for the acceptance of results from a single species as sufficient to mount a clinical trial is to accelerate the time between laboratory discovery and a clinical trial. However, such hasty action may jeopardize the clinical trial because of failure to optimize the treatment parameters, the patient population or the timing of the intervention. Because a negative finding on a clinical trial subtracts from the ability to conduct trials in the future, it is important that the urge to “fast-track” a laboratory finding to the clinic be tempered by a careful evaluation of the various parameters of the intervention. Failure to optimize the treatment parameters such as dosage on a clinical trial has the potential to jeopardize conduct of future trials of a different and potentially very effective dosage of the same treatment. Moreover, a negative clinical trial based on results from a single species also diminishes the ability in the future to argue for the relevance of that particular species as a viable model of amblyopia. It is also important to recognize that failure of a clinical trial provides ammunition for activist groups opposed to the use of all animal models. As it was perceived that treatment parameters on a clinical trial based upon results obtained from species other than NHP’s would need to be tweaked by data obtained from a second species, participants vacillated between various versions of the alternative viewpoint.

The viewpoint that discoveries from rodent or cat animal models be replicated first on NHP’s prior to a clinical trial inevitably introduces delay and reduces the number of possible treatments that could be explored. The suggestion of a two species “rule” with the caveat that two rodent species would probably not qualify, was considered a useful practical compromise as it increased substantially the ability to test numerous treatments. Upon progression to a cat model of a potential treatment based on findings in a rodent, a negative finding would make it unnecessary to proceed to tests on a NHP. An understanding of the efficacy and timing of manipulations in at least two species can elucidate how likely these treatments are to translate to humans. This consideration is made pointedly in consideration of pharmacological interventions in children. Because of the risk of off-target effects of pharmacological treatments, especially when prescribed to children with other brain regions likely undergoing sequential

critical periods, special care should be taken to understand the efficacy of these drugs across species prior to clinical trials, not only for safety, but also to ensure translation of the optimum timing and dosage from animal models to humans. An example of a “two-species” examination of the use of binocular retinal silencing with intra-vitreous injection of tetrodotoxin (TTX) as a replacement for darkness to treat the consequences of a prior early period of monocular deprivation has recently been published (Fong, Mitchell, Duffy *et al.*, 2016). The study was conducted in three different laboratories on two species (mouse and cat) and reported very similar outcomes and thus replication of the results in both species.

Fuelling the debate on this Recommendation were considerations of two main issues that related to the problem of translation of dosage and timing of interventions from animals to humans. The difficulty of testing dosage of treatments was emphasized by the recent report of the somewhat ambivalent preliminary findings of a clinical trial on the use of a re-purposed drug, the SSRI Citalopram, that was reported at the March 2016 meeting by Ben Thompson. The ability to employ a FDA-approved drug has many benefits, not the least being the ability to fast-track a clinical trial. On the other hand, because the prior approved use of the drug was for administration to adults, the dosage applied to children or young adults in the amblyopia trial was deliberately conservative. This raised the issue of how to interpret the results of the trial, since it is possible that the drug was ineffective at the dosage applied. It also pointed to the many difficulties associated with the establishment of dosage levels. With respect to translation of dosage levels from adults to children guidance may be sought from the calculations made for the effective dosage levels of drugs, such as cancer drugs, that are commonly used on humans from children to adults where multiple considerations including weight, allometric and metabolic measures are applied.

A related concern is the issue of how to translate developmental and susceptibility timelines across species to humans so that the timing of treatment would be optimal. During this discussion it was pointed out that, for example, the common 4:1 multiple applied to translate developmental timelines from macaques to humans applies only to resolution acuity and does not apply to relative eye growth where the ratio is 3:1. Development of an equivalent ratio between species for stereoacuity is hampered by the existence of too many isolated studies of monkeys on small numbers and the use of very different stereo measures. For example, only a single study exists (O’Dell and Boothe, 1997) of the time of emergence of stereopsis in macaques. Extrapolation of the developmental timeline of the cat to humans is complicated by the delay in eye opening as well as the slow disappearance of the hyaloid artery around the crystalline lens in cats.

Conclusions

In contrast to the comparative sparsity of animal models of many other neurological clinical disorders, study of the basis of amblyopia and pursuit of new avenues for its treatment are guided by a rich variety of animal models that employ a common set of experiential manipulations on divergent species. Our Recommendations for the application of Animal Models focused on the use of a monocular deprivation as the most common experiential manipulation, that has been employed widely with graded success across species from rodents to NHPs. The use of a common manipulation has aided comparison of the results across species but also permitted identification of gaps in our knowledge of the perceptual abilities of certain species including rodents and marmosets.

- Two of our Recommendations (1 & 2) suggest particular gaps in knowledge that need to be filled and also common methods of assessment that could be applied across species as an aid to comparison of perceptual performance. Sometimes assumptions are made about the perceptual abilities of a particular species without hard evidence. A case in point is the assumption that rodents do not possess stereopsis in the face of a lack of tangible evidence of their ability to employ stereoscopic vision. Tangible data on this issue would assist discussion of the role of binocular neurons in the rodent visual cortex. On the basis of the demonstration of large independent eye movements in freely moving rats, it has been suggested (Wallace, Greenberg, Sawinski *et al.*, 2013) that the main purpose of binocular neurons may be to ensure a large panoramic visual field above them to escape predation from raptors.
- Our third Recommendation for increased use of marmosets as a NHP species was suggested as a means to speed the path to clinical trials in situations where information from NHPs was deemed essential.
- Our final Recommendation could be deemed a principle or strategy to guide the path from results obtained from an animal model to a clinical trial. For translation to a clinical trial, participants advocated the principle of a “two-species replication” with the suggestion that the two species not both be rodents. Participants thought that a wider adoption of marmosets as models of amblyopia may lead eventually to the principle that one of the two species be a NHP.

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Chapter 7

Amblyopia: New Molecular/Pharmacological and Environmental Approaches

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Introduction

Emerging technologies are now giving us unprecedented access to manipulate brain circuits, shedding new light on treatments for amblyopia. This research is identifying key circuit elements that control brain plasticity and highlight potential therapeutic targets to promote rewiring in the visual system during and beyond early life. Here, we explore how such recent advancements may guide future pharmacological, genetic, and behavioral approaches to treat amblyopia. We will discuss how animal research, which allows us to probe and tap into the underlying circuit and synaptic mechanisms, should best be used to guide therapeutic strategies. Uncovering cellular and molecular pathways that can be safely targeted to promote recovery may pave the way for effective new amblyopia treatments across the lifespan.

New Molecular/Pharmacological and Genetic Approaches

Novel experimental approaches in neuroscience have recently identified promising molecular, pharmacological, and genetic avenues for amblyopia therapy. The common goal of these therapies is to harness the brain's inherent ability to restructure itself by tapping into specific brain circuits and cellular mechanisms that promote plasticity. Such targets include, for example, excitatory and inhibitory synaptic components, neuromodulators, and epigenetic regulators. Commonly used pharmacological agents, transcranial direct current and magnetic stimulation (tDCS/TMS), and behavioral therapies may act through these or other cellular pathways yet to be discovered. Understanding the precise cellular mechanisms that promote circuit changes in experimental animals promises to guide new therapeutic approaches for treating amblyopia in humans.

Targeting Inhibitory and Excitatory Synapses: Monocular visual deprivation during a critical period in early life remodels excitatory synapses extensively, inducing a rapid loss of dendritic spines and elimination of many axonal branches of geniculocortical afferents serving the deprived eye (Antonini and Stryker, 1993; Mataga, Mizaguchi and Hensch, 2004). These losses of input are followed by a progressive expansion of axons and potentiation of responses from the open eye (Antonini, Fagiolini and Stryker, 1999; Frenkel and Bear, 2004). Because these modifications are assumed to underlie development of amblyopia, excitatory synapses represent strong candidate targets for its treatment. Indeed, recent reports have revealed that changes in the levels of the excitatory postsynaptic density protein PSD-95 governs the duration of the critical period for ocular dominance plasticity in the

visual cortex, independent of changes in inhibitory circuits (Huang, Stodieck, Goetze *et al.*, 2015). PSD-95 expression increases in the visual cortex during the critical period for ocular dominance (OD) plasticity and promotes the progressive maturation of so-called “silent” synapses that contain only NMDA-type glutamate receptors and that lack AMPA receptors. Genetic loss of PSD-95 function leads to the persistence of silent synapses, allowing the juvenile form of OD plasticity to be maintained lifelong. Strikingly, using a viral gene-silencing approach to reduce PSD-95 in the visual cortex of adult mice rejuvenates excitatory synapses by reinstating silent synapses like those in immature cortex, and reopens a critical period for visual cortical plasticity (Huang, Stodieck, Goetze *et al.*, 2015).

Converging studies also point to intracortical inhibitory synapses as key regulatory sites of critical period plasticity (reviewed in Takesian and Hensch, 2013; see also Chapter 3). Reducing inhibitory synapse function by intracortical microperfusion of a GABA synthesis inhibitor or GABA_A receptor antagonist can enhance plasticity in rodent visual cortex during adulthood (Harauzov, Spolidoro, DiCristo *et al.*, 2010). However, this manipulation does not produce plasticity like that in the critical period, where responses in the deprived eye are dramatically reduced. Instead, it accelerates or enhances the adult form of plasticity seen in rodents, which increases the response to the open fellow eye with little or no effect on the deprived-eye responses. In contrast, transplantation of specific types of embryonic inhibitory neurons into postnatal visual cortex creates a second critical period of OD plasticity that follows the end of the normal one and is of similar duration (Southwell, Froemke, Alvarez-Buylla *et al.*, 2010; Tang, Stryker, Alvarez-Buylla *et al.*, 2014). The most prominent feature of this second critical period is the reduction of deprived-eye responses, exactly as in the normal critical period. Future work is needed to elucidate how the interplay of excitatory and inhibitory synaptic function across cortical cell types may control cortical network plasticity.

Targeting Neuromodulatory Systems: Evidence has accumulated that neuromodulatory systems are also key targets for inducing plasticity to improve amblyopia. Neuromodulators such as serotonin and acetylcholine are released in the visual cortex from projections arising from the raphe nuclei and basal forebrain. These inputs are normally activated by salient stimuli and specific behavioral states, such as reward acquisition, punishment, and exercise (Fu, Tucciarone, Espinosa *et al.*, 2014; Hangya, Ranade, Lorenc *et al.*, 2015). However, these neuromodulatory systems can also be pharmacologically targeted by drugs commonly used to treat depression, such as selective serotonin reuptake inhibitors (SSRIs), or Alzheimer’s disease, such as cholinesterase inhibitors. Interestingly, it has been found that these pharmacological agents promote recovery from amblyopia in rodent models. For example, chronic treatment with SSRIs to enhance serotonergic signaling reopens a period of plasticity in the visual cortex of adult amblyopic rats, allowing for recovery of visual acuity (Maya Vetencourt, Sale, Viegi *et al.*, 2008). Likewise, boosting acetylcholine signaling with a cholinesterase inhibitor enables recovery from amblyopia in the adult visual cortex (Morishita, Miwa, Heintz *et al.*, 2010). How do neuromodulators act within visual cortical circuits? Recent studies have uncovered a specific set of cortical inhibitory neurons that respond robustly to neuromodulators to enhance cortical plasticity (Letzkus, Wolff, Meyer *et al.*, 2011; Pi, Hangya, Kvitsiani *et al.*, 2013; Fu, Tucciarone, Espinosa *et al.*, 2014; Fu, Kaneko, Tang *et al.*, 2015). These GABAergic cells reside in the outermost layers of the cortex and are identified by the selective expression of vasoactive intestinal peptide (VIP). Optogenetic activation of VIP cells directly drives plasticity in the primary visual cortex of the adult mouse (Fu, Kaneko, Tang *et al.*, 2015). VIP cells are thought to augment cortical activity and plasticity through inhibition of other cortical GABAergic interneurons (Letzkus, Wolff, Meyer *et al.*, 2011; Pfeffer, Xue,

He *et al.*, 2013; Pi, Hangya, Kvitsiani *et al.*, 2013; Donato, Rompani, Caroni, 2013; Fu, Tucciarone, Espinosa *et al.*, 2014; Fu, Kaneko, Tang *et al.*, 2015). Other cell types in visual cortex may also have a role in plasticity induced by neuromodulators in adult mice. Pairing acetylcholine release in the visual cortex with specific visual stimuli enhances stimulus-selective responses of cortical neurons, by engaging astrocyte-dependent strengthening of excitatory synapses (Chen, Sugihara, Sharma *et al.*, 2012).

Ongoing clinical trials with drugs targeting these neuromodulatory systems highlight this approach as a promising avenue for amblyopia treatment in adult patient populations. SSRI treatment has been shown to augment visually-evoked potentials (VEPs) in normal human subjects (Normann, Schitz, Fürmaier *et al.*, 2007). In some adult patients with amblyopia, an SSRI (citalopram) enhanced visual acuity improvements when combined with two weeks of occlusion therapy (Lagas, Black, Stinear *et al.*, 2014). However, another study pairing SSRIs with video game training demonstrated no added value of the SSRI treatment (Uusitalo, 2013). It is possible that such behavioral and pharmacological manipulations reach a ceiling effect if they engage similar neuromodulatory pathways. Likewise, an ongoing clinical study at Boston Children's Hospital is using donepezil, a cholinesterase inhibitor that is typically used to treat Alzheimer's disease, to boost cholinergic signaling and recover vision in amblyopic patients (T. Hensch, personal communication).

Targeting Epigenetic Regulation Using Histone Deacetylase (HDAC) Inhibitors: Brain circuits respond to environmental signals via dynamic changes in DNA methylation and histone modifications that control gene transcription (Fagiolini, Jensen, Champagne, 2009). Visual stimulation during early life induces histone acetylation in the mouse visual cortex, but the same stimulation in adulthood has little effect. This age-related decline in the capacity for experience-dependent regulation of histone acetylation makes it a candidate to underlie the developmental reduction in visual cortical plasticity (Putignano, Lonetti, Cancedda *et al.*, 2007). In fact, increasing histone acetylation by inhibition of HDACs can reinstate plasticity in the adult visual cortex of rodents to allow recovery from amblyopia (Putignano, Lonetti, Cancedda *et al.*, 2007; Silingardi, Scali, Belluomini *et al.*, 2010). Thus, HDAC inhibitors may represent yet another class of drugs with the potential to improve visual acuity beyond early life.

The HDAC inhibitor valproate (VPA) has already been found in a clinical study to reopen a period of plasticity to learn absolute pitch (Gervain, Vines, Chen *et al.*, 2013). Absolute pitch, the ability to produce or identify a musical pitch without a reference sound, is possessed by only about 0.01% of the general population and acquired during a critical period in early life. Generally, absolute pitch is learned through musical training before 6 years of age, and is rarely, or perhaps never, acquired during adulthood (Van Hedger, Heald, Koch *et al.*, 2015). However, administration of VPA, a commonly used mood stabilizer, opened a window of opportunity for adults to learn absolute pitch. These findings suggest that epigenetic actions of VPA may re-set cortical circuitry to allow for juvenile-like plasticity. Future work will be required to reveal the cellular and circuit mechanisms underlying HDAC inhibitors such as VPA.

Transcranial Direct Current Stimulation (tDCS)/Transcranial Magnetic Stimulation (TMS): Pharmacological interventions aimed to stimulate cortical plasticity pose risks of side effects. Therefore, there is a great deal of interest in discovering novel and less invasive alternatives to activate endogenous

plasticity mechanisms. One promising strategy is to use transcranial direct current or magnetic stimulation, non-invasive brain stimulation techniques that can transiently alter neural excitability in targeted brain regions. Ongoing work is attempting to exploit this technique in adult patients with amblyopia to open a brief window of opportunity to improve visual function (B. Thompson, personal communication). A recent study found that a single session of tDCS can temporarily increase visual evoked potentials (VEPs) and contrast sensitivity driven by amblyopic eyes of adult patients (Ding, Li, Spiegel *et al.*, 2016), paving the way for future studies that will combine this stimulation technique with visual training for long-term improvements. Intriguingly, tDCS may increase excitability by a reduction in GABAergic inhibition (Stagg, Best, Stephenson *et al.*, 2009), a mechanism known to regulate adult visual cortical plasticity. It should be mentioned, however, that these current/magnetic stimulation protocols may have a problem of pathway specificity. It is therefore essential that measures be taken to ensure that changes are restricted to target circuits.

Combining Pharmacological Interventions & Behavioral Training: Across both human and animal studies, it is evident that pharmacological intervention alone is not generally sufficient for successful treatment of amblyopia. Instead, the research strongly supports the need to combine pharmacological approach with personalized behavioral training, with the goal of targeting plasticity within specific brain regions or specific cortical circuits. Interestingly, VPA treatment improved absolute pitch, but not other measures of auditory function (T. Hensch, personal communication), suggesting that VPA may not induce widespread effects, but instead focal plasticity in response to targeted training paradigms. In the mouse visual system, recovery of closed-eye responses following long-term monocular deprivation is preferentially enhanced to the particular visual stimuli presented during VIP cell activation induced by running (Kaneko and Stryker, 2014). Can these pharmacological approaches be used to shorten or enhance behavioral treatment? The next section will discuss promising behavioral treatments that could be used alone or in combination with pharmacological approaches.

Environmental and Behavioral Treatments

In an attempt to find novel non-invasive methods of stimulating visual plasticity in adulthood, a number of behavioral interventions have emerged that may help to treat amblyopia in humans. These include manipulations of the environment, such as exposure to an enriched environment or complete visual deprivation, both of which reactivate robust plasticity in the visual cortex. Similarly, engaging humans and animals in voluntary physical exercise (e.g., running wheels) and visuomotor tasks has led to remarkable increases in neuronal plasticity. Finally, novel vision training paradigms may result in more practical, less expensive therapies, and faster recovery. Here, we highlight some of these novel treatments, and discuss their success in both animals and humans.

Environmental Enrichment (EE) / Running. It is now evident that increased levels of environmental stimulation have a profound impact on experience-dependent plasticity within both the developing and adult brain. Experimental animals are generally raised in small cages with only their littermates, strongly limiting social interactions and physical exercise. Recent studies have found significant effects of environmentally-enriched cages in which the animals are housed in large groups, have access to running wheels and are exposed to a complex environment that elicits social and exploratory behaviors. Notably, adult amblyopic rats housed in enriched environments recovered from long-term monocular deprivation (Sale, Maya Vetencourt, Medini *et al.*, 2007). Moreover, raising mice in an enriched

environment extended the critical period for a juvenile form of OD plasticity into adulthood (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014), and allowed an adult form of OD plasticity to persist even throughout life (Greifzu, Kalogeraki, Löwel, 2016). Remarkably, placing standard-cage-reared mice into an enriched environment as adults restored OD plasticity, apparently rejuvenating the visual cortex (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014; Greifzu, Kalogeraki, Löwel, 2016). These effects of EE on plasticity seem largely to be due to the reduction of GABAergic inhibition to juvenile levels (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014) accompanied by decreased peri-neuronal nets in the visual cortex (Sale, Maya Vetencourt, Medini *et al.*, 2007). These studies highlight EE as a non-invasive means of harnessing known plasticity mechanisms (reduced intracortical inhibition) to promote visual recovery. Surprisingly, use of just one of the components of EE has recently been shown to preserve plasticity to older ages, namely a running wheel allows adult mice raised in standard cages to express OD-plasticity into adulthood (Kalogeraki, Greifzu, Haack *et al.*, 2014). Furthermore, even short-term running, just during the 7-day monocular deprivation period restored OD-plasticity to adult standard cage raised mice. Notably, it is important to distinguish the extension into adult life or the enhancement of the effects of deprivation (e.g., MD) from the enhancement of recovery of visual function because the underlying mechanisms may be different (see Chapter 3 for further discussion).

However, the human environment is generally much more ‘enriched’ than that of any experimental animal, raising the question of whether EE in rodents may be translated into a treatment protocol for humans. Social interactions, novelty, exercise, and engagement of the visuomotor systems are all components of EE that may engage distinct brain regions, circuits, and cellular mechanisms. Researchers using animal models or human subjects should strive towards identifying the key aspects of an EE that can be implemented to promote plasticity.

Dark Exposure. It has long been known that dark rearing from birth retains the visual cortex in an immature state and prolongs the critical period for ocular dominance plasticity (e.g., Cynader, 1983; Mower, Caplan, Christen *et al.*, 1985; Fox, Daw, Sato *et al.*, 1991). Recent work has shown that periods of total darkness that eliminate all visually-driven activity can reactivate robust plasticity in the adult visual cortex of rats (He, Hodos and Quinlan, 2006; He, Ray, Dennis *et al.*, 2007), mice (Stodieck, Greifzu, Goetze *et al.*, 2014) and in juvenile kittens (Duffy and Mitchell, 2013). The ability to promote synaptic plasticity in the adult visual cortex through dark exposure was predicted by the BCM sliding threshold theory of synaptic plasticity, which predicts that the loss of patterned visual experience would lower the value of the synaptic modification threshold and enable recovery of weakened deprived-eye inputs (Cooper and Bear, 2012). Indeed, dark exposure in adulthood stimulates the expression of the NMDA receptor a molecular switch known to lower the threshold for synaptic modification (Yashiro, Corlew and Philpot, 2005; He, Hodos and Quinlan, 2006; Philpot, Cho and Bear, 2007).

The plasticity that is reactivated by dark exposure can be harnessed to promote the recovery from amblyopia. For example, rats rendered amblyopic by chronic monocular deprivation initiated at eye opening recovered visual acuity when 10 days of dark exposure in young adulthood (P70-100) were followed by binocular vision or reverse occlusion (He, Ray, Dennis *et al.*, 2007). Remarkably, short-term dark exposure (10 days) can reinstate visual cortical plasticity even in very old mice (P535; Stodieck, Greifzu, Goetze *et al.*, 2014). However, the effects of dark exposure on adult plasticity may vary across species; while 10 days of darkness enhanced OD plasticity in juvenile kittens, this treatment failed to restore OD plasticity in adult cats (Duffy, Lingley, Holman *et al.*, 2016). The recovery from

amblyopia following dark exposure was rapid, occurring in kittens within just a week after removal from the darkness (Duffy and Mitchell, 2013). Importantly, repetitive performance of a visual task following dark exposure further improved the recovery of acuity, but delaying the visual stimulation for several weeks following dark exposure prevented the recovery of visual acuity (Eaton, Sheehan, Quinlan *et al.*, 2016). This finding suggests that a period of darkness opens a limited window of plasticity during which the cortex is more receptive to visual training, and may guide design of new therapies.

It may be puzzling that manipulations that on the surface appear to be very different - putting animals either in complete darkness or in an enriched environment - both stimulate robust plasticity in the adult cortex. It is possible that distinct neural mechanisms lead to a common outcome, possibly through a common substrate. A reduction in inhibitory synaptic transmission by dark exposure may be a feature in common with EE. As with environmental enrichment (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014), dark exposure may work through a rejuvenation of intracortical inhibition, including a reduction of excitatory drive onto fast-spiking interneurons (Huang, Gu, Quinlan *et al.*, 2010; Gu, Tran, Murase *et al.*, 2016) and a decrease in the number of parvalbumin-expressing inhibitory cells and surrounding peri-neuronal nets (Stodieck, Greifzu, Goetze *et al.*, 2014). Indeed, the adult recovery from long-term monocular deprivation can be stimulated by a reduction in the activity of inhibitory neurons (Kaneko and Stryker 2014). Dark exposure also reduces neurofilament protein levels, which is hypothesized to destabilize the neuronal cytoskeleton (Duffy and Mitchell, 2013) and promotes the recovery of thalamocortical synaptic transmission and the density of dendritic spines on pyramidal neurons in the primary visual cortex (Montey and Quinlan, 2011).

An exciting pilot study is now evaluating whether adult amblyopic humans will also recover visual function following a brief period in complete darkness (B. Backus and E. Quinlan, personal communication). Preliminary results suggest that 5 days of darkness is well tolerated, with no reports of anxiety or changes in physical health. An ongoing study is now evaluating the effects of 10 days of darkness on adult amblyopic patients. To exploit the transient period of plasticity immediately following darkness, the patients will undergo intensive visual training in the weeks following the dark exposure. The practicality in humans as a treatment for amblyopia must be addressed, as studies in rodents and kittens suggested that shorter periods of dark exposure are ineffective, and even brief periods of light exposure prevented the effects (He, Ray, Dennis *et al.*, 2007; Mitchell, MacNeill, Crowder *et al.*, 2016). Thus, access to completely dark environments for a sufficient duration of time with proper support to guarantee safety and well-being may pose challenges for widespread use. Interestingly, visual deprivation for several days by binocular intravitreal injections of the pufferfish toxin tetrodotoxin promotes similar fast visual recovery in amblyopic cats (Fong, Mitchell, Duffy *et al.*, 2016), raising the possibility of developing novel pharmacological techniques to transiently block vision, if adequate safety measures can reliably be ensured. Blindfolding may offer another solution – one study suggested that blindfolding normally sighted adults for 5 days leads to rapid changes in experience-dependent functional neural connectivity (Merabet, Hamilton, Schlaug *et al.*, 2008). However, it has been shown in amblyopic kittens that binocular lid deprivation does not recapitulate the recovery-promoting effects of darkness (Duffy, Bukhamseen, Smithen *et al.*, 2015). If it is established that 10 days of complete darkness promotes visual recovery in amblyopic humans, future studies in both humans and animal models may identify methods to shorten, segment, or facilitate binocular occlusion. A further understanding of the neural mechanisms underlying dark exposure may allow us to predict which combination of treatments will induce robust visual cortical plasticity. Dark exposure offers a novel and promising non-invasive approach for the recovery of vision.

Exercise & Visuomotor Engagement. Recent research links physical activity to profound adult plasticity in the visual cortex, providing another promising behavioral intervention for recovery from amblyopia. Remarkably, allowing adult mice to run on a treadmill potently enhances visual cortical activity (Niell and Stryker, 2010) and promotes recovery of vision following monocular deprivation (Kaneko and Stryker, 2014). Moreover, visual stimulation or running alone did not improve visual function, suggesting that plasticity is facilitated only in activated neural circuits during running (Kaneko and Stryker, 2014). Recent studies have identified the key cellular mechanism underlying the effects of locomotion - VIP cells (Fu, Tucciarone, Espinosa *et al.*, 2014). In fact, genetic silencing of VIP cells in amblyopic adult mice prevents the recovery of visual function by running, suggesting that the activity of these cells is necessary for the enhanced plasticity (Fu, Kaneko, Tang *et al.*, 2015).

The potential of physical activity to promote amblyopic recovery has caught the attention of the clinical field. Adult subjects who intermittently cycled on a stationary bicycle while watching a movie showed enhanced effects of transient eye patching compared to those subjects who watched the movie while sitting still (Lunghi and Sale, 2015). Moreover, tasks that directly engage both visual and motor circuits have achieved great success in reversing amblyopia. For example, recovery from amblyopia is expedited by tasks requiring coordination of hand and eye movements, such as having patients manipulate objects during visual training (reviewed in Daw, 2013). Patients with amblyopia exhibit impairments in oculomotor performance, including saccadic eye movements (Niechwiej-Szwedo, Goltz, Chandrakumar *et al.*, 2010; Niechwiej-Szwedo, Chandrakumar, Goltz *et al.*, 2012; McKee, Levi, Schor *et al.*, 2016; Perdziak, Witkowska, Gryncewicz *et al.*, 2016), smooth pursuit (Raashid, Liu, Blakeman *et al.*, 2016), fixation stability (González, Wong, Niechwiej-Szwedo *et al.*, 2012; Chung, Kumar, Li *et al.*, 2015), hand-eye coordination (Niechwiej-Szwedo, Goltz, Chandrakumar *et al.*, 2011; 2014), and execution of grasping movements (Grant, Melmoth, Morgan *et al.*, 2007; Suttle, Melmoth, Finlay *et al.*, 2011); thus targeting visuomotor circuits during treatment may help to alleviate some of these deficits.

Novel Visual Training Procedures. There is no doubt that conventional patch therapy, directed toward improving the visual function of the amblyopic eye while occluding the fellow eye, is effective in the majority of cases if initiated at the appropriate age. However, it is not always effective, and it has additional limitations: patching interferes with binocular input, which can lead to poor binocular outcome, and it often has poor compliance (reviewed in Birch, 2012; Hess and Thompson, 2015). When patch therapy is ineffective, there is no consensus on effective treatment. Since the 1970s, a number of alternative procedures, many inspired by the animal research literature, have been proposed. None of these has attained the degree of acceptance that would make them a new standard of care. We note a number of them here not as an endorsement but to foster a critical examination of the relationship between laboratory research findings and approaches to therapy for human patients.

Some alternative strategies use binocular exposure approaches to treat amblyopia. For example, a set of training paradigms called “monocular fixation in a binocular field” (MFBF), in which both eyes remain open while a vision task is accomplished by a single eye, have been reported to have some success (Brock, 1963; Cohen, 1981). One MFBF procedure places a red filter over the dominant eye such that both eyes receive light, but only the amblyopic eye can see the markings of a red pen used to perform various tasks (reviewed in Daw, 2013). The Cambridge Vision Stimulator (CAM) treatment combined short-term occlusion, visual stimulation using contours of all orientations and

training exercises, although efficacy was not demonstrated in a randomized clinical trial (Campbell, Hess, Watson *et al.*, 1978). Indeed, there are many reports of successful results of active vision therapy with only minimal amounts of occlusion (reviewed in Garzia, 1987). Some reports suggest that this approach may be particularly successful in older patients; for example, a group of older children and adult patients with anisometropic amblyopia achieved long-lasting improvements in visual acuity and binocular function following a treatment that combined active vision therapy with occlusion of only 2-5 hours per day, although the role, if any, of active vision was not demonstrated using a control group (Wick, Wingard, Cotter *et al.*, 1992).

Video game therapy has emerged as a form of active vision training aimed at improving both acuity and stereopsis in both amblyopic children and adults (reviewed in Hess and Thompson, 2015; Levi, Knill, Bavelier, 2015). Anaglyphic video games were developed for vision therapy more than 30 years ago (Press, 1981) and used as an MFBF approach for the treatment of amblyopia (Ludlam, 1992). The clinical finding that suppression – a reduced contribution of the amblyopic eye during binocular viewing – is an important part of the amblyopia syndrome and a greater understanding of the biological underpinnings of amblyopia has spurred new dichoptic approaches to promote “binocular re-balancing.” These approaches may reactivate latent binocular pathways by reducing inhibitory interactions, boosting attenuated excitatory function, and/or shifting the synaptic modification threshold in favor of potentiation (reviewed in Hess and Thompson, 2015). For example, recent studies have found that both amblyopic preschool children and adults can show enhanced improvements in visual and motor function by playing dichoptic iPad or iPod games (Hess, Mansouri, Thompson, 2011; Birch, Li, Jost *et al.*, 2015; Vedamurthy, Nahum, Huang *et al.*, 2015; Webber, Wood, Thompson *et al.*, 2016). Since lack of binocular function is a key risk factor for persistent amblyopia, the development of new binocular training strategies may have a substantial impact on improving acuity and recovering stereopsis (reviewed in Levi, Knill, Bavelier, 2015). Unfortunately, recent randomized clinical trials have failed to provide evidence that video games are any better than patching in older children and or that they improve binocular function (Holmes, Manh, Lazar *et al.*, 2016; Kelly, Jost, Dao *et al.*, 2016). Future work will be required to elucidate how monocular and dichoptic experience contribute to amblyopia recovery across distinct patient populations.

Elaborate visual training regimes have been shown in some cases by one of us (Paul Harris) to improve vision in amblyopic humans but are expensive and time-intensive. They have not yet been adopted widely because of a lack of controlled trials that demonstrate both efficacy and safety. Therefore, there is a continued need to seek novel, faster training approaches. For example, a study in adult amblyopic macaques suggested that implementing a ‘global’ training paradigm may lead to visual improvements that generalize beyond the trained stimulus (Kiorpes and Mangal, 2015). Moreover, training strategies that engage attentional and emotional processes, including movies and action video games, appear in some reports to be particularly successful (reviewed in Levi and Li, 2009, reviewed in Bavelier, Levi, Li *et al.*, 2010). Such training is likely to stimulate neuromodulatory systems that promote plasticity and to activate circuits encoding higher-order visual functions that are impaired in patients with amblyopia (reviewed in Kiorpes, 2006).

Use of Animal Models to Find Treatments for Amblyopia

With the advent of new technologies in neuroscience, the field continues to rely heavily on animal models to uncover the neural mechanisms underlying human pathologies such as amblyopia. The mouse in particular has emerged as an ideal model for taking advantage of genetic, optogenetic, physiological, and imaging tools that allow experimenters to label, manipulate, and monitor specific cell types with great precision. Nevertheless, it is not yet clear whether rodent models are appropriate for the study of amblyopia and its treatment in humans.

One potential limitation of mice for the study of amblyopia is that the mouse does not have a fovea, and therefore the entire mouse retina resembles the peripheral retina of the primate (Naarendorf, Esdaille, Banden *et al.*, 2010). Although amblyopia was first described as a deficit in foveal vision, amblyopic deficits have also been detected in the visual field periphery (Irene Gottlob, personal communication). In fact, patients with amblyopia have been shown to exhibit decreased motion and contrast detection through the amblyopic eye (Katz, Levi and Bedell, 1984; Levi, Klein and Aitsebaomo, 1984). Thus, the absence of foveal vision may not exclude the mouse *a priori* as a model to study human amblyopia. Furthermore, the mouse primary visual cortex exhibits binocular integration and disparity selectivity that support depth perception (Scholl, Burge and Priebe, 2013), potentially allowing the use of mice to study the loss of stereoscopic vision associated with amblyopia.

Another major consideration when evaluating the effectiveness of potential amblyopia treatments in mice is that the common forms of human amblyopia are not generally studied in the mouse. Basic research on amblyopia in rodents has focused almost exclusively on monocular deprivation. However, human patients with amblyopia exhibit various types of amblyopia that are generally classified as anisometropic, strabismic and deprivation amblyopia. Deprivation amblyopia is the least common form amblyopia, accounting for less than 5 per cent of cases (Holmes and Clarke 2006). Can we get closer to the human condition? Experiments in monkeys have employed more subtle forms of deprivation such as anisometropia and strabismus, revealing reduced binocularity and poorer spatial resolution in V1 neurons driven by the amblyopic eye (Movshon, Eggers, Gizzi *et al.*, 1987; Kiorpes, Kiper, O'Keefe *et al.*, 1998). In mice, one possibility is to take advantage of the available genetic models to identify genetic ocular defects that better approach the common human forms of amblyopia (Engle, 2007). However, while exploring new experimental models of amblyopia, it is also important to take advantage of the tremendous progress over the past 50 years in our understanding of monocular deprivation in mice as a premier model for understanding critical periods for cortical plasticity and the underlying regulating factors.

Assessing Outcomes of Amblyopia Treatment

What is the best test to assess amblyopia and amblyopia recovery in experimental models? In animal models, amblyopia is generally assessed using grating acuity. Animals are presented with drifting gratings at increasing spatial frequencies to determine optomotor, behavioral or neural thresholds. Grating acuity is a strong measure of degraded visual function associated with amblyopia, particularly deprivation amblyopia, and corresponds to measures of Snellen (optotype) acuity in humans (Levi and Klein, 1982). Thus, grating acuity is an appropriate and relatively easy way to assess acuity in animals. However, it should be noted that grating and optotype acuity are not equivalent. This may be

particularly important for the detection of crowding effects, which are marked in amblyopia. Moreover, not all grating tasks reveal the same acuity. In particular, using the visual water task in rodents, a visual discrimination task based on reinforcement learning (Prusky and Douglas, 2004; Prusky, West and Douglas, 2000) allows measurement of good perceptual thresholds of visual acuity.

In addition to assessing acuity as a recovery measure, it will be important to extend our evaluation to other visual deficits associated with amblyopia. For example, the loss of stereoptic depth perception may have the greatest impact on the quality of life for the amblyopic patient, impairing ability on visuomotor tasks and limiting career options (Levi, Knill and Bavelier, 2015). Moreover, adults who lack stereopsis tend to be refractory to therapy. Individuals with amblyopia, particularly strabismus, also exhibit oculomotor deficits (McKee, Levi, Schor *et al.*, 2016), and other visuomotor deficits during the performance of fine motor tasks (Grant and Moseley, 2011). Thus, developing novel methods of measuring trajectories for recovery of binocular vision and visuomotor ability in both experimental animal models and humans may provide valuable insight into the success of future amblyopia treatments.

Finally, there is a need to evaluate the effectiveness of amblyopia treatments on higher brain regions. Deficits in vision function associated with amblyopia may result from changes in extrastriate regions instead of or as well as primary visual cortex (reviewed in Kiorpes and McKee, 1999). Few studies assess the extent of impairment or recovery outside of the primary visual cortex. Using less V1-centric metrics to assess recovery of function by tapping into the higher-order dorsal and ventral streams will provide further insights into novel treatments. Studies outside of purely visual regions also have the potential to illuminate deficits in unexpected pathways, such as visuomotor and cross-modal connections (for example, visual-tactile interactions; Niechwiej-Szwedo, Chin, Wolfe *et al.*, 2016). So far, the study of the neural underpinnings of amblyopia and the molecular factors that promote recovery from amblyopia has been largely restricted to V1. Future work that determines whether other brain regions show critical periods and plasticity mechanisms that are similar to those observed in V1 will better inform us how and when to treat amblyopia.

Toward Identifying Common Pathways to Recovery

Novel molecular, pharmacological, and behavioral treatments are emerging to harness the brain's plasticity mechanisms for the recovery from amblyopia (see Figure 7.1). Ongoing challenges will be to determine how the various behavioral manipulations engage plasticity factors, and how these plasticity factors interact within the cortical networks to promote the desired re-wiring. A number of distinct candidate plasticity factors have been identified and ongoing work will address whether these factors operate independently or as components of a common mechanism. For example, exercise and video games may engage neuromodulatory systems to activate specific types of cortical inhibitory interneurons known to control plasticity. Identifying “hub” circuits, cells, or molecular mechanisms that promote visual plasticity promises to lead to better targeted treatment strategies that should mitigate side effects and accelerate recovery from amblyopia in human patients.

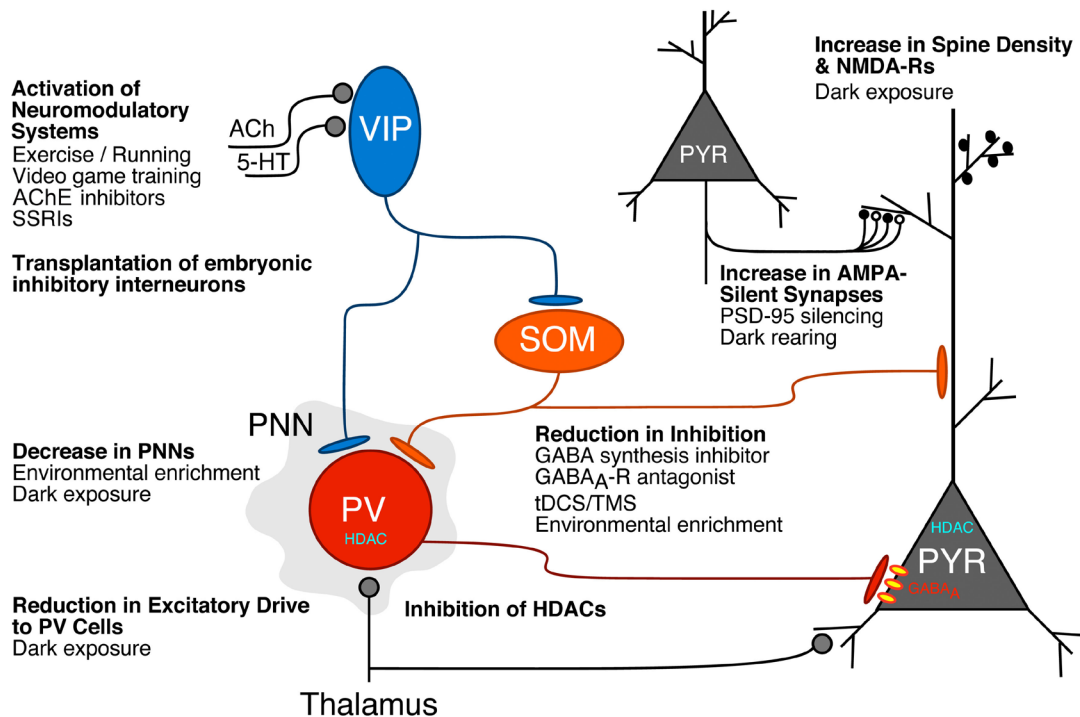


Figure 7.1. Cellular and circuit mechanisms underlying molecular, pharmacological, and environmental approaches to increase plasticity in the visual cortex.

Release of **neuromodulators** in the visual cortex, such as acetylcholine (ACh) or serotonin (5-HT=5-hydroxytryptophan), activate VIP (vasoactive intestinal polypeptide) inhibitory cells that promote cortical activity and plasticity by inhibiting other inhibitory interneuron subtypes, PV (parvalbumin) and SOM (somatostatin) cells (Letzkus, Wolff, Meyer *et al.*, 2011; Pi, Hangya, Kvitsiani *et al.*, 2013; Kaneko and Stryker, 2014; Fu, Tucciarone, Espinosa *et al.*, 2014; Fu, Kaneko, Tang *et al.*, 2015). These neuromodulatory systems can be activated by pharmacological treatments such as cholinesterase (AChE) inhibitors (Morishita, Miwa, Heintz *et al.*, 2010) or selective serotonin reuptake inhibitors (SSRIs; Maya Vetencourt, Sale, Viegi *et al.*, 2008; Lagas, Black, Stinear *et al.*, 2014) or behavioral therapies such as exercise (Fu, Kaneko, Tang *et al.*, 2015; Lunghi and Sale, 2015) and video game training (Bavelier, Levi, Li *et al.*, 2015). **Transplantation of embryonic inhibitory neurons** into postnatal visual cortex induces a second critical period of ocular dominance plasticity after the normal one (Southwell, Froemke, Alvarez-Buylla *et al.*, 2010; Tang, Stryker, Alvarez-Buylla *et al.*, 2014; Isstas, Teichert, Bolz *et al.*, 2017). Plasticity is also enhanced in the adult visual cortex by **decreasing perineuronal nets** (PNNs) that predominantly enwrap PV cells by pharmacological (Pizzorusso, Medini, Berardi *et al.*, 2002) or behavioral interventions, such as environmental enrichment (Sale, Maya Vetencourt, Medini *et al.*, 2007) or dark exposure (Stodieck, Greifzu, Goetze *et al.*, 2014). A **reduction in excitatory drive to PV cells** (Huang, Gu, Quinlan *et al.*, 2010; Gu, Tran, Murase *et al.*, 2016) and an **increase in spine density and NMDA-Rs** (Yashiro, Corlew, Philpot, 2005; He, Hodos, Quinlan, 2006; Philpot, Cho, Bear, 2007; Montey and Quinlan, 2011) may also contribute to the enhanced plasticity that occurs with dark exposure. Various manipulations that **reduce inhibitory synaptic function** have been found to enhance visual cortical plasticity, including drugs that inhibit GABA synthesis or GABA_A receptors (Harauzov, Spolidoro, DiCristo *et al.*, 2010), tDCS/TMS (Stagg, Best, Stepherson *et al.*, 2009) and environmental enrichment (Greifzu, Pielecka-Fortuna, Kalogeraki *et al.*, 2014). **Inhibition of HDACs** can also reinstate plasticity in the adult visual cortex to allow recovery from amblyopia (Putignano, Lonetti, Cancedda *et al.*, 2007; Silingardi, Scali, Belluomini *et al.*, 2010). Finally, an **increase in AMPA-silent synapses** (white synaptic boutons) underlies the heightened plasticity following knock-out or virus mediated gene silencing of PSD-95 (Huang, Stodieck, Goetze *et al.*, 2015). Silent synapses also persist into the adult visual cortex in dark-reared mice (Funahashi, Maruyama, Yoshimura *et al.*, 2013). Figure modified from one presented by Takao Hensch at the Lasker/IRRF Initiative on Amblyopia workshops, Woods Hole, MA, July/August, 2015.

Recommendations

- The emergence of novel genetic, imaging, and electrophysiological tools to probe circuit and cellular mechanisms is shedding light on new targets to promote plasticity in visual circuits. Pharmacological agents, transcranial direct current or magnetic stimulation (tDCS/TMS), and behavioral therapies may harness these plasticity mechanisms for amblyopia treatment. A focus on understanding how such treatments engage precise circuit, cellular, and molecular mechanisms will provide insight into focused strategies to promote recovery.
- Novel visual training paradigms that exploit our increased understanding of the biological underpinnings of amblyopia recovery are needed. Future work should continue to seek training strategies that are tailored to the individual patient to engage attentional, emotional, and visuomotor circuits for faster and more effective recovery.
- The use of animal models such as mice to study treatments for human amblyopia presents numerous challenges because of differences in both visual function and visual cortical circuits between species. Furthermore, mouse research has been largely confined to studying deprivation amblyopia, the least common form of human amblyopia. New experimental procedures mimicking the more common forms of human amblyopia in mice and other animal models would enhance progress for the treatment of amblyopia in humans. Nevertheless, knowledge gained over the past 50 years of animal research, with mice as the premier model of the last decade, should continue to inform our understanding of critical periods and their underlying cellular and molecular mechanisms.
- Grating acuity is a reliable measure of amblyopia that can be readily assessed in both animal models and humans. However, developing novel methods of measuring trajectories for recovery of other visual functions, including binocular vision and visuomotor ability, is likely to improve the success of amblyopia treatments in humans. Moreover, future work should be directed at determining whether brain regions outside of the primary visual cortex can additionally be targeted to promote recovery from amblyopia.

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Concluding Remarks

John Dowling

All agree that amblyopia is a disorder that affects visual structures beyond the eye. Many say simply that amblyopia is a brain disorder. But the retina is part of the brain, pushed out into the eye during development. As someone who has long studied the retina, I wonder if the retina is at all affected. At first glance, retinal function appears normal in amblyopia, but is it totally unaltered? I am not convinced, but as yet nothing of significance has been shown in this regard. The reasons I suggest this are three-fold: first, there are centrifugal fibers from higher visual structures that innervate the retina and alterations in higher visual pathways could very well affect the retina. Second, as noted many times in this report, there are many visual deficits that occur in amblyopia – it is not just a defect in visual acuity but in contrast sensitivity, accommodation, fixation, binocularity and so forth. Third, some visual alterations are seen in the other eye in unilateral amblyopia and suggest to me that a closer examination of retinal function in amblyopia might be useful to undertake.

I am not suggesting that retinal changes are the major alterations in amblyopia; clearly, the major effects of amblyopia are manifest in the cortex. But where in the cortex? Beginning in area V1, but certainly in higher visual areas as well and even, probably, in other non-visual areas. The bottom line is that amblyopia is a very complex disorder consisting of several different forms, each of which is expressed somewhat differently. Today, throughout the world, we are focusing enormous effort on studies of brain structure and function, and many of the major issues regarding how the brain functions are front and center in amblyopia research. In other words, findings in amblyopia research are instructive in terms of understanding brain function and vice-versa.

A prime example is that of brain plasticity – how hard-wired are our brains? Our views on this have changed dramatically over the past half-century, beginning with the pioneering studies of Wiesel and Hubel on monocular visual deprivation in cats and monkeys. Dramatic changes in structure and function occur in area V1 of the cortex (as shown schematically in the drawings on the cover to this volume), often after just a relatively short period of deprivation. And as was learned from the clinic, the changes in amblyopia occur most dramatically in the young, during the so-called critical period. It was also recognized in the clinic that if recovery was to be achieved, it happened most readily by interventions in the critical period. All of the above is certainly correct, but what has changed is our understanding of critical periods - when the nervous system is modifiable. Whereas it was once viewed that the critical period was finite, we now recognize that it is not. Brain plasticity can occur all of our lives, although as we grow older it does decline. As described in several of the chapters in this volume, critical periods can be extended and even reopened by various manipulations, by the administration of drugs and other neuroactive substances, by brain stimulation and even by environmental and behavioral treatments. Further, we now recognize that various visual phenomena have different critical periods in terms of timing. All of this research is beginning to have clinical impact. It was believed that recovery from amblyopia in humans could not happen in adulthood; however, this view has now softened and various approaches to this possibility are being explored. Clearly, there is great variability in the response of older individuals to these therapies, so we still have much to learn.

That our brain circuitry can be altered throughout our lives by neurons growing new dendrites and forming new synapses, research pioneered by visual deprivation studies, has impact far beyond amblyopia. Indeed, our view today is that the brain is continually changing by everything we do and experience. A paradigm shift in our thinking, it has immense implications on understanding how the brain develops, how it ages and on how we treat various brain disorders and degenerative diseases. We hope this report will be useful not only for those studying amblyopia but for brain science in general.

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Appendix 4 – Abbreviations

AMPA - α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid	GABA _A	MD – monocular deprivation
ARC – anomalous retinal correspondence		MFBF - monocular fixation in a binocular field
BMI – binocular motion integration		MT – region of the visual cortex (also called V5)
BPEDS – Baltimore Pediatric Eye Study		MEPEDS - Multi-ethnic Pediatric Eye Disease Study Group
CAM – Cambridge Vision Stimulator		NHP – non-human primates
CS – contrast sensitivity		NMDA – N-methyl-D-aspartate
EE – environmental enrichment		GABA _A
EEG – electroencephalography		OD – ocular dominance
FDA – US Food and Drug Administration		PSD-95 – excitatory post-synaptic density protein
fMRI – functional magnetic resonance imaging		PEDIG - The Pediatric Eye Disease Investigator Group
GABA – gamma-aminobutyric acid		PVS – pediatric vision scanner
HDAC – histone deacetylase inhibitor		RCTs – randomized clinical trials
HOTV - Pediatric eye chart in which only four letters are offered, and a child is allowed to match the letters with a card held in the lap		SSRI – selective serotonin reuptake inhibitor
LEA – chart designed with symbols of a circle, square, apple, and house so that each symbol measures visual acuity similarly; designed for young children who do not know how to read letters		SSVEP – steady state visually evoked potential
LogMAR – Logarithm of the Minimum Angle of Resolution chart designed to enable a more accurate estimate of acuity as compared to other charts		tDCS – transcranial direct current stimulation
LGN – lateral geniculate nucleus		TMS – transcranial magnetic stimulation
		V1, V2, V3, V4, V5, etc. – regions of the visual cortex
		VEP – visual evoked potential
		VIP – vasoactive intestinal peptide
		VIP-HIP - Vision in Preschoolers-Hyperopia in Preschoolers Study
		VPA – valproate

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