ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE
AND ENGINEERING

CONSIDERATIONS OF MODIFIED VISION AND CROWDING TESTS, FOR IMPROVED DETECTION OF AMBLYOPIA

LOUISA HAINE

A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

Submitted: May 2022

Dedication

To Peter, Bella, Erik, and Orson

Acknowledgements

First, I would like to acknowledge the VC studentship received from Anglia Ruskin University, without which, this research would not have been possible. Besides the VC Studentship that supported me during my studies (awarded to Dr Sarah Waugh when at ARU), financial support for the conduct of the first two experiments of this work is acknowledged from the ARU Quality Related (QR) research fund to Anglia Vision Research. Financial support for the conduct of the third experiment is acknowledged from a grant subcontract to Dr Waugh with NYU via the National Institute of Health (USA). Thanks also to the Director of ACPOS and clinicians within ACPOS or facilitating recruitment for this study.

Next, I would like to recognise the efforts of my supervisory team and advisors; Dr Monika Formankiewicz, Dr Sarah Lalor, Dr Sarah Waugh and of course Dr Akash Chima who have supported me throughout this rather unprecedented time-period.

Thank you to Diane Moore and my lovely colleagues at Anglia Ruskin University for their never-ending encouragement and support; and of course, to all my study participants for their time, efforts, and patience. I hope you all had lots of fun.

To my family and friends, thank you for never doubting me, even when I doubted myself. Your jokes, laughter and consideration mean more to me than you will ever know.

To my wonderful children, Bella, Erik and Orson, thank you for being my cheerleaders. Your infectious laughter, fun games, new interests, and cuddles have given me the strength and positivity to keep moving towards the finish line.

Finally, to my husband Peter, I simply wouldn't have gotten this far without you. Thank you, for your love, care and seemingly never-ending conveyer belt of snacks and drinks.

ANGLIA RUSKIN UNIVERSITY

ABSTRACT

FACULTY OF SCIENCE AND ENGINEERING

DOCTOR OF PHILOSOPHY

CONSIDERATIONS OF MODIFIED VISION AND CROWDING TESTS, FOR IMPROVED DETECTION OF AMBLYOPIA

LOUISA HAINE

MAY 2022

Amblyopia is a neurodevelopmental disorder characterised by deficits of visual acuity. It demonstrates greater sensitivity to visual crowding; the phenomena whereby accurate object identification is reduced due to the presence of nearby or flanking objects. Recent research suggests that the current visual acuity tests may not contain sufficiently crowded optotypes, potentially allowing amblyopes to avoid detection. Increasing crowding in acuity tests by using an optimised test arrangement may improve sensitivity to the detection of amblyopia by maximising interocular acuity differences (IOD). In the first experiment of this study, acuity is examined in both visually healthy and amblyopic children using an optimised arrangement and the acuities obtained compared with the Sonksen logMAR test (SLT). This 'Enhanced Cambridge crowding' (L-ECC) test demonstrated significantly increased crowding magnitudes for all groups examined (**p<.001).** L-ECC IODs were significantly larger for strabismic/mixed amblyopes, compared with the SLT (**p<.005**), but significantly smaller for anisometropic amblyopes (**p<.005**). Research with contrast-modulated stimuli has demonstrated increased crowding in adults, compared with luminance-generated stimuli. The second experiment examined the same paediatric cohort with a contrast-modulated 'Enhanced Cambridge crowding' CM-ECC test. While the CM-ECC yielded significantly higher acuities (**p<.001**), IODs were not significantly different from the SLT (p=.306). The third and final experiment in this study establishes the foveal crowding distances of amblyopic children, using the new 'Pelli' Optotype, for the first time. Pelli foveal crowding distances measured in both trigram and repeated formats showed that strabismic/mixed amblyopic eyes had significantly larger crowding distances than controls (**p<.005**) and anisometropic amblyopic eyes. (**p<.05**). Crowding distance IODs were also 20-30 times larger in strabismic/mixed amblyopes than controls. Crowding distance ratios (amblyopic eye/fellow eye) were larger than acuity ratios (**p<.001)** for strabismic/mixed amblyopes, but not anisometropic amblyopes. Crowding distance tests may therefore be more sensitive than acuity tests for detecting strabismic amblyopia.

Key words: amblyopia; visual acuity; visual crowding; visual screening; contrast-modulated optotypes; crowding distance

Table of Contents

List of Figures

List of Formulae

List of Tables

List of Abbreviations

Abbreviation	***Meaning***
AE	*Amblyopic eye*
CD	*Crowding distance*
CM	*Contrast modulated*
CM-ECC	*Contrast modulated Enhanced Cambridge Crowding test*
CM-Iso	*Contrast modulated isolated optotypes*
ECC	*Enhanced Cambridge Crowding test*
FE	*Fellow eye*
IOD	*Interocular difference*
Iso	*Isolated optotypes*
L	*Standard luminance*
LE	*Left eye*
L-ECC	*Luminance defined Enhanced Cambridge Crowding test*
L-Iso	*Luminance defined isolated optotypes*
RE	*Right eye*
SLT	*Sonksen logMAR test*
VA	*Visual acuity*

Chapter One – Introduction and literature review

1.1 Introduction

Amblyopia, or 'lazy eye' as it is commonly known, is defined as the reduction in best-corrected visual acuity (defined as the smallest resolvable detail in a target or pattern) of one or both eyes due to abnormal visual neurodevelopment during infancy or childhood (Flom and Neumaier, 1966; Von Noorden, 1974; Attebo et al., 1998; Holmes and Clarke, 2006; Levi and Li, 2009; Gunton, 2013). In addition to reduced visual acuity, amblyopia also results in decreased contrast sensitivity (the ability to detect luminance contrast) (Hess and Howell, 1977; Levi and Harwerth, 1977; Hess and Bradley, 1980; Bradley and Freeman, 1981; Howell, Mitchell and Keith, 1983; Abrahamsson and Sjostrand, 1988; Giaschi et al., 1993; McKee, Levi and Movshon, 2003; Huang et al., 2007), abnormal visual crowding (impairment of target identification due to surrounding/flanking objects) (Stuart and Burian, 1962; Jacobs, 1979; Giaschi et al., 1993; Simmers et al., 1999; Chung, Li and Levi, 2007, 2008a; Levi, 2008) and reduction in stereoacuity (Goodwin and Romano, 1985; Simmers et al., 1999; Birch, 2013).

Amblyopia occurs due to image deprivation or degradation in childhood and is predominantly unilateral (Wiesel and Hubel, 1963; Von Noorden, 1974; Holmes and Clarke, 2006; Daw, 2014), although bilateral presentation does occur approximately half as frequently as a unilateral presentation (Chia et al., 2010; Li et al., 2019; Meng et al., 2021). The most common amblyogenic factors are strabismus (misalignment of the visual axis), anisometropia (unequal refractive error between the two eyes) and a combination of both (Von Noorden, 1974; Simons, 2005; Holmes et al., 2006; Wu and Hunter, 2006; Williams, 2009; Hamm et al., 2014). In a multicentre randomised clinical trial of 409 amblyopic children aged between three and six years (Repka et al., 2002), 38% had strabismus, 37% anisometropia, and 24% had combined strabismus and anisometropia.

Stimulus deprivation amblyopia (produced by factors such as a cataract or significant upper lid ptosis) is less common, affecting around three percent of amblyopes (Hillis, Flynn and Hawkins, 1983; Shaw et al., 1988). This thesis will focus on the most common types of amblyopia; unilateral amblyopia with strabismus (both pure strabismic and strabismic/anisometropic mixed) and pure anisometropic amblyopia.

Amblyopia is a preventable and leading cause of paediatric monocular vision loss; with a prevalence of between 1% to 4% (Flom and Neumaier, 1966; Simons, 1996; Attebo et al., 1998; Simons, 2005; Carlton et al., 2008; Powell and Hatt, 2009; Van De Graaf et al., 2016). Early detection and treatment are successful in reducing these prevalence rates and improving patient visual outcomes (Oliver et al., 1986; Nucci et al., 1992; Noda, Hayasaka and Setogawa, 1993; Elder, 1994; Beardsell, Clarke and Hill, 1999; Newsham, 2000; Wang, 2015); however, few anisometropic amblyopes/amblyopes without any strabismus (~15%) are identified before age five, likely due to the absence of observable signs of visual dysfunction (Shaw et al., 1988). Strabismic amblyopes present youngest (median age 3.64 years), followed by combined strabismic-anisometropic (median age 4.68 years) and finally anisometropic (median age 6.27 years) (Shaw et al., 1988). Consequently, childhood vision screening programmes are used in the U.K. to help detect unidentified amblyopia, with children being screened between the ages of four and five years (Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015). Untreated, amblyopia persists into adulthood, increasing the lifetime risk of bilateral visual impairment (BVI) from 10% seen in non-amblyopes to 18% (van Leeuwen et al., 2007).

For optimal amblyopia detection, visual acuity tests used in vision screening should maximise the effects of amblyopic visual deficits. Recent research has concluded that modification of visual acuity tests by enhancing the presence of visual crowding may improve their sensitivity to the detection of amblyopia by increasing the interocular visual acuity difference (Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014; Lalor,

2018). In the following sections of this chapter, studies of normal paediatric development of visual acuity and crowding are reviewed, followed by the impact of amblyopia upon these two visual functions. Developments in visual crowding and contrast-modulated second-order visual stimuli are also discussed concerning the potentially improved identification of amblyopia within childhood visual assessment. Finally, the project aims are stated.

1.2 Visual Development

For normal visual development to occur, unhindered, equal, and corresponding visual input from each eye is required from birth through to cortical maturation, as deprivation or irregularities of visual input during this time can result in 'profound, perhaps complete impairment of vision' (Wiesel and Hubel, 1963. pg. 1006) (Daw, 1998; Thompson et al., 2015; Siu and Murphy, 2018).

Early work by Wiesel and Hubel (1963) examining the effect of two to three months of monocular deprivation in kittens has driven research into neuroplasticity and visual development. Monocular visual deprivation (light and form) of kittens from birth demonstrated a critical period of around three months during which the kitten was sensitive to the effects of the visual deprivation. This resulted in the reduction of vision in the affected eye leading to absence of form perception, hesitant gait and poor navigation when viewing monocularly with the deprived eye. Physiologically, within the lateral geniculate nucleus (LGN), cellular atrophy was noted as well as reduced cellular activity of the layers relating to the deprived eye (Hubel and Wiesel, 1962). In addition, single-unit recordings in the striate cortex revealed cortical changes in ocular dominance, with the non-deprived eye dominating cellular activity (83 of 84 cells recorded were 'completely uninfluenced by the deprived eye' Wiesel and Hubel; 1963: pg 1106). These undesirable physiological changes were partially mitigated if the kitten had one to two months of typical visual experience prior to monocular lid suture occlusion, and no changes were noted in cats that had reached

adulthood prior to monocular deprivation (Hubel and Wiesel, 1962; Wiesel and Hubel, 1963). These results suggested that while the neurological connections that underpin visual processing are present at birth, they are vulnerable to change owing to environmental input for a given period. Following this research, the term 'critical period' became commonly used to describe the period in which deprivation is effective in limiting visual function (Daw, 1998).

Visual critical periods are thought to occur from birth to around puberty, although specific critical periods are dependent upon the anatomical level being examined, the visual function being assessed and the visual experience of the subject in question (See review by Daw; 1998). In humans, as in cats, visual deprivation occurring beyond the close of the visual critical period shows a diminishing effect and does not result in the same neurological deficit that occurs if a visual disruption occurs during the critical period (Vaegan and Taylor, 1979; Daw, 1998). In adulthood, the same amblyopic sensory deprivations result in no permanent or limited neurological effects (Berardi, Pizzorusso and Maffei, 2000).

1.2.1 Development of Visual Acuity

Clinically, visual acuity (VA) is a measure of the finest resolvable detail within a high contrast pattern, e.g., black letters on white background (Song, 2009). VA scores using different systems of measurement are given in Table 1.01. In visually healthy individuals, acuity is finest at the fovea, deteriorating rapidly towards the periphery (Burton, 1959; Levi and Carney, 2011). Throughout visual development, an individual's acuity may be limited by factors such as visual optics and accommodation (Mohindra et al., 1978; Banks, 1980), as well as foveal immaturity and photoreceptor density (Green, 1970; Yuodelis and Hendrickson, 1986; Curcio et al., 1990).

Table 1.01*: Corresponding scores of different visual acuity measurements*

Metric	Imperial	Decimal	MAR (minutes of arc ‘)	logMAR	Degrees	Cycles per degree (cpd)
6/60	20/200	0.1	10	1.0	0.166	3.0
6/48	20/160	0.13	8	0.9	0.133	3.75
6/36	20/120	0.17	6	0.78	0.1	5.0
6/30	20/100	0.2	5	0.7	0.083	6.0
6/24	20/80	0.25	4	0.6	0.066	7.5
6/18	20/60	0.33	3	0.48	0.05	10
6/12	20/40	0.5	2	0.3	0.033	15
6/9	20/30	0.63	1.5	0.18	0.025	20
6/7.5	20/25	0.8	1.25	0.1	0.021	24
6/6	20/20	1.0	1.0	0.0	0.016	30
6/5	20/16	1.25	0.83	-0.1	0.014	36
6/4	20/12.5	1.6	0.66	-0.2	0.011	45
6/3	20/10	2.0	0.5	-0.3	0.008	60

Rapid visual acuity improvement occurs after birth (Spekreijse, 1983; Norcia and Tyler, 1985; Lai, Wang and Hsu, 2011; Guo et al., 2015), although tasks used to quantify this vary considerably. Different types of subjective assessment of acuity (briefly summarised in Table 1.02), various test designs and developmental maturation rates are discussed below.

Table 1.02*: Summary of different subjective visual acuity tasks*

Acuity Task	Definition	Example existing test
Detection	The ability to discern the presence or absence of a stimulus	Sinusoidal gratings
Resolution	The smallest size discriminable between to objects/ separate elements of a pattern	Sinusoidal / square wave gratings. Teller Acuity cards (Teller, 1979) Cardiff Acuity Cards
Vernier acuity	Minimum discernible misalignment position / difference in positioning	Displaced/ misaligned lines, Vernier Gratings
Recognition	Minimum sized object, picture or optotype that can be identified	Kay Pictures (Kay, 1983) ETDRS (Early Treatment Diabetic Retinopathy Study) logMAR chart, Cambridge Crowding Cards (Atkinson et al., 1988).

1.2.2 Detection, Resolution and Vernier acuity tasks

Detection tasks examine the individual's perception of a stimulus's presence or absence. Uniocular examination of spatial acuity in four to seven year-olds using vertical sinusoidal gratings displayed at spatial frequencies of 0.33, 0.5, 1, 2, 3, 5,10 and 20 cpd by Ellemberg and colleagues (1999) found no difference between right and left eyes for any age group. Monocular maturation of grating acuity detection occurred at six years of age.

Resolution tasks examine the smallest size discriminable between two separate elements of a pattern or target (Pointer, 2008; Heinrich and Bach, 2013). Resolution acuity can be assessed using targets such as a sinusoidal or square wave gratings (Teller, 1979) or by identifying a gap in a target such as the Tumbling-E test or Landolt-C test, also known as the broken ring test (Pointer, Gilmartin and Larke, 1980; Pointer, 2008; Lai, Wang and Hsu, 2011). In practicality, resolution acuity is less applicable to daily life and is not directly equivalent to recognition acuity (Droste, Archer and Helveston, 1991; Kushner, Lucchese and Morton, 1995), as resolution acuity demonstrates lower sensitivity to the presence of optical blur (Thorn and Schwartz, 1990; Howard and Firth, 2006), foveal abnormalities (Mayer, Fulton and Rodier, 1984) and amblyopia (Mayer, Fulton and Rodier, 1984; Kushner, Lucchese and Morton, 1995; Rydberg et al., 1999). Grating tests, however, are notably helpful for the examination of preliterate young children and infants (Teller, 1979; McDonald, Dobson and Sebris, 1985; McDonald, 1986) and offer a suitable alternative behavioural method of VA assessment to optotype recognition tasks (Drover et al., 2009). Using a two alternative forced-choice (2-AFC) method of VA assessment known as preferential looking, the examiner is required to make a subjective interpretation of the child's response to the stimuli (Dubowitz, Dubowitz and Morante, 1980; Dubowitz, 1980; Teller et al., 1986). A second type of resolution VA task involves the discrimination of gaps in optotypes, such as the Landolt-C; however, more frequently, these tests involve identifying the position of the break/gap and so could also be categorised under visual acuity recognition tasks. Again, these tests are helpful for preliterate children or non-verbal/non-native language speakers as they do not require recognition and identification of optotypes (Hyvärinen, Näsänen and Laurinen, 1980).

Bypassing optical aberrations of the eye, Campbell and Green (1965) reported maximum VA resolutions of 60 cpd in two visually healthy adults using sinusoidal gratings projected by a non-helium laser. A summary of resolution visual acuity maturation rates is given in Table 1.03, showing adult-like VA resolutions from three to six years of age.

Table 1.03: *Visual maturation ages according to studies examining tests of detection acuity, resolution acuity and vernier acuity; in ascending order of age of maturity.*

Study	Acuity	Task	Age of acuity maturation
Catford and Oliver (1973)	Resolution	Examination of optokinetic nystagmus using nystagmus drum targets	3 years
Kiorpes and Movshon (1989)	Vernier	Combination of preferential looking and operant methods in seven visually normal monkeys	30-40 weeks (~3 yrs)
Mayer and Dobson (1982)	Resolution	Preferential looking with square wave gratings	5 years
Stiers, Vanderkelen and Vandenbussche (2003)	Detection	Preferential looking with square wave gratings horizontally or vertically orientated, with a luminance matched grey card control	>5 years
Stiers, Vanderkelen and Vandenbussche (2003)	Resolution	Preferential looking with square wave gratings	>5 years
Birch et al. (1983)	Resolution	Preferential looking with horizontal and vertical gratings	>5 years
Ellemberg et al. (1999)	Detection	Detection of sinusoidal gratings	6 years
Carkeet, Levi and Manny (1997)	Vernier	Vernier acuity examined with uncrowded static stimuli and a 3AFC psychophysical paradigm	~ 6 years
Skoczenski and Norcia (2002)	Vernier	VEP measure of vernier acuity	10-14 years

Spatial localisation tasks involve perceiving differences in spatial positioning, such as discerning a displacement in a line or contour (Westheimer, 1975; Levi and Klein, 1983). This is also known as Vernier acuity and is a form of hyperacuity, the perception of spatial thresholds an order of magnitude smaller than the resolution limits of the eye (Westheimer, 1975, 1987, 2012). Studies of vernier acuity in infants (aged between 1 – 13 months) using 2AFC preferential looking techniques have demonstrated vernier acuity thresholds of 64 arc-mins at one month (Holmes and Archer, 1993), improving by three octaves up to six months of age (Manny and Klein, 1984), reaching four arc mins by 13 months of age (Holmes and Archer, 1993). Further improvements are demonstrated up to 14 years of age (Skoczenski and Norcia, 2002).

1.2.3 Recognition acuity tasks

This study focuses on visual acuity recognition tasks, which are the most commonly used clinical tests of visual acuity and involve the identification (verbal or via matching) of a target optotype or picture (Kay, 1983; Mayer, Fulton and Rodier, 1984; Pointer, 2008; Heinrich and Bach, 2013; Anstice and Thompson, 2014; O'Connor and Milling, 2020) or the cardinal direction of an optotype (Pointer, Gilmartin and Larke, 1980; Stiers, Vanderkelen and Vandenbussche, 2003; Pointer, 2008; Lai, Wang and Hsu, 2011). Children as young as two years of age have demonstrated the ability to undertake recognition acuity tasks using pictorial optotypes (Kay, 1983). However, practically, clinical assessment is considered to be most viable from age three years (82.8% testability rates with Lea Symbols) (Kvarnström and Jakobsson, 2005), with testability increasing with age to between 92.9%-96.5% using Lea Symbols at four years (Kvarnström and Jakobsson, 2005).

Optotype recognition acuity tasks appear in many formats. For example, optotypes may be presented individually (such as isolated Sloan letters (Sloan, 1959) or the Sheridan Gardiner test), in a line/linear format (such as the Sonksen logMAR test (Salt et al., 2007))

or in a descending resolution chart arrangement (such as the ETDRS (Bailey and Lovie, 1976)). Considering the simplest of the recognition tasks, identifying the direction of an optotype rotated into (usually) four cardinal points (Pointer, Gilmartin and Larke, 1980), these directional VA tests are useful for preliterate children, or non-verbal/non-native language speakers, as they do not require recognition and identification of the optotypes, only directional responses, either verbally (*'up, down, left, right'*) or by pointing. Caution must be taken when interpreting these thresholds, however, as children up to around age 7.5 years often exhibit left-right directional confusion (Davidson, 1934, 1935) and, to a lesser degree, up-down confusion (Davidson, 1935; Schaller and Harris, 1975).

Other pre-literate recognition tests of visual acuity involve the use of pictures or symbols. Tests developed for this purpose include, but are not limited to (in date order) the Osterberg Chart, also known as the Danish Pictorial Sight test (Østerberg, 1936), the Modified Pictograph Method (Fink, 1945), Allen Cards (Allen, 1957), Ffooks Symbols (Ffooks, 1965), Bealle Collins (Keith, Diamond and Stansfield, 1972), Lea Symbols (Hyvärinen, Näsänen and Laurinen, 1980), Kay Pictures (Kay, 1983; Milling et al., 2015; O'Connor and Milling, 2020), Wright Figures (Cem Mocan, Najera-Covarrubias and Wright, 2005), and more recently the Auckland Optotypes (Hamm et al., 2018). The lack of standardisation and shape cues may be problematic in the design structure and arrangement of pictorial acuity tests (Anstice and Thompson, 2014; Anstice et al., 2017). Despite a strong correlation being reported between the visual acuity scores of the original isolated Kay Picture test and Snellen VA in older children and adults (n=160) (90% of participants yielded equal acuities or differences no greater than one Snellen line) (Kay, 1983); later studies of Allen cards, Wright Figures and Kay Pictures have demonstrated over-estimation of VA thresholds in children (Cem Mocan, Najera-Covarrubias and Wright, 2005; Dobson et al., 2009; Anstice and Thompson, 2014; Milling et al., 2015; Anstice et al., 2017; O'Boyle, Chen and Little, 2017).

Recently, the Kay Picture test has been updated (see Figure 1.01) in order to bring the format closer in line with guidelines from the International Council of Ophthalmology (Consilium Ophthalmologicum Universale, 1984) (Figure 1.02), and the British Standards of Tests charts for clinical determination of distance visual acuity (British Standards Institution, 2003), thus ensuring consistency across clinical visual acuity tests (Milling et al., 2015; O'Connor and Milling, 2020). The guidelines are based on suggestions made by Bailey and Lovie (1976). Normative data values for the updated single optotype format Kay Pictures Test were established following monocular VA assessment at three metres, of 283 children aged 20-57 months (O'Connor and Milling, 2020). Results showed a statistically significant improvement in acuity with age ($p<.001$), with 100% testability seen in 48–53-month-old children and a mean right eye VA of +0.03±0.07 logMAR at 48-53 months. Unfortunately, the age of visual maturity with this new test was not evaluated within the study. However, age-matched comparisons against Lea symbols revealed similar improvements in acuity with age (0.22±0.12 logMAR at 36-41 months, to 0.1±0.13 logMAR at 60-65 months), although better acuity thresholds were achieved with the updated Kay Picture test (O'Connor and Milling, 2020).

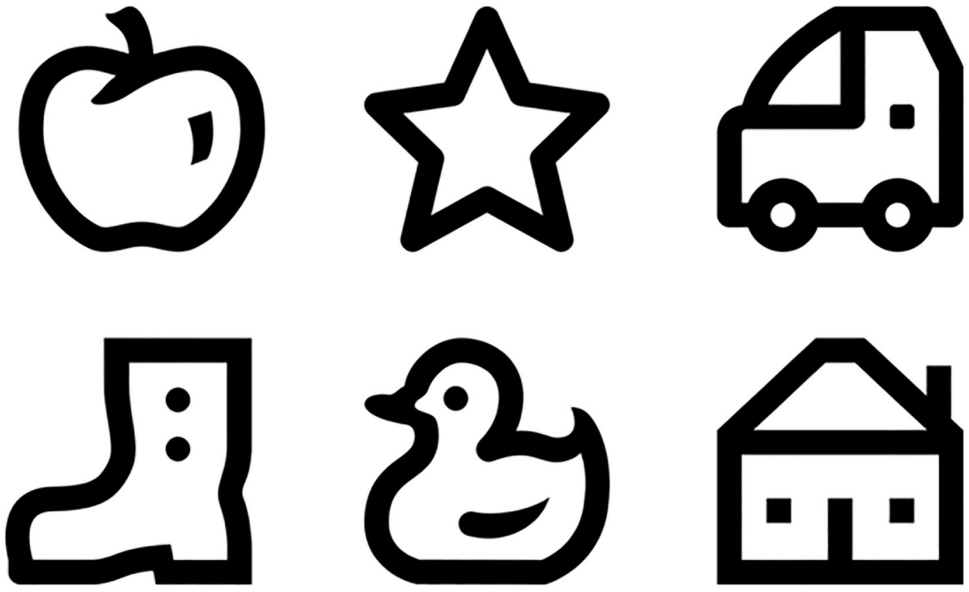


Figure 1.01: *Updated Kay Pictures test optotypes* (Milling et al., 2015; O'Connor and Milling, 2020)

1. Optotypes should be black on white background.
2. Crowding elements should be incorporated into the test.
3. Optotypes (either pictures or letters) should be of approximate equal legibility.
4. Optotypes (either pictures or letters) should be evenly spaced and centrally disposed. The gap between letters should be equal to the width of the letters.
5. At least five optotypes should be displayed on each line.
6. Optotype (either pictures or letters) sizes should have a geometrical progression of step sizes of 0.1 log units per line.

Figure 1.02: *Adapted summary of the International Visual Acuity Chart Guidelines* (Consilium Ophthalmologicum Universale, 1984; Anstice and Thompson, 2014)

Most recognisable to the layperson are optotype tests using letters derived from the English alphabet. These include, but are not limited to the Snellen test (Snellen, 1965), the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, the Bailey-Lovie chart (Bailey and Lovie, 1976), Sheridan-Gardner letters (Sheridan and Gardiner, 1970), the Keeler logMAR chart (previously Glasgow Acuity Cards) (Keeler, UK), HOTV (Lippmann, 1971), Cambridge crowding cards (Atkinson et al., 1986), and the Sonksen logMAR test (SLT) (Salt et al., 2007). Studies demonstrate maturation of isolated recognition acuity (Table 1.04) occurring between the ages of five and six years (Atkinson et al., 1986; Stiers, Vanderkelen and Vandenbussche, 2003; Jeon et al., 2010; Waugh et al., 2018). One study recorded adult-like acuities present within their eight-year-old participants, which were identified as significantly better than the thresholds obtained in the five-year-old group; however, no extrapolation was made regarding the age at which maturity occurred (Jeon et al., 2010).

Table 1.04: *Isolated visual acuity maturation ages according to various studies examining Recognition acuity tests, in ascending order of age of maturity.*

Study	Task	Age of acuity maturation
Atkinson et al. (1986)	Isolated Landolt-C	5 years
Stiers, Vanderkelen and Vandenbussche. (2003)	Isolated Landolt-C	>5 years
Jeon et al. (2010)	Isolated Tumbling-E	Between 5 and 8 years
Waugh et al. (2018)	Isolated Sloan letters	6 years

As one of the first clinical visual acuity assessments, the Snellen chart, is widely used and recognised, although it has numerous design imperfections. These include inadequate inter-optotype legibility (Bennett, 1965), inconsistent size of letter progression (Bailey and Lovie, 1976; McGraw, Winn and Whitaker, 1995), and inconsistent inter-optotype and inter-line spacing (Bailey and Lovie, 1976); all of which influence acuity outcomes (Norgett and Siderov, 2011; Lalor, 2018; Sailoganathan et al., 2018). The presence of flanking objects (e.g. bars, boxes or optotypes) also degrades visual acuity (Bouma, 1970; Fern et al., 1986; Simmers et al., 1999; Coates, Chin and Chung, 2013; Lalor, 2018; Sailoganathan et al., 2018). Crowded visual acuity tests are discussed further in Section 1.3.1. Differences between isolated and crowded acuity occur because of visual crowding, which will be discussed next, and further in chapters three and five.

1.3 Visual Crowding

Impaired visual identification of ‘crowded’ targets has been studied for nearly 100 years. For example, Korte (1923) observed that nonsense words presented to the visual periphery were less identifiable than single letters presented at the same location, demonstrating a deleterious interaction between the letters. Davage and Sumner (1950) examined this

further, finding that inspection of an isolated single-line on a Snellen chart yielded greater letter recognition than when the entire chart was exposed. Ehlers subsequently articulated this phenomenon, stating that "if the visual field is crowded with letters, the area of the visual field in which the letters can be recognized, narrows" (Ehlers, 1953).

This spatial interference phenomenon has been assigned many labels over the years, such as lateral masking (Loomis, 1978; Wolford and Chambers, 1983; Chambers and Wolford, 1983), contour interaction (Flom, Weymouth and Kahneman, 1963; Hess and Jacobs, 1979; Fern et al., 1986; Flom, 1991) and crowding (Stuart and Burian, 1962; Levi and Klein, 1983, 1985; Atkinson and Braddick, 1983; Atkinson et al., 1986; Doron, Spierer and Polat, 2015). Masking is considered to occur when a single feature detector is stimulated by both the target and mask, whilst in visual crowding, different feature detectors are stimulated by the target and mask, which are then combined by a single feature integrator (Pelli, Palomares and Majaj, 2004). The current study will focus on the concept of crowding, an important limiting factor in the performance of many critical visual tasks such as optotype identification (Bouma, 1970; Yu et al., 2007; Levi and Carney, 2011; Vejnović and Zdravković, 2015), saccadic visual search performance (Vlaskamp and Hooge, 2006; Yildirim, Meyer and Cornelissen, 2015) and reading speed (Chung, 2002; Levi, Song and Pelli, 2007; Chung, 2012). Crowding is an umbrella term covering the phenomenon of misidentification of a target and subsequent reduction in target threshold acuity due to the presence of nearby bars and contours (commonly known as contour interaction), as well as more complex objects such as pictures and optotypes (Flom, 1991; Pelli, Palomares and Majaj, 2004; Pelli, 2008; Levi, 2011). It is generally considered that crowding is a combination of contour interaction (Flom, Weymouth and Kahneman, 1963; Flom, 1991), inaccuracy of gaze control, and attention (Flom, 1991; Pelli, Palomares and Majaj, 2004; Danilova and Bondarko, 2007; Norgett and Siderov, 2011; Bedell et al., 2013; Hairol, Formankiewicz and Waugh, 2013).

Subject to Bouma's law (see Formula 1.01 and Figure 1.03) (Bouma, 1970) in the periphery of normal vision (Jacobs, 1979) and in amblyopic eyes (Schor, Terrell and Peterson, 1976; Kovács, Polat and Norcia, 1996; Simmers et al., 1999; Chandna et al., 2001; Hess et al., 2001), crowding is thought to demonstrate a "bottle-neck" of visual processing (Pelli, Palomares and Majaj, 2004; Levi, 2008; Herzog and Manassi, 2015). Target detection is preserved as observers can appreciate the existence of the target; however, the properties of the target are confused and rendered unidentifiable (Pelli, Palomares and Majaj, 2004; Pelli and Tillman, 2008; Harrison and Bex, 2015). This is considered representative of excessive integration and facilitation of composite image features (Pelli, Palomares and Majaj, 2004; Bulakowski, Post and Whitney, 2011; Herzog and Manassi, 2015). Identification of targets requires first detection of multiple features, followed by integration of these features to formulate object identity (Graham, 1980; Chubb, Olzak and Derrington, 2001; Pelli, Palomares and Majaj, 2004). Several mechanisms have been suggested as a source for crowding, including positional uncertainty (Levi, Klein and Yen Lee Yap, 1987), feature averaging (Parkes et al., 2001) and source confusion (Strasburger and Malania, 2013). Pelli and colleagues have suggested that crowding occurs when a target and flanker, which stimulate different feature detectors, are pooled excessively at the same feature integrator, causing target confusion and misidentification (Pelli, Palomares and Majaj, 2004).

$$\text{Distance}_{\text{flankers}} = 0.5\,\varphi°$$

Formula 1.01*: Bouma's law (Bouma, 1970) - In order for letter identification to occur, the spacing between the flankers and the target should be approximately 0.5x the eccentricity of the target from the fovea.*

Figure 1.03: *Based on Bouma's law (Bouma, 1970), if target 'T' is placed in the periphery at 10°, the zone of interference is expected to occur around 5° from the centre of the target, as represented by the red arrow. Target 'H falls within the 'zone of interference' and therefore will be unidentifiable. The centre of target 'E' falls at the 'critical distance' and therefore will be identifiable. Target 'L' falls outside of the 'zone of interference' and will be identifiable.*

Behavioural studies of crowding have indicated that it occurs due to an inability to isolate a target from its surrounding flankers, which instead are mistakenly integrated, causing object confusion and misidentification (Flom, Heath and Takahashi, 1963; Levi and Klein, 1985; Parkes et al., 2001; Levi, Hariharan and Klein, 2002a; Pelli, 2008; Strasburger and Malania, 2013). Participants in the Parkes et al (2001) study of orientation selectivity tasks demonstrated that in crowded conditions, participants were able to report the average combined orientation of the target and array, indicating that visual information is not lost via visual crowding but is instead 'pooled' within large receptive fields and averaged across the visual stimuli.

Evidence suggests that crowding is a cortical phenomenon, although the precise cortical location is yet to be determined and may involve multiple areas of the visual system (Louie, Bressler and Whitney, 2007; Whitney and Levi, 2011). The cortical location of visual crowding was determined in an early study by Flom, Heath and Takahashi, who demonstrated that the presence of flanking contours similarly reduced the resolution of the target Landolt-C, regardless of whether the flankers were presented monocularly or dichoptically (Flom, Heath and Takahashi, 1963). Visual crowding could arise as early as V1 (Liu et al., 2009; Polat, Sagi and Norcia, 1997; Neri and Levi, 2006; Pelli and Tillman,

2008; Nandy and Tjan, 2012), although other studies have further indicated neurological involvement beyond the primary visual cortex (He, Cavanagh and Intriligator, 1996), specifically V2 (Bi et al., 2009; He, Wang and Fang, 2019), V3 (Bi et al., 2009), V4 (Motter, 2006) or the ventral pathway (Freeman, Chakravarthi and Pelli, 2012). Identification and quantification of perceptual changes/deficits of visual crowding could therefore reflect abnormalities or maldevelopment within both the striate and extra-striate cortices.

1.3.1 Quantification of Visual Crowding

Quantification of crowding has used several terminologies. Firstly, magnitude of crowding refers to the difference in acuity threshold between flanked and unflanked targets (Chung, Levi and Legge, 2001; Livne and Sagi, 2007; Norgett and Siderov, 2011; Lalor, 2018). The spatial extent of crowding, sometimes referred to as 'critical spacing', is the smallest distance between a target and flanking objects where target identification is not impeded (Chung, 2002; Pelli, Palomares and Majaj, 2004; Martelli, Majaj and Pelli, 2005; Coates, Chin and Chung, 2013; Rosen, Chakravarthi and Pelli, 2014; Pelli et al., 2016; Ronconi, Bertoni and Bellacosa Marotti, 2016).

The incorporation of crowding with flanking contours, boxes and optotypes is frequently seen in commercial visual acuity tests, such as the Keeler logMAR test and Sonksen logMAR test, to highlight the presence of crowding limited conditions such as amblyopia (Simmers, Gray and Spowart, 1997; Bradfield, 2013; Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015). Comparisons of commercial crowded and uncrowded tests in 103 children aged between four and nine years of age clearly demonstrate the differences in acuity when tested with isolated optotypes and linearly presented crowded optotypes (Norgett and Siderov, 2011) (see Figure 1.04 for the acuity tests examined). While no significant difference was seen between the isolated Single Kay Picture and Sheridan Gardiner tests for both younger (mean: 5 years 9 months, n=39) and older children (mean:

8 years 7 months, n=64), significant effects of test upon acuity threshold were seen when using the crowded linear tests (p<.001, for both younger and older children). Furthermore, when crowding magnitude (referred to as relative acuity loss within this study) was examined, younger children showed greater acuity losses compared with older children, demonstrating that magnitude of the crowding effect was greater at younger ages (Atkinson et al., 1988; Langaas, 2011; Norgett and Siderov, 2011; Lalor, 2018).

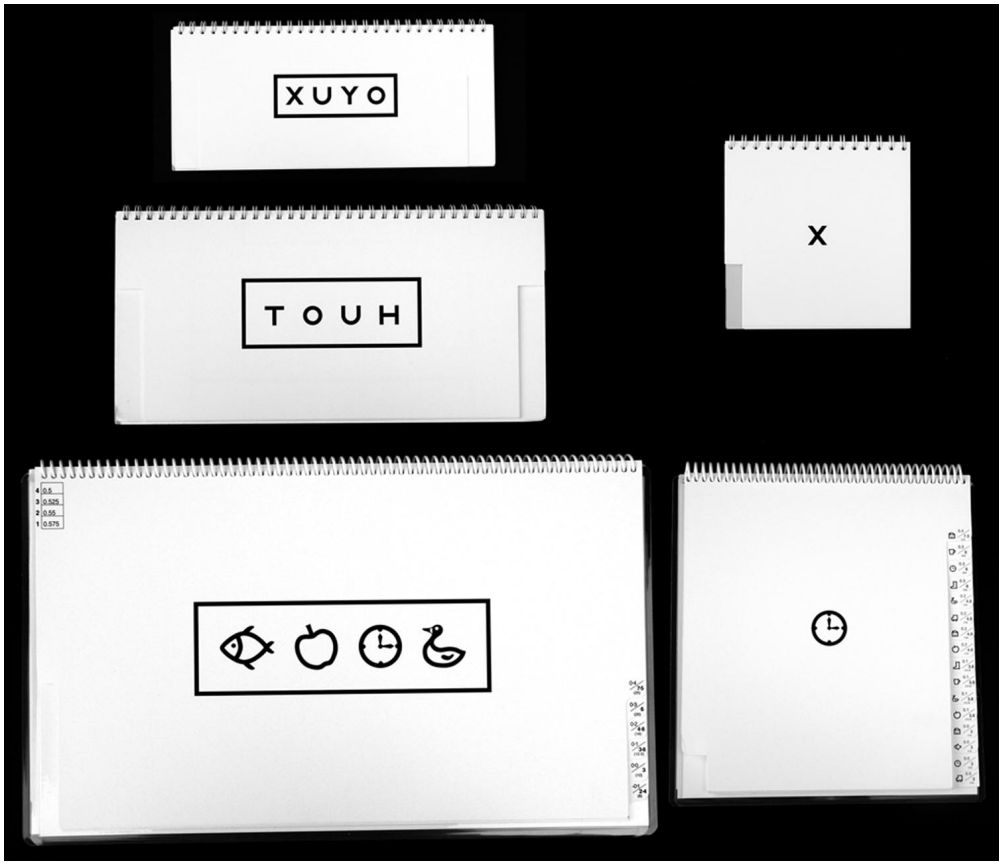


Figure 1.04: *Visual acuity tests examined by Norgett and Siderov* (2011)

This is further evidence that acuity for isolated letters matures between age five to six years (see Table 1.04 for a summary) however crowded acuity (acuity tests which incorporate visual crowding features) demonstrates later maturity of between 5 and >11 years of age depending upon the test used and its design (see Table 1.05 for a summary) (Atkinson and Braddick, 1983; Atkinson et al., 1986; Simons, 1983; Ellemberg et al., 1999; Drover et al.,

2008; Sonksen et al., 2008; Pan et al., 2009; Jeon et al., 2010; Lai, Wang and Hsu, 2011; Langaas, 2011; Guo et al., 2015; Almoqbel, Irving and Leat, 2017; Lalor, 2018; Siu and Murphy, 2018).

Table 1.05: *Crowded acuity maturation ages according to various studies examining recognition acuity tests, in ascending order of age of maturity.*

Study	Task	Age of acuity maturation
Lai, Wang and Hsu et al. (2011c)	Linear Tumbling-E	5-6 years
Lai, Wang and Hsu et al. (2011c)	Linear Landolt-C	5-6 years
Atkinson and Braddick (1983)	Crowded Landolt-C	>5 years
Atkinson et al. (1986)	Crowded Landolt-C	>5 years
Simons (1983)	Crowded Landolt-C	6 years
Pan et al. (2009)	HOTV	>6 years
Lalor (2018b)	Modified Cambridge Crowding Test	~ 7 years
Drover et al. (2008)	Linear crowded HOTV	Between 7 and 8-10 years
Sonksen et al. (2008)	Sonksen logMAR test	~ 8 years
Langaas (2011)	Crowded logMAR	9-10 years
Jeon et al. (2010)	Crowded Tumbling-E	>11 years

1.3.2 Optimal crowding features

Many factors can influence the magnitude of the crowding effect (see Table 1.06 for a summary). Target-flanker similarity and inter-optotype spacing are the most pertinent to this discussion of test design for assessment in children. Clear evidence of these factors is demonstrated in a comparison of two studies (Vision in Preschoolers (VIP) Study Group, 2003; Cyert, 2004). In the 2003 study by the Vision in Preschoolers (VIP) group, researchers concluded that the letter based HOTV test (with four flanking bars) yielded 0.25 logMAR lines better visual acuity than Lea Symbols in 3 to 3.5-year-olds (Vision in Preschoolers (VIP) Study Group, 2003).

In contrast, Cyert, (2004) reported that HOTV yielded slightly worse visual acuity thresholds than Lea Symbols in their study of 1253 three to five-year-olds. Differences between the thresholds achieved can be likely attributed to the different formats of the presented tests. While Cyert (2004) utilised comparable linear presentations of crowded single lines, the VIP study group employed a single HOTV optotype flanked by bars and a crowded 'ETDRS' style Lea optotype chart. The Lea chart in the VIP study demonstrated increased crowding effects due to the linear presentation, presence of surrounding complex and increased target-flanker complexity and similarity, resulting in artificially poorer acuity thresholds for the Lea Symbols, compared with the flanked single HOTV (Vision in Preschoolers (VIP) Study Group, 2003).

The Cyert (2004) study, with its comparable presentation format, highlights another crowding influencing feature; crowded letters lead to poorer acuities than crowded pictures. Kay Picture optotypes have been shown to significantly overestimate visual acuity ($p<.001$) in visually healthy children and adults (Anstice et al., 2017). Overestimations of approximately 0.1 logMAR ($p<.001$) are seen in amblyopic children aged four to six years when Kay acuities were compared with the Keeler letter chart (O'Boyle, Chen and Little,

2017). It is considered that this overestimation occurs because Kay Picture optotypes yield shape cues that provide additional identification information, which are not as prominent in letter optotypes (Little, Molloy and Saunders, 2012; O'Boyle, Chen and Little, 2017). As such, letter charts rather than picture charts are preferable for the testing of children; however, picture optotypes may be more approachable and engaging for younger/non-literate participants (Allen, 1957; Kay, 1983; Woodhouse et al., 1992; Milling et al., 2015).

This overestimation of visual acuity with the Kay Pictures test appears to be consistent and predictable (O'Boyle, Chen and Little, 2017); therefore, it can provide a realistic estimation of crowded letter acuity should a child be unable to complete a crowded letter optotype task.

A comprehensive study of crowding effects was recently conducted by Lalor, Formankiewicz and Waugh (2016), who compared the effects of different flanking contours and optotypes, in addition to different target-flanker spacings, to establish the ideal design and spatial features of an optimally crowded visual acuity test. Tests examined were Crowded Kay Pictures, Lea Symbols, HOTV and Cambridge Crowding test. As per the VIP study group (2003), the greatest acuity diminishing effects were seen in the five adult participants with flanking optotypes using the Cambridge crowding test, compared to tests employing flanking boxes (Kay Pictures, Lea Symbols and HOTV – displayed in Figure 1.05). In addition, lowest (best) visual acuities (averaged across all participants, stimuli, and target-flanker spacings) were obtained with the Crowded Kay Picture test (mean acuity -0.27$\pm$0.11 logMAR) and highest (poorest) acuities occurred with the optotype flanked Cambridge Crowding test (mean acuity -0.10$\pm$0.08 logMAR) ($p<.001$) (Lalor, Formankiewicz and Waugh, 2016).

Table 1.06: *Factors which influence the magnitude of visual crowding*

Condition	Effect
Attention	Attention and target location cues reduce the effects of crowding (Strasburger, 2005; Freeman and Pelli, 2007; Chakravarthi and Cavanagh, 2007; Dakin et al., 2009; Yeshurun and Rashal, 2010)
Flanker grouping	Flankers that 'group' separately from the target, can reduce or eliminate crowding (Livne and Sagi, 2007; Levi and Carney, 2009; Livne and Sagi, 2010; Saarela, Westheimer and Herzog, 2010)
Flanker masking and suppression	Crowding can be reduced or abolished when the surrounding flankers are themselves masked or suppressed (Chakravarthi and Cavanagh, 2009; Wallis and Bex, 2011).
Holistic / contextual processing	When a targets objects context is incorrect e.g. Faces presented upside down, the effect of crowding is reduced (Farzin, Rivera and Whitney, 2009).
Target-flanker distance	Reduced target-flanker/inter-optotype spacing increases crowding magnitude (Atkinson et al., 1988; Norgett and Siderov, 2011; Lalor, 2018)
Target-flanker similarity	Similarity in target-flanker complexity (Bernard and Chung, 2011), shape and size (Kooi et al., 1994), spatial frequency (Chung, Levi and Legge, 2001), colour (Kooi et al., 1994; Waugh and Formankiewicz, 2019), and orientation (Levi, Hariharan and Klein, 2002b; Hariharan, Levi and Klein, 2005), have all been shown to increase the crowding effect.

Maximal crowding and contour interaction effects were seen when target-flanker spacing was decreased to zero stroke widths (abutting) or one stroke width, with crowding effects diminishing as spacings increased. As abutting contours and flankers would not be suitable for acuity evaluation, one stroke width target-flanker spacing was concluded to provide optimal crowding. Implementation of this reduced target-flanker spacing would induce maximum possible crowding, resulting in poorer acuity scores, thereby enhancing the test's sensitivity to crowding sensitive conditions. This target-flanker spacing is considerably narrower than current crowded visual acuity tests, where inter-optotype spacing currently varies from 0.5 optotype widths (Cambridge Crowding cards, Kay Pictures and Keeler logMAR) to 1.0 optotype widths (HOTV and Lea Symbols) (see Table 1.07 for a summary of crowded visual acuity tests and their features).

Test	Uncrowded optotypes used in the main experiment	Example of a crowded display
Kay Pictures		
Lea Symbols		
HOTV	H O T V	V
Cambridge Crowding Cards	H O T V	U A H L C

Figure 1.05: *Uncrowded and crowded optotypes examined by Lalor, Formankiewicz and Waugh, (2016)*

In summary, examination with crowded acuity tests is preferable to examination with isolated optotypes, as crowded tests show steeper and later development than isolated tests and, therefore, may be more sensitive to developmental abnormalities. Optimal test design can maximise crowding effects within acuity tests, by utilising target letters rather than pictures, flanking optotypes rather than bars or boxes (ideally in a Cambridge crowding style arrangement), and reduced target-flanker/inter-optotype spacing of one stroke width.

Due to the variety of features seen within crowded recognition acuity tasks, care should be taken to not generalise normative crowded acuity values but instead be age and test specific for use in clinical diagnostic situations. While these test design recommendations reflect a significant improvement in the optimisation of utilisation of visual crowding within acuity tests, calculation of crowding magnitude is a somewhat circuitous method of clinically quantifying the amount of visual crowding present, requiring the examiner to measure both isolated and optimally crowded acuities to ascertain the magnitude of crowding present. Assessment of crowding in isolation from acuity would allow for distinct quantification of these two visual functions.

1.3.3 Critical spacing of crowding, or crowding distance.

An alternative measure of visual crowding is the minimum distance by which vision is unaffected by the presence of surrounding objects and is known as the ‘critical spacing of crowding’ or the ‘crowding distance’ (CD), a term coined by Waugh et al., (2018).

Table 1.07: *Crowded paediatric visual acuity tests and their various features*

Test	Letters/Symbols	Design	Optotypes per row	Inter-optotype spacing measured edge to edge (optotype units)	Image
Original Crowded Kay Pictures	8 Kay Pictures	Linear with a box surround	4	0.5	
Redeveloped Crowded Kay Pictures	6 Kay Pictures	Linear with box surrounding each picture	5	0.6 (between optotypes) 0.3 (between contour and optotype)	
Sonksen logMAR test	H O T U V X	Linear with a box surround	4	1.0	

Keeler logMAR crowded test (previously Glasgow acuity cards)	H O U V X Y	Linear with a box surround	4	0.5
Cambridge Crowding Cards	Targets: H O T V X Flankers: A C L U	Single optotype surrounded by 4 different flanking optotypes	NA	0.5
Crowded HOTV	H O T V	Linear with a box surround	5	1.0
Crowded Lea Symbols	4 Lea Symbols	Linear with a box surround	5	1.0

When surrounding objects breach the crowding distance, the target may not be correctly identified as both target and flankers are then placed in the same interference zones, as first described by Bouma and discussed above (Bouma, 1970; Pelli and Tillman, 2008; Whitney and Levi, 2011). As these 'interference zones' increase in size with eccentricity from the fovea, crowding distances are easier to quantify in the periphery (Bouma, 1970). Foveal crowding distance is challenging to measure due to its small size and the presence of confounding variables such as optical aberrations, as corneal and lens abnormalities blur the light falling on the fovea, producing pre-neural restrictions to fine spatial perception (Ogle, 1960; Shah, Dakin and Anderson, 2012; Song, Levi and Pelli, 2014).

Previous studies of foveal visual crowding fail to distinguish between lateral masking and visual crowding or used optotypes like standard letters that are spatially too large to assess small crowding distances, such as those found in the fovea. Experimental quantifications of the crowding distance vary from 0.013 to 0.046 degrees measured from the edge of one optotype to the edge of another (studies summarised in Table 1.08). Clinically, it is not possible to use optotypes such as Sloan letters (1:1 width: height ratio) to quantify crowding distance, as the optotype proportions would result in letter overlap at such small distances (measured centre-to-centre) (See Figure 1.06 for an example).

To quantify the crowding distance within the fovea, optotypes of different proportions would need to be designed to allow optotypes to be placed closer together without overlapping. The 'Pelli" optotype (Figure 1.07), with height: width proportions of 5:1, allows for closer optotype positioning (measured centre-to-centre), allowing for the quantification of crowding distance (see Figure 1.08 for an example).

Table 1.08: *A summary of foveal crowding distances according to various previous studies, in ascending date order*.

Study	Target	Flankers	Crowding Distance (CD) in deg (measured edge-to-edge)
Toet and Levi., (1992)	T	T's	0.02 – 0.04 (based on foveal target thresholds of 0.02-0.04°)
Danilova and Bondarko., (2007)	Tumbling E	Tumbling E's	0.029-0.035
Lev, Yehezkel and Polat (2014)	E	E's	0.046
Pelli et al (2016)	Pelli number – single target	Pelli numbers	0.020
Pelli et al (2016)	Pelli numbers – Repeated targets	Pelli numbers	0.027
Coates et al (2018)	Tumbling E	Tumbling E's	0.013-0.022

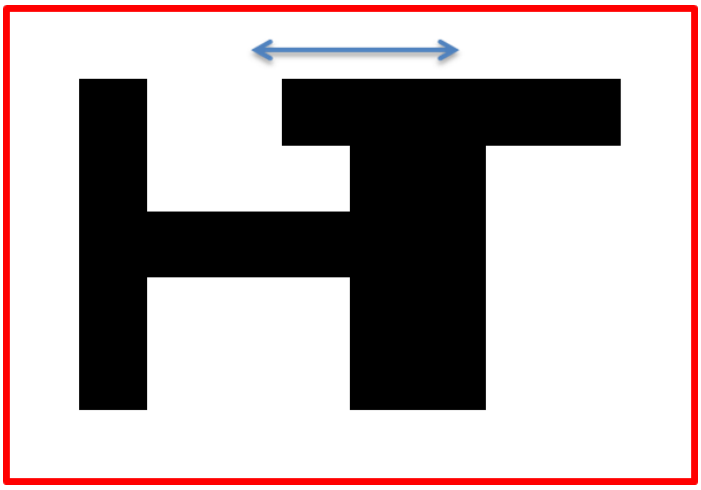


Figure 1.06: *Example of critical spacing of crowding using Sloan letters, measured centre-to-centre, for a small crowding distance.*

Figure 1.07: *The "Pelli" and "Sloan" optotypes, with their respective design proportions – reproduced from Pelli et al (2016)*

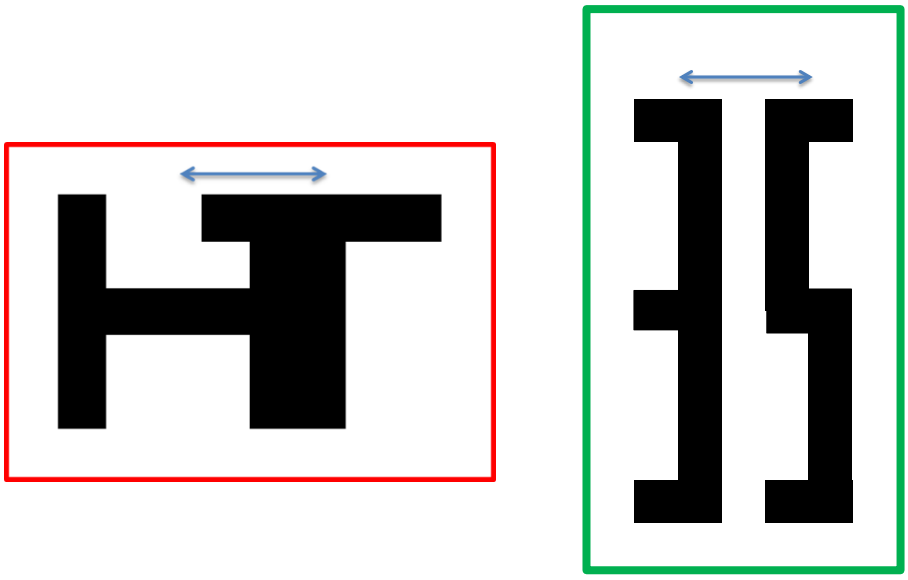


Figure 1.08: *Comparison of critical spacing of crowding using Sloan letters and Pelli optotypes, measured centre-to-centre for a small crowding distance*

Initial investigation of over 200 school-aged children using the Pelli optotype demonstrated maturation of crowding distance to occur around age eight (Waugh et al., 2018). Additionally, Waugh et al, (2018) also measured isolated acuity thresholds for each participant, revealing a difference in the developmental time courses of about two years for these two different visual functions (A summary of crowding distance maturity rate studies can be found in Table 1.09). This disparity in maturation age adds further credibility to the idea that crowding, and acuity are distinct visual functions and, therefore, may be impeded to different extents depending upon the aetiology of the visual dysfunction. The Pelli optotype and crowding distance are discussed in more detail in Chapter five.

In a comprehensive study by Song, Levi and Pelli (2014), the authors also discussed a 'double dissociation' between acuity and crowding. Examination of visually normal participants (*n*=3), pure anisometropic amblyopes (*n*=6), strabismic amblyopes (with and without anisometropia; *n*=12), along with additional data from apperceptive agnosia patients (*n*=20, provided by Strappini et al., 2017) were used to establish and compare isolated foveal visual acuity thresholds (*A*) and threshold spacing (*S*). After examining all participants, the authors established critical threshold values for both acuity (0.15 deg) and spacing/acuity ratio (1.84), which, if exceeded, identified erroneous visual function. Song, Levi and Pelli conclusively demonstrated that acuity and crowding can be differently affected with only impaired acuity (>0.15 deg) seen in anisometropic amblyopia, but only impaired spacing/acuity ratios (>1.84) seen in apperceptive agnosia. Independent assessment of acuity and crowding would allow for a more straightforward differential diagnosis of crowding and/or acuity limited visual conditions, including amblyopia. However, no data currently exists on the crowding distance of paediatric amblyopes using this new Pelli optotype.

Table 1.09: *A summary of foveal crowding distance maturation age according to various previous studies, in ascending order of age*.

Study	Participants	Target and flankers	Age of foveal crowding maturation
Waugh et al., (2018)	201 children (age range 3-11 years)	Target: Pelli numbers Flankers: Pelli numbers	8 years
Semenov, Chernova and Bondarko (2000)	140 children (age range 3-9 years)	Target: Landolt C Flankers: Bars	9 years
Bondarko and Semenov., (2005)	292 children (age range: 8-17 years)	Target: Landolt C Flankers: bars	10 years
Jeon et al., (2010)	78 participants (age range 5 years – adult)	Target: Sloan E Flankers: Bars	>11 years
Bondarko and Semenov., (2005)	292 children (age range: 8-17 years)	Target: Sloan E Flankers: Sloan E	12 years
Bondarko and Semenov., (2005)	292 children (age range: 8-17 years)	Target: Gratings Flankers: Gratings	12 years
Semenov, Chernova and Bondarko., (2002)	215 participants (age range 3-20 years)	Target: Gratings Flankers: Gratings	Gradual decrease in CD up to age 20 years

1.4 Amblyopia

Amblyopia is characterised as a loss in visual acuity of the affected eye(s), as well as compromised or absent binocular single vision, without any underlying ocular disease, irregularity or visual pathway abnormality (Von Noorden, 1974; Hillis, Flynn and Hawkins, 1983; Attebo et al., 1998; Holmes and Clarke, 2006; Sengpiel, 2013; Solebo and Rahi, 2013; Hess and Thompson, 2015). It is a neurodevelopmental visual disorder, a form of cerebral visual impairment caused by blurred images or loss of binocular single vision in the affected eye (McKee, Levi and Movshon, 2003; Snowling and Davidoff, 1992), the most common cause of childhood visual impairment (Holmes and Clarke, 2006; Wong, 2012; Solebo and Rahi, 2013; Daw, 2014), and the presence of amblyopia increases an individual's lifetime risk of bilateral visual impairment from 10% to 18% (van Leeuwen et al., 2007). In addition to visual dysfunction, children with amblyopia demonstrate reduced fine and gross motor skills (Webber et al., 2008b; Webber, Wood and Thompson, 2016; Niechwiej-Szwedo, Colpa and Wong, 2019), compromised reading proficiency (Birch et al., 2015; Kelly et al., 2017; Webber, 2018) as well as lower self-esteem and reduced self-perceived social acceptance (Webber et al., 2008a; Birch et al., 2019).

It is considered that the factors that contribute to the development of amblyopia change with age; with strabismus being the predominant factor in infants (<1yr) (Birch and Holmes, 2010) and anisometropia and mixed presentations increasing with age (Repka et al., 2002; Birch and Holmes, 2010; Birch, 2013) (See Table 1.10 for a summary of amblyopia presentation proportions). Rarely an amblyogenic factor cannot be found; therefore, amblyopia is a diagnosis of exclusion, only being diagnosed once all other structural irregularities of the eye and visual pathway have been discounted (Flom and Neumaier, 1966).

Successful treatment of amblyopic vision relies on early intervention and good treatment compliance (Nucci et al., 1992; Loudon, Polling and Simonsz, 2003; Fronius et al., 2014; Vagge and Nelson, 2017); with denser amblyopia and late diagnosis associated with poorer treatment compliance (Oliver et al., 1986; Nucci et al., 1992) and poor compliance leading to reduced visual improvement (Woodruff et al., 1994; Newsham, 2000; Awan, Proudlock and Gottlob, 2005). Current evidence-based amblyopia treatment regimens consist of refractive correction (where required), followed by occlusion or optical/pharmacological penalisation of the non-amblyopic eye to strengthen the visual acuity of the amblyopic eye (Repka et al., 2002; Cotter, 2006; PEDIG, 2010; Gunton, 2013).

In the UK, timely detection of amblyopia is mediated via paediatric vision screening programmes (Rahi et al., 2002; Solebo, Cumberland and Rahi, 2015; Bruce et al., 2016), with orthoptic-led visual screening considered the 'gold-standard' (Bolger et al., 1991; Spowart, Simmers and Tappin, 1998); although with additional training, nurse-led visual screening also demonstrates high sensitivity and specificity for amblyopia detection (Mathers, Keyes and Wright, 2010). A systematic review (Schmucker et al., 2009) of 27 studies of visual screening programmes indicated that sensitivity to detection of amblyopia increases with age; however, amblyopia treatment outcomes correspondingly decrease with the age of treatment initiation (Chou, Dana and Bougatsos, 2011), therefore visually screening children at four to five years of age is considered both clinically effective and financially viable (Tailor et al., 2016), compared with earlier, more intensive visual screening (Williams et al., 2001). In addition, examination of these older, school-aged children (age four to five) offers benefits of increased levels of attention and letter identification compared with younger children; beyond age four to five, these same testing benefits offer no further advantage and yield poorer treatment outcomes, especially for severe and moderate amblyopes (Solebo and Rahi, 2013).

While younger treatment initiation yields better visual outcomes in amblyopes, compliance with visual acuity tests can be variable and inconsistent in younger children (<36months) (Chia et al., 2010). Isolated optotype testing can deliver greater engagement and, therefore, testability (Stuart and Burian, 1962; Flom, Weymouth and Kahneman, 1963; Woodruff, 1972; Rodier, Mayer and Fulton, 1985; Morad, Werker and Nemet, 1999). However, in individuals with amblyopia, studies have repeatedly demonstrated increased visual crowding resulting in greater reductions in visual acuity when targets are crowded, compared with visually normal individuals (Mayer and Gross, 1990; Morad, Werker and Nemet, 1999; Hess et al., 2001; Levi, Hariharan and Klein, 2002b).

This increased amblyopic sensitivity to visual crowding within crowded acuity tests results in poorer acuity thresholds and, consequently, amplified interocular differences. This highlights the presence of the amblyopia, which is ideal for early amblyopia identification and referral; therefore, crowded logMAR-based acuity tests are recommended for vision screening (Spowart, Simmers and Tappin, 1998; Solebo and Rahi, 2013; Cotter et al., 2015). Suitable pre-literate crowded visual acuity tests could further enhance amblyopia detection within visual screening programmes; however, there are currently no commercially available crowded visual acuity tests that meet the recommendations given by Lalor (2018) (i.e., letter optotype identification, in a Cambridge crowding style arrangement, with one stroke width (edge-to-edge) target-flanker spacing (see Table 1.07 above)).

1.4.1 Amblyopic subtypes

Due to the variety of amblyopic aetiologies, differences in amblyopic visual function loss patterns are seen between different subgroups (Levi and Klein, 1982a; b, 1985). As such, amblyopia cannot be considered a single condition defined solely by reduced visual acuity (McKee, Levi and Movshon, 2003; Roper-Hall, 2007). A selection of affected visual

functions are briefly explored below, focusing on the recognised differences in acuity and crowding between uniocular anisometropic, strabismic, and mixed strabismic and anisometropic amblyopia subtypes.

1.4.2 Acuity and crowding differences between amblyopia subtypes

Differences in the severity and pattern of visual acuity loss have been noted for strabismic versus anisometropia amblyopia (Gestalder and Green, 1971; Levi and Klein, 1982a; b; Mayer, Fulton and Rodier, 1984; Levi, Klein and Yen Lee Yap, 1987; Levi and Klein, 1990; Birch and Swanson, 2000; McKee, Levi and Movshon, 2003; Hamm et al., 2014). In a large sample study by Mckee, Levi and Movshon (2003), participants (n=495) were grouped by clinical features (see Figure 1.09), and unilateral examination of grating, optotype and vernier acuities was conducted. As per previous studies, while anisometropic amblyopes demonstrated deficits in grating acuity that were almost proportional to optotype and vernier acuity, strabismic amblyopes show greater losses of optotype and vernier, compared with their losses of grating acuity (Levi and Klein, 1982a; b; McKee, Levi and Movshon, 2003).

These functional differences in visual acuity performance mirror the results of both Levi and Klein (1982c) and Birch and Swanson (2000), and are considered to occur due to differences in the development of binocular single vision (McKee, Levi and Movshon, 2003). Evidence for theory this can be seen in observations of anisometropic and strabismic amblyopes, with severe visual loss. In the presence of dense amblyopia, the retained binocular vision functions usually seen in mild and moderate anisometropic amblyopes, are disrupted. This subsequently results in poorer vernier/hyperacuity functions, resembling more closely the visual functions of strabismic amblyopes (McKee, Levi and Movshon, 2003).

Table 1.10: *Proportional presentation of unilateral amblyopia subtypes, as reported by various studies in ascending date order.*

Study	Participants	Anisometropia	Strabismus	Stimulus deprivation	Mixed	Other
Quah et al.,(1991)	n=48 amblyopic adults aged 18-19 years	50%	18.7%	-	16.7%	14.5% astigmatism
Attebo et al.,(1998)	*n*=3647 adults	50%	19%	4%	27%	-
Repka et al.,(2002)	*n*=409; Children 2.6 – 6.9 years	~40%	~40%	-	~20%	-
Friedman et al., (2009)	*n*=18 uniocular amblyopic children aged 6 – 71 months	33%	33%	5%	11%	16% Isoametropic amblyopia
Birch and Holmes (2010)	*n*=250; Children <3yrs	5%	82%	-	13%	-
Chia et al., (2010)	n=14 amblyopic children aged 6-72 months	79%	21%	0%	0%	-
Yekta et al.,(2017)	Total n = 1130; Children aged 6 – 15 years	45.2%	29%	-	16.1%	9.7% Isometropic amblyopia
Hansen et al.,(2019)	N=20 amblyopes; Children 10.6-12.8years	37%	35%	-	-	28% astigmatism

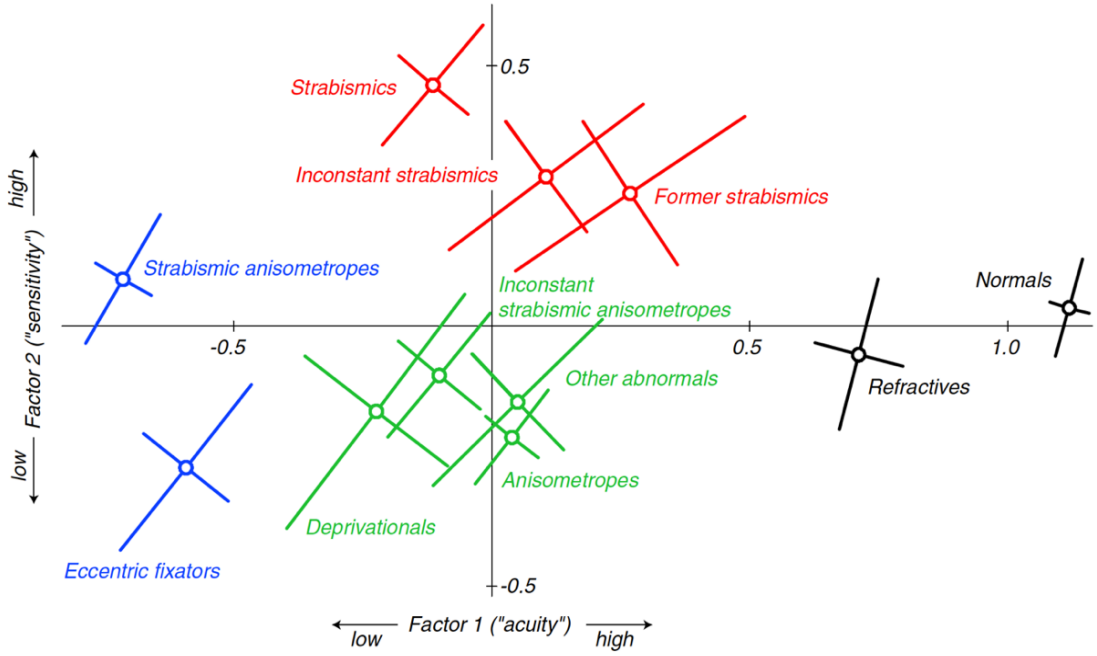


Figure 1.09: *Behavioural locations of 11 different clinical groups (compiled into four key categories: normal/near-normal (black), moderate acuity loss with superior contrast sensitivity (red), moderate acuity loss with impaired contrast sensitivity (green) and severe acuity loss (blue)), respective to visual acuity and contrast sensitivity. Reproduced from McKee et al. (2003).*

Finally, regarding mixed strabismic-anisometropic amblyopes, mixed strabismic-anisometropic amblyopes disproportionately represent amblyopes with dense amblyopia (Ciuffreda, Levi and Selenow, 1991; McKee, Levi and Movshon, 2003). This increased acuity loss can be explained via the combination of acuity disrupting mechanisms experienced by this subgroup, namely generalised optical blur (seen in anisometropic amblyopes) and central retinotopic suppression, with disruption of binocularly driven cortical neurons (seen in strabismic amblyopes) (Wiesel and Hubel, 1963; Sireteanu and Fronius, 1981). Therefore, the different amblyogenic factors result in differing neuro-anatomical adaptations during visual development, leading to distinctive functional visual acuity anomalies.

In addition to acuity, research has also demonstrated that subtypes of amblyopia respond differently to the presence of crowding. While strabismic and mixed amblyopes demonstrate statistically significant crowding (calculated as acuity threshold elevation between isolated and crowded test presentations), anisometropic amblyopes do not (Morad, Werker and Nemet, 1999; Bonneh, Sagi and Polat, 2004; Formankiewicz and Waugh, 2013). Furthermore, using dioptric blur (0-4D) to simulate anisometropia amblyopia, Formankiewicz and Waugh (2013) examined crowding effects in six commercially available (two isolated (Sheridan-gardener and single Kay Pictures), four crowded (LogMAR, Crowded Kay pictures, Cambridge Crowding cards and Sonksen logMAR test)) visual acuity tests. Foveal crowding magnitude reduced significantly in the presence of blur ($p=0.031$), with the greatest foveal crowding effects seen in the unblurred fovea with abutting flankers (target flanker separation=0). The authors concluded that visual acuity in simulated anisometropic amblyopes is not mediated by crowding but instead is more akin to dioptric blur of the normal fovea. This blur/crowding disparity or 'double-dissociation' between these two common amblyopic subtypes was also exposed by Song, Levi and Pelli (2014), who demonstrated increased spacing/acuity ratios ($S/A > 1.84$) in strabismic and mixed strabismic-anisometropic amblyopes, comparable to the visuospatial behaviour of normal peripheral fixation. Anisometropic amblyopes, in comparison, consistently demonstrated a smaller influence of crowding, with spacing/acuity ratios <1.84.

In summary, perceptual differences occur between anisometropic and strabismic amblyopes; anisometropic visual deficits arise due to chronic retinal blur and under-sampling of the retinal image, while strabismic amblyopes visual deficits result from increased visual crowding, and positional uncertainty due to loss of binocularity (Blakemore and Vital-Durand, 1992; Kiorpes and McKee, 1999; Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014).

1.5 Beyond first-order; Second-order stimuli

Commercial visual acuity charts display black optotypes of various sizes on white backgrounds, which are visible due to the luminance difference between the object and the background. These are known as first-order images and can be seen clearly on Fourier spectral analysis (Sutter, Sperling and Chubb, 1995) (Figure 1.10).

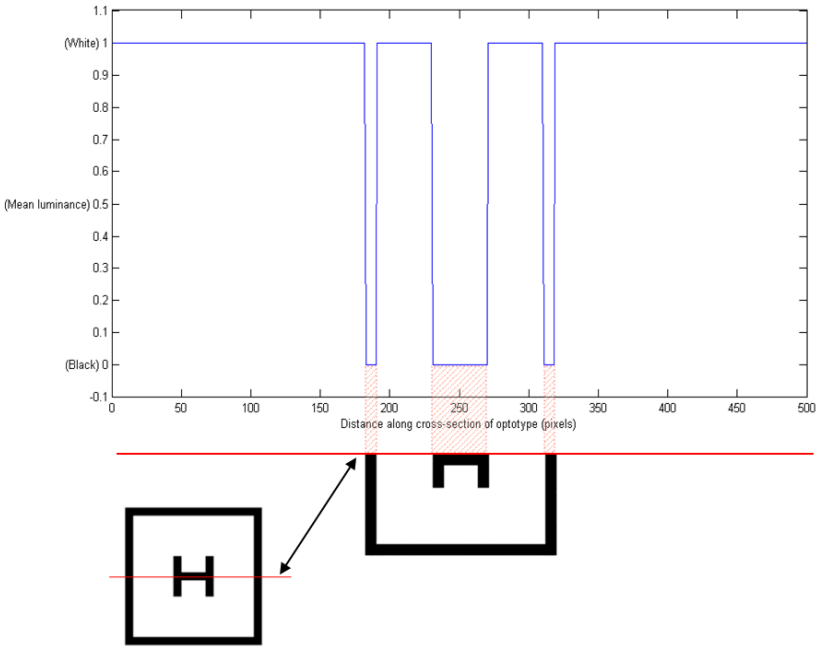


Figure 1.10*: Luminance profile of a first-order luminance-defined letter "H" with box surround. Reproduced with permission from Lalor (2018).*

While high contrast luminance charts provide valuable data about spatial resolution, we must consider that objects in the natural world are defined by more than 'spatiotemporal variations of luminance' (Vaina, Cowey and Kennedy, 1999). Second-order stimuli, defined by variations in other features, such as contrast, motion and texture, offer additional information to the viewer (Chubb and Sperling, 1988; Cavanagh and Mather, 1989; Baker, Boulton and Mullen, 1998; Baker, 1999; Vaina, Cowey and Kennedy, 1999; Baker and

Mareschal, 2001; Wong, Levi and McGraw, 2001; Sukumar and Waugh, 2007; Hairol, Formankiewicz and Waugh, 2013). Within certain environmental visual conditions, such as shadows (Daugman and Downing, 1995) or the perception of glossy surfaces (Motoyoshi et al., 2007), this second-order information is extremely valuable to image perception; however, additional extra-striate neural processing is required for second-order stimulus perception due to its increased complexity (Chubb and Sperling, 1988; Cavanagh and Mather, 1989; Faubert, 2002; Larsson, Landy and Heeger, 2006; Li et al., 2014).

Contrast-defined images display no mean luminance differences between the background and the target object (Figure 1.11). Consequently, optotypes can only be extracted by the visual system using differences in contrast without consistent luminance cues. Studies examining the perception of contrast-modulated stimuli have identified binocularly driven cortical areas (such as V2) (Von Der Heydt, Peterhans and Baumgartner, 1984; Leventhal et al., 1998; Bullier, 2001; Larsson, Landy and Heeger, 2006; Schira et al., 2009) as the potential extra-striate loci; evidence for this arises from feline (Zhou and Baker, 1994; Leventhal et al., 1998; Mareschal and Baker, 1998), primate single-cell response studies (Leventhal et al., 1998; Baker and Mareschal, 2001; Li et al., 2014), as well as human VEP (Calvert et al., 2005), psychophysical (Wong, Levi and McGraw, 2005; Hairol and Waugh, 2010b) and fMRI studies (Larsson, Landy and Heeger, 2006; Ashida et al., 2007).

As cells within the striate cortex (V1) are spatially selective for luminance, they are unable to detect second-order contrast-defined stimuli in isolation (Faubert, 2002). Second-order stimuli, therefore, require additional extra/post striate processing, involving areas such as V2 (Li et al., 2014), V4 and MT; VO1, V3A/B and LO1 (Larsson, Landy and Heeger, 2006). This additional processing requirement results in elevated visual thresholds (Habak and Faubert, 2000).

1.5.1 CM-optotype acuity thresholds

Isolated acuity thresholds are shown to be significantly poorer for CM, than luminance-modulated (LM) optotypes ($p<.001$) in both visually normal children (Lalor, 2018) and adults (Woi et al., 2016). Later adult-like visual acuity maturation is seen for CM optotypes (9.7 ± 1.2 years) than for L (8.0 ± 1.1 years) and LM optotypes (7.9 ± 1.1 years) (Lalor, 2018). Studies of young and elderly adults also demonstrate earlier, but slower decline of CM defined optotype visual acuity thresholds compared with LM (Tang and Zhou, 2009), with reduced binocular CM visual acuity thresholds seen in elderly participants (54.0±1.83 years old) compared with younger participants (25.4±1.29 years old) (Woi et al., 2016; Woi, Sharanjeet-Kaur and Hairol, 2019).

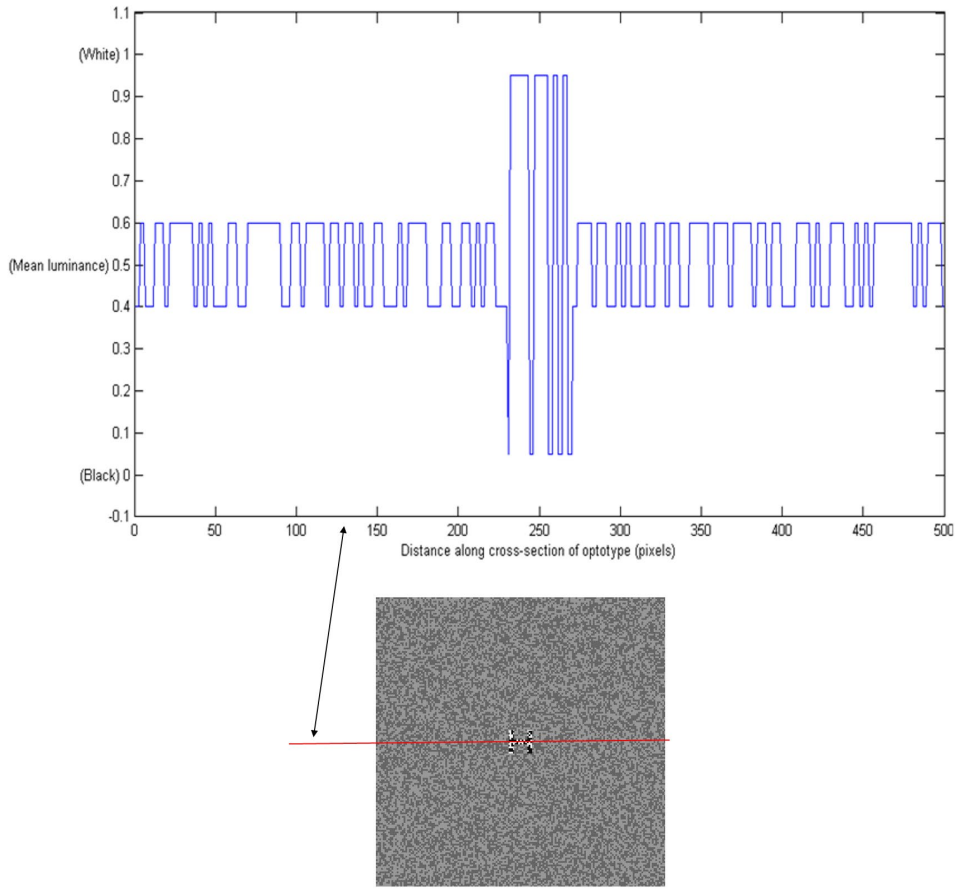


Figure 1.11: *Luminance profile of a second-order contrast-defined isolated letter "H". Reproduced with permission from Lalor (2018).*

Differences in visual acuity development and deterioration between first and second-order optotypes, support Daw's theory (Daw, 1998) that increasingly complex, extra-striate visual processing develops later, and therefore may be more sensitive to anomalous visual input. The use of contrast-modulated optotypes may therefore be a more sensitive measure of higher neurological visual deficits, than first-order luminance defined optotypes.

1.5.2 Second-order processing in amblyopia

Recent evidence suggests that amblyopia is not confined to the primary visual cortex, but that visual processing deficits due to amblyopia extend to the extra-striate dorsal ('where') and ventral ('what') processing pathways in amblyopes (Simmers et al., 2006; Gao et al., 2015) and may be more exaggerated within the extra-striate cortex (Wong, Levi and McGraw, 2001). Studies of second-order stimuli in amblyopes have aimed to discover whether amblyopes display deficits of detecting second-order stimuli. In 2001, Wong, Levi and McGraw's examination of contrast thresholds of both first and second-order sinusoid stimuli in five amblyopic adults, demonstrated some evidence for increased loss of second-order spatial information, compared with first-order spatial input, with the authors suggesting a possible magnification of neurological deficits seen in early processing (V1). Deficits of spatial stimuli defined by second-order features of contrast, orientation and motion have all been identified in amblyopic adults (Gao et al., 2015), although psychophysical (Simmers et al., 2003, 2006; Simmers, Ledgeway and Hess, 2005) and fMRI imaging studies (Barnes et al., 2001; Li et al., 2007) support findings that the extent to which these different second-order functions are diminished to, is varied (Simmers et al., 2011). Second-order visual dysfunctions are considered to occur in amblyopes due to defective output fed forward from the linear processing of spatial luminance in V1, which itself develops abnormally because of anomalous childhood visual input (Sharma, Levi and Klein, 2000; Wong, Levi and McGraw, 2001; Hariharan, Levi and Klein, 2005). It is further hypothesised that normal binocular single vision is required for the development of "optimal second-order visual

processing" (Wong, Levi and McGraw, 2001; Thibault et al., 2007), and consequently it is theorised that amblyopes may show greater deficits within extra-striate areas in response to abnormal visual input (Chung, Li and Levi, 2007, 2008a; Chima, Formankiewicz and Waugh, 2015).

1.5.3 Crowding in Second-Order Stimuli

As visual crowding is exaggerated in amblyopic eyes using first-order luminance-based optotypes, several studies examining visual crowding using second-order stimuli have been conducted to ascertain their usefulness in amblyopia diagnosis and monitoring. Examination of six amblyopes, five visually normal controls and two non-amblyopic strabismics (all adults) by Wong, Levi and McGraw (2005) demonstrated a disparity of responses to the collinearly or orthogonally flanked second-order contrast-defined target. While target facilitation was recorded for visually normal participants at target-flanker separations of 4λ (13% ± 4% for collinear flanks and 11% ± 4% for orthogonal flanks), the amblyopic and strabismic observers demonstrated increased contrast threshold ratio's (suppression), at the same separation (Wong, Levi and McGraw, 2005). Non-amblyopic/fellow eyes also showed suppression of similar magnitude, with binocular amblyopes demonstrating the lowest suppressive effects. These differences in second-order spatial interaction in amblyopes reflect deficits in the principally binocular V2 cortical area (Hubel and Livingstone, 1987; Wong, Levi and McGraw, 2005).

Moving onto discriminability of crowded CM targets, studies by Chung and colleagues (Chung, Li and Levi, 2007, 2008a) have revealed that in visually normal and amblyopic adults, a greater magnitude of crowding occurs for second-order target stimuli compared with first-order stimuli. Specifically, in amblyopes, calculations of crowding magnitude demonstrated more substantial crowding effects for CM stimuli (1.44±0.15 and 1.45±0.21 for amblyopic and fellow eyes, respectively) than for first-order stimuli (1.11±0.03 and

1.03±0.03 for amblyopic and fellow eyes respectively). This enhanced crowding seen with CM presentation may be useful for amblyopic visual screening purposes, to amplify interocular differences and further highlight the presence of amblyopia. However, it is worth noting that this study utilises a small number of adult amblyopic participants, which may not accurately reflect the visual behaviours of amblyopic children still undergoing visual maturation. It is also difficult to distinguish between the behaviours of the different amblyopic subtypes. In the case of Chung, Li and Levi (2008a), of the seven amblyopes observed, all but one demonstrated a manifest strabismus, meaning these results may not be generalisable to all amblyopes but may be more biased towards those amblyopes with defective/absent binocularity.

More recently, crowded CM optotypes have been examined within visually healthy children, using the same optotypes and arrangements as seen in Figure 1.05 (Lalor, 2018). While crowded acuity thresholds were higher for CM optotypes than L and LM optotypes, CM crowding magnitude was not significantly different from the magnitude of contour interaction nor significantly different from adult crowding magnitude. Initial studies into amblyopic crowded CM perception have so far only occurred in adults. To establish the usefulness of second-order crowded acuity thresholds in amblyopia detection and management, it is essential to establish the CM psychophysical visual acuity responses of amblyopic children and to further distinguish whether a difference in response is seen between binocular (pure anisometropic) and non-binocular (strabismic and mixed) amblyopic subtypes.

1.6 Summary

In this project, suggestions put forth by Lalor, Formankiewicz and Waugh (Lalor, Formankiewicz and Waugh, 2016) regarding the enhanced inter-optotype distance of one stroke width within an acuity test will be examined for the first time in an amblyopic population and compared to a current commercially available crowded acuity test. The

following studies aim to establish whether reduced optotype spacing might lead to enhanced detection of amblyopia. While crowding magnitudes of both luminance (L) and contrast-modulated (CM) Cambridge crowding test stimuli have been established for normal children aged 3-16 by Lalor (2018), the effects of enhanced crowding on strabismic, anisometropic and mixed amblyopes have not yet been studied. Therefore, it would be advantageous to establish whether decreased optotype spacing and second-order optotype presentation result in stronger crowding in amblyopes than a control group of age-matched visually healthy children. This information could help inform future visual acuity test design by maximising their sensitivity to crowding, such as in amblyopia. Furthermore, improving earlier detection will help to provide better visual outcomes for amblyopic individuals.

In the first of two experiments, visual acuity is measured in both amblyopic and visually healthy children using both isolated letters and a modified Cambridge-crowding test with a target-flanker spacing of one stroke width, using optotypes displayed with standard luminance (L); referred to as the enhanced Cambridge crowding test (L-ECC). In the second experiment, visual acuity is examined again using both isolated letters and a modified Cambridge-crowding test displayed with contrast-modulation (CM), with target-flanker spacing set at a spacing of one stroke width; referred to as the contrast-modulated Cambridge crowding test (CM-ECC).

Foveal crowding distances of amblyopic children have not yet been examined using the ‘Pelli’ optotype. Therefore, in the third and final experiment, foveal crowding distance is measured using the new and novel ‘Pelli’ optotype (displayed in both a trigram and repeated optotype format), using the same cohort of children enrolled in Experiments one and two. Comparing the amblyopic thresholds with those of the visually healthy control group will establish the usefulness of this test as a clinical tool for diagnosing and monitoring amblyopia for the first time.

1.7 Primary research questions

Research question:

"Do new vision test modifications offer better sensitivity to detection of interocular difference leading to improved detection of amblyopia, than the Sonksen logMAR test (SLT)?

Hypotheses

Null hypothesis one ($H1_O$): The new crowding tests do not offer better sensitivity to interocular differences in acuity in amblyopic children, than the Sonksen logMAR test (SLT).

Alternative hypothesis one ($H1_a$): The new crowding tests offer better sensitivity to interocular differences in acuity in amblyopic children, than the Sonksen logMAR test (SLT).

Null hypothesis two ($H2_O$): There is no difference in the sensitivities of the new tests to detecting strabismic amblyopia, versus anisometropic amblyopia.

Alternative hypothesis two ($H2_a$): There is a difference in the sensitivities of the new tests to detecting strabismic amblyopia, versus anisometropic amblyopia.

Chapter Two – General Methodology

2.1 Introduction

The purpose of the following studies was to psychophysically assess visual acuity and foveal crowding distance in visually healthy and amblyopic children. This chapter describes methods for the three main experiments examining the L-ECC, CM-ECC and the Pelli foveal crowding distance test. Details of any pilot studies can be found in each corresponding dedicated experimental chapter.

Visually healthy and amblyopic (anisometropic and strabismic/mixed) children were sought to complete all three main studies. This allowed for the recruitment and testing of sufficient numbers of amblyopic participants to satisfy the power calculations for all three studies within a limited time period (see **2.2.2** for power calculations). It also allowed for the additional benefit of direct comparison between the three studies.

2.2 Materials and Methodology

2.2.1 Apparatus

Presentation and control of visual stimuli used custom-written MATLAB programmes (MathWorks™, Natick, USA), the 'Psychophysics Toolbox Version 3 (PTB-3)' and a 15-inch MacBook Pro with Retina display (spatial resolution: 2880x1800 pixels, framerate: 60Hz) with operating system macOS HighSierra (version 10.13.6). Previous research has used Cathode ray tube (CRT) monitors; however, liquid crystal display (LCD) monitors are now considered suitable replacements for CRT as they have demonstrated consistency of brightness for both horizontally and vertically orientated gratings (Wang and Nikolić, 2011), as well as requiring shorter warm-up periods to obtain stability of luminance (Fletcher and

Sutherland, 2009; Poth and Horstmann, 2017). CRT displays' physical size and weight are disadvantages compared to the lighter and more portable LCDs for visual screening.

2.2.1.1 Gamma correction

To present the optotypes at the required contrast levels, gamma correction of the MacBook pro screen was essential to conduct. Gamma correction was performed with a ColorCal MKII Colorimeter (Cambridge Research Systems), measuring 768 luminance levels to ensure correct screen calibration and a linear output of luminance's. For the CM stimuli, dynamic noise was used to reduce further the potential of luminance artefacts affecting psychophysical measures (Smith and Ledgeway, 1997; Bertone et al., 2010). A lack of gamma calibration and its influence on acuity thresholds was tested in one participant (LH) to demonstrate the importance of careful calibration. Visual acuities were measured using calibrated and non-calibrated Isolated-CM (CM-Iso) and CM-ECC stimuli. Acuities reported (see Table 2.01) are the mean of four runs of the acuity program.

Table 2.01: *Mean and SE acuity thresholds (logMAR) of CM-iso and CM-ECC presentations for two different display formats.*

	CM-Iso threshold	**CM-ECC threshold**
Gamma corrected staircase	0.150±0.010	0.279±0.011
Non-gamma corrected staircase	-0.293±0.006	-0.032±0.007

Comparisons revealed that non-gamma corrected acuities were significantly lower (better) (**p<.001**) than gamma-corrected acuities for both isolated (by 0.443±0.025 logMAR) and crowded (by 0.311±0.30 logMAR) arrangements, highlighting the importance of careful calibration.

Without careful calibration of computer screens, acuity measured using CM stimuli yields luminance artefacts, which allows for much higher acuity levels to be attained. These are not, then, CM acuities at all.

Other studies comparing visual acuity thresholds for LM and CM stimuli also indicate differences between 0.3 - 0.5 logMAR, worse with CM stimuli (Woi et al., 2016; Lalor, 2018). This fundamental difference is due to increased spatial summation areas for CM versus LM stimuli (Sukumar and Waugh, 2007). All further results obtained in this study were obtained with careful calibration and gamma correction.

For all three main experiments, a wireless remote keyboard was employed to allow the examiner to sit close to the participants while inputting participant responses.

A PlusoptiX A12C auto-refractor [Plusoptix GmbH 2017; Nuremberg, Germany] (Figure 2.01) was used to screen for the presence of any significant undiagnosed refractive errors as defined by the ABCD criterion (Clausen and Arnold, 2007) (Table 2.02), in control participants who did not have a habitual prescription. In addition, all amblyopes had undergone cycloplegic refraction performed by a qualified optometrist or ophthalmologist.

The Sonksen logMAR Test (Salt et al., 2007; Sonksen et al., 2008) (booklets C and D -linear displays) and corresponding matching card (Figure 2.02) was used to assess recognition visual acuity and provide a baseline clinical visual acuity with a commercially available acuity test. The SLT is also the acuity test primarily utilised for the visual acuity assessment of children at Addenbrookes Hospital.

Figure 2.01*: Left: Patient facing aspect of the PlusoptiX A12C auto-refractor, Right: Examiner facing aspect of the PlusoptiX A12C auto-refractor.*

Table 2.02*: ABCD-preferred autorefraction referral criteria* (Clausen and Arnold, 2007)

	Hypermetropia	Myopia	Astigmatism	Anisometropia
Referral criteria	>2.50D	>2.00D	>1.50D	≥1.00D

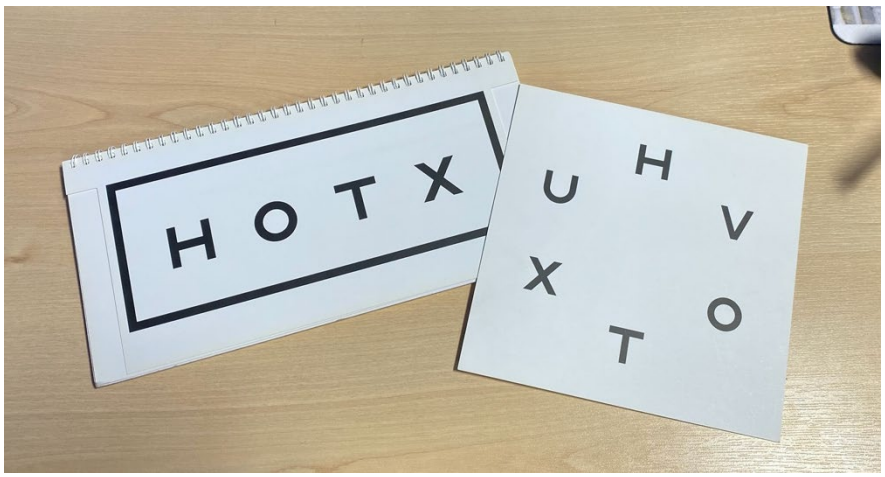


Figure 2.02*: Sonksen logMAR linear test and corresponding matching card.*

Optotypes for the three test presentations, the 'Enhanced Cambridge Crowding Test' (L-ECC), 'Contrast-modulated Cambridge Crowding Test' (CM-ECC) and the Pelli 'Crowding Distance Test' were each printed onto respective matching cards (Appendix one), allowing participants who did not wish to verbalise their response, a way of indicating it simply (Ciner et al., 2003; Shah et al., 2012; Anstice and Thompson, 2014).

The commercially available Frisby Stereotest (Stereotest Ltd, Sheffield, U.K) was also used to quantify each participant's stereoacuity.

All acuity and crowding distance measures were conducted monocularly. A variety of total occlusion options were available to participants (Figure 2.03), as this allowed for greater compliance with occlusive wear for the duration of the study.

Figure 2.03*: Children modelling three different occlusion options.*

As with a previous study conducted in a primary school in Cambridge (Waugh et al., 2018), a 'research passport' and stickers were given/offered to all participants to improve engagement and compliance with the examination. Stamps and stickers were added as each presentation was completed.

2.2.2 Sample Size

G*Power (version 3.1.9.2) and a one-way ANOVA were used to estimate the required sample size. Using an effect size, *Cohen's f* is set at 0.4 (for a "large" effect size), the significance level or probability of committing a type 1 error (incorrect rejection of null hypothesis) was set at 0.05; a minimum sample size of *n=21* participants in each group (Control, anisometropic amblyopes and strabismic/mixed amblyopes) was required to achieve a study power of 80%, for a total minimum sample size of *n=63*.

2.2.3 Participants

Inclusion and exclusion criteria for all experiments can be seen in Table 2.03. In total, 76 participants (*n=51* amblyopes (n=*26* anisometropic amblyopes and n=*25* strabismic/mixed amblyopes) and *n*=25 visually healthy controls) were recruited. All participants were children (mean age 6.4 years, range 3-11 years). Eight participants (one control and seven amblyopes) were excluded. The excluded control had high bilateral hypermetropia and had previously been under the observation of an orthoptic department for possible bilateral amblyopia. Seven amblyopes were excluded due to poor compliance with their refractive correction, insufficient anisometropia, Ectopia Lentis, Haemangioma, alternation of amblyopic eye and non-compliance with testing.

Participants in the control group (n=24) were visually healthy volunteers recruited via social media and word-of-mouth. Control participants had no known visual dysfunction and wore their habitual prescription if previously prescribed.

Amblyopic participants (n=44) were recruited from the Addenbrookes Clinical Paediatric Ophthalmic Service (ACPOS). Each participant had a diagnosis of unilateral amblyopia by the Addenbrookes Hospital (Cambridge) Ophthalmology team, $\geq$0.1 logMAR interocular

visual acuity difference (Cleary and Reinecke, 2001; Stewart et al., 2002, 2004; Lai et al., 2011; Hansen et al., 2019) and had undertaken a minimum of six weeks of refractive adaptation (Flynn and Cassady, 1978; Clarke et al., 2003).

Table 2.03: *Inclusion and exclusion criteria*

Inclusion Criteria	**Amblyopic participants:** Male and female 3 to 11-year-old children diagnosed by ACPOS clinicians as amblyopic (anisometropic or strabismic/mixed). Participants must have completed 6 weeks (or more) of refractive adaption, if correction required. **Control Participants:** Male and female 3 to 11-year-old children who have been falsely referred to the Hospital Eye Service (ACPOS) by the visual screening service or healthy volunteers who have satisfactory visual functions, as per the national screening guidelines. Must have completed 6 weeks (or more) of refractive adaption if correction required. **All participants** must be able to complete the Sonksen logMAR Test (SLT) either verbally or via the use of a matching card.
Exclusion criteria	Uncorrected refractive error. The presence of any other vision limiting medical conditions not listed in the inclusion criteria. Any prior or existing medical history of epilepsy or seizures.

Classification criteria of amblyopia subtypes can be seen in Table 2.04. All amblyopic participants were required to wear their prescribed refractive correction throughout the examination (if given) and had a satisfactory fundus examination undertaken by either an Ophthalmologist or Optometrist.

Clinical information for all included participants (*n=68*) can be seen in Table 2.05.

Table 2.04: *Classification criteria for the amblyopic groups*

Amblyopia	≥0.1 logMAR interocular difference (Elliott and Firth, 2009)
Anisometropic amblyopia	Presence, or recorded history of ≥1.00 dioptre interocular difference, in any meridian (Ingram, 1979; Attebo et al., 1998; Huynh et al., 2006; Friedman et al., 2009; Barrett, Bradley and Candy, 2013). No manifest strabismus with prescribed refraction.
Strabismic/mixed amblyopia	Presence of constant or intermittent manifest strabismus, for near and/or distance fixation with prescribed refraction. With or without the presence (or recorded history) of ≥1.00 dioptre anisometropia, in any meridian (Ingram, 1979; Attebo et al., 1998; Huynh et al., 2006; Friedman et al., 2009; Barrett, Bradley and Candy, 2013).

Table 2.05: *Background Information for all included paediatric participants.*

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses if worn)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
1	3	M	Anisometropic		Near – No apparent deviation (NAD) Distance - NAD	R: +5.50/-0.25x90 L: +1.75/-0.25x180	+0.125 +0.025	0.100	170
2	8	F	Control			RPO: +0.50/-0.50x1 LPO: +0.50/-0.25x55	-0.100 -0.075	0.025	40
3	6	M	Control			RPO: +0.75/-0.50x101 LPO: +1.25/-0.75x79	+0.050 +0.050	0.000	170
4	9	M	Strabismic	Left esotropia	Near - 15^LET Distance - 15^LET	R: +0.50DS (not prescribed) L: +1.00/-0.50x170 (not prescribed)	-0.175 +0.475	0.650	negative
5	8	F	Control			R: -1.25DS L: -1.00DS	-0.075 -0.075	0.000	55
6	4	M	Control			RPO: Plano LPO: Plano	-0.025 0.000	0.025	40
7	10	F	Control			RPO: Plano/-0.25x62 LPO: +0.25/-0.25x78	-0.200 -0.200	0.000	40
8	10	F	Control			RPO: -0.50DS LPO: -0.50DS	-0.150 -0.150	0.000	55

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
9	6	M	Anisometropic		Near - minimal X with good recovery	R: +1.50/-3.50x25	+0.250	0.325	40
					Distance - minimal X with good recovery	L: +0.75/-0.50x180	-0.075		
10	8	M	Strabismic (Mixed – history of anisometropia)	Left intermittent distance exotropia	Near - 15^ Int LXT with 6^ intermittent L hypertropia	R: +8.00/-0.50x95	+0.100	0.325	negative
					Distance - 10^ LXT with 6^ L hypertropia	L: +8.00/-0.50x80	+0.425		
11	5	F	Anisometropic		Near - minimal X	R: +0.75DS	-0.100	0.300	85
					Distance - minimal X	L: +2.50DS	+0.200		
13	5	F	Anisometropic (history of anisometropia)		Near - 6^ x with rapid recovery	R: +1.25/-0.75x180	-0.050	0.175	55
					Distance – NAD	L: +1.75/-0.50x160	+0.125		

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
14	6	M	Strabismic (mixed)	Left esotropia	Near - 20^ LET Distance - 12^LET	R: +2.50/-0.50x70 L: +3.75/-0.50x90	-0.100 +0.125	0.225	negative
15	4	F	Control			RPO: Plano/-0.25x100 LPO: Plano	0.000 0.000	0.000	55
16	10	F	Control			RPO: +0.25/-0.50x103 LPO: +0.25/-0.50x82	-0.200 -0.200	0.000	20
17	6	F	Control			RPO: Plano/-0.25x34 LPO: +0.25/-0.50x1	-0.075 -0.100	0.025	40
18	8	F	Control			RPO: +0.25/-0.25x139 LPO: +0.25/-0.25x51	-0.050 -0.100	0.050	55
20	6	F	Anisometropic		Near - E with good recovery Distance - E with good recovery	R: +4DS L: +5DS	+0.050 +0.375	0.325	600
21	6	F	Strabismic (mixed)	Right exotropia	Near - 8^RXT Distance - Alternating XT	R: +3.25/-0.50x180 L: +1.50DS	+0.050 -0.050	0.100	negative

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
22	5	F	Anisometropic		Near - NAD Distance - NAD	R: +5.00/-1.50x25 L: +5.00/-1.00x150	+0.275 +0.450	0.175	negative
25	8	M	Strabismic (mixed)	Left intermittent distance exotropia	Near - 14^ X Distance - 14^ L/alternating Int XT	R: -2DS L: -3.00/-0.50x165	0.000 +0.175	0.175	55
26	6	M	Anisometropic		Near - minimal X with rapid recovery Distance - NAD	R: +2.50/-2.25x15 L: +0.25DS	+0.200 -0.050	0.250	110
27	6	F	Anisometropic		Near - NAD Distance - NAD	R: +3.50/-4.25x110 L: +1.00DS	+0.150 -0.075	0.225	40
28	9	M	Control			RPO: Plano LPO: +0.25DS	-0.150 -0.125	0.025	55
29	7	M	Strabismic	Partially accommodative right esotropia with ARC	Near - 6^ RET Distance - 4^E	R: +6.25/-1.75x180 L: +6.25/-1.50x175	+0.450 -0.025	0.475	negative
30	8	F	Strabismic	Partially accommodative right esotropia	Near - 12^RET Distance - RET	R: +2.50/-0.50x20 L: +1.50DS	+0.175 -0.100	0.275	negative
31	5	F	Anisometropic		Near - NAD Distance - NAD	R: +0.50/-2.00x50 L: +0.50DS	+0.150 -0.025	0.175	55
32	6	M	Anisometropic	Fully accommodative right esotropia	Near - 6^E Distance - 2^E	R: +1.00DS L: +2.25/-0.25x180	-0.050 0.200	0.250	110

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
33	9	F	Control			R: +0.25DS L: +0.50/-0.75x10	-0.225 -0.175	0.050	40
34	6	M	Anisometropic		Near - Orthophoric Distance - NAD	R: +4.00/-3.75x15 L: +1.50/-1.25x170	0.050 -0.100	0.150	55
35	5	M	Anisometropic		Near - Orthophoric Distance - NAD	R: +2.50/-0.50x180 L: +5.00/-1.25x165	-0.075 0.150	0.225	55
36	6	F	Control			RPO: +0.50/-0.50x70 LPO: Plano	-0.125 -0.125	0.000	55
37	8	F	Control			R: +0.75DS L: +0.75DS	-0.175 -0.175	0.000	40
39	5	M	Strabismic (mixed)	Partially accommodative left esotropia	Near - 8^ intermittent LET Distance - 6^ intermittent LET	R: +0.50DS L: +1.50DS	0.050 0.275	0.225	340
40	6	F	Strabismic (mixed)	Fully accommodative left esotropia controlled to microtropia without identity	Near - 10^LET Distance - 10^LET	R: +4.50/+1.25x90 L: +6.00/+2.50x90	0.000 0.375	0.375	negative

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
41	7	M	Control			RPO: Plano/-0.25x79 LPO: Plano/-0.25x102	-0.175 -0.175	0.000	55
42	8	F	Strabismic	Accommodative right esotropia controlled to Int. distance exotropia	Near - 6^ RET Distance - 10^ RXT	R: +8.00/-1.00x10 L: +8.00/-1.25x180	+0.275 0.000	0.275	negative
43	6	F	Anisometropic (history of anisometropia)		Near - Minimal X Distance - NAD	R: +4.50/-1.75x175 L: +5.25/-2.25x5	-0.050 +0.050	0.100	85
44	8	F	Control			RPO: -0.25DS LPO: Plano/-0.25x30	-0.025 -0.050	0.025	110
45	4	M	Control			RPO: -1.00/-0.25x30 LPO: -1.00DS	+0.025 +0.025	0.000	85
46	9	M	Control			RPO: Plano/-0.25x1 LPO: +0.25/-0.50x9	-0.200 -0.250	0.050	40
47	6	M	Control			RPO: +0.25/-0.25x84 LPO: +0.25DS	-0.125 -0.150	0.025	55
48	4	F	Control			RPO: Plano LPO: Plano	+0.050 +0.025	0.025	40
49	4	F	Control			RPO: +0.25/-0.25x45 LPO: Plano/-0.25x158	0.000 +0.025	0.025	85

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
50	6	F	Control			RPO: -0.25DS LPO: Plano/-0.25x1	+0.050 +0.050	0.000	55
51	9	F	Control			RPO: +0.25/-0.25x64 LPO: +0.50/-0.25x117	+0.050 +0.050	0.000	85
52	7	M	Strabismic (mixed)	Residual partially accommodative left esotropia	Near - 20^ LET+ 6^ LHT Distance - 12^ LET + 2^ LHT	R: +1.50DS L: +5.00/-0.75x170	-0.100 +0.775	0.875	negative
53	6	M	Strabismic (mixed)	Fully accommodative right esotropia controlled to R microtropia without identity	Near - 2^ E Distance - 2^ E	R: +2.50/-0.75x180 L: +1.50/-0.50x90	+0.325 +0.050	0.275	170
54	5	F	Anisometropic		Near - Orthophoric Distance - NAD	R: +2.00/-0.75x180 L: +3.50/-1.50x180	+0.050 +0.275	0.225	85
55	6	M	Strabismic (mixed)	Residual left esotropia and hypotropia	Near - 4^ LET + 20^ LHoT Distance - 4^ LET + 12-15^ LHoT	R: +2.50DS L: +2.25/-0.75x180	+0.000 +0.150	0.150	340
56	6	M	Anisometropic		Near – NAD Distance - NAD	R: -0.50DS L: -0.50/-1.00x180	-0.025 +0.100	0.125	55

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
57	7	M	Strabismic (mixed)	Right exotropia	Near - 12^RXT Distance - 25^RXT	R: +5.50/-0.75x90 L: +2.50DS	0.875 -0.075	0.950	negative
58	5	F	Anisometropic		Near – Orthophoric Distance – NAD	R: +3.50/-0.50x180 L: +4.50/-1.00x180	-0.050 0.100	0.150	55
60	7	F	Anisometropic		Near – Orthophoric Distance – NAD	R: +1.00/-0.50x15 L: +1.75DS	-0.125 0.000	0.125	55
61	4	F	Strabismic (mixed)	Fully accommodative left esotropia controlled to microtropia without identity	Near - 10^ int LET Distance - 6^ E	R: +3.50DS L: +7.00/-1.00x180	0.125 0.375	0.250	negative
62	6	M	Anisometropic	Fully accommodative right esotropia	Near - 2^ E with rapid recovery Distance - NAD	R: +8.50/-1.50x10 L: +7.00/-0.75x20	0.250 0.025	0.225	55
63	4	M	Anisometropic		Near - Orthophoric Distance - Orthophoric	R: +1.75DS L: +5.50DS	0.050 0.375	0.325	55
65	6	M	Anisometropic		Near - 2^X Distance - 2^X	R: +2.50/-2.50x60 L: +0.25DS	0.225 -0.075	0.300	85
66	7	F	Strabismic	Partially accommodative right esotropia	Near - 10-12^ RET Distance - 10^ RET	R: +7.25/-1.75x155 L: +7.25/-2.25x180	0.325 0.050	0.275	negative

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Cover test (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
67	10	F	Anisometropic		Near - 2^ X Distance – NAD	R: +4.50/-1.25x180 L: +0.25/-0.75x180	+0.250 -0.100	0.350	55
68	5	F	Anisometropic		Near - 4^ X Distance - 2^ X	R: +5.00/-0.25x69 L: +1.25/-0.25x173	+0.250 -0.150	0.400	55
69	5	M	Strabismic (mixed)	Partially accommodative left esotropia	Near - 6^ LET Distance - 4-6^ LET	R: +5.50/-1.50x120 L: +7.25/-1.75x120	-0.125 +0.425	0.550	negative
70	10	M	Strabismic	Left esotropia	Near - 8^ LET Distance - 6^ LET	R: -6.00/-1.25x55 L: -6.50/-0.75x125	-0.075 +0.150	0.225	110
72	5	F	Strabismic	Partially accommodative right esotropia	Near - 12^ RET Distance - 8^ RET	R: +7.00/-1.25x180 L: +6.50/-1.25x160	+0.375 +0.100	0.275	negative
73	7	F	Strabismic (mixed)	Partially accommodative right esotropia	Near - 20^ RET with 2^ hypertropia Distance - 12^ RET	R: +2.50/-0.75x170 L: +1.00DS	+0.250 +0.000	0.250	negative
74	4	F	Strabismic (mixed)	Partially accommodative left esotropia	Near - 15^ RET Distance - 15^ RET	R: +3.50/-1.25x90 L: +6.25/-0.25x180	+0.050 +0.850	0.800	negative
75	4	F	Control			RPO: Plano/-0.25x165 LPO: Plano/-0.25x177	+0.000 +0.000	0.000	55

Participant number	Age (Years)	Sex	Recruited group	Clinical strabismus diagnosis	Deviation (With glasses)	Current refraction / Plusoptix estimate	SLT VA (logMAR)	IOD (logMAR)	Frisby Stereoacuity (seconds of arc)
76	8	M	Strabismic (mixed)	Convergence excess left esotropia	Near - 25^LET Near with bifocal segment - 9^ int LET Distance - 3^E	R: +4.50/-1.50x180 (+2.50 near add) L: +6.25/-1.50x180 (+2.50 near add)	-0.125 +0.075	0.200	with bifocal; 360", without bifocals; negative

Key

^ - Prism Dioptres
R – Right Eye
L – Left Eye
RPO – Right eye plusoptix result
LPO – Left eye plusoptix result

Int – intermittent
E – esophoria
X – exophoria
ET – esotropia
XT – exotropia

HT – hypertropia
HoT - hypotropia

2.2.4 Procedure

The study procedure is summarised in Figure 2.04. Prospective participants were identified by their clinician at their ACPOS clinical appointment and given guardian information sheets (Appendix two) and age-appropriate patient information sheets (Appendix three). Healthy volunteers recruited via word-of-mouth were accepted as control participants. All participants were required to provide informed assent (Appendix four), and their parent/guardian provided written informed consent (Appendix five) prior to participation. Recruited individuals were allocated a participant number, and all results were recorded using this participant number to preserve anonymity, and participants could leave the study at any time without prejudice.

Detailed ocular history was acquired from examinations of participants' clinical records and conversations with parents and guardians. This information was used to confirm whether inclusion criteria were met and identify to which group the participant was allocated (control, anisometropic amblyope or strabismic/mixed amblyope). Ocular history for amblyopic participants can be seen in Appendix six.

Following recruitment, within-group covariate adaptive randomisation using minimisation (a method of adaptive stratified sampling to balance the presence of confounding variables between groups) was adopted to select which eye was examined first, to avoid eye bias (Hu et al., 2014). Amblyopes had either their amblyopic eye (AE) or fellow eye (FE) assessed first for all three experiments, while control participants had either their right eye (RE) or left eye (LE) assessed first.

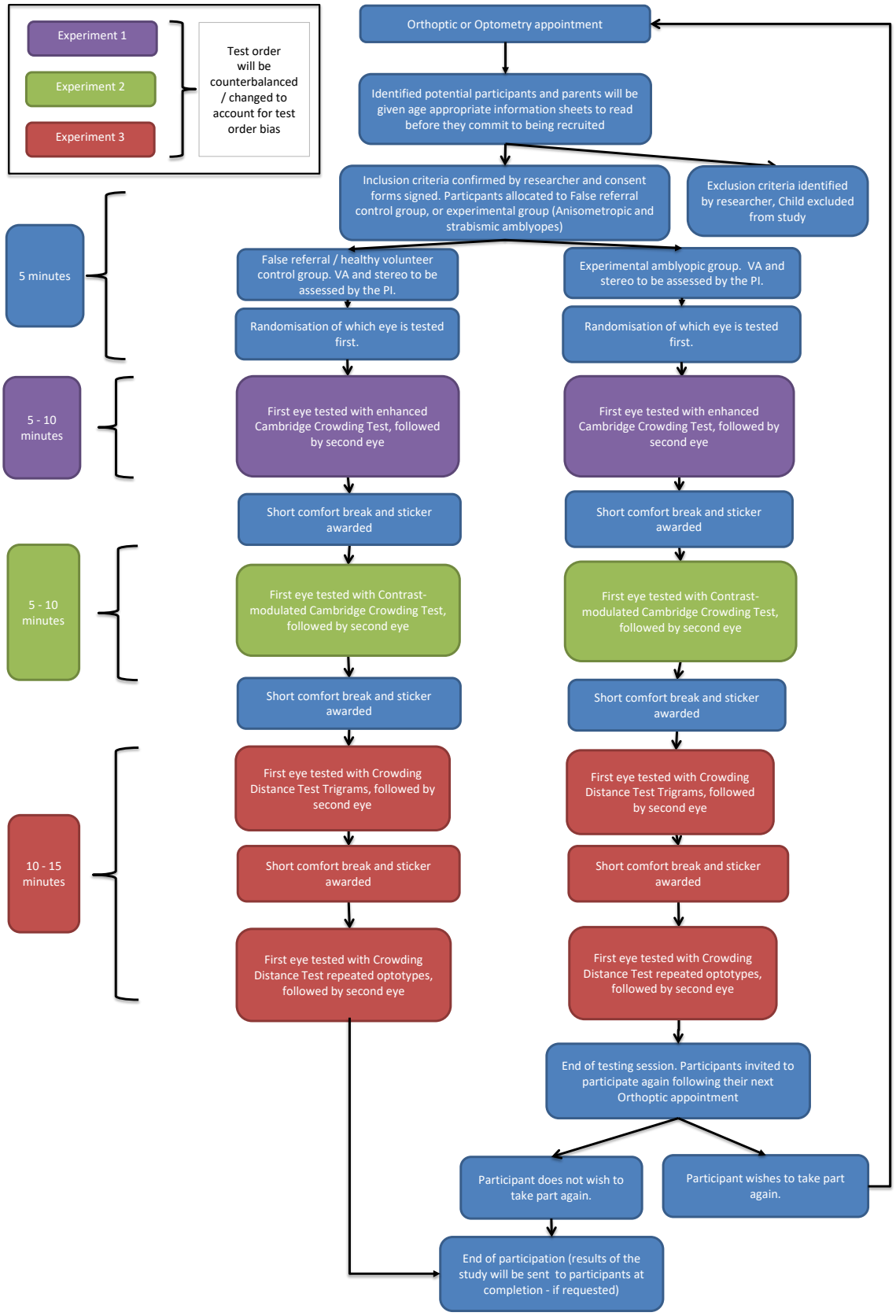


Figure 2.04: *Study test procedure flowchart*

Minimisation ensures equal distribution of possible confounding variables, which in this study were identified as amblyopia severity (mild <0.3logMAR IOD, moderate 0.3 to 0.6 logMAR and marked >0.6 logMAR) (Stewart et al., 2005), strabismus presentation (esotropia or exotropia), current occlusion treatment (patch or atropine), gender (Male or Female), age (36-90 months or 91-143 months) and refractive error (hypermetropia or myopia). One point was allocated for each confounding variable present per person, and the eye chosen to be tested first per participant, was allocated to the eye with the lowest cumulative number of those confounding variables to maintain an overall balance between which eye was tested first (Table 2.06). Equal distribution of these possible confounding variables within each group resulted in six minimised groups (Control: RE tested first, Control: LE tested first, anisometropic amblyope: AE tested first, anisometropic amblyope: FE tested first, strabismic/mixed amblyope: AE tested first, strabismic/mixed amblyope: FE tested first). Minimisation provides fairer statistical analysis and extrapolation of results by reducing type 1 errors and improving power (Kernan et al., 1999).

Following minimisation, each minimised group underwent counterbalancing of the order of experiments to prevent test order bias. In this study, the examination of the three different experimental tests resulted in 12 different test order permutations (Table 2.07). Counterbalancing occurred prior to testing and was achieved by asking each participant to blindly select a permutation number from a bag pertaining to their allocated minimised group (Figure 2.05). This combined use of stratified randomisation and counterbalancing ensured the prevention of testing order bias between the eyes, test order presentation bias, and ensured that thresholds obtained were not influenced due to practice, attention, or fatigue effects.

a)

Prognostic factors	AE	FE
Myopia	0	0
Hypermetropia	5	5
Esotropia	0	0
Exotropia	0	0
Mild	5	4
Moderate	0	1
Marked	0	0
Male	3	3
Female	2	2
36-90 months	5	5
91-143 months	0	0
Patching	2	2
Atropine	0	0
Total	22	22
Allocated		

b)

Prognostic factors	AE	FE
Myopia		
Hypermetropia	5	5
Esotropia		
Exotropia		
Mild	5	4
Moderate		
Marked		
Male		
Female	2	2
36-90 months	5	5
91-143 months		
Patching		
Atropine		
Total	17	16
Allocated		

c)

Prognostic factors	AE	FE
Myopia	0	0
Hypermetropia	5	6
Esotropia	0	0
Exotropia	0	0
Mild	5	5
Moderate	0	1
Marked	0	0
Male	3	3
Female	2	3
36-90 months	5	6
91-143 months	0	0
Patching	2	2
Atropine	0	0
Total	22	26
Allocated		

Table 2.06: *Example of minimisation*

a) Equal numbers of total confounding variables are present for AE or FE tested first minimisations.

b) The next participant has four confounding variables (highlighted). Examination of these variables shows that FE leading has fewer of these variables, so the participant is allocated to the FE leading set.

c) These four variables are added to the FE cumulative total.

Table 2.07: *Test order permutations*

Permutation	1st Test	2nd Test	3rd Test
1	L-ECC	CM-ECC	Pelli trigram format followed by repeated format
2	L-ECC	CM-ECC	Pelli repeated format followed by trigram format
3	L-ECC	Pelli trigram format followed by repeated format	CM-ECC
4	L-ECC	Pelli repeated format followed by trigram format	CM-ECC
5	CM-ECC	Pelli trigram format followed by repeated format	L-ECC
6	CM-ECC	Pelli repeated format followed by trigram format	L-ECC
7	CM-ECC	L-ECC	Pelli trigram format followed by repeated format
8	CM-ECC	L-ECC	Pelli repeated format followed by trigram format
9	Pelli trigram format followed by repeated format	L-ECC	CM-ECC
10	Pelli repeated format followed by trigram format	L-ECC	CM-ECC
11	Pelli trigram format followed by repeated format	CM-ECC	L-ECC
12	Pelli repeated format followed by trigram format	CM-ECC	L-ECC

Figure 2.05: *Counterbalancing token bags for the six minimised groups. C (control), S (strabismic/mixed amblyope), A (anisometropic amblyope), RE (right eye 1st), LE (left eye 1st), AE (amblyopic eye 1st), NAE (non-amblyopic eye 1st).*

The study took place in a first floor modified clinical room with daylight fluorescent lighting within the University Eye Clinic on Bradmore Street, Cambridge.

Appropriate seating was included to allow a participant to be examined, as well as sufficient and comfortable space for the presence of the researcher and the participant's parent/guardian (Figure 2.06). Anglia Ruskin University evaluated the clinical suitability of these rooms for visual acuity assessment, and an appropriate risk assessment was completed. Participants faced the screen as close to a 90 degree angle as possible, to help prevent variations in contrast perception (O'Connor et al., 2007).

Figure 2.06: *Panoramic image of the experimental space*

All tests were conducted with the child's corrective lenses if required. Each complete session took an expected time of 45 to 60 minutes, depending upon the attention span and cooperation of the child, including regular breaks and relaxation points. It was not possible to blind the researcher as to which group test participants were allocated, as those patients with strabismic/mixed amblyopia were easily identified due to the presence of their strabismus. However, testing was conducted using psychophysical computer protocols (e.g., staircases) that relied on participant responses to determine stimulus size presentation order. These psychophysical procedures were the same for every participant.

All participants had the tests explained to them in the form of an astronaut story, and they completed tasks (the three modified vision tests) to help the astronaut undertake the missions successfully. This ensured that the examination was fun and engaging to improve compliance and concentration. The eye not being tested was occluded with an eye patch or a patch that clings to a spectacle lens (Figure 2.03 above).

2.2.5 Testing distances

Testing was completed at 3m, except for amblyopic eyes. To ensure that the presentation size of optotypes was appropriate, testing distances were scaled for the amblyopic eyes of participants. This process is used routinely throughout vision science studies examining amblyopia, with participants being positioned proportionally closer to the stimulus by the order of magnitude difference in their interocular acuity (Table 2.08). This process ensures that each eye receives the same range of physical visual stimulation and presentation of optotypes on the computer screen, with the visual score obtained adjusted accordingly for the decrease in distance.

Table 2.08: *Scaled acuity and test distance calculations*

Interocular difference (logMAR)	Amblyopia density	Order of magnitude difference	Fellow eye test distance (m)	Amblyopic eye test distance (m)
0	NA	1	3	**3**
0.1	Mild	1.258925412	3	2.382984704
0.2	Mild	1.584893192	3	1.892872033
0.3	***Moderate***	1.995262315	3	***1.503561701***
0.4	Moderate	2.511886432	3	1.194321512
0.5	Moderate	3.16227766	3	0.948683298
0.6	***Marked***	3.981071706	3	***0.753565929***
0.7	Marked	5.011872336	3	0.598578694
0.8	Marked	6.309573445	3	0.475467958
0.9	Marked	7.943282347	3	0.377677624
1	Marked	10	3	0.3

Examination of the adjusted testing distances revealed a halving of testing distance at intervals of 0.3 logMAR, which coincided with increasing amblyopic density categories (Stewart et al., 2005). To simplify this scaling process, the scaled amblyopic eye test

distances were grouped as per amblyopic density (Table 2.09). For marked amblyopes, test distance was rounded up to 1m to coincide with other minimum distances currently used in other distance visual acuity tests such as Snellen (Stevens, 2007; Marsden, Stevens and Ebri, 2019) and ETDRS (Peh, Agelis and Chen, 2012).

Table 2.09: *Scaled test distances per amblyopic density*

Interocular difference and corresponding amblyopic density (logMAR)	Fellow eye test distance (m)	Amblyopic eye test distance (m)
0.100 - 0.275 (Mild)	3m	3m
0.300 - 0.575 (Mod)	3m	1.5m
>0.600 (Marked)	3m	1m

This quick and straightforward scaling method is useful as it is easily replicable within a clinical situation while preventing 'topping out' (threshold not reached even with the largest optotype presentable) in eyes with poorer acuity. Scaling also ensures that repeated optotype presentations display appropriately, as unnecessarily large presentations would reduce repeatability. Maximum and minimum sizes of presentable optotypes at each distance are given in Table 2.10.

Table 2.10: *Maximum and minimum sizes of presentable optotypes at the three different testing distances*

Testing distance (m)	Maximum presentable optotype size (logMAR)	Minimum presentable optotype size (logMAR)
3m	+0.99	-0.89
1.5m	+1.29	-0.59
1m	+1.59	-0.29

2.2.6 Stimuli

To produce computer-generated L-ECC and CM-ECC optotypes, every size of the optotype was carefully checked and calibrated to ensure accurate physical size. Targets (H, O, T, V) and flanking (U, C, L, A) optotypes for the ECC and isolated acuity examination were constructed using MATLAB (MathWorksTM, Natick, USA) matrices and scaled to allow for an extensive range of stimulus sizes to fit on the screen. Optotype generation and presentation was controlled in MATLAB via the Psychophysics toolbox (Pelli, 1997). In combination with participant viewing distances, the generated optotype sizes were used to calculate accurate logMAR acuity scores. The custom-written program ensured that all stimuli were constructed in multiples of whole numbers of pixels so that exact logMAR scores were calculated (not estimated). The lower limit of optotype size was restricted by the minimum number of pixels required to form each optotype adequately. In contrast, the upper limit was restricted by both test distance and display screen size.

Second-order contrast-modulated optotypes were created using dynamic noise (Smith and Ledgeway, 1997) to ensure that static luminance clumps are not present to facilitate optotype identification. For each stimulus frame, different binary (black or white) noise checks are drawn onto the screen, and the noise is multiplied by a square-wave optotype profile. The screen cycles through one noise page every four temporal frames so that the noise appears to twinkle in time. The size of the black and white noise checks on the stimulus screen was scaled to the overall optotype size, such that each letter consists of 15 noise checks, which is most effective for the measurement of vision (Pelli and Farell, 1999) and ensures that the noise is resolvable at all optotype sizes.

2.2.7 Establishing baseline thresholds

Prior to the research experiments, visual acuity was assessed using the Sonksen logMAR Test (SLT) (Salt et al., 2007; Sonksen et al., 2008). Stereoacuity was also assessed with the Frisby test (Stereotest Ltd, Sheffield, U.K). If the participant had attended the clinic immediately prior to their research appointment, the SLT and Frisby threshold data obtained during their clinical appointment (by an Orthoptist) were retrieved from their notes. Otherwise, the researcher measured them using the standard testing protocols, as did the clinical orthoptists involved in ACPOS.

Uniocular SLT visual acuities were obtained via the following method. Children were asked to verbally identify the letters on a matching card to ascertain their literacy skills and understanding. Those unable to verbally identify the required letters were allowed to match. All participants were allowed to hold the matching card for reference. The left eye was then occluded with an appropriate patch/glasses. Testing occurred at 3m and followed the SLT testing procedure (see Salt et al. 2007 for details). Testing terminated once three consecutive letters were incorrectly identified, then the right eye was occluded and the left eye acuity was measured.

Frisby stereoacuity thresholds were measured following the standard Frisby testing procedure (Stereotest Ltd, Sheffield, U.K). Starting at 50cms, participants were presented with the largest (6mm) plate and asked to identify the target's position (Figure 2.07). Three correct responses precipitated a change to the 3mm plate and finally the 1.5mm plate. If the participant was able to identify the 1.5mm target at 50 cms, the test distance was increased by 10 cms, and the test was repeated. If the participant is unable to identify the target at 3mm or 1.5 mm, then the clinician or researcher returned to the last plate that the participant correctly identified and increased the distance until the threshold was

established. If no target was detectable, motion parallax was used to establish the child's understanding of the test. A negative result was recorded if the child could not identify a target when the 6mm plate was motionless but could identify the target once movement was introduced. All participants were carefully observed throughout to ensure testing distances were maintained.

Figure 2.07: *Undertaking a Frisby test*

2.2.8 Experimental procedures

Staircase procedures were used to establish acuity recognition and crowding distance thresholds for the three experimental presentations (Pelli et al., 2016; Lalor, 2018; Waugh et al., 2018). The staircase is a quick, efficient and popular method of determining an accurate visual acuity threshold; it is, therefore, an appropriate method of evaluating visual acuity thresholds with young children in clinical settings (Cornsweet, 1962; Corwin, Kintz and Beaty, 1979; Witton, Talcott and Henning, 2017).

For both first- and second-order presentations of the enhanced Cambridge Crowding Test, a self-paced method staircase with a four-alternative-forced-choice (4AFC) psychophysics procedure was used (Hess and Jacobs, 1979; Leat, Li and Epp, 1999; Tripathy and Cavanagh, 2002; Hariharan, Levi and Klein, 2005; Wong, Levi and McGraw, 2005; Hairol, Formankiewicz and Waugh, 2013). Participants were instructed to identify which of the four possible target optotypes (H, O, T, V) were presented and positioned centrally on the screen. The two presentation types (isolated and crowded) were interleaved into a single experiment, which helped to provide variety and novelty for the participants, as well as mitigating for fatigue and practice effects.

A two-down, one-up procedure was used to establish recognition acuity thresholds (Pelli et al., 2016; Lalor, 2018). Two correct responses resulted in the staircase descending by 0.1 logMAR, and an incorrect response increased optotype sizes by 0.1 logMAR. The two-down, one-up staircase also yields a low probability of 6.25% that the staircase descended due to guessing, thereby safeguarding the accuracy of results in this potentially tricky to examine young cohort (Lalor, 2018). The staircase terminated following a pre-set number of staircase reversals, usually six, although this could be reduced to four to decrease testing time if necessary, depending upon the child's age, and pre-test observations of their concentration, and attention span. Thresholds were calculated by averaging the acuities at each reversal; however, the first two reversals were disregarded to prevent overestimation of the visual acuity threshold in the event of an accidental incorrect response early on in the staircase (Witton, Talcott and Henning, 2017; Lalor, 2018). To motivate the child and reduce anxiety often associated with threshold level stimuli, the staircase procedures also included "catch" trials every sixth presentation using optotypes larger than the anticipated acuity threshold (either the starting acuity or the largest acuity displayed). These catch trials did not contribute to threshold calculations and reduced the predictability of the test (Bach, 1996). An example staircase for the L-ECC can be seen in Figure 2.08.

For the crowding distance test, a QUEST adaptive staircase with 20 trials per arrangement was employed to establish both isolated Sloan visual acuity and Pelli optotype crowding distance thresholds (Pelli and Watson, 1983).

For all experiments, all participants were carefully observed throughout to ensure testing distances were maintained and unlimited response time was provided for each trial, however, prompt answers were encouraged. Guesses were required when the target optotype was near the participant's threshold. No feedback was given about the accuracy of their response, but positive reinforcement was given throughout to encourage testing compliance.

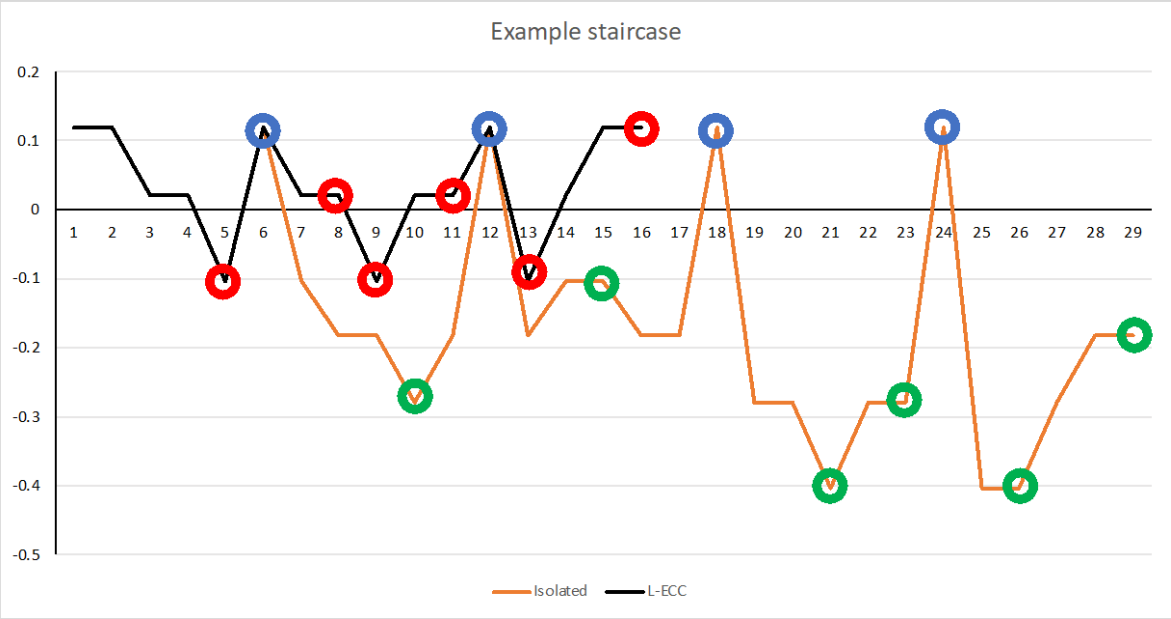


Figure 2.08: *A descending staircase for interleaved presentations of the isolated and enhanced Cambridge crowding (L-ECC) acuity tests. Six reversals are seen for the isolated (green circles) and L-ECC (red circles) format. The first two reversals of each format are discarded prior to threshold averaging. Catch trials are shown (blue circles).*

2.2.8.1 Experiment one – Enhanced Cambridge Crowding Test

The participant was shown the enhanced Cambridge Crowding Test (L-ECC) letter matching card before the start of the experiment and asked to name the letters with

whatever names they wished to use. If they could not do this easily, they were allowed to match what they saw by pointing to an optotype on the matching card (see Figure 2.09).

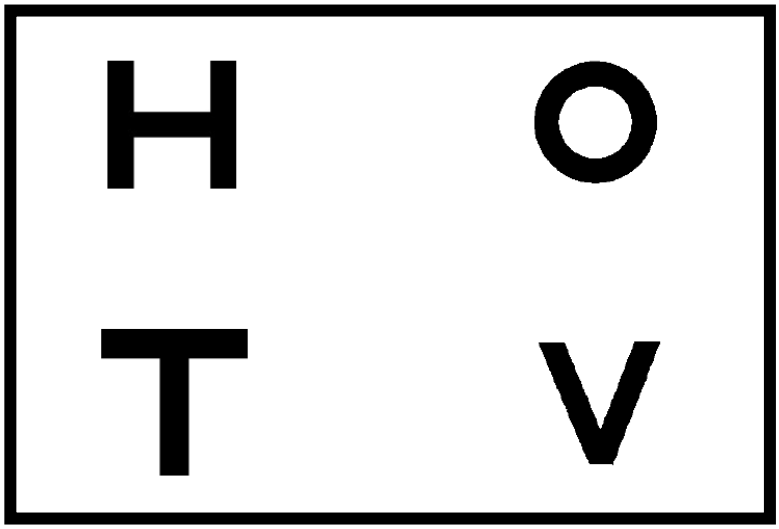


Figure 2.09: *"Enhanced Cambridge Crowding test" optotypes*

Using the corresponding eye's SLT acuity threshold results, supra-threshold starting points (+0.200 logMAR, rounded to the nearest 0.1 logMAR) were used to decrease test duration, reduce frustration and encourage engagement with the examinations (Salt et al., 2007; Lalor, 2018).

A laterally-reversible target letter (e.g., H, O, T or V) was displayed on the computer screen in two presentations, either isolated or surrounded by four other letters (U, A, L, C) as per the Cambridge Crowding Test (Atkinson et al., 1988). For the crowded presentation, the surrounding letters were placed one stroke width away from the target letter. The child was requested to identify the isolated letter or central letter only. Responses were recorded by the examiner using the Bluetooth Apple "Magic Keyboard", and input of the participant's response precipitated the programme to move on to the subsequent trial. The two presentations were randomly interleaved for the duration of the experiment, and each

presentation terminated at the appropriate number of reversals (four or six). After a short break, the second eye was examined in the same manner.

2.2.8.2 - Experiment two – Contrast-modulated Cambridge Crowding Test

The procedure for Experiment 2, examining visual acuity thresholds using the second-order contrast-modulated enhanced Cambridge crowding test, was the same as experiment one. Previous studies demonstrate that CM acuity (Figure 2.10) is approximately 0.500 logMAR poorer than acuity for first-order luminance-defined optotypes. Therefore, staircases commenced at +0.700 logMAR above the corresponding eye SLT threshold (round to the nearest 0.1 logMAR).

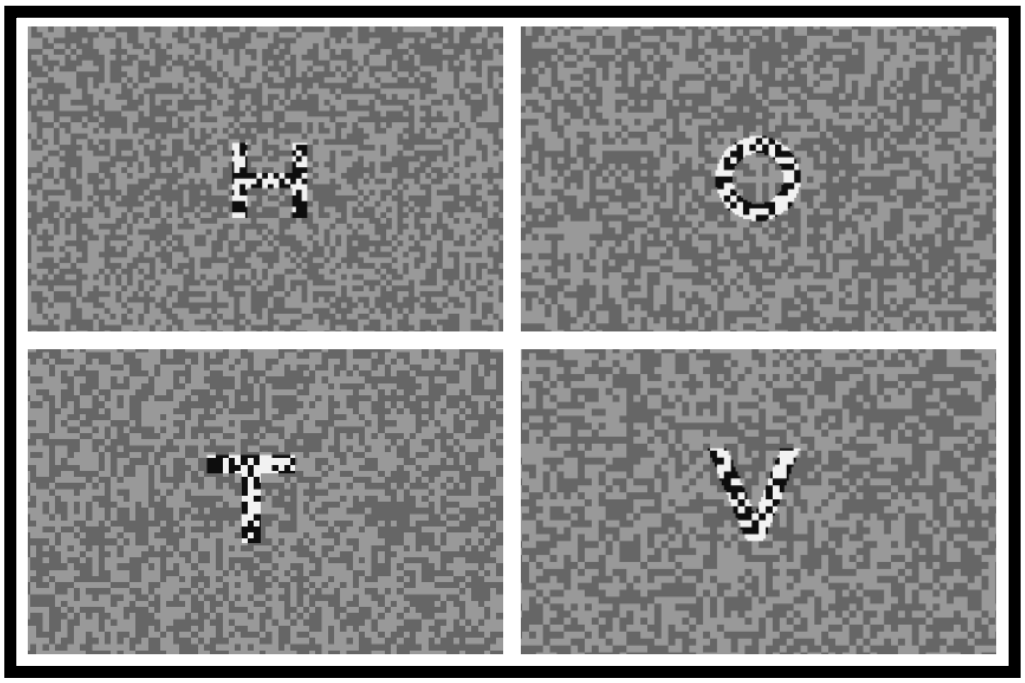


Figure 2.10: *"Contrast-modulated enhanced Cambridge crowding test" optotypes*

2.2.8.3 - Experiment three – "Crowding Distance Test."

The participant was shown the Pelli Optotypes (Figure 2.11) prior to the experiment and asked to name the numbers. If they could not do this easily, they were allowed to match what they saw by pointing to an optotype on the matching card (see Appendix one).

Figure 2.11: *"Crowding Distance Test" Pelli optotypes*

Foveal crowding distance using the Pelli trigram arrangement or the Pelli single line arrangement was assessed first (depending upon the permutation selected), with participants being asked to identify either the central target (Trigram) or both presented optotypes (repeated) (Figure 2.12). Horizontal inter-optotype spacing was covaried with optotype size and was proportionally set at 1.4x (measured here centre-to-centre). Finally, visual acuity thresholds were measured using nine isolated Sloan letters and participants were asked to identify the single target optotype. Clear verbal or matching responses using a matching card containing the nine target options were accepted. Throughout the study, participants were offered stickers and stamps on a 'passport' to maintain interest and concentration and make the study fun and engaging. In addition, soft toy teddies and aliens decorated the room and were available to 'help' the child. This is helpful, particularly with young children (Waugh et al., 2018).

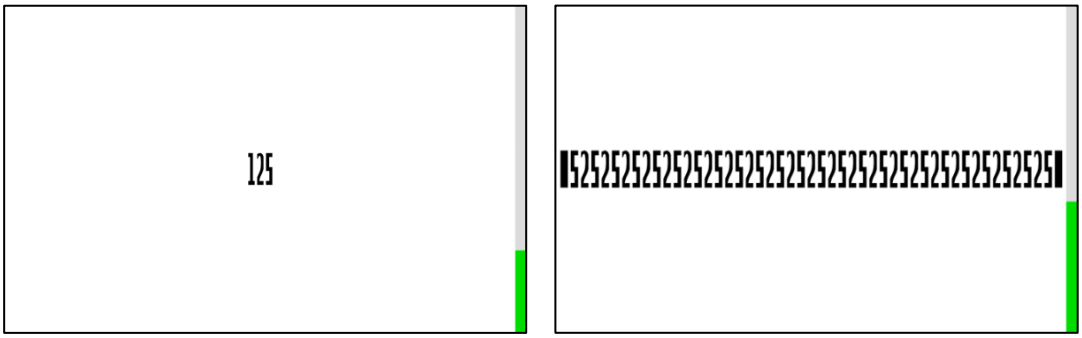


Figure 2.12: *Left - Pelli optotypes in a trigram arrangement. Right - Repeated Pelli optotypes in a single line arrangement. Reproduced with permission from Haine, Waugh, Formankiewicz and Pelli* (2019).

Following completion of the vision tests, participants were given a £10 Amazon gift card and asked if they wished to be involved again with the study. If they did, they were tested again following their next Orthoptic review with the ACPOS clinic or at a time that suited the family best. If not, this was the end of their participation.

2.3 Ethical considerations

This research was conducted in full conformation with the principles of the "Declaration of Helsinki", Good Clinical Practice (GCP) and within the laws and regulations of the United Kingdom; and was approved by Anglia Ruskin University and Cambridge South research ethics committee (REC) and the HRA on 08/08/2018.

2.4 Analysis

As amblyopia is diagnosed by significant interocular difference, collecting data from both eyes is necessary. However, one must be careful when analysing data from both eyes as this violates statistical assumptions of independence of observation (Karakosta et al., 2012; Armstrong, 2013). As this study involves three different groups (one control and two test groups), options for data analysis comparing the test eyes to the control eyes include using

data from one control eye only or averaging the threshold results of the control eyes and comparing this ‘control threshold’ to the amblyopic and fellow eyes of the anisometropic and strabismic/mixed amblyopes. To retain all data, control right eyes were compared to amblyopic eyes, and control left eyes were compared to fellow eyes, following confirmation of statistical insignificance between the right and left eye thresholds. Data analysis was conducted using IBM SPSS Statistics v28.0 (SPSS Inc, Chicago) with Repeated Measures Analysis of Variance models. Confirmation of normality and sphericity was assessed with a Shapiro-Wilk test, and Mauchley’s Test of Sphericity. Where necessary, error rates were adjusted with a Huynd-Feldt Correction to ensure that type 1 errors (incorrect rejection of the null hypothesis) were less likely. Post-hoc and planned analyses were conducted using a Tukey test where appropriate.

Chapter Three - Enhanced Cambridge Crowding Test

3.1 Introduction

Despite amblyopia resulting in a variety of visual deficits, visual acuity remains the principal tool of amblyopia diagnosis (Flom and Neumaier, 1966; Williamson et al., 1995; Simmers, Gray and Spowart, 1997; Donaghy and Larson, 2015). The acuity diminishing phenomenon of visual crowding is amplified in amblyopes, with increased crowding magnitudes and larger spatial extents of crowding seen when compared with visually healthy individuals (Stuart and Burian, 1962; Flom, Weymouth and Kahneman, 1963; Mayer and Gross, 1990; Morad, Werker and Nemet, 1999; Hess et al., 2001; Levi, Hariharan and Klein, 2002b). This knowledge has resulted in the incorporation of flanking bars, boxes or optotypes into various visual acuity tests (Hilton and Stanley, 1972; Atkinson et al., 1988; Simmers, Gray and Spowart, 1997; McGraw and Winn, 1993; McGraw et al., 2000; Schlenker, Christakis and Braga-Mele, 2010), to further diminish the visual acuity of amblyopic eyes, thereby highlighting the presence of amblyopia via increased interocular acuity differences (Atkinson et al., 1988; Rydberg et al., 1999; Greenwood et al., 2012). These differences in interocular visual acuity are a vital factor for amblyopia diagnosis and treatment monitoring (Flom and Neumaier, 1966; Attebo et al., 1998; Flynn et al., 1998, 1999; Simons, 2005; Holmes and Clarke, 2006; Holmes et al., 2006; Wu and Hunter, 2006; Lalor, Formankiewicz and Waugh, 2016; O'Boyle, Chen and Little, 2017).

Within the U.K., whole-population vision screening programmes to detect childhood visual conditions such as amblyopia recommend using logMAR-based, crowded visual acuity tests (Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015; Cotter et al., 2015). While the value/cost-effectiveness of visual screening is often debated (Snowdon and Stewart-Brown, 1997; Rahi and Dezateux, 1997; Williams et al., 1998; Rahi, Cumberland and

Peckham, 2006), population-based randomised control trials have demonstrated that visual screening reduces the prevalence of amblyopia by up to 60% (Williams et al., 2001), although efficacy within the population depends upon attendance rate (Newman and East, 2000). The U.K. National Screening Committee's recommendation is that testing should be 'Orthoptic led', utilise logMAR-based crowded acuity tests, and be conducted at four to five years of age, as earlier screening risks higher rates of false positives (McCullough and Saunders, 2019), while later screening risks poorer visual outcomes for moderate and severely amblyopic children (Kirk et al., 2008; Holmes et al., 2011; Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015). Existing logMAR-based acuity charts include, but are not limited to, ETDRS, Cambridge Crowding test, HOTV, Kay Pictures, Lea Symbols, and the Sonksen logMAR Test (SLT) (See Chapter 1, Section 1.3.2 for a more detailed review of acuity charts and their arrangements). While this U.K. committee recommends using logMAR-based crowded tests to assess visual acuity, a specific test is not named. Internationally, HOTV and Lea Symbols have been previously recommended by the US Prevention Service Task Force as the most appropriate tests for the vision screening of children under five years of age (Chou, Dana and Bougatsos, 2011).

Current pre-literate clinical visual acuity tests demonstrate a variety of optotype-flanker arrangements (Livne and Sagi, 2007, 2010; Glen and Dakin, 2013; Lalor, Formankiewicz and Waugh, 2016; Norgett and Siderov, 2017), optotype legibility (Bailey and Lovie, 1976; McGraw and Winn, 1993; Zhang et al., 2009; Candy et al., 2011), flanker type and spatial complexity (quantified by stroke frequency and perimetric complexity, defined as ″the square of the inside and outside perimeter of a symbol, divided by the "ink" area; (Bernard and Chung, 2011)) (Pelli et al., 2006; Dakin et al., 2010; Grainger, Tydgat and Isselé, 2010; Bernard and Chung, 2011; Chanceaux, Mathôt and Grainger, 2014; Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016; Norgett and Siderov, 2017) and target-flanker spacings (Levi and Carney, 2011; Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016) which collectively influence the acuity result.

Studies of visually healthy children and adults show that current pre-literate acuity tests result in only minimal crowding (Formankiewicz and Waugh, 2013; Lalor, Formankiewicz and Waugh, 2016; Hairol, Omair and Kaur, 2016). Modification and enhancement of crowding using existing logMAR based pre-literate visual acuity tests could provide additional sensitivity to the detection of amblyopia (Newman and East, 1999; Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016). Some simple optotype adjustments could provide this enhanced sensitivity with relative ease within traditional first-order tests that use black optotypes on a white background (Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016). These adjustments include flanking features which display greater similarity to the target optotype through increased perceptual grouping (Kooi et al., 1994; Bernard and Chung, 2011; Manassi, Sayim and Herzog, 2012; Song, Levi and Pelli, 2014; Chakravarthi and Herbert, 2019), combined with optimal positioning of flankers such that maximal crowding occurs throughout testing (Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016; Chakravarthi and Herbert, 2019).

3.1.1 Crowded test flanking features

For children under the age of six years, single crowded optotypes are considered the ‘gold standard’ visual assessment (Solebo and Rahi, 2013; Cotter et al., 2015; Solebo, Cumberland and Rahi, 2015), as this decreases the potential impact of eye movement abnormalities upon the identification of linear optotypes arrangements (Flom, Weymouth and Kahneman, 1963) However, the flanking feature type is unspecified within the U.K. guidelines. Studies by Lalor, Formankiewicz and Waugh (2016) and Hairol, Omair and Kaur (2016) aimed to clarify the optimal flanking feature by measuring the effects of contour interaction and crowding upon visual acuity thresholds.

Four pre-literate visual acuity tests (Kay Picture Test (Kay Pictures Ltd, Tring UK) (Kay, 1983), Lea Symbols (Good-Lite, Illionois, USA) (Hyvärinen, Näsänen and Laurinen, 1980), HOTV (Precision Vision, Illionois, USA) (Lippmann, 1971) and the Cambridge Crowding test (Clement Clarke, Harlow, UK) (Atkinson et al., 1988) (See Figure 1.05, pg. 23)) were modified for single optotype target display by Lalor, Formankiewicz and Waugh (2016). Monocular visual acuity thresholds were obtained for the dominant eye in five visually healthy adult participants (mean age: 23.8 years, range 22-25 years). Isolated and crowded optotypes were displayed at nine metres, with crowded optotypes featuring edge-to-edge target-flanker separation of zero or abutting, 1, 2, 3, 4 and 5 stroke widths. Within this adult cohort, the magnitude of crowding seen in the Cambridge Crowding cards was significantly greater (***p*<.05**) than the magnitude of contour interaction seen in the other three pre-literate tests when a box or bars were abutting the target. When target-flanker separations were set at one and two stroke widths from the target, Cambridge Crowding cards demonstrated significantly greater crowding than Lea Symbols. Beyond three stroke widths target-flanker separation, no significant difference in magnitude of crowding was seen between all tests ($p>.05$).

In a separate study, Hairol, Omair and Kaur (2016) also concluded that relative performance (% correct responses) was maximally decreased when flanking letters and bars were used, compared with flanking boxes. The magnitude and spatial extent of crowding were also greatest with vertically arranged or surrounding (on four sides) optotype flankers (Hairol, Omair and Kaur, 2016). While both studies reach similar conclusions, caution must be taken in their interpretation regarding childhood amblyopia screening as both studies utilised visually healthy adult participants. Contour interaction and crowding effects in pre-literate acuity tests have been reported to be greater in visually healthy children than adults (Norgett and Siderov, 2014; Lalor, 2018). In a study involving amblyopic adults with strabismus, Norgett and Siderov (2017) found flanking letters superior to bar flankers regarding crowding magnitude.

The increased target-flanker similarity and spatial complexity yielded by optotype flankers heightens the effect of visual crowding and may be the optimal flanking feature for amblyopes (Kooi et al., 1994; Bernard and Chung, 2011; Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014; Norgett and Siderov, 2017; Lalor, 2018). The only letter-based pre-literate commercial acuity test with optotype flankers surrounding all-four sides is the Cambridge Crowding test. Further optimisation of this test for amblyopic visual screening is achievable via modification of the target-flanker spacing.

3.1.2 Target-flanker spacing

Studies of visually healthy adults and children (Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014; Lalor, 2018) and amblyopic adults (Song, Levi and Pelli, 2014; Lalor, 2018) indicate that much smaller target-flanker distances are required than are currently provided by existing commercial tests, to induce maximum crowding effects.

Of note, units to quantify flanker distance separation remain unstandardized, with optotype widths, stroke widths and minutes of arc all variably employed to describe this spatial separation (Simmers, Gray and Winn, 2000; Hess et al., 2001; Bedell et al., 2013; Chung, 2016; Lalor, Formankiewicz and Waugh, 2016). ‘Stroke widths’ refers to the thickness of the line weight used to draw the optotype. Edge-to edge and centre-to-centre target-flanker measurements have also been keenly debated as to their suitability (Pelli, Palomares and Majaj, 2004; Lalor, Formankiewicz and Waugh, 2016). In the study by Lalor, Formankiewicz and Waugh (2016), the spatial extent of crowding, defined as the closest target-flanker separation where flanked acuity did not differ from isolated acuity, was examined in terms of optotype-width, stroke width and arc-mins. When expressed in optotype widths, the variance in crowding extent was up to 30% greater than expression in stroke widths or arcmin. Stroke widths demonstrated the smallest variance at 26%. This indicates that stroke

widths best define the description of crowded optotype separation, and as such, target-flanker spacing will be considered in terms of stroke widths throughout this study.

In consideration of appropriate inter-optotype spacing distances within visual screening tests, studies have been numerous and varied. Early studies of the deleterious effects of contours can be found in the 1963 study by Flom, Weymouth and Kahneman, where examination of the perception of a Landolt C revealed the greatest detrimental effects occurred when bar contours were positioned 0.4 optotype widths (two stroke widths) away from the target. Negligible deleterious effects occurred beyond one optotype width (five stroke widths), measured edge-to-edge (Flom, Weymouth and Kahneman, 1963). These findings led to the commercial adoption of one optotype width (five stroke width) spacing seen in the Bailey-Lovie Chart, ETDRS and SLT (Bailey and Lovie, 1976; Salt et al., 2007; Sonksen et al., 2008). While the use of a five stroke width inter-optotype spacing allows clinicians to obtain an accurate assessment of acuity without significant crowding effects (Bailey and Lovie, 1976; Salt et al., 2007), this inter-optotype spacing is unfavourable for identifying amblyopia, where heightened visual crowding, instigating increased interocular acuity differences, is a critical diagnostic device (Hyvärinen, Näsänen and Laurinen, 1980; McGraw et al., 2000). Optimising inter-optotype and target-flanker spacing to enhance the crowding effect would be beneficial for the detection of crowding limited conditions such as amblyopia (Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014). Research has, however, remained unclear as to the optimal spatial separation required for maximal crowding magnitude.

Following on from their initial findings both, Lalor, Formankiewicz and Waugh (2016) and Hairol, Omair and Kaur (2016) examined the effect of target-flanker spacings on acuity thresholds. Both studies independently discovered statistically significant effects of flanker position in visually healthy adults, with peak magnitude of crowding (calculated as the difference between isolated acuity and ‘crowded’ acuity threshold) occurring either when

the flanking optotypes were either abutting the target or placed one stroke width away (measured edge-to-edge) (Formankiewicz and Waugh, 2013; Lalor, Formankiewicz and Waugh, 2016; Hairol, Omair and Kaur, 2016). Lalor (2018) further examined the effects of this reduced inter-optotype spacing (one stroke width) in 91 visually healthy children aged between 3 and 16 years of age, as visually healthy children have demonstrated both higher crowding magnitudes (Atkinson et al., 1988; Norgett and Siderov, 2011; Doron, Spierer and Polat, 2015) and larger spatial extents of crowding (Semenov, Chernova and Bondarko, 2000; Jeon et al., 2010; Waugh et al., 2018; Haine et al., 2021) than visually healthy adults. Furthermore, comparisons across all test arrangements revealed no significant difference in the magnitude of contour interaction (as induced within Kay Pictures, Lea Symbols and HOTV) between three to four-year-olds and adults, further demonstrating that these test formats may not be especially useful in the detection of crowding limited conditions during visual development. However, tests incorporating visual crowding (Cambridge Crowding Test) demonstrated rapid decreases in crowding magnitude from +0.40±0.05 logMAR seen in early years (three to four years) to +0.17±0.03 logMAR in 12-16-year-olds. As a result, crowded visual acuity reached maturation levels equivalent to those of adults, around age eight-years (Lalor, 2018), in agreement with psychophysical evidence that visual crowding matures at around age eight-years-of age in visually healthy children (Waugh et al., 2018; Haine et al., 2021). This decrease in crowding magnitude and rapid acuity threshold change seen in crowding limited tests, can therefore reflect the development of the ability to resolve crowded stimuli.

Based on these results, it is considered that one stroke width is the optimum target flanker spacing to facilitate maximum crowding magnitude effects in children without the optotypes abutting. Currently, no logMAR based, pre-literate acuity test meets this criterion. Therefore, for individuals with crowding-limited acuity, the Cambridge Crowding Test with one stroke width inter-optotype separation is suggested to be an ideal arrangement for the diagnostic purposes of increasing interocular acuity differences. This modified Cambridge Crowding

test is referred to as the Enhanced Cambridge Crowding Test (ECC). The ECC test may provide optimal spatial requirements for detecting crowding limited conditions, such as amblyopia; however, the effects of this modified test upon the visual acuity thresholds of amblyopes have not yet been examined. Therefore, this study assesses the application of this enhanced test format upon an amblyopic paediatric population for the first time to inform future visual screening recommendations.

3.1.3 Differences between amblyopic subtypes

Amblyopia is not uniform in its aetiology or neurological effects, and the disparity in visual deficits seen between amblyopic subtypes extend into their responses to visually crowded stimuli (Levi and Klein, 1982a, 1985; Abrahamsson and Sjostrand, 1988; McKee, Levi and Movshon, 2003; Song, Levi and Pelli, 2014; Doron, Spierer and Polat, 2015). Investigations of the most common amblyopic subtypes (strabismic, anisometropic and mixed strabismic-anisometropic) (Bradfield, 2013) reveal that amblyopes with strabismus demonstrate significant spatial crowding deficits (Stuart and Burian, 1962; Levi and Klein, 1982a, 1985; McKee, Levi and Movshon, 2003; Bonneh, Sagi and Polat, 2007; Hamm et al., 2014; Song, Levi and Pelli, 2014) with strabismics demonstrating visual acuity similar to that seen in the normal visual periphery (Levi, Klein and Yen Lee Yap, 1987; Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014). In comparison, acuity loss seen in anisometropic amblyopia is akin to normal foveal dioptric blur (Formankiewicz and Waugh, 2013; Hairol et al., 2014), with blur depressing contrast sensitivity for high and mid-level spatial frequencies (Hess and Howell, 1977; Howell, Mitchell and Keith, 1983) and reducing the peak magnitude of visual crowding (McKee, Levi and Movshon, 2003; Formankiewicz and Waugh, 2013; Hamm et al., 2014; Song, Levi and Pelli, 2014).

The optimally crowded ECC test may demonstrate greater benefits for the detection of individuals with strabismic or mixed amblyopia compared with anisometropic amblyopes.

This study, therefore, additionally aims to examine the visual acuity responses to the ECC from pure anisometropic amblyopes, strabismic amblyopes and mixed anisometropic-strabismic amblyopes. For the purposes of analysis, these shall be organised into two functional groups: those amblyopes with manifest strabismus (strabismic amblyopia and mixed amblyopia) and those without manifest strabismus (anisometropic amblyopia).

3.2 Study aims

Within this experiment, the aims were to;

1. Examine and compare visual acuity threshold estimates, interocular differences and crowding magnitudes of visually healthy children and clinically diagnosed amblyopes, aged between 3 and 11-years-old using isolated optotypes, the enhanced Cambridge Crowding Test (ECC) and the Sonksen logMAR test (SLT) presented in standard luminance (L); to determine whether the ECC provides greater sensitivity to amblyopia than the SLT.

2. Determine if differences exist in the visual acuity threshold estimates, interocular differences and crowding magnitudes, between strabismic/mixed and pure anisometropic amblyope subtypes, using the L-ECC.

3.3 Methodology

Full details of apparatus, stimuli and testing procedure can be found in Chapter 2, sections **2.2.1, 2.2.6, 2.2.4 and 2.2.8.1.**

3.3.1 Participants

Full participant details can be found in **2.2.3.** Participant demographics for L-ECC examination are summarised in Table 3.01.

3.3.2 Analysis

Full details of planned data analysis can be found in Chapter 2, section **2.4.**

Table 3.01: *Participant demographics for L-ECC examination*

	Sex		Age (Years)		Number per age group (years)			Total
	Male	Female	Mean	Range	3 - 4	5 - 7	8 -11	
Controls	**7**	**17**	**7.4**	**4-10**	**6**	**6**	**12**	**24**
Amblyopes	**23**	**21**	**6.6**	**3-10**	**4**	**32**	**8**	**44**
Anisometropes	*10*	*12*	*6.2*	*3-10*	*2*	*19*	*1*	*22*
Strabismic / mixed	*13*	*9*	*7.1*	*4-10*	*2*	*13*	*7*	*22*

3.4 Results

3.4.1 Visual Acuity Thresholds

Visual acuity threshold data for the three different tests (Isolated optotypes (L-Iso), SLT, and Enhanced Cambridge Crowding test (L-ECC)), averaged within each eye (RE, LE, Amblyopic eye, Fellow eye) and test group (controls, anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Figure 3.01 and Table 3.02. Data are mean±1 standard error unless stated otherwise. One-way repeated measures ANOVA demonstrated no statistically significant differences between right and left control eyes for all tests (p>.05) (Table 3.03), therefore control RE data were analysed against amblyopic eyes, and control LE data were analysed against fellow eyes. Re-analysis with the control eyes interchanged, did not yield any changes in significance.

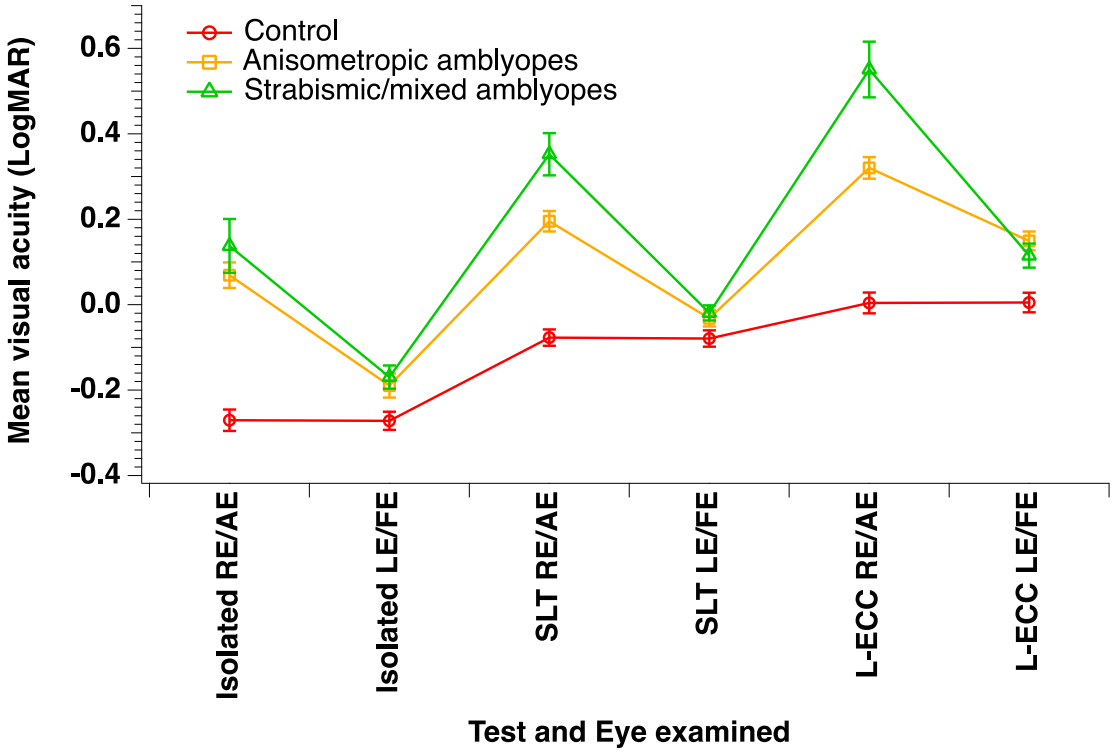


Figure 3.01: *Mean visual acuity thresholds for L-Isolated, SLT and L-ECC presentations in both eyes (RE-right eye, LE-left eye, AE-amblyopic eye, FE-fellow eye) of controls, anisometropic amblyopes and strabismic/mixed amblyopes. Error bars show ±1SE*

Table 3.02: *Mean visual acuity thresholds (logMAR) with standard error, for each group, eye, and acuity test.*

	n	L-lso	SLT	L-ECC
Controls – Right Eye	24	-0.270±0.025	-0.077±0.019	0.004±0.025
Controls – Left Eye	24	-0.272±0.021	-0.079±0.019	0.005±0.023
Anisometropic amblyopes – Amblyopic eye	22	0.069±0.030	0.195±0.024	0.320±0.024
Anisometropic amblyopes – Fellow eye	22	-0.189±0.028	-0.032±0.019	0.149±0.028
Strabismic/mixed amblyopes – Amblyopic eye	22	0.138±0.063	0.352±0.049	0.551±0.065
Strabismic/mixed amblyopes – Fellow eye	22	-0.169±0.027	-0.019±0.018	0.115±0.028

Table 3.03: *One-way repeated measures ANOVA examining acuity thresholds of right and left control eyes, per test format.*

	df	F	Sig	μ^2
L-lso	1	0.004	0.950	0.000
Error	23			
SLT	1	0.193	0.664	0.008
Error	23			
L- ECC	1	0.003	0.954	0.000
Error	23			

Visual acuities, as shown in Figure 3.01, were higher (worse) with SLT and L-ECC tests than L-Iso. A 3 (group) x 3 (test-format: L-Iso, SLT, L-ECC) x 2 (eye: RE/AE, LE/FE) repeated measures ANOVA (featuring one between factor (group) and two within factors (test-format and eye)) (see Table 3.04) showed this to be statistically significant [$F(2, 130) = 463.111$, **p<.001**]. This effect was significantly greater with the strabismic/mixed and anisometropic amblyopes than with the controls [$F(4, 130) = 4.614$, **p=.002**]. Visual acuities were also found to be significantly higher (worse) with the amblyopic eye than the fellow eyes, but no significant difference was seen between the eyes of the control participants [$F(2, 65) = 31.306$, **p<.001**]. The difference in the visual acuities between the eyes of participants was not significantly affected by test format [F(1.636,3.272)=0.412, p=.622]. These effects and interactions are explored below.

Table 3.04: *Repeated measures ANOVA with three test formats (Isolated, SLT, and L-ECC), three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) and eyes (Right eye, Left eye, Amblyopic eye and Fellow eye).*

	df	**F**	**Sig**	**μ^2**
Test format	**2**	**463.111**	**<0.001**	**0.877**
Error	130			
Group	**2**	**35.475**	**<0.001**	**0.522**
Error	65			
Eye	**1**	**103.132**	**<0.001**	**0.613**
Error	65			
Test format * group	**4**	**4.614**	**0.002**	**0.124**
Error	130			
Test format * eye	1.636	0.412	0.622	0.006
Error	3.272			
Eye * group	**2**	**31.306**	**<0.001**	**0.491**
Error	65			
Test format * group * eye	**3.272**	**7.906**	**<0.001**	**0.196**
Error	106.334			

3.4.1.1 Examination of effects between test-groups

Interaction of the three test groups (control, anisometropic amblyopes and strabismic/mixed amblyopes) and three test formats (Isolated, SLT and L-ECC) was seen on the visual acuity thresholds achieved; with significant effects of group seen for all three tests (**p<.001**) (Table 3.05).

Table 3.05: *Repeated measures ANOVA examining the effect of test group (controls, anisometropic amblyopes and strabismic/mixed amblyopes) on each test format (Isolated, SLT and L-ECC).*

	df	**F**	**Sig**	**μ^2**
Isolated format	**2**	**24.016**	**<0.001**	**0.425**
Error	65			
SLT format	**2**	**33.051**	**<0.001**	**0.504**
Error	65			
L-ECC	**2**	**36.529**	**<0.001**	**0.529**
Error	65			

To compare thresholds between groups, planned comparisons were made separately for each test, between the right eye of the control group and amblyopic eyes of the anisometropic and strabismic/mixed group, and between the left eye of the control group and fellow eyes of the anisometropic and strabismic/mixed group.

Pairwise comparisons revealed that for the isolated format both strabismic/mixed amblyopic eyes and anisometropic amblyopic eyes yielded significantly poorer thresholds than control eyes (+0.408±0.059, **p<.001** and +0.339±0.059, **p<.001** respectively), but that strabismic/mixed amblyopic did not differ significantly from anisometropic amblyopic eyes (+0.069±0.060, p=.779).

For the SLT format, both strabismic/mixed amblyopic eyes and anisometropic amblyopic eyes yielded significantly poorer thresholds than controls (+0.429±0.046, **p<.001** and +0.273±0.046, **p<.001** respectively), and strabismic/mixed amblyopic eyes were significantly larger than anisometropic amblyopic eyes (+0.157±0.047, **p=.004**).

For the L-ECC format, again, both strabismic/mixed amblyopes and anisometropic amblyopes yielded significantly poorer thresholds than controls (+0.546±0.059, **p<.001** and +0.316±0.059, **p<.001**), and strabismic/mixed amblyopic eyes were again significantly larger than anisometropic amblyopic eyes (+0.230±0.060, **p<.001**) (Figure 3.02).

Pairwise comparisons examining control eyes and fellow amblyopic eyes revealed that for the isolated format, strabismic/mixed fellow eyes yielded significantly poorer thresholds than control eyes (+0.102±0.036, **p=.017**), but that anisometropic fellow eyes did not differ significantly from either control eyes (+0.082±0.036, p=.074) or strabismic/mixed fellow eyes (-0.020±0.03, p=1.000).

For the SLT format, neither strabismic/mixed fellow eyes nor anisometropic fellow eyes demonstrated significantly different thresholds than control eyes (+0.060±0.026, p=.077 and +0.047±0.026, p=.227 respectively), and strabismic/mixed fellow eyes did not differ significantly from anisometropic fellow eyes (+0.012±0.027, p=1.000).

For the L-ECC format, both strabismic/mixed fellow eyes and anisometropic fellow eyes yielded significantly poorer thresholds than control eyes (+0.110±0.034, **p=.006** and +0.144±0.034, **p<.001**), while strabismic/mixed fellow eyes did not differ significantly from anisometropic fellow eyes (-0.034±0.035, p=.997) (Figure 3.03)

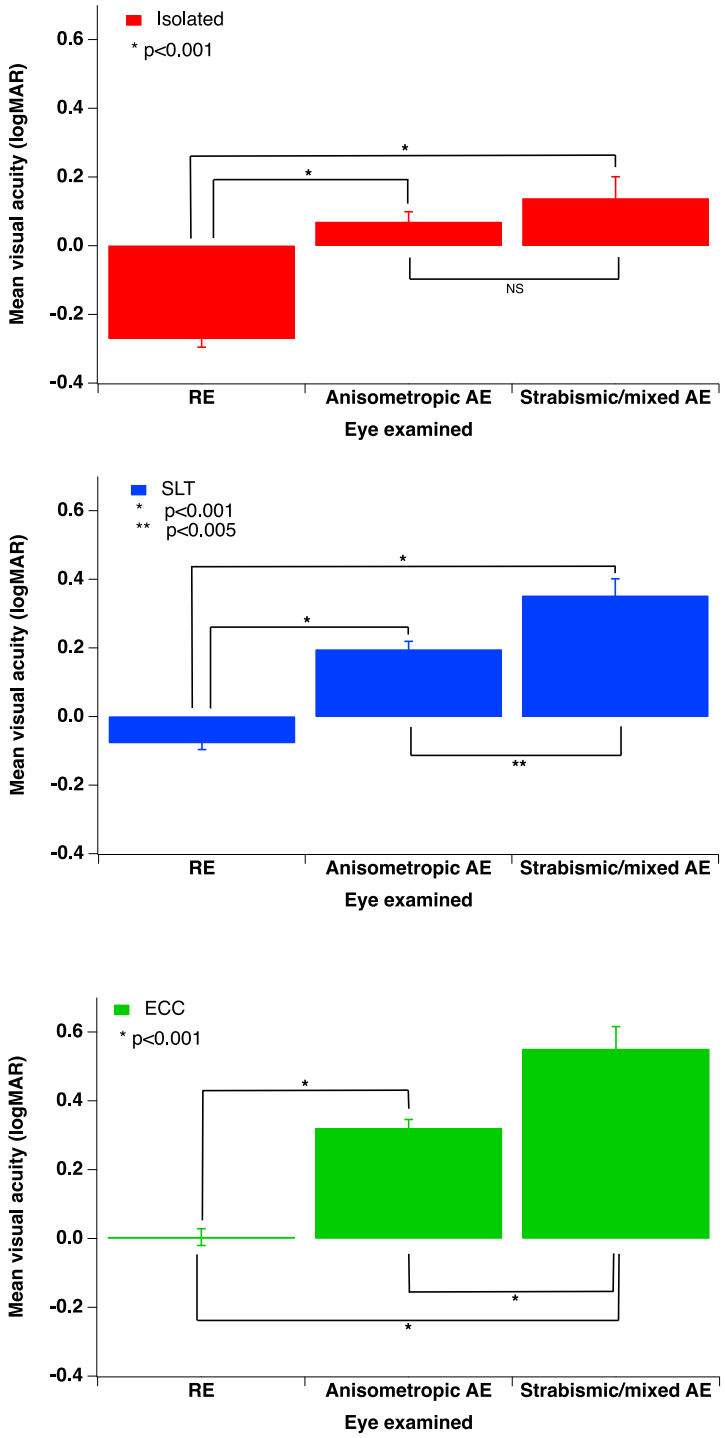


Figure 3.02: *Mean acuity thresholds for control right eyes, and anisometropic and strabismic/mixed amblyopic eyes, with significance bars. Top – Mean isolated acuity thresholds per eye. Middle – Mean SLT acuity thresholds per eye. Bottom – Mean L-ECC acuity thresholds averaged per eye. Error bars show ±1SE.*

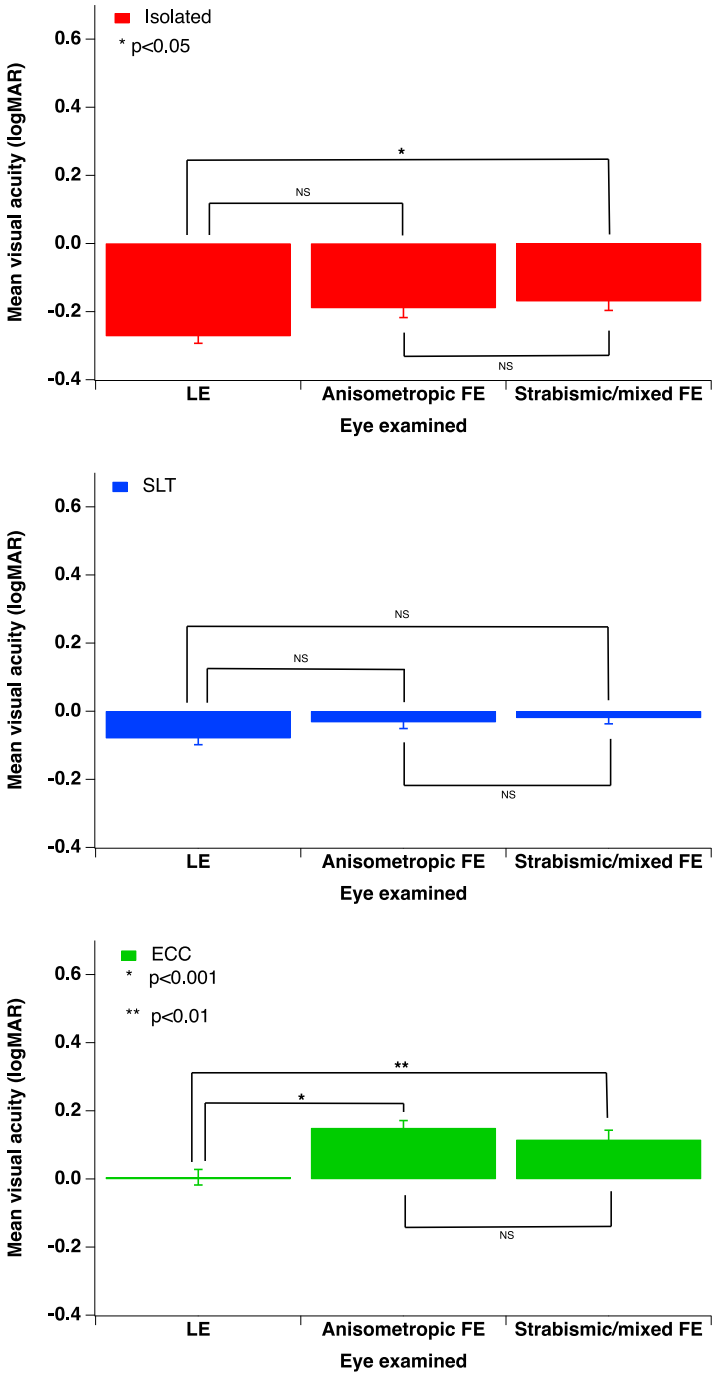


Figure 3.03: *Mean acuity thresholds for control left eyes, and anisometropic and strabismic/mixed fellow eyes, with significance bars. Top – Mean Isolated acuity thresholds per eye. Middle – Mean SLT acuity thresholds per eye. Bottom – Mean L-ECC acuity thresholds averaged per eye. Error bars show ±1SE.*

3.4.1.2 Effects within each test-group

Effect of test format (L-Iso, SLT, L-ECC) per group (Controls, anisometropic amblyopes and strabismic/mixed amblyopes)

All groups showed a significant effect of test (**p<.001**) (Table 3.06 and Figure 3.04). Pairwise comparison revealed that the L-ECC demonstrated significantly greater thresholds than SLT for both right and left control eyes (+0.081±0.19, **p<.001** and +0.084±0.018, **p<.001**). For anisometropic amblyopes, acuity increased by +0.125±0.020, (**p<.001**) for amblyopic eyes, and by +0.181±0.019 (**p<.001**) for fellow eyes. In strabismic/mixed amblyopes, amblyopic eyes increased by +0.198±0.020 (**p<.001**) and fellow eyes by +0.134±0.019 (**p<.001**).

Table 3.06: *One-way ANOVA (repeated measures) examining main effects of test format (Isolated, SLT and L-ECC) within each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	**2**	**175.005**	**<0.001**	**0.884**
Error	46			
Anisometropic amblyopes	**1.719**	**120.034**	**<0.001**	**0.851**
Error	36.101			
Strabismic/mixed amblyopes	**2**	**180.200**	**<0.001**	**0.896**
Error	42			

When these acuity changes were calculated as a percentage change (Table 3.07), the L-ECC demonstrates over twice the acuity diminishing effects of SLT (i.e., Iso to L-ECC compared with Iso to SLT) for both anisometropic and strabismic/mixed amblyopes. Interestingly, the amblyopic eyes of anisometropic amblyopes demonstrated the lowest change in thresholds overall.

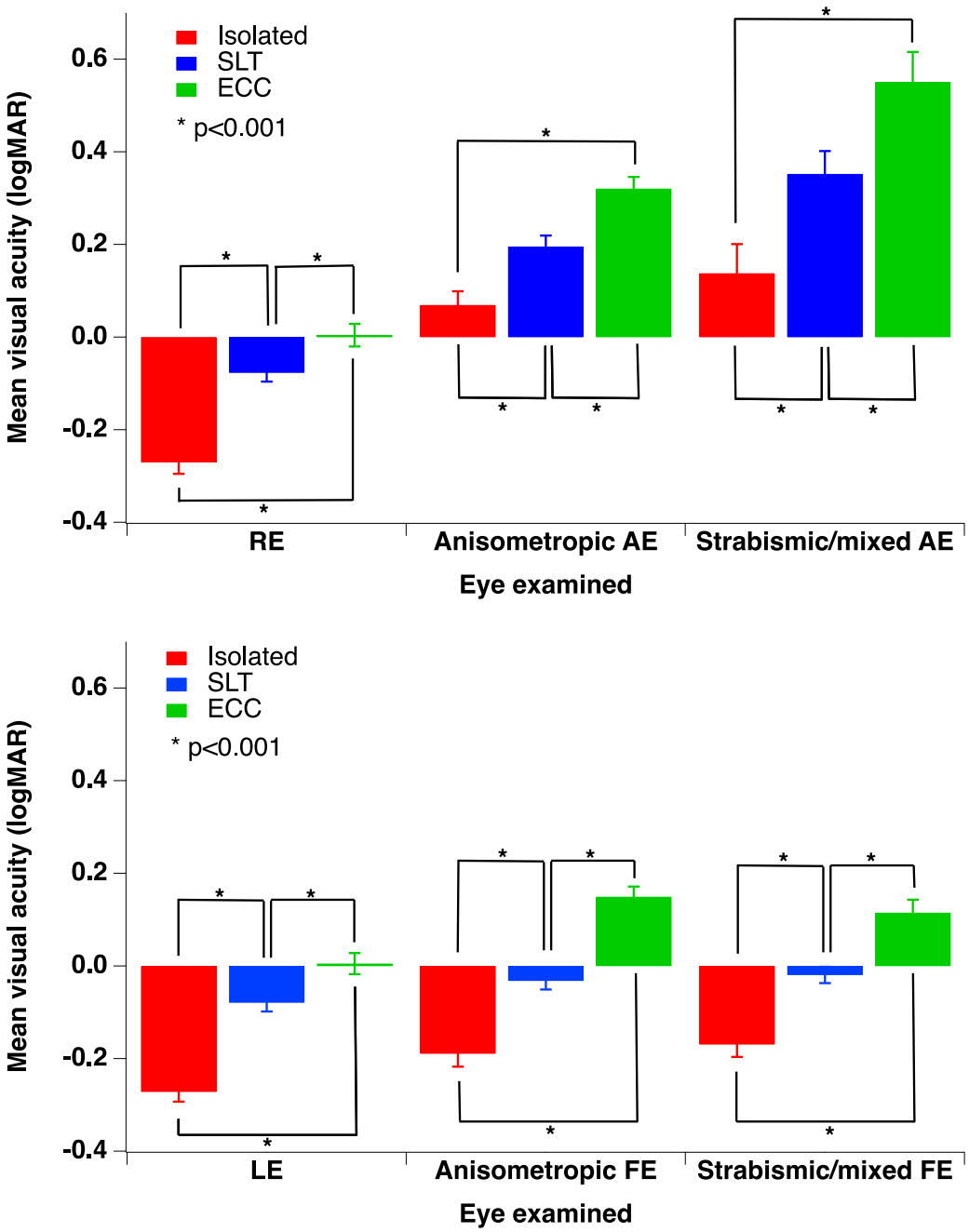


Figure 3.04: *Top – Acuity thresholds averaged for all tests within right control eyes and amblyopic eyes of each amblyopic subgroups, with significance bars. Bottom – Acuity thresholds averaged for all tests within left control eyes, and fellow eyes of each amblyopic subgroups, with significance bars. Error bars show ±1SE.*

Table 3.07: *Percentage change in acuity threshold between different test formats, per eye.*

	Iso to SLT	SLT to L-ECC	Iso to L-ECC
Controls RE	+56%	+21%	+88%
Controls LE	+56%	+21%	+89%
Anisometropic AE	+34%	+33%	**+78%**
Anisometropic FE	+44%	+52%	+118%
Strabismic/mixed AE	+64%	+58%	**+159%**
Strabismic/mixed FE	+41%	+36%	+92%

Effect of eye per group (Controls, anisometropic amblyopies and strabismic/mixed amblyopes)

Anisometropic and strabismic/mixed amblyopes had a significant difference in the visual acuities obtained between their two eyes (**p<.001**) but control participants did not (p>.05) (see Table 3.08). Pairwise comparisons showed that the amblyopic eye of anisometropic and strabismic/mixed amblyopes were significantly poorer than the fellow eye for the isolated (+0.258±0.041, **p<.001** and +0.307±0.04, **p<.001**, respectively), SLT (+0.227±0.031, **p<.001** and +0.372±0.31, **p<.001** respectively) and L-ECC format (+0.171±0.040, **p<.001** and +0.436±0.040, **p<.001** respectively) test formats.

Table 3.08: *One-way ANOVA (repeated measures) examining simple main effects of eye (RE, LE, amblyopic eye and fellow eye) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	F	Sig	μ^2
Controls	1	0.012	0.913	0.001
Error	23			
Anisometropic amblyopes	**1**	**84.612**	**<0.001**	**0.801**
Error	21			
Strabismic/mixed amblyopes	**1**	**46.712**	**<0.001**	**0.690**
Error	21			

3.4.2 Interocular differences (IOD)

Interocular difference (IOD) was calculated as the absolute difference (recorded in logMAR) between the acuities of each eye. Mean IODs for the three different tests (Isolated, SLT, and L-ECC), and test groups (controls, anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Figure 3.05 and Table 3.09. Data are mean±1 standard error unless stated otherwise.

The L-ECC did not produce larger IODs than the SLT. A 3 (group) x 3 (test-format) repeated measures ANOVA (featuring one between factor (group) and one within factor (test-format)) revealed IOD differences between the three test formats (Isolated, SLT, L-ECC) to be insignificant [F(1.783, 115.916) = 0.770, p=.452], however, the difference in IODs obtained by each group were significantly affected by the test format used [F(3.567, 115.916) = 12.141, **p<.001**] (Table 3.10).

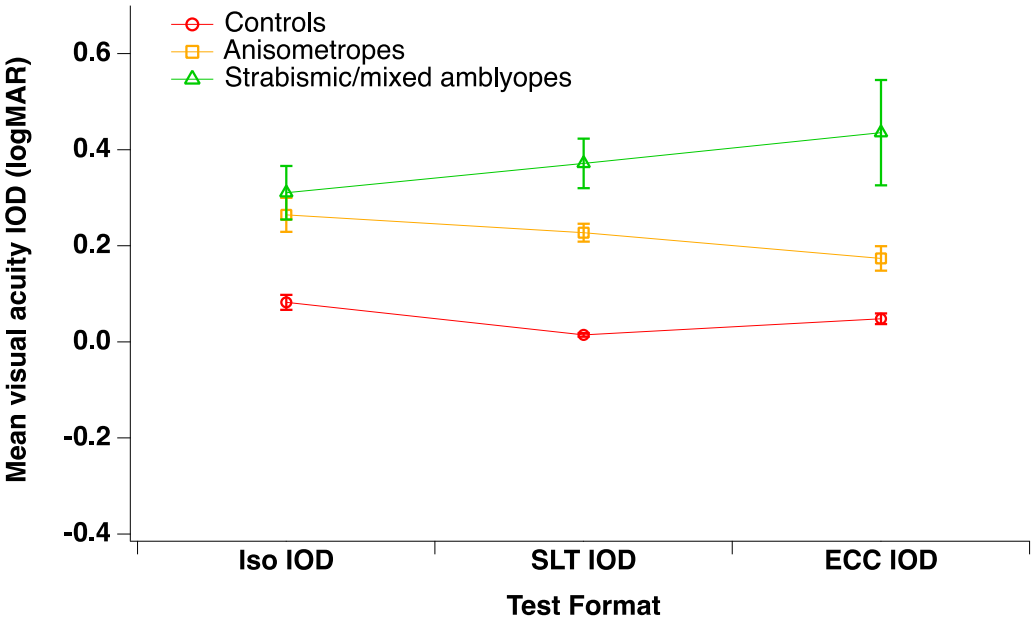


Figure 3.05: *Mean IOD (logMAR) for each acuity test (Isolated, SLT and ECC) per group (controls, anisometropic and strabismic/mixed amblyopes). Error bars show ±1SE.*

Table 3.09: *Mean visual acuity interocular differences (logMAR) with standard error, for each group and acuity test.*

	n	Iso IOD	SLT IOD	ECC IOD
Controls	24	0.082±0.016	0.015±0.004	0.048±0.011
Anisometropic amblyopes	22	0.264±0.035	0.227±0.019	0.174±0.026
Strabismic/mixed amblyopes	22	0.311±0.056	0.372±0.052	0.436±0.062

Table 3.10: *Repeated measures ANOVA examining differences in IOD with three test formats (Isolated, SLT and LM-ECC) for three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	F	Sig	μ^2
Test format	1.783	0.770	0.465	0.012
Error	115.916			
Group	**2**	**24.241**	**<0.001**	**0.427**
Error	65			
Test format * group	**3.567**	**12.141**	**<0.001**	**0.272**
Error	115.916			

3.4.2.1 Examination of effects between test-groups

Repeated measures analysis of each test demonstrated statistically significant differences in IOD between groups (Table 3.11 and Figure 3.06) for all tests (**p<.001** respectively). Pairwise comparisons revealed that for the isolated format both strabismic/mixed eyes and anisometropic eyes yielded significantly larger IODs than control eyes (+0.228±0.054, **p<.001** and +0.182±0.054, **p=.003** respectively), but strabismic/mixed eyes did not differ from anisometropic eyes (+0.046±0.055, p=1.000).

For the SLT format, both strabismic/mixed eyes and anisometropic eyes yielded significantly higher IODs than controls (+0.357±0.043, **p<.001** and +0.213±0.043, **p<.001** respectively), and strabismic/mixed eyes also demonstrated significantly larger IODs than anisometropic eyes (+0.144±0.044, **p=.005**).

For the L-ECC format, strabismic/mixed amblyopes yielded significantly larger IODs thresholds than controls (+0.387±0.054, **p<.001)** but anisometropic amblyopes did not (+0.126±0.054, p=.068). Strabismic/mixed amblyopic eyes again also demonstrated significantly larger IODs than anisometropic eyes (+0.262±0.055, **p<.001**).

Table 3.11: Repeated measures ANOVA examining the effect of group (controls, anisometropic amblyopes and strabismic/mixed amblyopes) for each test format (Isolated, SLT and L-ECC) on IODs.

	df	**F**	**Sig**	**μ^2**
Isolated IOD	**2**	**10.275**	**<0.001**	**0.240**
Error	65			
SLT IOD	**2**	**34.783**	**<0.001**	**0.517**
Error	65			
L-ECC IOD	**2**	**26.690**	**<0.001**	**0.451**
Error	65			

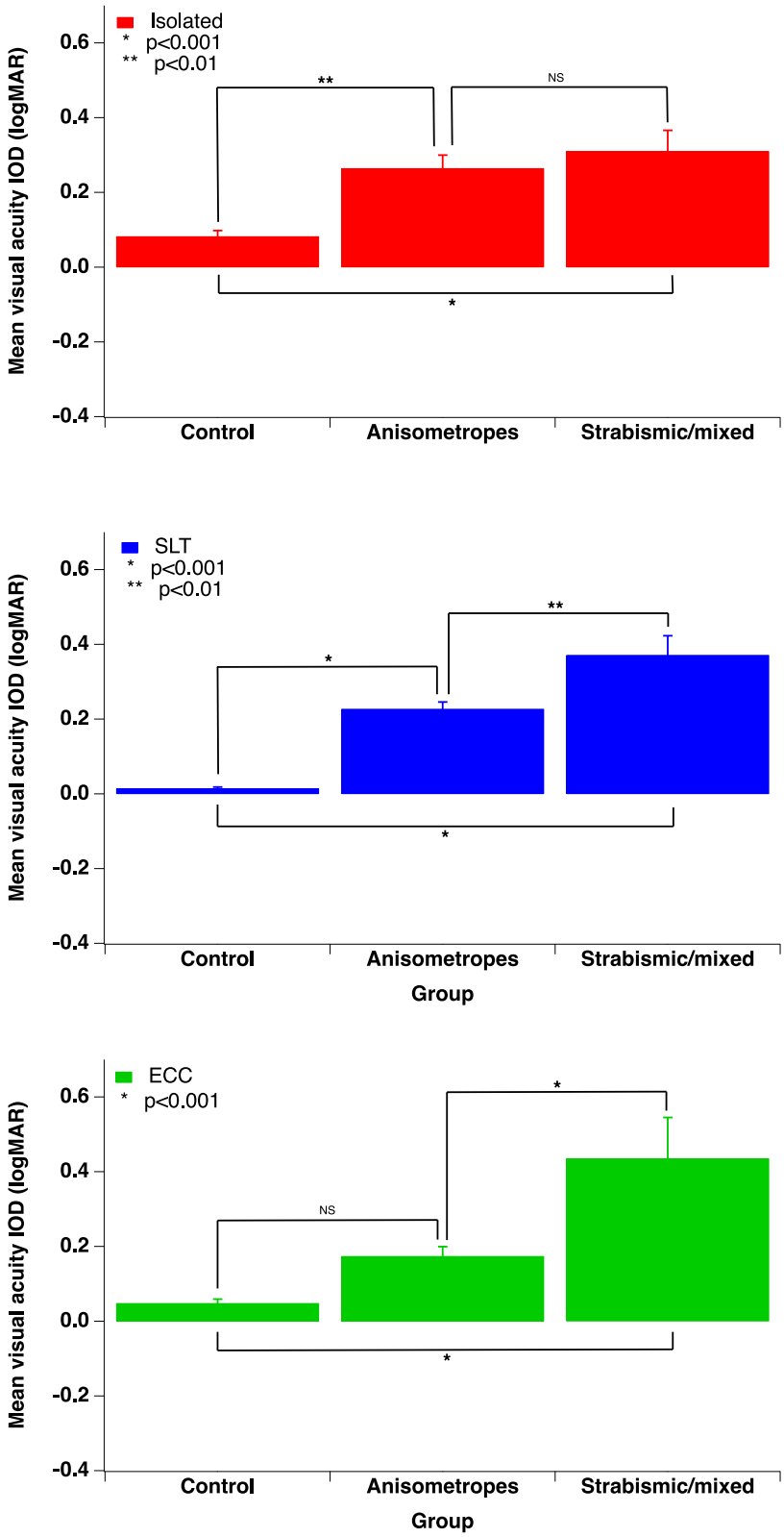


Figure 3.06: *Mean IODs for each test, per group, with significance bars. Top – Isolated IODs. Middle – SLT IODs. Bottom - ECC IODs. Error bars show ±1SE.*

3.4.2.2 Effects within each test-group

Effect of test format (Isolated, SLT and L-ECC) per group (Controls, anisometropic amblyopes and strabismic/mixed amblyopes)

Examination of test effects within groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) revealed statistically significant differences in IOD for all groups [Controls: $F(2, 46) = 8.781$, **p<.001;** Anisometropic amblyopes: $F(1.636, 34.347) = 6.624$, **p=.006**; Strabismic/mixed amblyopes; $F(2, 42) = 9.300$, **p<.001**] (Table 3.12), with decreased target-flanker spacing resulted in increasing IODs for strabismic/mixed amblyopes and decreasing IODs for anisometropic amblyopes (Figure 3.07).

Table 3.12: *Repeated measures ANOVA examining effect of test (Isolated, SLT and L-ECC) within each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	**2**	**8.781**	**<0.001**	**0.276**
Error	46			
Anisometropic amblyopes	**1.636**	**6.624**	**0.006**	**0.240**
Error	34.347			
Strabismic/mixed amblyopes	**2**	**9.300**	**<0.001**	**0.307**
Error	42			

Controls demonstrated statistically significantly larger IODs with isolated optotypes than with SLT (+0.068±0.023*,* **p=.012)**, but no significant difference in IOD was seen between SLT and L-ECC formats (p=.217), or isolated optotypes and the L-ECC format (p=.628). While this difference in IODs between isolated and SLT format was statistically significant, neither format resulted in a clinically significant IOD (i.e., $\geq$0.100 logMAR).

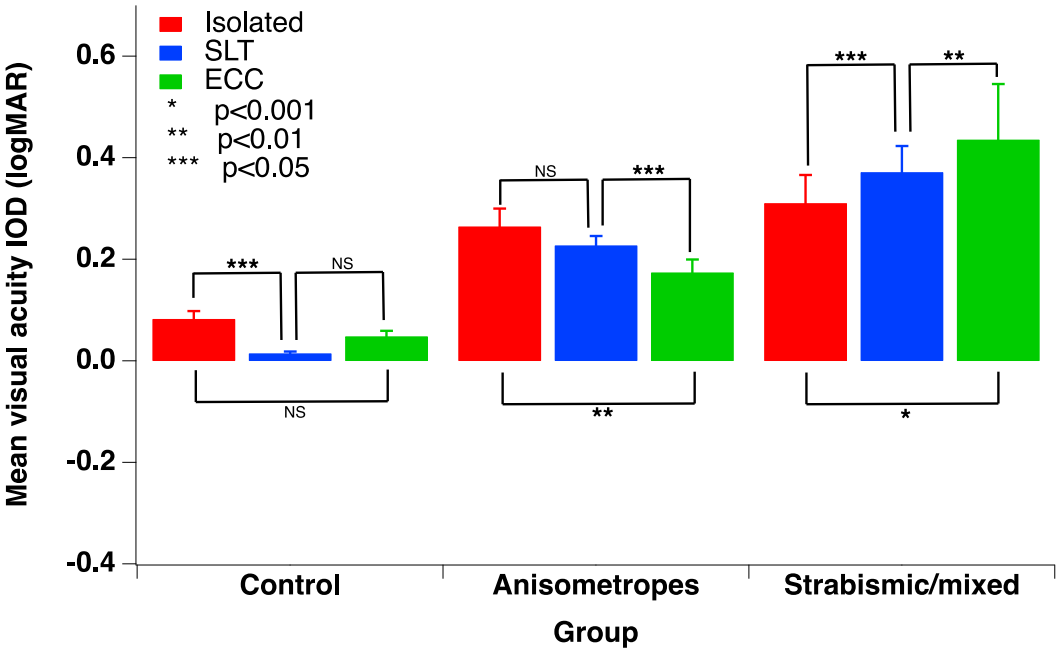


Figure 3.07: *Mean IODs for all tests (Isolated, SLT and L-ECC) within control, anisometropic and strabismic/mixed amblyopic groups. Error bars show ±1SE.*

Anisometropes demonstrated a downward trend whereby decreasing target-flanker spacing decreased IODs. No significant difference in IOD was seen between the Isolated and SLT formats (p=.364), however the L-ECC yielded significantly lower IODs than both the Isolated (-0.091±0.028, **p=.006**) and SLT format (-0.053±0.019, **p=.021**).

Comparably, strabismic/mixed amblyopes demonstrated an upward trend whereby decreasing target-flanker spacing resulted in increased IODs experienced. SLT IODs were significantly larger than those of the isolated format (+0.061±0.024, **p=.037**), and L-ECC IODs were significantly larger than those of the SLT (+0.064±0.019, **p=.004**), and isolated optotypes (+0.125±0.028, **p<.001**).

3.4.3 Crowding magnitude

Magnitude of crowding was calculated as the acuity difference in logMAR between thresholds achieved with the isolated format and each of the crowded formats (SLT and L-ECC) (i.e. SLT threshold – isolated, and L-ECC threshold – isolated). Crowding magnitude data for the two different crowded tests (SLT and L-ECC), averaged within each eye (RE, LE, Amblyopic eye, Fellow eye) and test group (Controls, Anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Figure 3.08 and Table 3.13. Data are mean±1 standard error unless stated otherwise. Crowding magnitudes were lowest in the amblyopic eyes of anisometropic amblyopes (SLT: +0.126±0.027, L-ECC: +0.251±0.025) and highest in the amblyopic eyes of strabismic/mixed amblyopes (SLT: +0.215±0.028, L-ECC: +0.413±0.030).

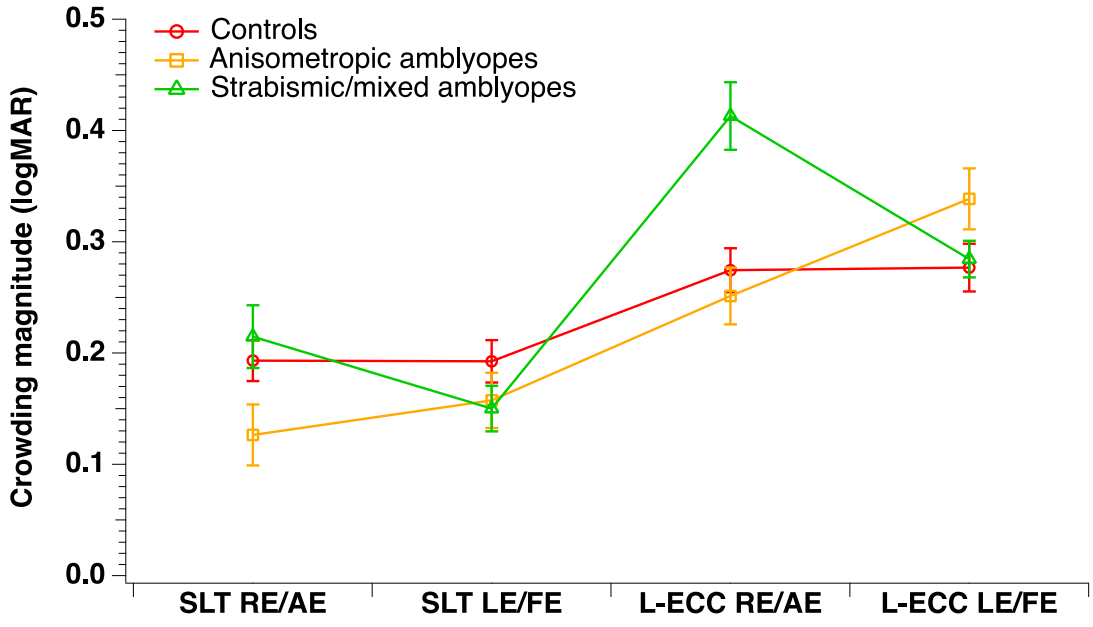


Figure 3.08: *Mean crowding magnitudes of the SLT and L-ECC (given in logMAR) for each eye (RE-right eye, LE-left eye, AE-amblyopic eye, FE-Fellow eye) of all groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes). Error bars show ±1SE*

Table 3.13: *Mean crowding magnitudes of the SLT and L-ECC (given in logMAR) with standard error, for each group (controls, anisometropic and strabismic/mixed amblyopes) and eye.*

	n	SLT	ECC
Controls – Right eye	24	+0.193±0.018	+0.274±0.020
Controls – Left Eye	24	+0.193±0.019	+0.277±0.022
Anisometropic amblyopes – Amblyopic eye	22	+0.126±0.027	+0.251±0.025
Anisometropic amblyopes – Fellow eye	22	+0.157±0.025	+0.336±0.027
Strabismic/mixed amblyopes – Amblyopic eye	22	+0.215±0.028	+0.413±0.030
Strabismic/mixed amblyopes – Fellow eye	22	+0.150±0.020	+0.284±0.016

One-way repeated measures ANOVA demonstrated no statistically significant differences between right and left control eyes for all tests ($p>.05$) (Table 3.14), therefore control RE data were analysed against amblyopic eyes, and control LE data were analysed against fellow eyes. Re-analysis with the control eyes interchanged, did not yield any changes in significance.

Table 3.14: *One-way repeated measures ANOVA examining the crowding magnitudes of right and left control eyes, per test format.*

	df	F	Sig	μ^2
SLT crowding magnitudes	1	0.001	0.979	0.000
Error	23			
ECC crowding magnitudes	1	0.006	0.941	0.000
Error	23			

The L-ECC demonstrated larger crowding magnitudes than SLT, which a 3 (group) x 2 (test-format) x 2 (eye) repeated measures ANOVA (featuring one between factor (group) and two within factors (test-format and eye)) revealed to be statistically significant [$F(1, 65) = 204.222$, **p<.001**] (Table 3.15). This effect was significantly larger in strabismic/mixed amblyopes, than anisometropic amblyopes and controls [$F(2, 65) = 7.860$, **p<.001**]. Compared with their fellow eyes, the crowding magnitudes of the amblyopic eye were larger in strabismic/mixed amblyopes, but smaller in the amblyopic eyes of anisometropic amblyopes; no difference was seen between the eyes of controls [$F(2, 65) = 7.550$, **p=.001**]. The difference in crowding magnitudes between the eyes of participants was not significantly affected by the test format [F(1,65)=0.018, p=.892].

Table 3.15: *Repeated measures ANOVA examining crowding magnitudes, with two test formats (SLT, and L-ECC), three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) and eyes (Right eye, Left eye, Amblyopic eye and Fellow eye).*

	df	**F**	**Sig**	**μ^2**
Test format	**1**	**204.022**	**<0.001**	**0.758**
Error	65			
Group	2	2.149	0.125	0.062
Error	65			
Eye	1	0.559	0.457	0.009
Error	65			
Test format * group	**2**	**7.860**	**<0.001**	**0.195**
Error	65			
Test format * eye	1	0.018	0.892	0.000
Error	65			
Eye * group	**2**	**7.550**	**0.001**	**0.189**
Error	65			
Test format * group * eye	**2**	**8.859**	**<0.001**	**0.214**
Error	65			

3.4.3.1 Examination of effects between groups

An effect of test format (SLT and L-ECC) was seen on the crowding magnitudes of the three test groups (control, anisometropic amblyopes and strabismic/mixed amblyopes) (**p<.001**), with significant differences in crowding magnitude seen between the three groups for the L-ECC format (**p=.011**), but not the SLT (p>.05) (Table 3.16).

Table 3.16: *Repeated measures ANOVA examining the simple effects of group (controls, anisometropic amblyopes and strabismic/mixed amblyopes), upon each test format (SLT and L-ECC).*

	df	**F**	**Sig**	**μ^2**
SLT magnitude	2	2.031	0.139	0.059
Error	65			
L-ECC magnitude	**2**	**4.822**	**0.011**	**0.129**
Error	65			

Further examination of the L-ECC format revealed significantly larger crowding magnitudes between strabismic/mixed amblyopic eyes and control eyes (+0.139±0.035, **p<.001**), strabismic/mixed and anisometropic amblyopic eyes (+0.162±0.036, **p<.001**), but not between anisometropic amblyopic eyes and control eyes (-0.023±0.035, p=1.000). No statistically significant difference in crowding magnitude was noted either between strabismic/mixed fellow eyes and control eyes (+0.008±0.031, p=1.000), strabismic/mixed and anisometropic fellow eyes (-0.054±0.032, p=.282), or anisometropic fellow eyes and control eyes (+0.062±0.031, p=.156) for the L-ECC format (Figure 3.09).

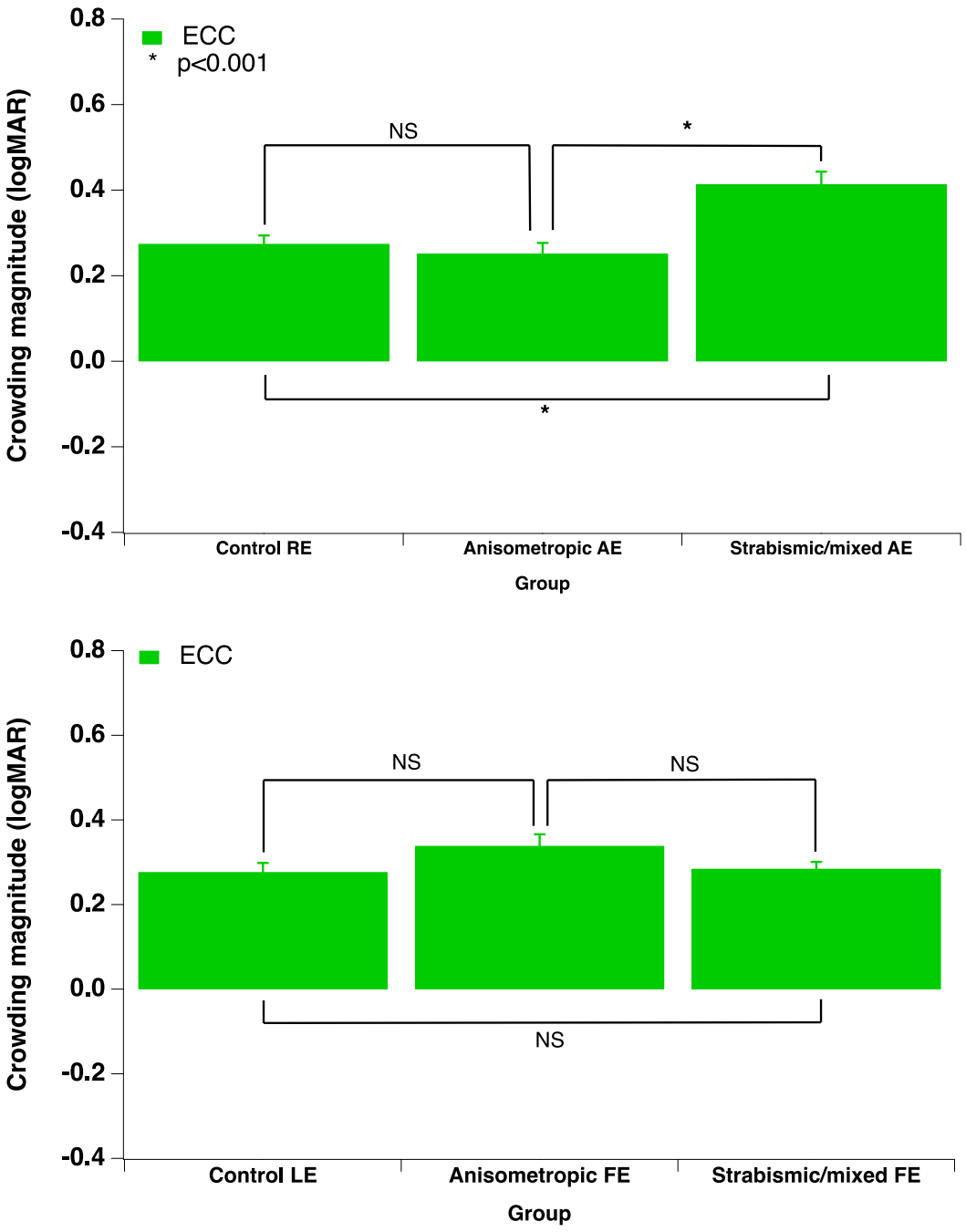


Figure 3.09: *Mean ECC crowding magnitudes, with significance bars. Top – Control right eyes, anisometropic and strabismic/mixed amblyopic eyes, Bottom – Control left eyes, anisometropic and strabismic/mixed amblyopic eyes. Error bars show ±1SE.*

3.4.3.2 Effects within each test-group

Examination of test format within each group

All groups showed a significantly larger crowding magnitudes with the L-ECC compared with the SLT (**p<.001**) (Table 3.17). Pairwise comparisons revealed significantly larger crowding magnitudes for the ECC compared with the SLT, for all groups (Control: +0.091±0.016, **p<.001;** Anisometropic amblyopes: +0.115±0.031, **p<.001;** Strabismic/mixed amblyopes: +0.191±0.021, **p<.001**). Crowding magnitudes were larger using the L-ECC than SLT for both the eyes of controls (RE: +0.092±0.019, **p<.001**; LE: +0.090±0.018, **p<.001**), anisometropic amblyopes (Amblyopic eye: +0.110±0.037, **p=.004**; Fellow eye: +0.120±0.035, **p=.001**) and strabismic/mixed amblyopes (Amblyopic eye: +0.225±0.025, **p<.001**; Fellow eye: +0.157±0.024, **p<.001**) (Figure 3.10).

Table 3.17: *One-way ANOVA (repeated measures) examining the simple main effects of test format (SLT and L-ECC) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	**1**	**23.707**	**<0.001**	**0.508**
Error	23			
Anisometropic amblyopes	**1**	**126.810**	**<0.001**	**0.858**
Error	21			
Strabismic/mixed amblyopes	**1**	**88.840**	**<0.001**	**0.809**
Error	21			

When calculated as a percentage change, the increases in crowding magnitude from SLT to the L-ECC, are clearly identified within each eye; with the largest changes in crowding magnitude occurring in the amblyopic eyes of strabismic/mixed amblyopes (+57.85%), followed by the fellow eyes of both anisometropic amblyopes and strabismic/mixed amblyopes (51.74%) (Table 3.18).

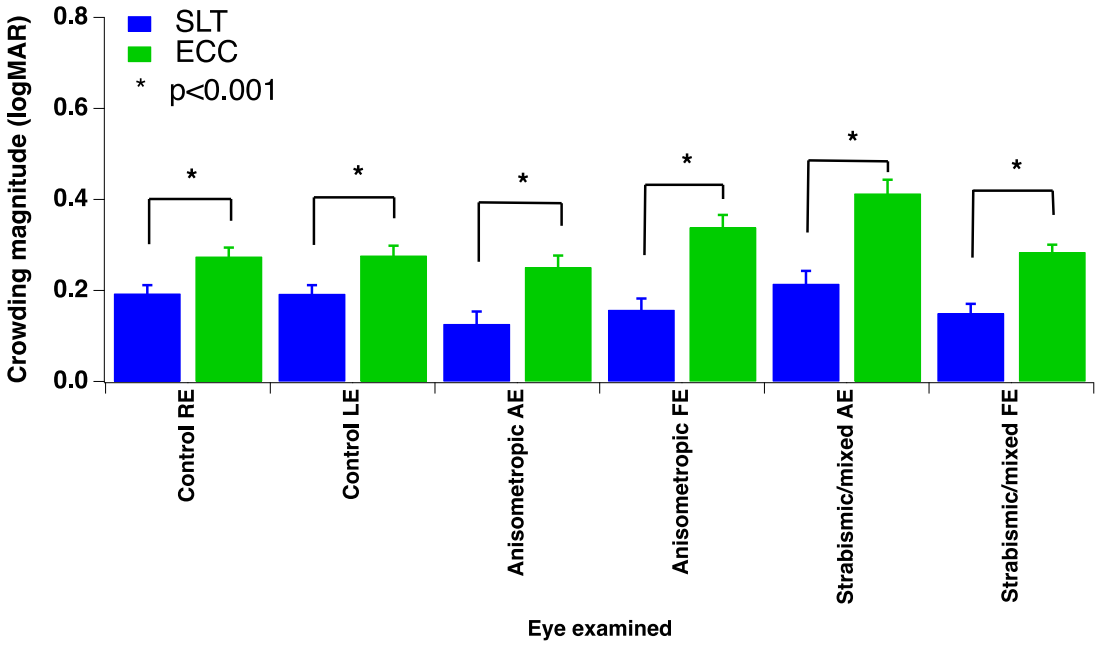


Figure 3.10: *Mean crowding magnitudes for SLT and L-ECC presentations in all eyes (RE-right eye, LE-left eye) of controls, anisometropic amblyopes and strabismic/mixed amblyopes, with significance bars. Error bars show ±1SE*

Table 3.18*: Percentage change in crowding magnitude between SLT and L-ECC, per eye.*

	SLT to L-ECC
Controls RE	+20.56%
Controls LE	+21.39%
Anisometropic AE	+33.31%
Anisometropic FE	+51.74%
Strabismic/mixed AE	+57.85%
Strabismic/mixed FE	+51.74%

Effect of eye within each group, examined per test

Strabismic/mixed amblyopes showed larger crowding magnitudes with their amblyopic eye compared with their fellow eye [$F(1,21) = 11.162$, **p=.003**], but anisometropic amblyopes and controls did not show such effects (p>.05) (Table 3.19). Pairwise comparisons showed that the amblyopic eye of strabismic/mixed amblyopes yielded significantly larger crowding magnitudes than the fellow eye for both the SLT format (+0.065±0.027, p=.**021**) and the L-ECC format (+0.129±0.033, **p<.001**) (Figure 3.11). Anisometropic amblyopes came close to a significant effect of eye; with the amblyopic eye of anisometropic amblyopes yielding significantly smaller crowding magnitudes than the fellow eye for the L-ECC format (-0.87±0.033, **p=.011**), but not SLT (-0.031±0.027, p=.259).

Table 3.19: *Repeated measures ANOVA examining simple main effects of eye (RE, LE, amblyopic eye and fellow eye) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	1	0.001	0.974	0.000
Error	23			
Anisometropic amblyopes	1	3.974	0.059	0.159
Error	21			
Strabismic/mixed amblyopes	**1**	**11.162**	**0.003**	**0.347**
Error	21			

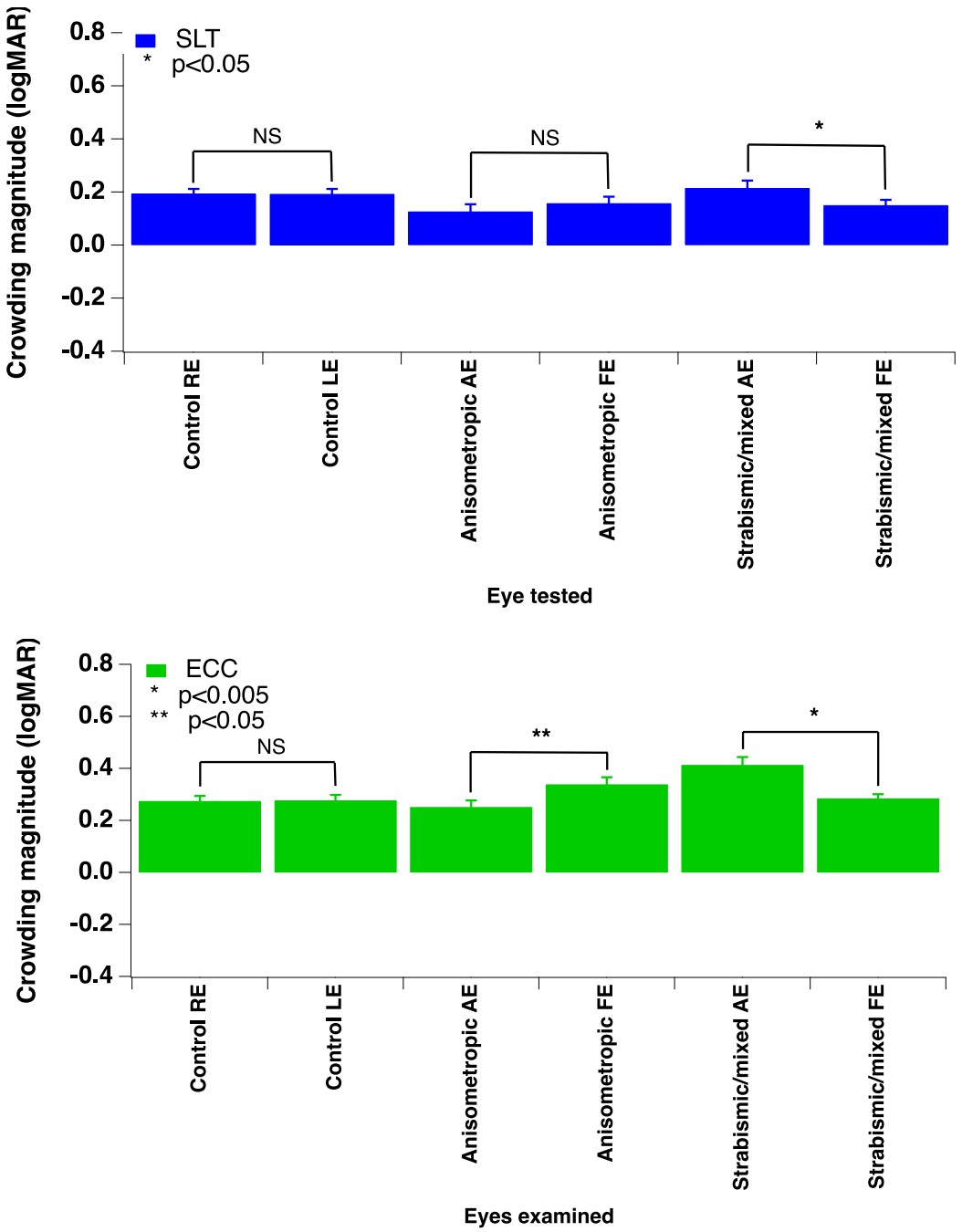


Figure 3.11*: Mean crowding magnitudes for SLT and L-ECC presentations in all eyes (RE-right eye, LE-left eye) of controls, anisometropic amblyopes and strabismic/mixed amblyopes (AE- amblyopic eye, FE – fellow eye). Top – SLT crowding magnitudes, Bottom – L-ECC crowding magnitudes. Error bars show ±1SE*

3.4.4 Interocular difference in crowding magnitude

Interocular difference of crowding magnitude was calculated as the relative difference in crowding magnitude (calculated as the difference in logMAR between the acuity thresholds of the isolated format and each of the crowded formats (SLT and L-ECC)), between the two eyes (i.e., RE-LE or AE-FE). Data regarding the interocular difference of crowding magnitude for the two different crowded tests (SLT and L-ECC), and three test groups (Controls, Anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Figure 3.12 and Table 3.20. Data are mean±1 standard error unless stated otherwise.

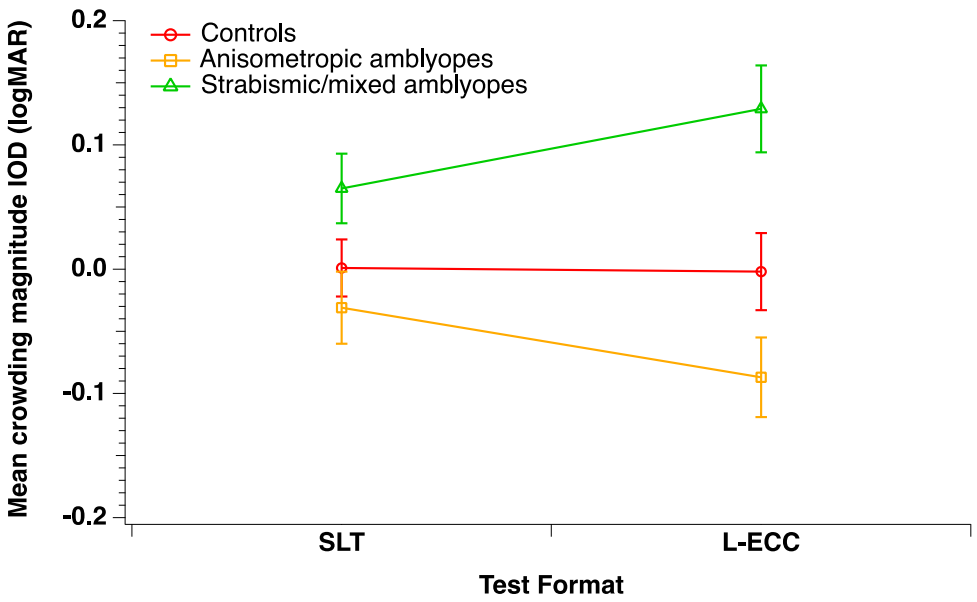


Figure 3.12: *Mean interocular difference of crowding magnitude (logMAR) for each acuity test (SLT and ECC) per group (controls, anisometropic and strabismic/mixed amblyopes). Error bars show ±1SE.*

While controls demonstrate less than one letter difference (<0.020 logMAR) in crowding magnitude IOD, strabismic/mixed amblyopes demonstrate positive values of crowding magnitude IOD for both tests, indicating that crowding magnitude is larger in amblyopic eye. Comparatively, anisometropic amblyopes show negative values of crowding magnitude IOD for both tests, demonstrating that higher crowding magnitudes are seen in fellow eye of this amblyopic subgroup.

Table 3.20: *Mean interocular differences in crowding magnitudes of the SLT and L-ECC (calculated as right eye crowding magnitude – left eye crowding magnitude; or amblyopic eye crowding magnitude – fellow eye crowding magnitude, given in logMAR), for each group (controls, anisometropic and strabismic/mixed amblyopes).*

	n	SLT	ECC
Controls	24	+0.001±0.023	-0.002±0.031
Anisometropic amblyopes	22	-0.031±0.029	-0.087±0.032
Strabismic/mixed amblyopes	22	+0.065±0.028	+0.129±0.035

A 3 (group) x 2 (test-format) repeated measures ANOVA (featuring one between factor (group) and one within factor (test-format)) revealed a statistically significant effect of test format on the interocular differences in crowding magnitude, for each group [$F(2, 65) = 8.859$, **p<.001**]. The highest interocular differences of crowding magnitude were seen in strabismic/mixed amblyopes, while the lowest differences were seen in anisometropic amblyopes [$F(2, 65) = 7.550$, **p=.001**]. No simple main effect of test format (SLT, L-ECC) was seen [$F(1, 65) = 0.018$, p=.892] (Table 3.21).

Table 3.21: *Repeated measures ANOVA examining interocular crowding magnitudes with two test formats (SLT and LM-ECC) for three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	F	Sig	μ^2
Test format	1	0.018	0.892	0.000
Error	65			
Group	**2**	**7.550**	**0.001**	**0.189**
Error	65			
Test format * group	**2**	**8.859**	**<0.001**	**0.214**
Error	65			

3.4.4.1 Between group analysis

Effect of test format (SLT and L-ECC) between group (Controls, anisometropic amblyopes and strabismic/mixed amblyopes)

Repeated measures analysis of each test demonstrated statistically significant differences in crowding magnitude IOD between groups (Table 3.21 and Figure 3.12) for all tests (**p<.001** respectively) (Table 3.22).

Pairwise comparisons revealed that for the SLT format, strabismic/mixed eyes yielded significantly higher crowding magnitude IODs than anisometropic amblyopic eyes (+0.096±0.039, **p=.047).** Neither anisometropic eyes, nor strabismic/mixed eyes showed significant differences to control eyes (-0.032±0.038, p=1.000 and +0.064±0.038, p=.283, respectively). For the L-ECC format, strabismic/mixed eyes yielded significantly higher crowding magnitude IODs than anisometropic amblyopic eyes (+0.216±0.047, **p<.001**) and control eyes (+0.131±0.047, **p=.018**). No significant difference was seen between anisometropic eyes, and control eyes (-0.085±0.046, p=.208)

Table 3.22: Repeated measures ANOVA examining the effect of group (controls, anisometropic amblyopes and strabismic/mixed amblyopes) for each test format (Isolated, SLT and L-ECC).

	df	**F**	**Sig**	**μ^2**
SLT crowding magnitude IOD	**2**	**3.210**	**<0.047**	**0.090**
Error	65			
L-ECC crowding magnitude IOD	**2**	**10.707**	**<0.001**	**0.248**
Error	65			

3.4.4.2 Within test group analysis

Effect of test format (SLT and L-ECC) per group (Controls, anisometropic amblyopes and strabismic/mixed amblyopes)

Examination of test effects within groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) revealed statistically significant decreases in crowding magnitude IOD for anisometropic amblyopes: (F(1, 21) = 9.982, **p=.005)**, statistically significant increases in crowding magnitude IOD for strabismic/mixed amblyopes (F(1, 21) = 6.368, **p=.020**), and no differences for controls (F(1, 23) = 0.034, p=.855) (Table 3.22), with the L-ECC compared with SLT (Table 3.23 & Figure 3.13).

Table 3.23: *Repeated measures ANOVA examining effect of test (SLT and L-ECC) within each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	1	0.034	0.855	0.001
Error	23			
Anisometropic amblyopes	**1**	**9.982**	**0.005**	**0.322**
Error	21			
Strabismic/mixed amblyopes	**1**	**6.368**	**0.020**	**0.233**
Error	21			

Anisometropes demonstrated a downward trend whereby decreasing target-flanker spacing decreased crowding magnitude IODs, with the L-ECC demonstrating significantly lower crowding magnitude IODs than the SLT (-0.056±0.020*,* **p=.007**). Comparably, strabismic/mixed amblyopes demonstrated an upward trend whereby decreasing target-flanker spacing resulted in increased crowding magnitude IODs experienced. L-ECC crowding magnitude IODs were significantly larger than those of the those of the SLT (+0.064±0.020*,* **p=.002**).

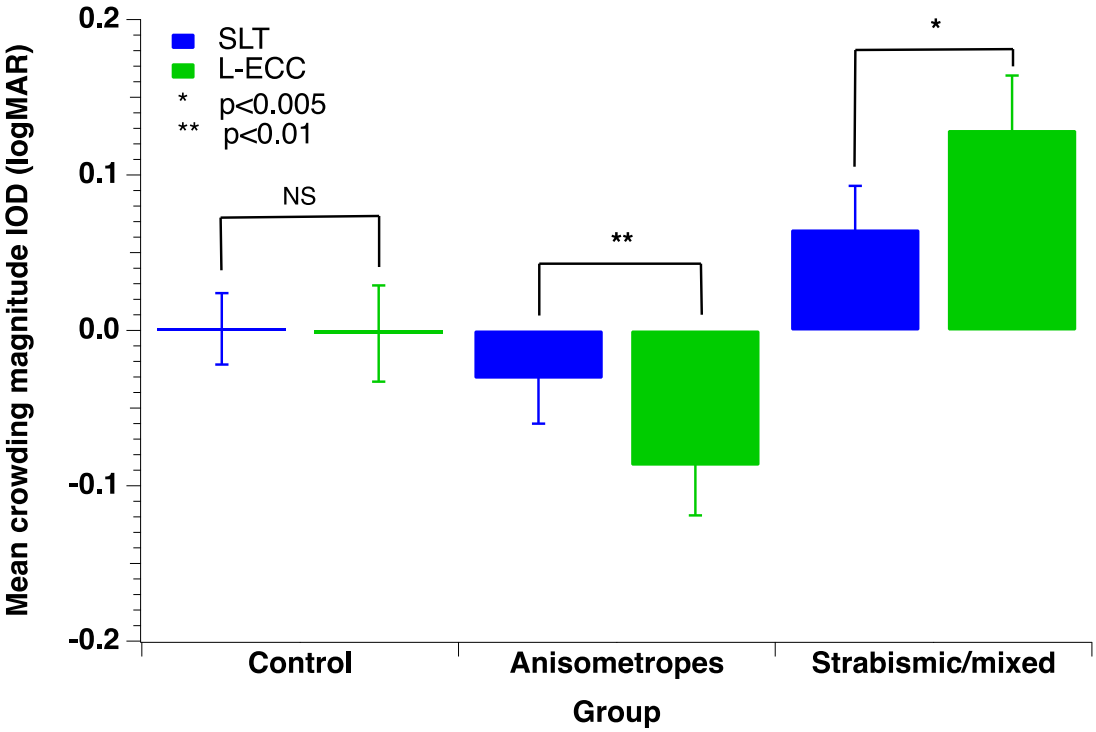


Figure 3.13: *Mean crowding magnitude IODs for each test, per group, with significance bars. Error bars show ±1SE.*

3.5 Discussion

This study aimed to compare the visual crowding effects of the L-ECC compared to the SLT for amblyopia detection. The results showed that the L-ECC demonstrates significantly larger crowding magnitudes than the SLT for all eyes; however, distinctions in threshold elevations and interocular differences are seen between anisometropic amblyopes and strabismic/mixed amblyopes. While anisometropic amblyopes demonstrated decreases in IOD of acuity, strabismic/mixed amblyopes demonstrated increases.

Crowding magnitude effects

In the current study, when decreased inter-optotype spacing was applied to the Cambridge crowding test to create the L-ECC, significantly larger crowding magnitudes were seen for

all eyes of paediatric controls and amblyopes than with the SLT. This result is in line with data from previous studies of visually healthy adults and children, which determined that crowding magnitude effects are stronger with reduced inter-optotype spacing (Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016; Lalor, 2018), and when a target letter is flanked by other letters compared to more simple flankers, such as boxes or bars (Norgett and Siderov, 2014; Hairol, Omair and Kaur, 2016; Lalor, Formankiewicz and Waugh, 2016). The crowding magnitudes of the paediatric control eyes displayed in this study were comparable to those obtained by Lalor (2018), despite the different testing distances (3m in this study vs 9m in Lalor's) (Figure 3.14).

Strabismic/mixed amblyope crowding magnitudes

When calculated as a percentage, the amblyopic eyes of strabismic/mixed amblyopes demonstrated the largest increases in crowding magnitude (compared with the SLT) at 57.9%, followed by the corresponding fellow eye at 51.7%. Crowding is greater in the periphery compared with the fovea, particularly when targets and flankers occur from the same perceptual group (Kooi et al., 1994). Studies of strabismic/mixed amblyopic adults as well visually healthy adults have shown increased crowding effects present in the fovea of amblyopes with strabismus, akin to those present in the periphery of visually normal adults (Formankiewicz and Waugh, 2013; Norgett and Siderov, 2017). Norgett and Siderov suggest that this reflects a similarity in processing, with strabismic/mixed exhibiting increased target-flanker integration within the fovea, occurring at a rate greater than predicted by chance (Norgett and Siderov, 2017). Further evidence for this strabismic amblyopic vision is demonstrable using crowded targets within peripheral vision (Formankiewicz and Waugh, 2013). Strabismic amblyopic vision is limited by 'non-local' long-range inhibitory crowding mechanisms (Bonneh, Sagi and Polat, 2004) and also demonstrates increased crowding because of positional uncertainty (Flom, 1991; Formankiewicz and Waugh, 2013)

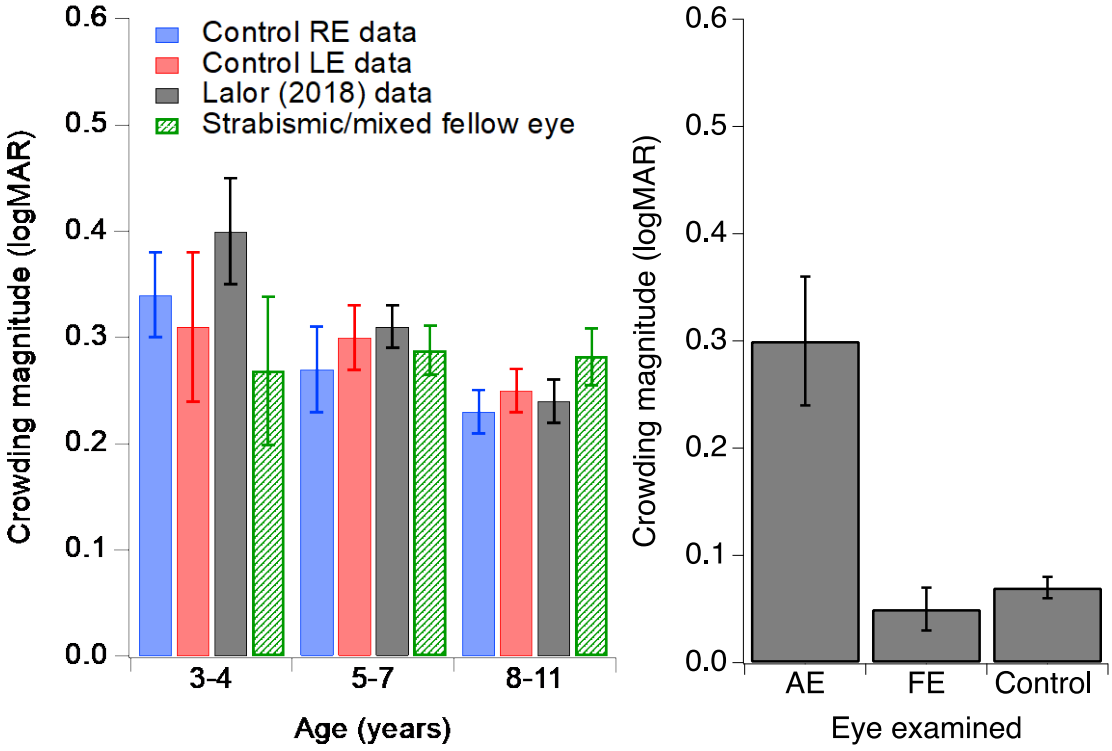


Figure 3.14: *Mean crowding magnitudes for Cambridge Crowding arrangements. Left: Data from this study (n=24 controls and n=22 strabismic/mixed amblyopes) and data extracted from Lalor (2018)(n=24), comparing the crowding magnitudes (per age group) seen in the fellow eyes of paediatric strabismic/mixed amblyopes with control eyes, with an inter-optotype spacing of one stroke width. Right: Data extrapolated from Norgett and Siderov (2017) (Adult strabismic/mixed amblyopes – AE and FE, n=11) and Lalor (2018) (visually healthy control adults, n=5), comparing the crowding magnitudes seen in the fellow eyes of adult strabismic/mixed amblyopes with control eyes, with an inter-optotype spacing of 2.5 stroke widths. Error bars show ±1SE.*

Examination of paediatric participants within this study and that of Lalor (2018) using a Cambridge Crowding arrangement with 0.2 letter widths (one stroke width) inter-optotype spacing shows that the fellow eyes of strabismic/mixed amblyopes demonstrate comparable crowding magnitudes to those of visually healthy controls (Figure 3.14 - Left). This effect has also been seen in strabismic/mixed amblyopic adults (Norgett and Siderov, 2017; Lalor, 2018) (Figure 3.14 - Right).

Anisometropic amblyope crowding magnitudes

In contrast, for anisometropic amblyopes, while the SLT yielded no differences in crowding magnitude between the amblyopic and fellow eye, the L-ECC demonstrated significantly larger crowding magnitudes in the fellow eye compared with the amblyopic eye.

Interestingly, of all eyes tested in this study, crowding magnitude was lowest for amblyopic eyes of anisometropic amblyopes. While the amblyopic eyes of strabismic/mixed amblyopes show significantly increased target-flanker foveal integration, anisometropic amblyopic eyes do not. The reduction of inter-optotype spacing and increase in target-flanker similarity did not increase crowding magnitudes in either the amblyopic or fellow eye of anisometropic amblyopes, demonstrating that anisometropic visual acuity loss is not limited by visual crowding.

Instead, the visual behaviour of anisometropic amblyopes can be modelled on optical blur (Formankiewicz and Waugh, 2013; Song, Levi and Pelli, 2014), as optically, the presence of blur reduces the contrast of the spatial frequency of the target (Simpson, Barbeito and Bedell, 1986), which is vital for optotype recognition (Kwon and Legge, 2013). This contrast reduction has been shown to diminish and even negate crowding effects for adult and paediatric amblyopes when linear optotypes were presented in low contrast (11%) compared with high contrast (96%) (Giaschi et al., 1993).

Additionally, Simmers and colleagues (1999) likewise reported acuity diminishing contour interaction effects in adult amblyopes (anisometropic, strabismic and mixed) that were negated in the presence of +1.50DS of blur. The authors hypothesised that contour interaction, a key component of visual crowding, depends upon optotype contrast. Evidence for this can be seen within this paediatric cohort, in the lower crowding magnitudes present

for the amblyopic eyes of anisometropic amblyopes, compared with the crowding magnitudes of the visually normal control group.

Unfortunately, neither Giaschi nor Simmers examined for different effects of amblyopic subtypes, primarily due to limited numbers of amblyopic participants (Giaschi et al., 1993; Simmers et al., 1999). However, a comparison of anisometropic and strabismic/mixed amblyopes as distinct sub-groups (akin to this study) did occur in the study by Bonneh, Sagi and Polat (2004). Using both single and crowded optotype formats, the authors revealed smaller crowding magnitudes occurring in anisometropes than in strabismic/mixed participants. Regrettably, no fellow eye or control eye data were collected for this study, so conclusions regarding visual behaviour compared to visually normal individuals or the fellow eyes of each amblyopic subtype were unable to be drawn.

A neurological basis for this phenomenon within anisometropic amblyopes is proposed in long-range neural connections involved in contour interaction. Dependent upon stimulus factors such as orientation, inter-optotype spacing and contrast (Polat and Sagi, 1993, 1994a; b; Polat and Norcia, 1996; Zenger and Sagi, 1996; Polat, Sagi and Norcia, 1997; Polat et al., 1998; Bonneh, Sagi and Polat, 2007) these long range-connections can behave in either an excitatory or inhibitory manner on a target within a single cortical receptive field. Polat and colleagues (1998) demonstrated suppression of observed cortical fields in a single-cell study of 83 cells from five adult cats when high contrast Gabor patch targets were flanked horizontally with high contrast (80%) collinear Gabor patches. Comparatively, facilitation of target detection occurred when mid and low-level contrast targets were presented. The early presence of uncorrected unilateral blur in anisometropic amblyopes reduces the contrast and spatial frequency of visual stimuli in the affected eyes of developing children. Subsequently, only mid or low spatial frequencies are perceived and processed during this early critical period leading to high spatial frequency deficits of contrast sensitivity. Furthermore, based on the findings of Polat and colleagues (1998), it

is likely that only the facilitatory mid and low contrast long-range lateral connections involved in contour interaction and perceptual organisation develop correctly, with the high contrast long-range lateral connections responsible for cortical inhibitory responses, failing to mature adequately. As such, even once optically corrected, the anisometropic amblyopic eye neurologically behaves as if it is blurred (Simmers et al., 1999; Formankiewicz and Waugh, 2013) and is less affected by the presence of surrounding optotypes in crowded conditions than visually normal individuals.

In the study by Bonneh, Sagi and Polat (2004) significant linear correlation between contrast masking and visual crowding was seen. While anisometropic amblyopes displayed both low lateral suppression and weak crowding, strabismic amblyopes showed high lateral suppression and crowding (Bonneh, Sagi and Polat, 2004; Doron, Spierer and Polat, 2015). Therefore, it appears that visual crowding and lateral facilitation have a reciprocal relationship, whereby crowding limits facilitation and increases suppression, but a reduction in crowding reduces lateral suppression, enabling lateral facilitation (Doron, Spierer and Polat, 2015). In this study, lower crowding magnitudes (although not significantly so) are seen in the amblyopic eyes of anisometropes than control eyes, and significantly higher magnitudes are seen in the amblyopic eyes of strabismic/mixed amblyopes. Anisometropic and strabismic/mixed amblyopes could be considered opposite ends of a ‘Crowding and Masking Spectrum’, with visually normal individuals representing a balance of crowding and masking phenomenon (Figure 3.15)

Interestingly, within this study, the L-ECC yielded larger crowding magnitudes in the fellow eye of anisometropic amblyopes compared with their amblyopic eye, an effect not seen in the less crowded SLT (Figure 3.11). Crowding magnitudes of the fellow eye in these anisometropic amblyopes were also larger (although not significantly so) than in control eyes or the fellow eyes of strabismic /mixed amblyopes.

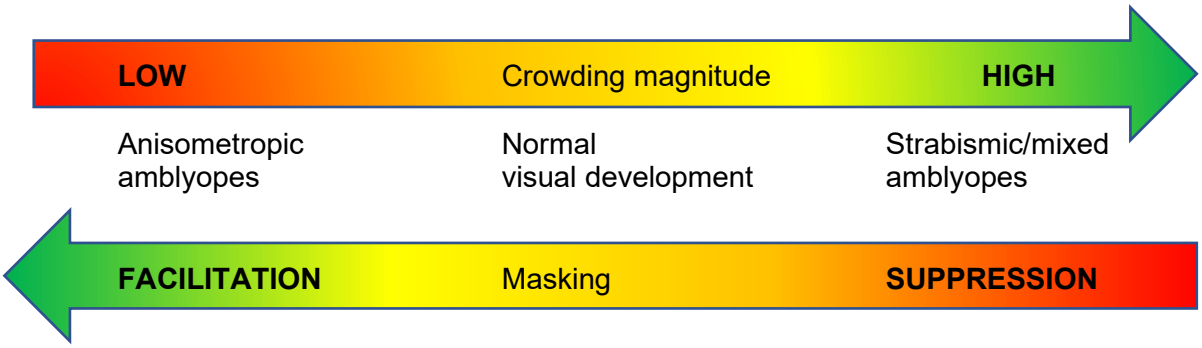


Figure 3.15: *Proposed 'Crowding and Masking Spectrum'.*

This curious visual behaviour of the fellow eye in anisometropic amblyopes has been alluded to in other studies, with fellow eye instability being correlated with reduced reading rates in anisometropic amblyopes (Kelly et al., 2017; Birch and Kelly, 2017). Several elements influence the crowding phenomenon: contour interaction, fixation stability and attention. Flom (1991) considered the oculomotor component to be the most important and visually influential; therefore, fixation instability may explain the increased crowding magnitude seen in the fellow eyes of these anisometropes, although further study is needed in this area.

Using an optimally crowded test such as the L-ECC, larger differences in crowding magnitude between the amblyopic and fellow eye are seen for these two amblyopic subtypes compared with SLT. Therefore, calculation of the interocular difference of crowding magnitude as conducted above (***amblyopic eye crowding magnitude – fellow eye crowding magnitude***) using an optimally crowded test such as the L-ECC could help to establish the subtype. A positive value would indicate the presence of strabismus and a negative value would indicate anisometropic amblyopia. This may be useful in a clinical situation where binocular status is difficult to examine/establish.

Due to the opposing crowding magnitude behaviours seen in these two amblyopic subtypes, care must be taken to consider these as separate conditions (i.e., those with strabismus and without strabismus) rather than amalgamating them under the single umbrella term of 'amblyopia'. Further analysis of the amblyopic data collapsed into a single group, examining the main effects of group (controls vs amblyopes) showed no statistically significant differences in crowding magnitudes for amblyopic eyes compared with right eyes ($p>.05$ for all test formats) or fellow eyes compared with control left eyes ($p>.05$ for all test formats) (Appendix seven). Further examination of the main effects of eye within two groups (controls vs amblyopes) also showed no statistically significant differences in crowding magnitudes for right eyes compared with left eyes ($p>.05$ for all test formats) or amblyopic eyes compared with fellow eyes ($p>.05$ for all test formats) (Appendix seven). This study, therefore, provides further evidence that considering amblyopes as a single homogenous group is not ideal.

Acuity thresholds and IODs

The influence of crowding upon acuity threshold and consequently IOD is important for amblyopia identification and diagnosis. Interocular difference is the key diagnostic feature used to determine the presence of amblyopia, with all amblyopes in this study demonstrating significantly higher IODs than controls for existing acuity test formats (Isolated and SLT). However, when decreased inter-optotype spacing was applied to the Cambridge crowding test to create the L-ECC, significant increases in IOD were seen in strabismic/mixed amblyopes compared with SLT, but significant decreases in IOD were seen with anisometropic amblyopes, compared with SLT.

For strabismic/mixed amblyopes within this study, increased crowding magnitudes in both eyes (amblyopic eye > fellow eye) resulted in reduced acuity that was greater in the amblyopic eye than the fellow eye resulting in increased IOD in acuity. This would benefit

amblyopia detection in vision screening, as this highlights the presence of amblyopia. Comparatively, increased crowding magnitudes in both eyes (fellow eye > amblyopic eye) resulted in reduced acuity that was greater in the fellow eye than the amblyopic eye, resulting in a decreased IOD. This is disadvantageous for screening, as milder cases of anisometropic amblyopia may go undiagnosed.

As screening utilises visual acuity testing to identify potential amblyopia, further statistical and post hoc analysis of the amblyopic data collapsed into a single group was conducted, examining the main effects of group (controls vs amblyopes, see Appendix eight for details) on acuity thresholds, showing that significantly larger acuity thresholds occurred for amblyopic eyes compared with control eyes for all test formats (Isolated: +0.374±0.051, SLT: +0.352±0.043, L-ECC: +0.431±0.056, **p<.001 respectively**) and significantly larger thresholds for fellow eyes compared with control eyes (Isolated: +0.092±0.031, **p=.004**; SLT: +0.054±0.022, **p=.020**; L-ECC:+0.127±0.030, **p<.001**). The increased thresholds seen with the L-ECC could be beneficial for amblyopia detection; however, care must be taken into consideration for anisometropic amblyopes, as the use of an optimally crowded test such as the L-ECC would increase thresholds further in the fellow eye, than the amblyopic eye. In mild, anisometropic amblyopes, this may result in a false-negative screening result.

As IOD is a vital measurement for the diagnosis and management of amblyopia, further statistical analysis and post hoc analysis examining the effects of group (controls vs amblyopes, see Appendix nine for details) on acuity IODs was conducted, and a demonstrated significantly larger IODs for amblyopic eyes compared with control eyes for all test formats (Isolated: +0.205±0.046, SLT: +0.285±0.040, L-ECC: +0.257±0.053, **p<.001 respectively**). A 2 (group) x 3 (test format) repeated measures ANOVA revealed no two-way interaction between group (controls and amblyopes) and test-format (isolated, SLT, and L-ECC) [$F(1.6, 105.614) = 3.102$, p=.083) and no significant main effect of test (isolated, SLT and L-ECC) was seen [$F(1.6, 105.614) = 1.557$, p=.218]. Amblyopes, considered as

a single group, consistently demonstrated larger IODs than control participants, regardless of the test format used. However, again care must be taken, particularly when considering anisometropic amblyopes specifically, as IOD decreases significantly for this amblyopic subgroup when using an optimally crowded test such as the L-ECC, compared with the SLT and isolated optotypes. Therefore, this could reduce the IOD to a 'non-amblyopic' level in mild anisometropic amblyopes.

3.6 Conclusion

The L-ECC significantly increased the crowding magnitudes and visual acuity thresholds of all paediatric participants within this study. When amblyopes were considered as a single homogenous group, their IODs were not significantly greater with the L-ECC than with the commercially available SLT. However, when examined as aetiological subgroups, anisometropic and strabismic/mixed amblyopes yielded significantly different behaviours when presented with the closer positioned L-ECC, with anisometropic amblyopic eyes demonstrating decreasing crowding, which reduces IODs; while strabismic/mixed amblyopic eyes demonstrated increased crowding magnitudes, which increased IODs. The differences in response to enhanced crowded stimuli seen between anisometropic and strabismic/mixed amblyopes may arise due to differences in the development of suppressive, high spatial frequency, long-range cortical connections.

This knowledge could be used clinically as a differential diagnostic tool to aid the of amblyopic subtypes. Calculation of crowding magnitude IOD using an optimally crowded test such as the L-ECC along with an isolated optotype test can be undertaken quickly and easily within a clinical environment. In the presence of an IOD $\geq$0.100 logMAR, a positive result could indicate the presence of strabismus, whereas a negative result could indicate anisometropic amblyopia only.

Chapter Four – CM Enhanced Cambridge Crowding Test

4.1 Introduction

First-order (or luminance) contrast detection loss is a key feature of amblyopia due to neural deficits within the striate cortex (V1) (Kiorpes and McKee, 1999); however, some studies have reported increased losses of visual function when amblyopes are examined with second-order stimuli (Ellemberg et al., 2002, 2005; Faubert, 2002). Identification of non-luminance-defined stimuli such as contrast-modulated (CM) stimuli are considered to require more complex, non-linear processing than stimuli created with luminance boundaries (Faubert, 2002; Tang and Zhou, 2009; Bertone et al., 2010). These are also thought to operate over larger spatial scales than those processing first-order stimuli (Sukumar and Waugh, 2007). Visual acuity of non-luminance defined stimuli such as contrast-modulated optotypes (letters) also demonstrate later development. Adult-like acuity thresholds for CM optotypes are attained by 9.2±0.4 years of age, whereas acuity for luminance-defined (L) optotypes is adult-like at 7.4±1.0 years of age (Lalor, 2018). Studies of second-order motion detection have also demonstrated earlier visual maturity for first-order defined stimuli, than second-order defined stimuli, in both three-month-old infants and five-year-old children (Ellemberg et al., 2003, 2005).

Due to the increased processing requirements (Chubb and Sperling, 1988; Derrington, Badcock and Henning, 1993) and longer developmental critical periods (Daw, 1998; Ellemberg et al., 2003) of second-order stimuli, they are considered to have increased vulnerability to abnormal visual input or neurological deterioration compared with luminance-defined stimuli (Faubert, 2002; Wong, Levi and McGraw, 2005; Bertone et al., 2010). Monocular and binocular visual acuity thresholds using CM-optotypes in visually healthy adults have demonstrated smaller binocular summation ratios in older adults (54.0±1.83 years) compared with younger adults (25.4±1.29 years), demonstrating a

decline in binocular processing for CM stimuli, not replicated for the same stimuli defined by luminance modulation (LM) (Woi et al., 2016). It is therefore considered that neurodevelopmental disorders such as amblyopia could be enhanced by examination using second-order stimuli (Wong, Levi and McGraw, 2001; Ellemberg et al., 2005; Thibault et al., 2007)

Evidence for extra-striate amblyopic deficits has been revealed more recently by numerous studies of second-order processing (Sharma, Levi and Klein, 2000; Wong, Levi and McGraw, 2001; Simmers et al., 2003; Mansouri, Allen and Hess, 2005; Wong, Levi and McGraw, 2005; Wong and Levi, 2005). In the study by Wong, Levi and McGraw (2001) examination of first-order and second-order sinusoid contrast detection thresholds in amblyopic and fellow eyes of five adult amblyopes (four strabismic and one anisometropic), both revealed greater losses of sensitivity to second-order stimuli, than first-order stimuli, relative to those of dominant eyes in three visually healthy adult controls. The authors speculated that this difference may have been due to disruption of visual processing within V2 due to poor binocular visual input (Wong, Levi and McGraw, 2001). Further support for a specific binocular impairment in neurones processing second-order information is described by Mansouri et al. (2005), who reported poorer orientation selectivity for second-order visual stimuli than first-order stimuli in both the amblyopic and fellow eyes of individuals with unilateral strabismic and anisometropic amblyopia.

Results of studies of human imaging (Li et al., 2007) as well as those from neurophysiological (Merigan, Nealey and Maunsell, 1993; Zhou and Baker, 1994; Mareschal and Baker, 1998; O'Keefe and Movshon, 1998) and psychophysical (Chubb and Sperling, 1988; Ledgeway and Smith, 1994) studies, suggest different loci of visual processing for first-order and second-order stimuli. While the striate-cortex is firmly established as the locus of first-order visual processing (Larsson, Landy and Heeger, 2006), studies of second-order stimuli have implicated increasingly binocular extra-striate areas

(Wong, Levi and McGraw, 2001; Wong and Levi, 2005; Hairol and Waugh, 2010a; Waugh, Lalor and Hairol, 2010) such as V2 (Wong, Levi and McGraw, 2001), V4 (Motter, 2006) or MT (O'Keefe and Movshon, 1998; El-Shamayleh et al., 2010; Thompson et al., 2012) as neurological sites of interest. It has been suggested that the processing pathways of first- and second-order visual stimuli may exhibit complete independence from one another (Schofield and Georgeson, 1999) or represent a single pathway capable of processing both stimulus types concurrently (Benton, Johnston and McOwan, 2000). In addition, increasing evidence suggests initially separate processing streams which may interact but then combine at a later stage of visual processing (Rivest and Cavanagh, 1996; Chung, Li and Levi, 2007; Hairol and Waugh, 2010b). Evidence for this can be found in studies of lateral interaction that used test and reference flankers and examined the order (first or second) of reference flankers upon central target detection thresholds (Chung, Li and Levi, 2007).

4.1.1 Second-order crowding in amblyopia

As increased crowding magnitude is a key feature of luminance-defined amblyopic thresholds (See chapter three), studies of second-order spatial interactions presented promising possibilities for the detection and monitoring of amblyopia (Chung, Levi and Legge, 2001). Wong, Levi and McGraw followed up their 2001 study by examining the influence of flankers on the detection of second-order targets (Wong, Levi and McGraw, 2005). While in visually normal adults, collinear and orthogonal flankers demonstrated facilitation of second-order target detection thresholds; in amblyopic adults and non-amblyopic adults with strabismus (so those without good binocularity), suppression of second-order target detection occurred for both collinear and orthogonal flankers that diminished with increasing target-flanker spacing. Furthermore, crowding magnitude in all seven adult amblyopes tested using second-order optotypes arranged in a trigram was significantly higher than that measured for first-order optotypes. (Chung, Li and Levi, 2008a).

4.1.2 CM contour interaction and crowding effects in children

Contour interaction and crowding effects of second-order stimuli have been reasonably well studied in adult visual systems; however, information about these effects in children is more limited and even more so for amblyopic children. Amblyopic visual acuity outcomes are improved when diagnosis and management are achieved at an early age, with diagnosis often mediated by visual screening programmes aimed at children in their first year of school, aged between four and five years old. If CM stimuli were suggested to be important and useful for the detection of amblyopia in children, then the presentation of CM stimuli for the purposes of screening is important, although it must be accessible for children, as well as quick and easy to perform.

Examination of pre-literate 'crowded' optotypes displayed in L, LM and CM formats was studied by Lalor (2018) in visually healthy children (3-16 years) and adults. A comparison of contour interaction (HOTV optotypes surrounded by a box) and crowding (Enhanced Cambridge Crowding test) effects using first- and second-order stimuli showed that CM visual acuity was significantly poorer than LM visual acuity for visually healthy children (by +0.51±0.01 logMAR). Examination of L, LM and CM contour interaction and crowding magnitudes produced lower crowding with CM optotypes than with L or LM optotypes (Lalor, 2018). There was no significant difference between the effects of contour interaction and crowding (0.03±0.01 logMAR difference) for CM stimuli; however, the magnitude of crowding was greater than that of contour interaction for L and LM stimuli by 0.13±0.03 and 0.09±0.03 logMAR, respectively.

In conclusion, adults with amblyopia have demonstrated a greater loss of sensitivity to CM stimuli compared to that found using LM stimuli (Wong, Levi and McGraw, 2001, 2005; Chung, Li and Levi, 2008a; b; Hairol, Formankiewicz and Waugh, 2013). Therefore, it is

possible that the use of crowded contrast-modulated stimuli could be helpful in amblyopia screening.

Crowding magnitudes using CM-optotypes may provide greater sensitivity to the detection of amblyopia than luminance-based visual acuity tests. However, amblyopic children have not yet been examined using CM acuity stimuli. Therefore, this study aims to establish whether using an Enhanced Cambridge Crowding test presented with CM optotypes (CM-ECC) offers greater sensitivity for the detection of amblyopia in children than the commercially available SLT. Amblyopic children from two groups will be examined: those without a manifest strabismus and a refractive difference between their eyes (anisometropic amblyopes) and those with a manifest strabismus (strabismic/mixed amblyopes).

4.2 Study aims

Within this experiment, the aims were to:

1. Examine and compare visual acuity, interocular differences in visual acuity and crowding magnitudes in visually healthy children and clinically diagnosed amblyopic children aged between 3 and 11-years-old using the contrast-modulated enhanced Cambridge Crowding Test (CM-ECC) and the Sonksen logMAR test (SLT); to determine whether the CM-ECC provides greater sensitivity to amblyopia than the SLT.

2. Determine if differences exist in visual acuities, interocular acuity differences and crowding magnitudes between strabismic/mixed and anisometropic amblyope subtypes, using the CM-ECC

4.3 Methodology

Full details of apparatus, stimuli and testing procedure can be found in Chapter 2 sections **2.2.1**, **2.2.6, 2.2.4 and 2.2.8**.

4.3.1 Participant demographics

Full participant details can be found in **2.2.3**. One strabismic/mixed participant of this cohort did not complete the CM-ECC and was therefore excluded from the analysis. In total, sixty-seven participants completed the examination with the CM-ECC (mean age 6.93 years, range 3-11years). Participant demographics for the CM-ECC examination are summarised in Table 4.01.

4.3.2 Analysis

Full details of the planned data analysis can be found in Chapter 2, section **2.4**.

Table 4.01*: Participant demographics for L-ECC examination*

	Sex		Age (Years)		Number per age group (years)			Total
	Male	**Female**	**Mean**	**Range**	**3-4**	**5-7**	**8-11**	
Controls	**7**	**17**	**7.4**	**4-10**	**6**	**6**	**12**	**24**
Amblyopes	**22**	**21**	**6.6**	**3-10**	**4**	**31**	**8**	**43**
Anisometropes	*10*	*12*	*6.2*	*3-10*	*2*	*19*	*1*	*22*
Strabismic / mixed	*12*	*9*	*7.1*	*4-10*	*2*	*12*	*7*	*21*

4.4 Results

4.4.1 Visual Acuity Thresholds

Visual acuity data for the three different tests (SLT, CM-Isolated optotypes and CM-Enhanced Cambridge Crowding test), averaged for each eye (RE, LE, amblyopic eye AE, fellow eye FE) and test group (controls, anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Figure 4.01 and Table 4.02. Data points represent mean±1 standard error unless stated otherwise. One-way repeated measures ANOVA demonstrated no statistically significant differences between right and left eyes for all tests in the control group ($p>.05$) (Table 4.03), therefore control RE data were compared against amblyopic eyes, and control LE data were analysed against fellow eyes in subsequent analyses.

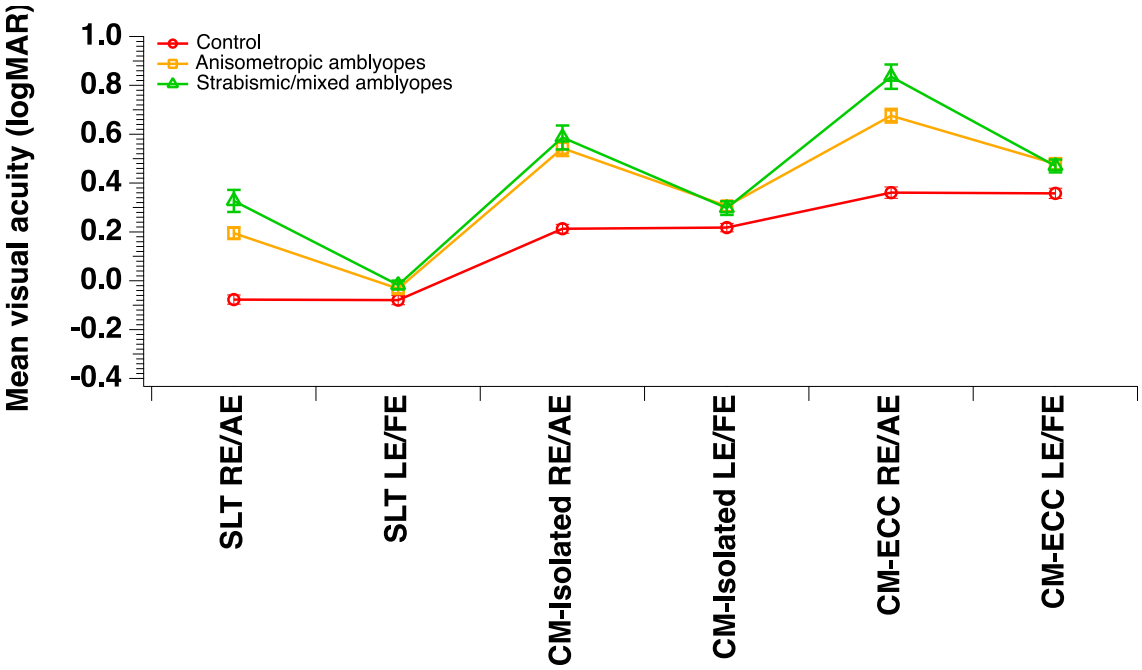


Figure 4.01*: Mean visual acuity thresholds for L-Isolated, SLT, L-ECC, CM-Isolated and CM-ECC presentations in both eyes (RE-right eye, LE-left eye, AE-amblyopic eye, FE-fellow eye) of controls, anisometropic amblyopes and strabismic/mixed amblyopes. Error bars show ±1SE .*

Table 4.02: *Mean visual acuity thresholds (logMAR) with standard error, for each group, eye, and acuity test.*

	n	SLT	CM-Iso	CM-ECC
Controls - Right eye	24	-0.077±0.019	+0.213±0.018	+0.361±0.023
Controls - Left eye	24	-0.079±0.019	+0.218±0.017	+0.358±0.021
Anisometropic amblyopes - amblyopic eye	22	+0.195±0.024	+0.543±0.032	+0.676±0.028
Anisometropic amblyopes - fellow eye	22	-0.032±0.019	+0.306±0.021	+0.479±0.023
Strabismic/mixed amblyopes- amblyopic eye	21	+0.327±0.045	+0.587±0.049	+0.836±0.050
Strabismic/mixed amblyopes - fellow eye	21	-0.017±0.018	+0.298±0.028	+0.470±0.026

Table 4.03: *One-way repeated measures ANOVA examining differences between the acuity thresholds of right and left control eyes, for each test presentation (SLT, CM-Iso and CM-ECC).*

	df	F	Sig	μ^2
SLT	1	0.193	0.664	0.008
Error	23			
CM-Iso	1	0.135	0.717	0.006
Error	23			
CM-ECC	1	0.039	0.845	0.002
Error	23			

Larger (poorer) thresholds were seen for contrast-modulated optotypes (CM-Iso and CM_ECC compared with SLT. A 3 (group) x 3 (test-format: SLT, CM iso, CM-ECC) x 2 (eye: RE/AE, LE/FE) repeated measures ANOVA (featuring one between factor (group) and two within factors (test-format and eye)) revealed this effect to be highly significant [*F*(1.938, 124.060) = 1355.902, **p<.001**]. This effect was significantly greater for strabismic/mixed and anisometropic amblyopes than with controls for with the contrast-modulated tests [*F*(3.877, 124.060) = 4.422, **p=.002**], with significantly higher (worse) acuities seen for the amblyopic eye compared with the fellow eye but no significant difference between the eyes of control participants [*F*(2, 64) = 33.203, **p<.001**]. The difference in acuities between the eyes of participants was not significantly affected by the test format [*F*(2, 128) = 0.760, p=.470], (Table 4.04)

Table 4.04: *Repeated measures ANOVA with three test formats (SLT, CM-ISO and CM-ECC), three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) and eyes (Right eye, Left eye, Amblyopic eye and Fellow eye).*

	df	**F**	**Sig**	**μ^2**
Test format	**2**	**1355.902**	**<0.001**	**0.955**
Error	128			
Group	**2**	**43.612**	**<0.001**	**0.577**
Error	64			
Eye	**1**	**115.647**	**<0.001**	**0.644**
Error	64			
Test format * group	**3.877**	**4.422**	**0.002**	**0.121**
Error	124.060			
Test format * eye	2	0.760	0.470	0.012
Error	128			
Eye * group	**2**	**33.203**	**<0.001**	**0.509**
Error	64			
Test format * group * eye	4	2.353	0.057	0.069
Error	128			

4.4.1.1 Examination of effects between test-groups

A two-way interaction was seen between the three test groups (control, anisometropic amblyopes and strabismic/mixed amblyopes) and three test formats (SLT, CM-iso, CM-ECC) and significant effects of group were seen for all three tests (**p<.001**) (Table 4.05).

To compare thresholds between groups, planned comparisons were made separately for each test, between the right eye of the control group and amblyopic eyes of the anisometropic and strabismic/mixed groups, and between the left eye of the control group and fellow eyes of the anisometropic and strabismic/mixed groups.

Table 4.05: *Repeated measures ANOVA examining effects of three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) upon each test format (SLT, CM-iso and CM-ECC)*

	df	**F**	**Sig**	**μ^2**
SLT format	**2**	**32.188**	**<0.001**	**0.501**
Error	64			
CM-Iso	**2**	**32.148**	**<0.001**	**0.501**
Error	64			
CM-ECC	**2**	**45.296**	**<0.001**	**0.586**
Error	64			

Comparisons of control, anisometropic and strabismic/mixed amblyopic eye thresholds

Pairwise comparisons revealed that for the SLT format, both strabismic/mixed amblyopic eyes and anisometropic amblyopic eyes yielded significantly poorer thresholds than control eyes (+0.404±0.043, **p<.001** and +0.273±0.042, **p<.001** respectively), and strabismic/mixed

amblyopic eye thresholds were significantly poorer than anisometropic amblyopic eyes (+0.132±0.044, **p=.011**).

For the CM-Iso format, both strabismic/mixed amblyopic eyes and anisometropic amblyopic eyes yielded significantly poorer thresholds than controls (+0.374±0.048, **p<.001** and +0.330±0.047, **p<.001** respectively), although strabismic/mixed amblyopic eyes did not differ significantly from anisometropic amblyopic eyes (+0.045±0.049, p=1.000).

For the CM-ECC format, again both strabismic/mixed amblyopes and anisometropic amblyopes yielded significantly poorer thresholds than controls (+0.476±0.049, **p<.001** and +0.316±0.048, **p<.001**), and strabismic/mixed amblyopic eyes also differed significantly from anisometropic amblyopic eyes (+0.160±0.050, **p=.006**) (Figure 4.02).

Comparisons of control, anisometropic and strabismic/mixed fellow eye thresholds

Pairwise comparisons examining control eyes and fellow amblyopic eyes revealed that for the SLT format, strabismic/mixed fellow visual acuity thresholds differed from neither control eyes (+0.062±0.027, p=.067) nor anisometropic fellow eyes (+0.015±0.027, p=1.000). Anisometropic fellow eyes also did not differ significantly from control eyes (+0.047±0.026, p=.231).

For the CM-iso format, strabismic/mixed fellow eyes demonstrated significantly poorer thresholds than control eyes (+0.079±0.031, **p=.040**) but not anisometropic fellow eyes (-0.008±0.032, p=1.000). Anisometropic fellow eyes amblyopes also demonstrated significantly poorer thresholds than control eyes (+0.087±0.031, **p=.018**).

For the CM-ECC format, both strabismic/mixed fellow eyes and anisometropic fellow eyes yielded significantly poorer thresholds than controls (+0.113±0.033, **p=.003** and

+0.121±0.033, **p=.001**), although strabismic/mixed fellow eyes did not differ significantly from anisometropic fellow eyes (-0.008±0.034, p=1.000) (Figure 4.02).

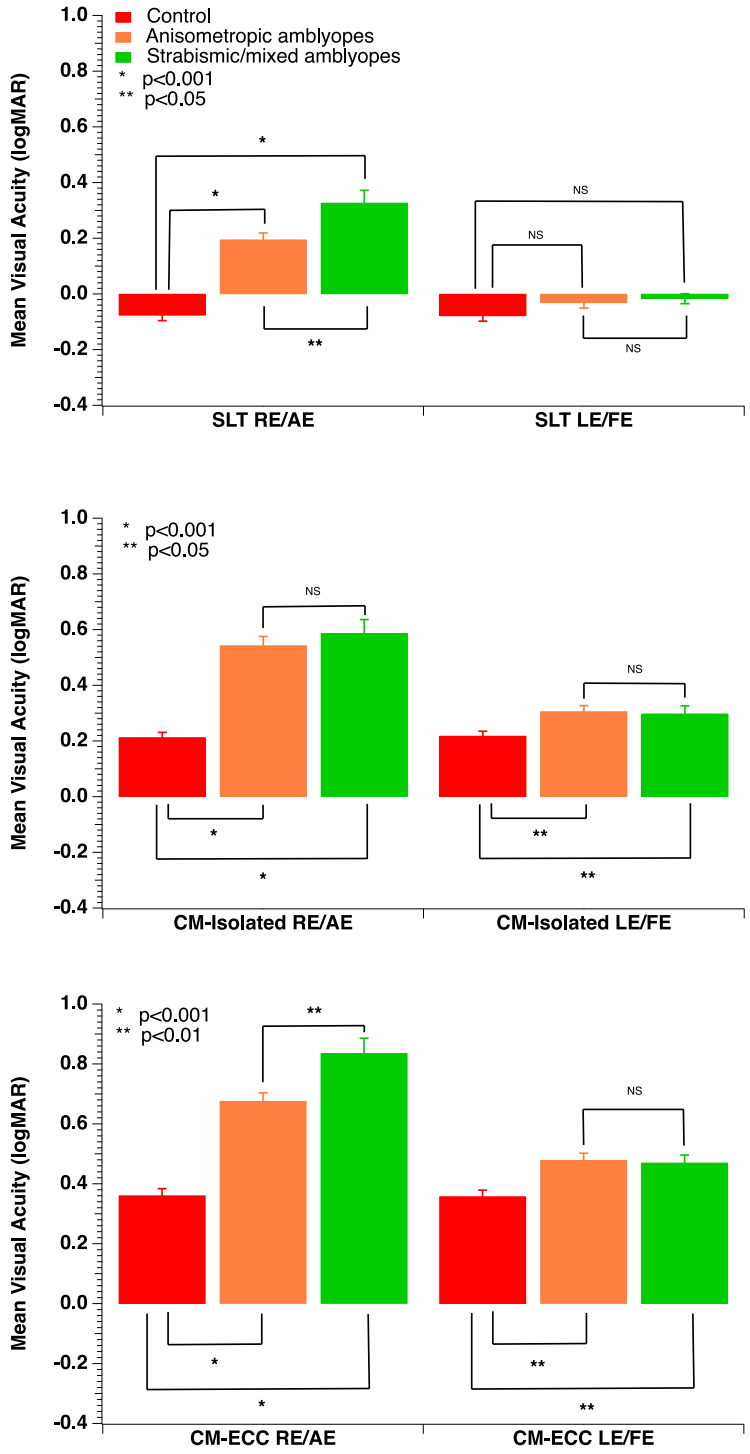


Figure 4.02: *Visual acuity thresholds averaged within each eye category, with significance bars. Top – SLT format. Middle – CM-iso. Bottom – CM-ECC format. Error bars show ±1SE.*

4.4.1.2 Effects within each test-group

Effect of test format (SLT, CM iso, CM-ECC) per group (controls, anisometropic amblyopes and strabismic/mixed amblyopes)

All groups showed a significant effect of test (**p<.001**) (Table 4.06 and Figure 4.03). Pairwise comparison revealed that CM-ECC demonstrated significantly greater thresholds than SLT for both right and left control eyes (+0.438±0.017, **p<.001** and +0.437±0.018, **p<.001**). For anisometropic amblyopes, thresholds increased by +0.481±0.027, (**p<.001**) between SLT and CM-ECC for amblyopic eyes, and by +0.510±0.015 (**p<.001**) for fellow eyes. In strabismic/mixed amblyopes, amblyopic eyes increased by +0.509±0.025, **p<.001** between SLT and CM-ECC and fellow eyes by +0.487±0.017 (**p<.001**).

Table 4.06*: One-way ANOVA (repeated measures) examining main effects of test format (SLT, CM-Iso and CM-ECC) for each group (controls, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	**2**	**503.123**	**<0.001**	**0.956**
Error	46			
Anisometropic amblyopes	**2**	**554.851**	**<0.001**	**0.964**
Error	42			
Strabismic/mixed amblyopes	**2**	**344.340**	**<0.001**	**0.945**
Error	40			

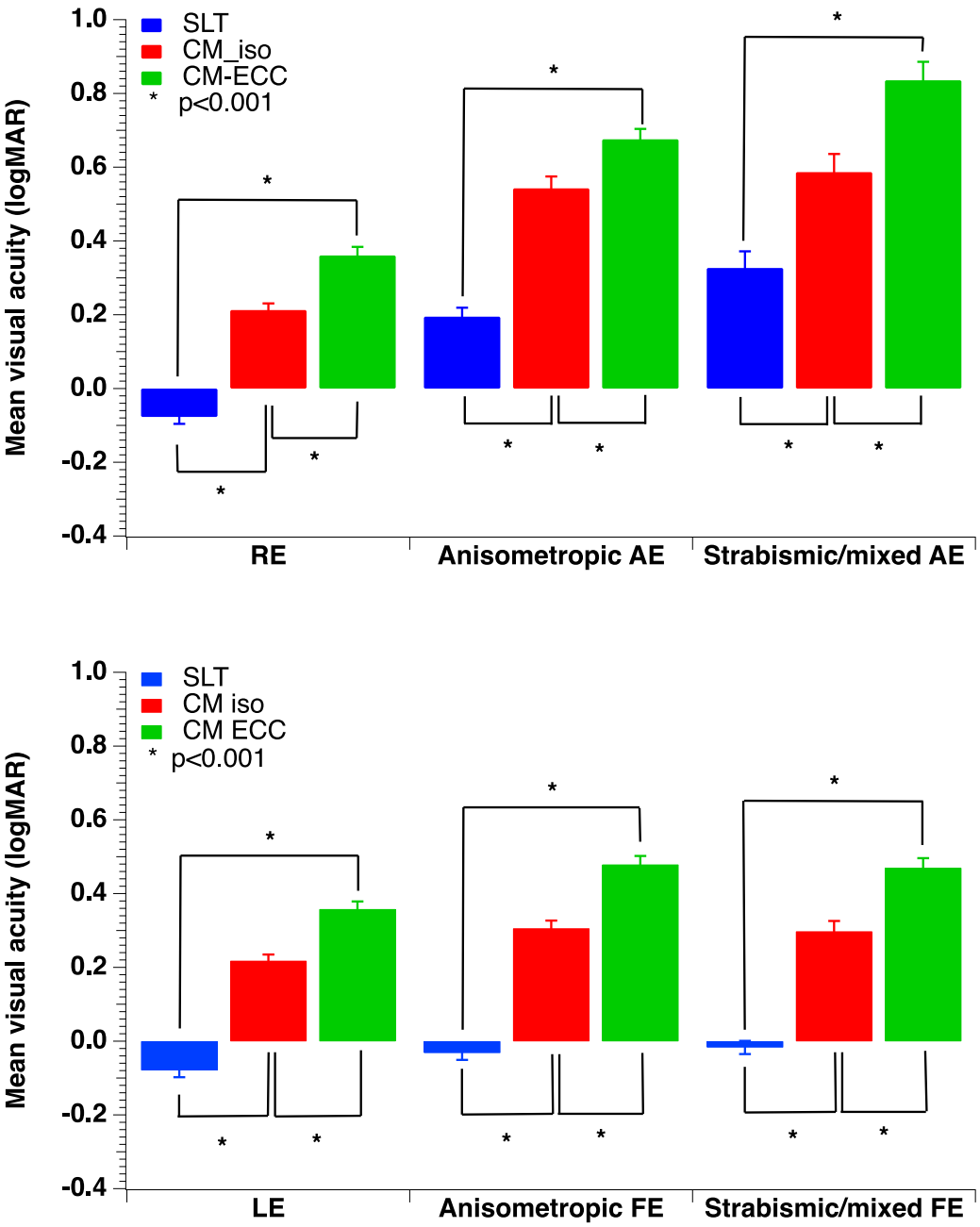


Figure 4.03: *Top – Visual acuity thresholds averaged for all tests within right control eyes (RE) and amblyopic eyes (AE) of both amblyopic subgroups, with significance bars. Bottom – Visual acuity thresholds averaged for all tests within left control eyes (LE) and fellow eyes (FE) of both amblyopic subgroups, with significance bars Error bars show ±1SE.*

Effect of eye per group (Controls, anisometropic amblyopies and strabismic/mixed amblyopes)

One-way repeated measures ANOVA revealed that both anisometropic and strabismic/mixed amblyopes showed main effects of eye (**p<.001**), but controls did not (p>.05) (Table 4.07). Pairwise comparisons showed that that amblyopic eye of anisometropic amblyopes yielded significantly higher thresholds than the fellow eye for the SLT format (+0.227±0.019, **p<.001),** the CM-iso format (+0.237±0.039, **p<.001**) and the CM-ECC format (+0.198±0.039, **p<.001**). The same effect occurred for strabismic/mixed amblyopes, with significantly higher thresholds occurring in the amblyopic eye compared with the fellow eye for the SLT format (+0.344±0.046, **p<.001),** the CM-iso format (+0.290±0.047, **p<.001**) and the CM-ECC format (+0.365±0.052, **p<.001**) (Figure 4.04).

Table 4.07: *One-way ANOVA (repeated measures) examining simple main effects of eye (RE, LE, amblyopic eye and fellow eye) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	1	0.000	0.990	0.000
Error	23			
Anisometropic amblyopes	**1**	**58.931**	**<0.001**	**0.737**
Error	21			
Strabismic/mixed amblyopes	**1**	**55.544**	**<0.001**	**0.133**
Error	20			

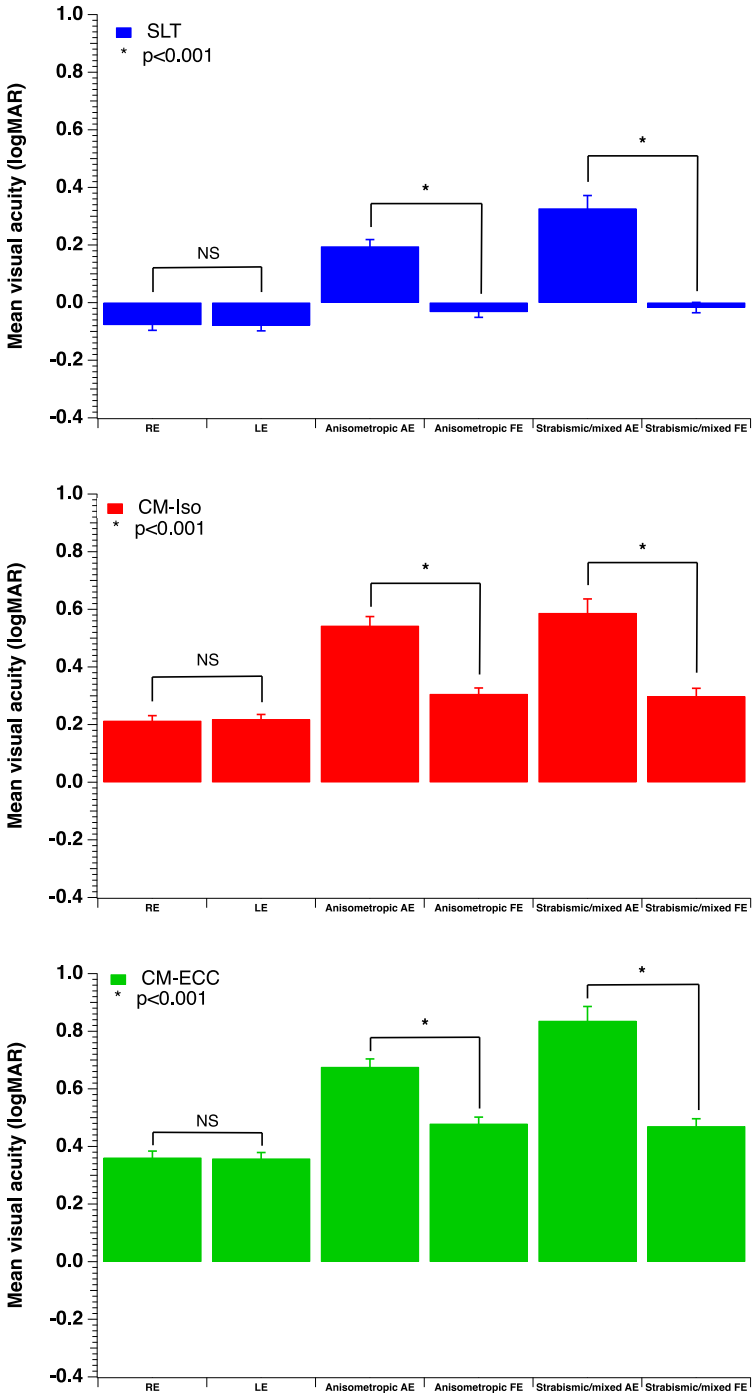


Figure 4.04: *Top - Isolated visual acuity thresholds averaged within each eye category. Middle – SLT visual acuity thresholds averaged within each eye category. Bottom - ECC visual acuity thresholds averaged within each eye category. Error bars show ±1SE.*

4.4.2 Interocular differences (IOD)

Interocular difference was calculated as the difference (recorded in logMAR) between the thresholds between eyes. Mean IODs for the three different tests (SLT, CM-iso and CM-ECC), and test groups (controls, anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Table 4.08. Mean IOD per group for the three visual acuity tests, are shown in Figure 4.05. Data are mean±1 standard error unless stated otherwise.

A 3 (group) x 3 (test-format) repeated measures ANOVA (featuring one between factor (group) and one within factor (test-format)) showed that differences in IOD seen between the three test formats were insignificant [$F(2, 128) = 1.196$, p=0.306]. Significantly larger IODs were seen for both strabismic/mixed and anisometropic amblyopes compared with controls [$F(2, 64) = 27.654$, **p<.001**]. This effect was significantly different between the three tests [$F(4, 128) = 3.238$, **p=0.014**], and is explored below (Table 4.09).

Table 4.08: *Mean visual acuity interocular differences (logMAR) with standard error, for each group and acuity test.*

	n	SLT IOD	CM-Iso IOD	CM-ECC IOD
Controls	24	+0.015±0.004	+0.054±0.009	+0.059±0.009
Anisometropic amblyopes	22	+0.227±0.019	+0.248±0.036	+0.221±0.033
Strabismic/mixed amblyopes	21	+0.344±0.046	+0.290±0.047	+0.365±0.052

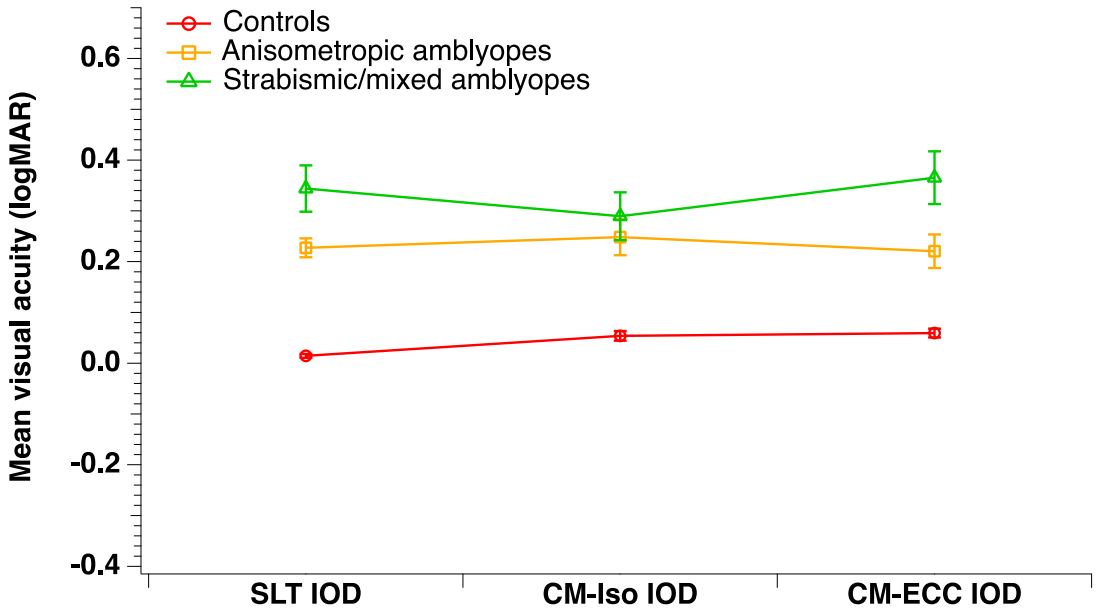


Figure 4.05: *Mean IOD (logMAR) for each acuity test (SLT, CM-iso and CM-ECC) per group (controls, anisometropic and strabismic/mixed amblyopes). Error bars show ±1SE.*

Table 4.09*: Repeated measures ANOVA examining differences in IOD with three test formats (SLT, CM-iso and CM-ECC) for three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Test format	2	1.196	0.306	0.018
Error	128			
Group	**2**	**27.654**	**<0.001**	**0.464**
Error	64			
Test format * group	**4**	**3.238**	**0.014**	**0.092**
Error	116			

4.4.2.1 Examination of effects between test-groups

A two-way interaction was seen between the three test groups (control, anisometropic amblyopes and strabismic/mixed amblyopes) and three test formats (SLT, CM-iso, CM-ECC) and significant effects of group were seen for all three tests (**p<.001**) (Table 4.10).

Table 4.10: *Repeated measures ANOVA examining effects of three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) upon each test format (SLT, CM-iso and CM-ECC)*

	df	**F**	**Sig**	**μ^2**
SLT	**2**	**38.952**	**<0.001**	**0.549**
Error	64			
CM-Iso	**2**	**14.905**	**<0.001**	**0.318**
Error	64			
CM-ECC	**2**	**20.093**	**<0.001**	**0.386**
Error	64			

Pairwise comparisons revealed that for the SLT format, both strabismic/mixed amblyopes and anisometropic amblyopes yielded significantly larger IODs than controls (+0.329±0.038, **p<.001** and +0.213±0.038, **p<.001** respectively), and strabismic/mixed amblyope IODs were significantly larger than those of anisometropic amblyopes (+0.117±0.039, **p=.011**).

For the CM-Iso format, both strabismic/mixed amblyopes and anisometropic amblyopes yielded significantly larger IODs than controls (+0.236±0.047, **p<.001** and +0.195±0.046, **p<.001** respectively), although strabismic/mixed amblyopes did not differ significantly from anisometropic amblyopes (+0.041±0.048, p=1.000).

For the CM-ECC format, again both strabismic/mixed amblyopes and anisometropic amblyopes yielded significantly larger IODs than controls (+0.306±0.048, **p<.001** and +0.161±0.048, **p=.004**), and strabismic/mixed amblyopes also differed significantly from anisometropic amblyopes (+0.145±0.049, **p=.014**) (Figure 4.06).

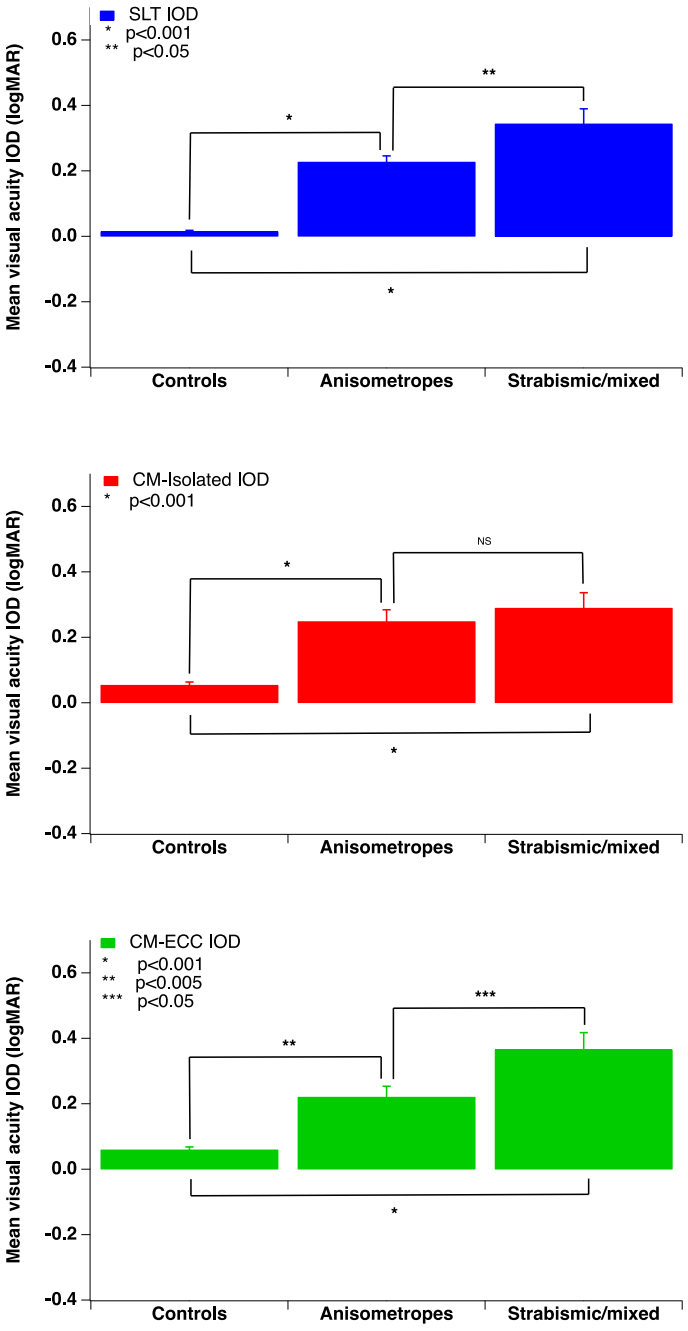


Figure 4.06: *Mean IODs (logMAR) for each test, per group, with significance bars. Top – SLT IODs, Middle – CM-Isolated IODs. Bottom – CM-ECC IODs. Error bars show ±1SE.*

4.4.3 Crowding magnitude

Magnitude of crowding is calculated as the difference in logMAR between the acuity thresholds of the crowded and isolated formats. For the SLT test, this was calculated as in chapter three (SLT threshold – L-Isolated optotype threshold). For the CM-ECC this was calculated as CM-ECC threshold – CM isolated threshold. Crowding magnitudes for the two different tests (SLT and CM-ECC), eyes (RE, LE, Amblyopic eye, Fellow eye) and test groups (controls, anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Table 4.11 and Figure 4.07. Data are mean±1 standard error unless stated otherwise. One-way repeated measures ANOVA demonstrated no statistically significant differences in crowding magnitude between right and left control eyes for all tests ($p>.05$) (Table 4.12), therefore control RE data were analysed against amblyopic eyes, and control LE data were analysed against fellow eyes.

Table 4.11: *Mean crowding magnitudes of the SLT and CM-ECC (given in logMAR) with standard error, for each group (controls, anisometropic and strabismic/mixed amblyopes) and eye.*

	n	SLT	CM-ECC
Controls – Right Eye	24	0.193±0.018	0.148±0.017
Controls – Left Eye	24	0.193±0.019	0.139±0.014
Anisometropic amblyopes – Amblyopic eye	22	0.126±0.027	0.133±0.019
Anisometropic amblyopes – Fellow eye	22	0.157±0.025	0.173±0.018
Strabismic/mixed amblyopes – Amblyopic eye	21	0.226±0.027	0.249±0.026
Strabismic/mixed amblyopes – Fellow eye	21	0.153±0.021	0.173±0.019

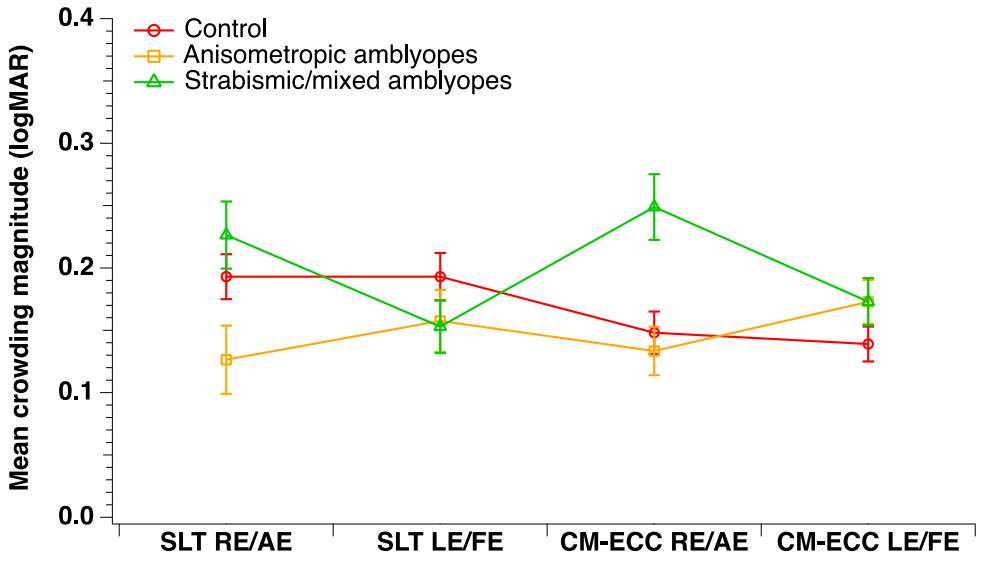


Figure 4.07*: Mean crowding magnitudes for SLT and CM-ECC presentations in both eyes (RE-right eye, LE-left eye, AE-amblyopic eye, FE-fellow eye) of controls, anisometropic amblyopes and strabismic/mixed amblyopes. Error bars show ±1SE*

A 3 (group) x 3 (test-format) x 2 (eye) repeated measures ANOVA (featuring one between factor (group) and two within factors (test-format and eye)) demonstrated no statistically significant effect of test format [$F(1, 64) = 0.288$, p=.635] or eye [$F(1, 64) = 2.076$, p=.155] on crowding magnitudes. Largest crowding magnitudes were seen with strabismic/mixed amblyopes [$F(2, 64) = 3.958$, **p=.024**], with this effect being greatest when using the CM-ECC test format [$F(2, 64) = 9.616$, **p<.001**], or when examining the amblyopic eye [$F(2, 64) = 3.510$, **p=.036**] (Table 4.13).

Table 4.12: *One-way repeated measures ANOVA examining differences between the crowding magnitudes of right and left control eyes, for each test presentation (SLT and CM-ECC.*

	df	F	Sig	μ^2
SLT	1	0.001	0.979	0.000
Error	23			
CM-ECC	1	0.278	0.603	0.012
Error	23			

Table 4.13: *Repeated measures ANOVA examining crowding magnitudes of two test formats (SLT and CM-ECC), three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) and two eyes (Right eye/Left eye, Amblyopic eye/Fellow eye).*

	df	**F**	**Sig**	**μ^2**
Test format	1	0.228	0.635	0.004
Error	64			
Group	***2***	***3.958***	***0.024***	***0.110***
Error	64			
Eye	1	2.076	0.155	0.31
Error	64			
Test format * group	***2***	***3.510***	***0.036***	***0.099***
Error	64			
Test format * eye	1	0.001	0.977	0.000
Error	64			
Eye * group	***2***	***9.616***	***<0.001***	***0.000***
Error	64			
Test format * group * eye	2	0.051	0.951	0.002
Error	64			

4.4.3.1 Effect of group

A two-way interaction was seen between the three test groups (control, anisometropic amblyopes and strabismic/mixed amblyopes) and two test formats (SLT, CM-ECC). Significant simple effects of group were seen for ECC (**p<.001**), but not SLT (p>.05) (Table 4.14).

Table 4.14: *Repeated measures ANOVA examining effects of three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) upon each test format (SLT and CM-ECC)*

	df	**F**	**Sig**	**μ^2**
SLT	2	2.324	0.106	0.068
Error	64			
CM-ECC	***2***	***6.218***	***0.003***	***0.163***
Error	64			

To compare crowding magnitudes between groups, planned comparisons were made separately for each test, between the right eye of the control group and amblyopic eyes of the anisometropic and strabismic/mixed group, and between the left eye of the control group and fellow eyes of the anisometropic and strabismic/mixed group.

Control crowding magnitudes compared with anisometropic amblyopic eye magnitudes and strabismic/mixed amblyopic eye magnitudes

Planned pairwise comparisons revealed that for the SLT format, control eye crowding magnitudes did not differ significantly from that in strabismic/mixed amblyopic eyes or anisometropic amblyopic eyes (-0.033±0.034, p=1.000 and +0.067±0.034, p=.156 respectively). Strabismic/mixed amblyopic eye crowding magnitudes were significantly larger than anisometropic amblyopic eyes (+0.100±0.035, **p=.017**). For the CM-ECC format, strabismic/mixed amblyopic eye crowding magnitudes were significantly larger than both control eyes and anisometropic amblyopic eyes (+0.101±0.029, **p=.003** and +0.116±0.030, **p<.001** respectively). No significant difference was seen between control eyes and anisometropic amblyopic eyes (+0.014±0.029, p=1.000) (Figure 4.08).

Control crowding magnitudes compared with anisometropic fellow eye magnitudes and strabismic/mixed fellow eye magnitudes

Pairwise comparisons examining control eyes and fellow amblyopic eyes revealed that for the SLT format, control eye crowding magnitudes did not differ significantly from that in

strabismic/mixed amblyopic eyes or anisometropic amblyopic eyes (+0.040±0.031, p=.600 and +0.035±0.030, p=.751 respectively). Strabismic/mixed amblyopic eye crowding magnitudes were not significantly smaller than anisometropic amblyopic eyes (-0.005±0.031, p=1.000). For the CM-ECC format, crowding magnitudes did not differ between control left eyes, strabismic/mixed fellow eyes and anisometropic fellow eyes (p>.05) (Figure 4.08).

4.4.3.2 Effects within each test-group

Effect of test format per group (controls, anisometropic amblyopes and strabismic/mixed amblyopes)

Control participants showed significant lower crowding magnitude with the CM-ECC, compared to the SLT (**p=.015**), but anisometropic and strabismic/mixed amblyopes did not (p>.05) (Table 4.15 and Figure 4.09). Pairwise comparison revealed no significant differences in crowding magnitude between the two tests, for the right eyes of controls (-0.046±0.025, p=.086), but the crowding magnitude of CM-ECC was significantly lower for control left eyes (-0.053±0.022, **p=.025**). The difference in crowding magnitudes seen between the right and left eyes represents a difference of -0.007 logMAR, which while statistically significant, would not be considered clinically significant.

Effect of eye per group (controls, anisometropic amblyopies and strabismic/mixed amblyopes)

One-way repeated measures ANOVA revealed that strabismic/mixed amblyopes showed main effects of eye (**p<.001** respectively), but controls and anisometropic amblyopes did not (p>.05) (Table 4.16). Pairwise comparisons showed that the amblyopic eye of strabismic/mixed amblyopes yielded significantly larger crowding magnitudes than the fellow eye for the SLT format (+0.073±0.028, **p=.017),** and the CM-ECC format (+0.076±0.028, **p=.014**) (Figure 4.10).

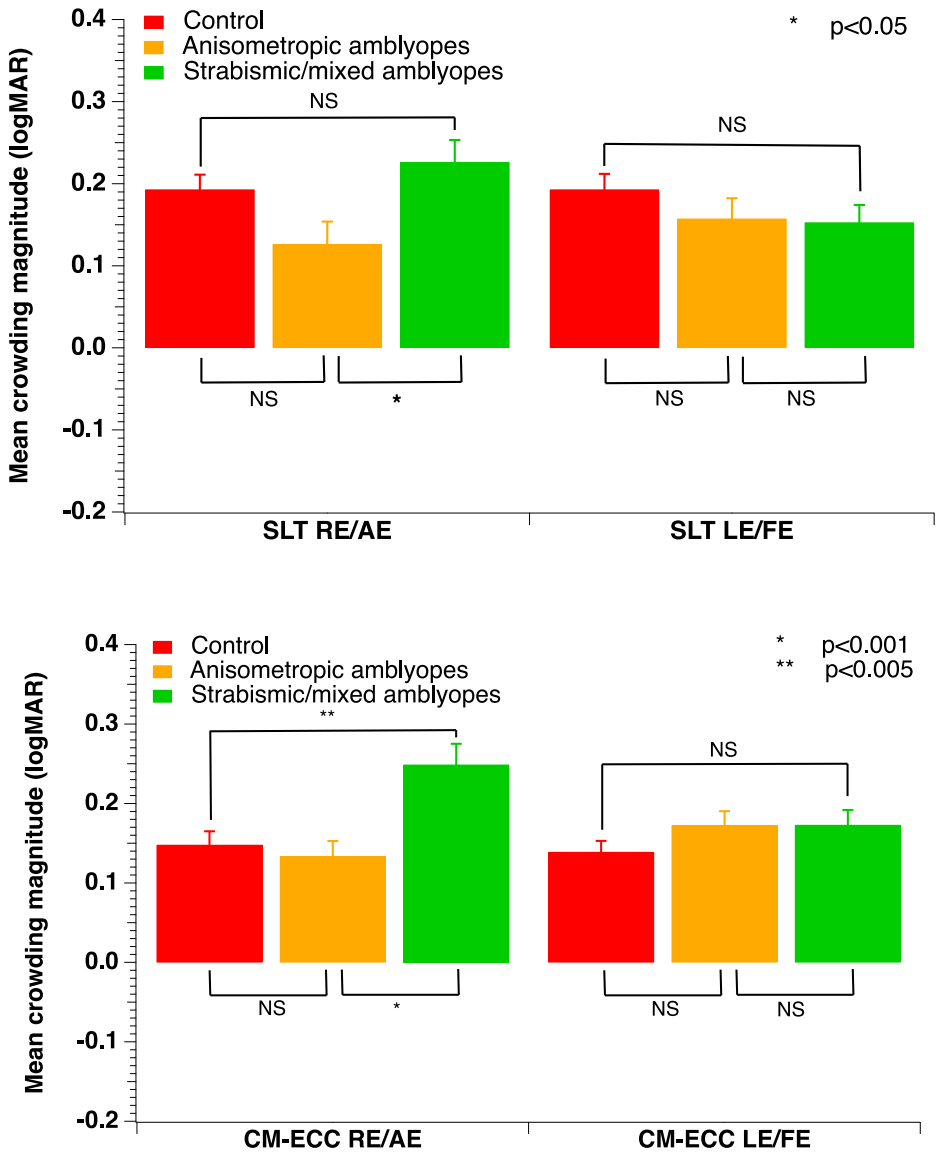


Figure 4.08: *Mean crowding magnitudes within each eye category, with significance bars. Top – SLT format. Bottom – CM-ECC format. Error bars show ±1SE.*

Table 4.15: *One-way ANOVA (repeated measures) examining main effects of test format (SLT and CM-ECC) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	F	Sig	μ^2
Controls	***1***	***6.961***	***0.015***	***0.232***
Error	23			
Anisometropic amblyopes	1	0.256	0.618	0.0.012
Error	21			
Strabismic/mixed amblyopes	1	0.966	0.337	0.046
Error	20			

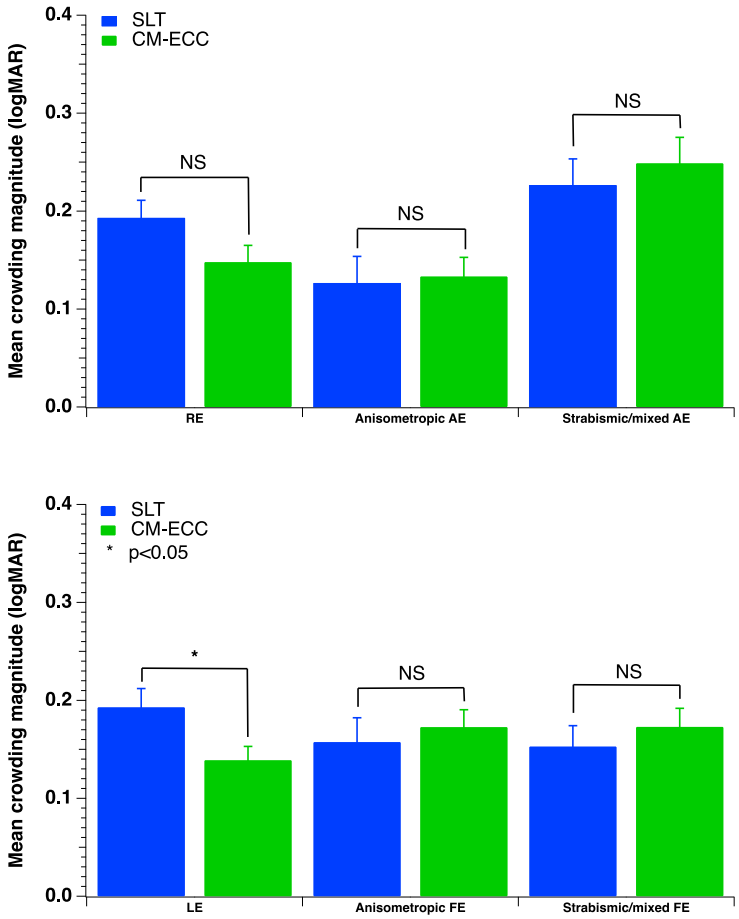


Figure 4.09: *Top – Mean crowding magnitudes for both tests (SLT and CM-ECC) compared within right control eyes (RE), anisometropic amblyopic eyes (AE) and strabismic/mixed amblyopic eyes, with significance bars. Bottom - Mean crowding magnitudes for both tests (SLT and CM-ECC) compared within left control eyes (LE), anisometropic fellow eyes (FE) and strabismic/mixed fellow eyes, with significance bars. Error bars show ±1SE.*

Table 4.16: *One-way ANOVA (repeated measures) examining simple main effects of eye (RE, LE, amblyopic eye and fellow eye) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	F	Sig	μ^2
Controls	1	0.112	0.741	0.005
Error	23			
Anisometropic amblyopes	1	3.229	0.087	0.133
Error	21			
Strabismic/mixed amblyopes	**1**	**14.170**	**0.001**	**0.415**
Error	20			

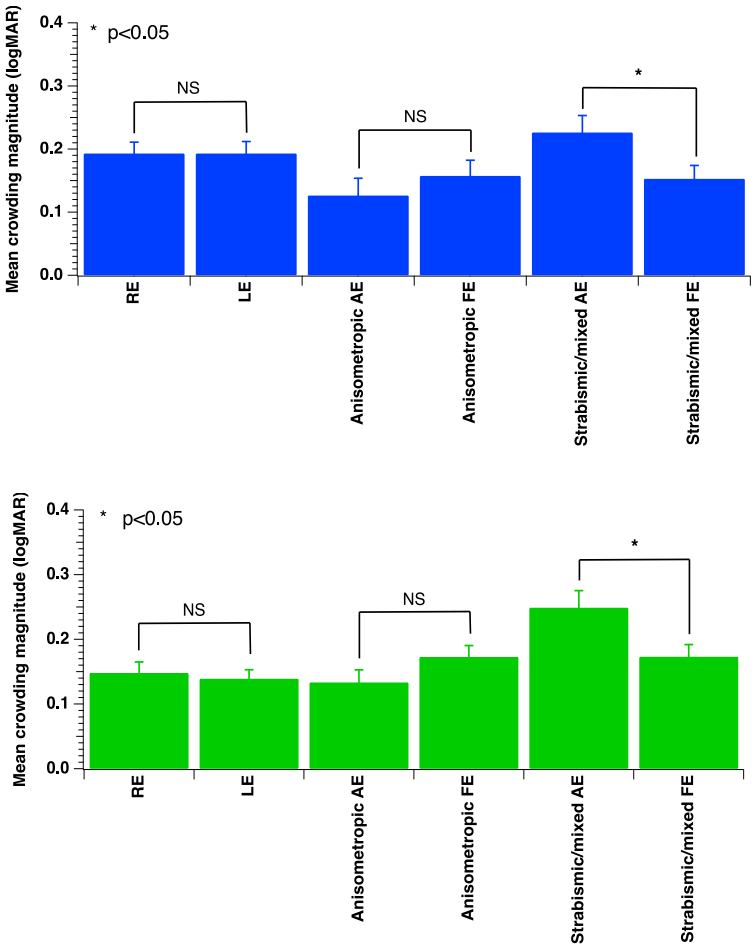


Figure 4.10: *Top – Mean SLT crowding magnitudes averaged within each eye category (Right eye – RE, Left eye - LE, Amblyopic eye – AE, Fellow eye – FE) with significance bars. Bottom – Mean CM-ECC crowding magnitudes averaged within each eye category, with significance bars. Error bars show ±1SE.*

4.5 Discussion

The aim of this study was to compare the visual crowding effects of the CM-ECC to the SLT for the purposes of amblyopia detection. The results showed that while the CM-ECC did demonstrate slightly larger crowding magnitudes than the SLT for the amblyopic and fellow eyes of anisometropic and strabismic/mixed amblyopes, this increase did not reach significance. In comparison, crowding magnitudes for controls eyes were smaller for the CM-ECC than for the SLT.

4.5.1 Crowding magnitudes

In their study, Lalor (2018) found lower crowding magnitudes using CM-ECC format in visually healthy children than for L-ECC, results which are replicated in the visually healthy control children in this study (Figure 4.11). Additionally, CM-ECC crowding magnitudes in this study were lower than those experienced with the SLT with controls. Comparison between contour interaction and crowding effects in Lalor's study demonstrated no significant differences for CM presented optotypes and did not offer any significant advantages over optotypes presented via luminance (L/LM). Lalor discussed how these results cast doubt on the benefit of CM optotypes for assessment of crowding; however, there may be some benefit for crowding limited conditions such as amblyopia, as slightly greater contour interaction and crowding effects have been demonstrated for CM than LM stimuli (Chung, Li and Levi, 2007; Hairol, Formankiewicz and Waugh, 2013). Initial investigation by Lalor using the ECC format with a limited number of amblyopic adults (unpublished) have demonstrated stronger CM crowding effects than CM-contour interaction effects, although only one anisometropic amblyope was present within that cohort.

CM-ECC crowding magnitudes of anisometropic and strabismic/mixed amblyopes within this cohort were minimally but insignificantly larger than those seen using SLT for both amblyopic and fellow eyes. This is contrary to the findings of Lalor (2018), who hypothesised that enhanced CM crowding could be diagnostic of a binocular anomaly, as the same effect was seen here in anisometropic amblyopes, as well as strabismic/mixed amblyopes.

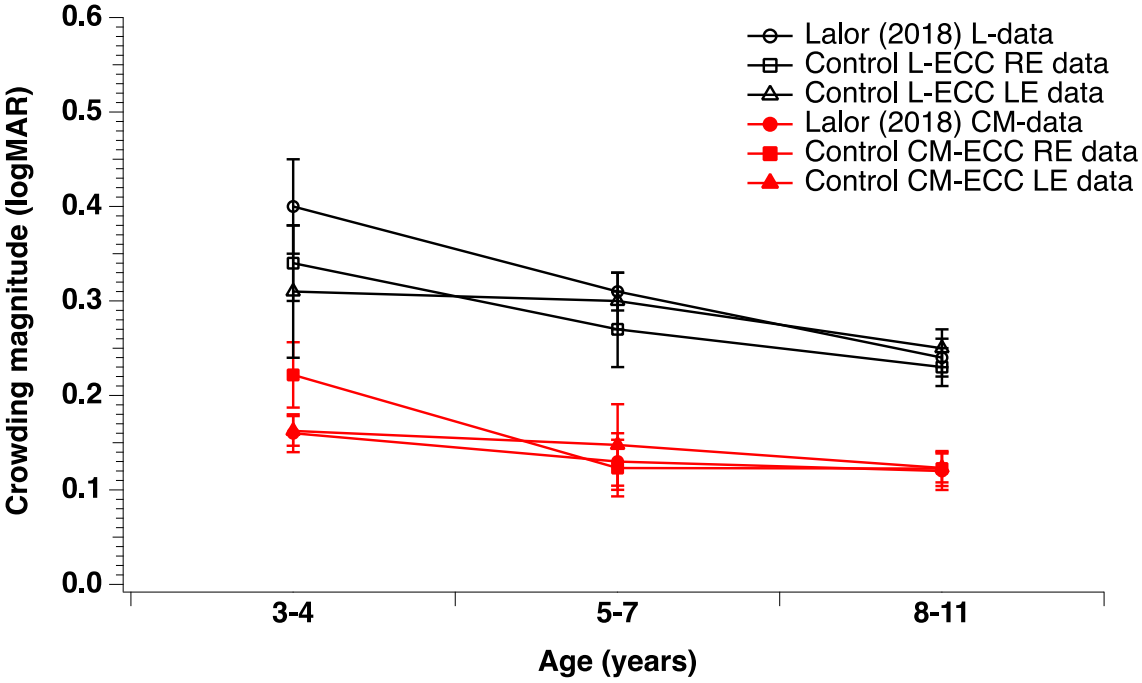


Figure 4.11: *Control group right and left eye crowding magnitude data of this study and data of visually healthy children (Lalor, 2018) for the L-ECC (see chapter three for results) and CM-ECC. Error bars show ±1SE.*

An explanation for these findings may possibly be found in the examination of CM visual processing. Contrast-modulated stimuli are considered to be processed in increasingly binocular neural regions of the brain (Wong, Levi and McGraw, 2001; Hairol, Formankiewicz and Waugh, 2013). Visual signals are transferred from the striate cortex to the extrastriate cortex, converging on binocular centres with large receptive fields, such as MT (Maunsell and Van Essen, 1983a; b; Maunsell and van Essen, 1987; Ellemberg et al., 2002) or V2

(Sheth et al., 1996; Wong, Levi and McGraw, 2001). In the study by Ellemberg et al (2002), the examination of motion perception by adults who experienced early monocular visual deprivation (congenital cataracts) demonstrated slightly diminished global motion perception for both eyes. In comparison, early binocular visual deprivation resulted in significantly greater global motion perception losses. The authors hypothesised that disruption to vision mediated by these binocularly driven extrastriate areas might be less disturbed by monocular deprivation, as the initial development of these extrastriate areas can be facilitated by input from the unaffected fellow eye.

Furthermore, interocular transfer effects are noted to occur increasingly within higher visual processing areas (i.e., beyond the primary visual cortex (PVC)) (Paradiso, Shimojo and Nakayama, 1989; Raymond, 1993; McColl and Mitchell, 1998). Evidence for this can be seen in examining the threshold data for the fellow eyes in this study. When examined with the SLT, no significant differences were seen between the acuity thresholds of the control eyes and the fellow eyes of either amblyopic group. However, once stimuli were presented in a higher CM-form (either isolated or CM-ECC), these fellow eyes now demonstrate significantly larger thresholds than the control eyes, demonstrating a deficit that was not present when optotypes were displayed in a luminance format. This additional deficit in the fellow eye threshold probably arises because of interocular transfer from the affected amblyopic eye.

4.5.2 Thresholds and IOD

Elevated visual acuity thresholds of around 0.3-0.5 logMAR are expected for CM presented optotypes, compared with luminance presented optotypes, as V2 receptive field sizes (Smith et al., 2001) and consequently, spatial summation areas (Sukumar and Waugh, 2007) are demonstrated to be around two to three times larger than those of V1. Mean

threshold elevation differences between L and CM presentations for both isolated and ECC formats used in this study are shown in Table 4.17 and were no exception to this rule.

For all eyes, threshold acuity was best with the SLT, poorer with the CM-Isolated format, and poorest with the CM-ECC. At first glance, this could reflect greater crowding effects; however, when we look at the difference between L presentations and CM-presentations (CM-isolated – L-isolated; CM-ECC – L-ECC), it is apparent that presentation in CM yields greater acuity diminishing effects for isolated optotypes than for optimally crowded arrangements, for all eyes.

Table 4.17*: Threshold elevations, given in logMAR, for isolated and ECC formats, when presented in CM (Mean CM format thresholds– Mean L format thresholds).*

	Isolated	**ECC**
Controls - Right eye	+0.483	+0.357
Controls - Left eye	+0.489	+0.350
Anisometropic amblyopes - amblyopic eye	+0.474	+0.356
Anisometropic amblyopes -fellow eye	+0.495	+0.352
Strabismic/mixed amblyopes- amblyopic eye	+0.449	+0.286
Strabismic/mixed amblyopes - fellow eye	+0.464	+0.355

Crowding is no exception to interocular transfer effects, with crowding effects demonstrated when even flanking bars are presented contralateral to the eye fixing on the target (Flom, Heath and Takahashi, 1963). Evidence for increased crowding effects with the L-ECC for both anisometropic and strabismic/mixed amblyopes was demonstrated in chapter three, with the largest crowding magnitudes seen in the amblyopic eyes of strabismic/mixed amblyopes, followed by the fellow eyes of anisometropic amblyopes. It is possible that

these larger luminance-defined crowding magnitudes, irrespective of whether they arise from the amblyopic or fellow eye, are weakened via presentation in contrast-modulation by the interocular transfer effects of the contralateral eye within the binocularly driven extrastriate area. Recent evidence supports the notion of visual functional improvement when utilising higher-order binocular processing areas, as anisometropic amblyopes appear to utilise second-order processing of coarse stereoacuity information to obtain satisfactory stereoacuity results with a Frisby Near Stereotest. However, these satisfactory scores are difficult to replicate when using a test (TNO), which requires excellent first-order processing of fine detail (Ateiza and Davis, 2019).

When the ECC was presented in CM, this reduction in crowding effects resulted in no significant difference in IOD (the key diagnostic criteria for amblyopia) between the existing SLT format and the CM-ECC. Therefore, as a visual screening tool, the CM-ECC provides no additional diagnostic benefit over the existing SLT.

4.5.3 The role of noise

It is considered that the incorporation of noise into acuity tests above the estimated internal noise raises the acuity detection threshold (Pelli and Farell, 1999; Levi, 2020). A comparison of L-optotypes to LM-optotypes by Lalor (2018) revealed threshold increases of approximately half a logMAR line on an acuity chart (0.06±0.01 logMAR), similar to those found in visually normal adults (Pelli, Levin and Chung, 2004). Reduced high noise efficiency has been demonstrated in amblyopic adults (anisometropic, strabismic and anisometropic/strabismic mixed (Pelli, Levi and Chung, 2004), resulting in a mean equivalent input noise elevation of 1.4x that of visually normal adults, equating to a decrease of one to two logMAR lines with noise. Interestingly, at high spatial frequencies (7.8cpd), all mild amblyopes displayed lower equivalent noise than visually healthy controls (Pelli, Levi and Chung, 2004). High noise efficiency was not able to be examined or compared in the

paediatric amblyopes within this experiment as luminance generated stimuli with noise (LM) was not examined. Examination using LM-optimally crowded in paediatric amblyopes would help establish how much of the threshold elevations seen here were due to noise and how much is due to presentation of the optotypes using second-order contrast.

4.6 Conclusions

While non-luminance defined stimuli are considered to have later critical periods of development (Daw, 1998) and therefore could be considered to be at greater risk to neurodevelopmental disorders such as amblyopia (Bertone et al., 2010); monocular deprivation may be less harmful to contrast-modulated optotype recognition in paediatric amblyopes, than previously thought. The presentation of the ECC using CM stimuli did not yield significantly increased crowding magnitudes for any of the amblyopic eyes examined, compared to the SLT; therefore, as a visual screening tool, the CM-ECC provides no additional diagnostic benefit over the existing SLT. Larger crowding effects have previously been seen when the ECC was presented using luminance compared to when generated here with contrast-modulation.

Future examination of paediatric amblyopes using LM optotypes will help establish how much the threshold elevation seen using CM-optotypes is due to internal noise; and whether differences in noise are seen between different amblyopic aetiologies in paediatric patients.

Chapter Five - Foveal crowding distances in normal and amblyopic children

5.1 Introduction

The critical spacing of crowding (defined as the minimum distance, centre-to-centre, between target and flankers that allows for accurate target identification (Pelli and Tillman, 2008)) increases proportionally with eccentricity (Bouma, 1970; Levi, Hariharan and Klein, 2002a; Pelli and Tillman, 2008; Gurnsey, Roddy and Chanab, 2011; Song, Levi and Pelli, 2014). However, the critical spacing of crowding within the fovea extends only over small distances of around 0.05 degrees (Bedell et al., 2013; Danilova and Bondarko, 2007; Siderov, Waugh and Bedell, 2013; Lalor, Formankiewicz and Waugh, 2016).

Amblyopia demonstrates exaggerated visual crowding in central vision (Rodier, Mayer and Fulton, 1985; Mayer and Gross, 1990; Morad, Werker and Nemet, 1999; Hess et al., 2001; Levi, Hariharan and Klein, 2002b); therefore, 'crowded' visual acuity tests are utilised to capitalise on this effect for the detection of amblyopia by making the visual acuity threshold poorer in the amblyopic eye and increasing the visual acuity interocular difference (Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015; Lalor, Formankiewicz and Waugh, 2016). However, this approach examines the deleterious effect of crowding magnitude upon a target but does not denote the individuals' 'crowding distance' or 'critical spacing of crowding'.

Clinical assessment of crowding distance in isolation could also provide vital diagnostic evidence for the diagnosis, treatment and monitoring of other physiological conditions where crowding deficits occur, but visual acuity is spared or less severely affected (Martelli et al., 2009; Robol et al., 2013; Crutch, 2014; Song, Levi and Pelli, 2014; Pelli et al., 2016).

These include conditions such as posterior cortical atrophy (McMonagle et al., 2006; Crutch and Warrington, 2007; Beh et al., 2015; Maia da Silva et al., 2017), apperceptive visual agnosia (Song, Levi and Pelli, 2014; Strappini et al., 2017) and schizophrenia (Kraehenmann et al., 2012). Following measurement of both crowding distance and isolated acuity in visually healthy adults, amblyopes and individuals with apperceptive agnosia, increased spacing:acuity ratios (SA<1.84) were seen in individuals with apperceptive agnosia and strabismic amblyopia (Song, Levi and Pelli, 2014). This increase occurred mainly in the absence of visual acuity loss for apperceptive agnostic patients but occurred in combination with acuity loss for strabismic amblyopes (Table 5.01)

Table 5.01: *Acuity in degrees compared with spacing/acuity ratios and the categorisation of visual behaviours. Adapted from Song, Levi and Pelli* (2014).

		Spacing/acuity ratio	
		SA< 1.84	SA>1.84
Acuity (deg)	<0.15	Visually normal individuals	Apperceptive agnosia
	>0.15	Anisometropic amblyopia	Strabismic Amblyopia

Problems arise with current clinical optotypes if an assessment of crowding distance in central vision is required. First, at around 0.05 degrees (three arcmins or 0.48 logMAR), the foveal crowding distance is smaller than the spatial extent of a current standard clinical optotype at the limits of visual resolution (Figure 5.01) (Pelli and Tillman, 2008; Pelli et al., 2016). Existing clinical optotypes are, therefore, simply too wide for foveal crowding distance measures. A second factor that affects detection thresholds (and therefore acuity

thresholds) is lateral (overlap) masking, which is most prominent in central vision, compared with visual crowding, which dominates in the periphery.

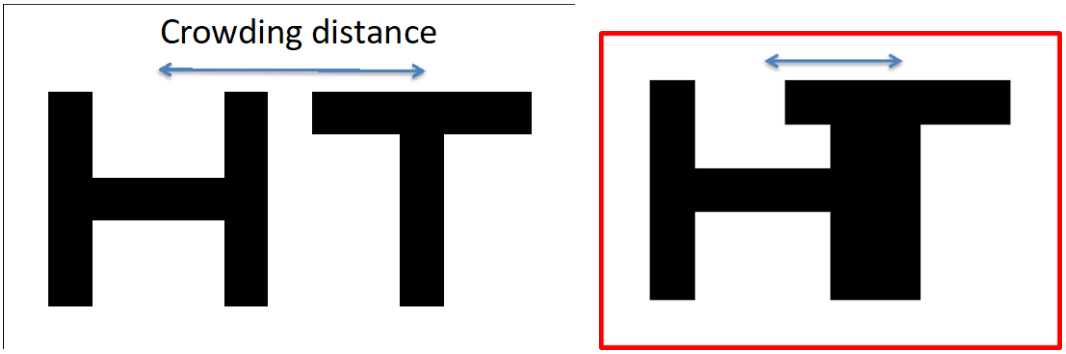


Figure 5.01: *Left – A diagrammatic example of centre-to-centre crowding distance measurement using Sloan style letters. Right – Over smaller crowding distances such as those in the fovea, existing optotypes are too wide and begin to overlap. Reproduced with permission from Haine, Waugh, Formankiewicz and Pelli* (2019).

Pelli et al. (2016) suggested that to measure foveal crowding distance, an optotype and its test arrangement should satisfy the following three conditions.

1. The optotype must be larger than the limits of visual resolution to allow it to be seen.
2. The optotype must be narrower than the foveal / fixation point crowding distance.
3. Inter-optotype spacing must be sufficiently large to prevent the effects of lateral masking.

5.1.1 The ‘Pelli’ optotype

The creation of the “Pelli” optotype satisfies these three conditions (Figure 5.02, (also seen in Chapter 1, Figure 1.07)). Proportionally it is taller but thinner than current clinical optotypes, with a 5:1 height/width ratio and a 2:1 width/stroke ratio. With a visual resolution limit of 0.02 degrees width (0.01 degrees stroke width (0.6 arcmins), it provides a smaller centre-to-centre distance, so flanking Pelli optotypes can be presented next to each other within the foveal crowding distance of 0.05 degrees, unlike standard 1:1 height/width optotypes (Figure 5.03, also seen in chapter one, Figure 1.08)) (Pelli et al., 2016). Therefore, the proportions of these new optotypes satisfy the first two spatial requirements for foveal crowding distance assessment.

Figure 5.02: *The Pelli optotypes with proportional measurements of height, width, and stroke width. Reproduced from Pelli, Waugh, Martelli et al., (2016).*

Evaluation of the Pelli optotype using a series of inter-optotype spacings revealed that lateral masking effects were minimised with a 1.4x spacing to width ratio (Pelli et al., 2016), satisfying the final third spatial condition for clinical assessment of foveal crowding distance.

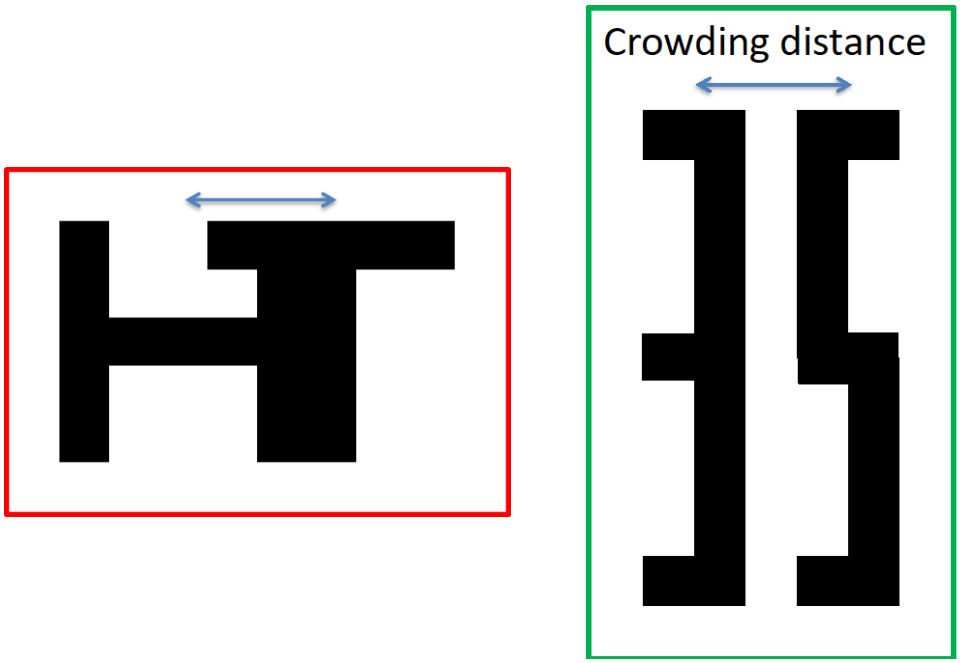


Figure 5.03: *An example of centre-to-centre crowding distances for Sloan style letters over a small crowding distance. The Pelli optotype (right) composition allows for closer placement over smaller crowding distances than Sloan letters (left). Reproduced with permission from Haine, Waugh, Formankiewicz and Pelli* (2019*).*

Crowding distance in over 200 British primary school children aged between 3 and 11 years of age was measured with this new "Pelli" optotype and published in a study by Waugh et al. (2018). Foveal crowding distances were measured using the 'Pelli" font in two arrangements: a trigram and repeated full-screen, and compared to measures of isolated Sloan acuity (Pelli et al., 2016; Waugh et al., 2018) (Figure 5.04).

Examination of the data revealed that the development of isolated visual acuity occurred at a rate of 1.4x (i.e. at age three mean acuity threshold was 1.4x higher than acuity at maturation (defined as 1.5x mean adult acuity threshold)) from age three until maturity about age six, similar to the results of previous visual acuity maturation studies (Atkinson and Braddick, 1983; Birch et al., 1983; Ellemberg et al., 1999; Stiers, Vanderkelen and Vandenbussche, 2003). Steeper rates of crowding distance with development were seen (improving at a rate of 3x) from age three to age eight years (Waugh et al., 2018).

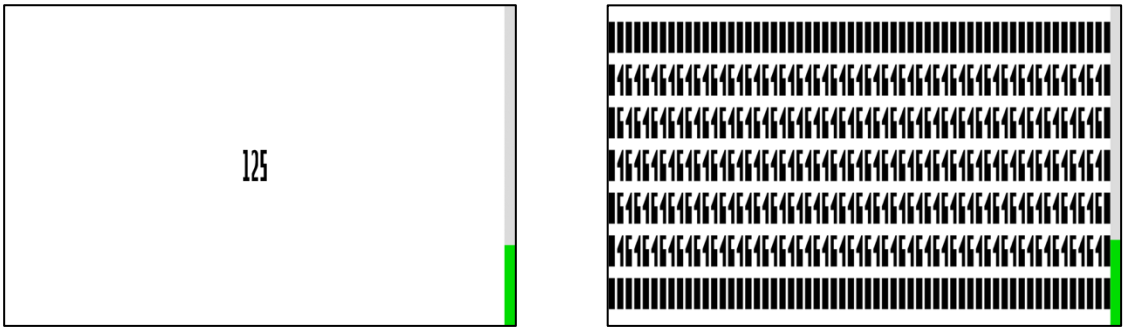


Figure 5.04*: The Pelli optotypes in two different arrangements. Left - Trigram, Right - a full-screen of repeated optotypes (two alternating Pelli optotypes repeated over the full-screen and surrounded by solid bars). Images reproduced with permission from Haine, Waugh, Formankiewicz and Pelli.* (2019*).*

These results support the theory that acuity and crowding are "functionally distinct and separately modifiable aspects of letter recognition" (Sternberg, 2003; Song, Levi and Pelli, 2014; Waugh et al., 2018). The steeper development and, therefore, enhanced sensitivity of spatial crowding compared with spatial acuity as a visual development assessment tool may mean that measurement of visual crowding has benefits for visual screening and amblyopia detection. Furthermore, with primary developmental data now available regarding crowding distances of visually normal children, identification of irregularities in visually developing children is now achievable. Used within a clinical environment, this test could help to highlight and identify conditions which demonstrate impaired visual crowding, such as amblyopia, posterior cortical atrophy and schizophrenia (Crutch and Warrington, 2007; Kwon, Legge and Dubbels, 2007; Pelli and Tillman, 2008; Kraehenmann et al., 2012; Robol et al., 2013; Maia da Silva et al., 2017).

5.1.2 Foveal crowding and repeated optotype assessment

Foveal crowding is subject to the influences of attention, fixation anomalies and gaze control (Flom, Weymouth and Kahneman, 1963). In conditions such as amblyopia, monocular fixation with the amblyopic eye is poorer than that seen in the eyes of visually healthy

individuals (Flom, Weymouth and Kahneman, 1963; Srebro, 1983; Kelly et al., 2019) due to deficits in neurological positioning (McKee et al., 2016). Both abnormal fixational eye movements and gaze control anomalies have been demonstrated in strabismic amblyopes (Srebro, 1983; Chung et al., 2015).

Repeated optotype presentation has been considered a method for managing the assessment of vision in the presence of fixation and gaze control instabilities (Kothe and Regan, 1990; Regan et al., 1992; Pardhan, 1997). With repeated optotypes, the eye continually passes over the same optotype, increasing the viewing time and number of viewing opportunities of the target (Regan et al., 1992; Facchin et al., 2019). Crowding distance estimates with repeated 'Pelli" optotypes have been shown to be beneficial in a small number of adult amblyopes (Waugh et al., 2018). They may also be helpful in amblyopic children. The repeated arrangement offers no advantage over the trigram arrangement for the measurement of crowding distance in visually healthy adults (Waugh et al., 2018). Nethertheless, a full screen of repeated optotypes can appear overwhelming and may affect compliance with visual assessment, especially in children (Atkinson et al., 1988). While the children in the Waugh et al. (2018) study were able to complete the repeated optotype arrangement, researchers noted that eight percent of children found the full-screen presentation challenging. Quantitatively, staircase variability (calculated as; $\boldsymbol{SD}(\%) = \frac{\boldsymbol{SD}\ (\boldsymbol{deg}\ )}{\boldsymbol{Crowding\ or\ Letter\ size\ threshold}\ (\boldsymbol{deg}\ )} \times \mathbf{100}$) of crowding distance for the full-screen repeated was significantly larger than the trigram format (**p=.020**) or isolated acuity (**p<.001**) (Waugh et al., 2018; Haine et al., 2021).

If amblyopic children are to be assessed, vision tests must be even more accessible, and user friendly as the vision screening age demographic in the U.K. is four to five-years of age (Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015; BIOS, 2020). Simplifying the repeated optotype arrangement could provide greater compliance, so in a pilot study,

examination of simpler repeat formats was conducted before testing was completed on amblyopic children. Full details of the apparatus can be found in **2.2.1.**

These pilot data were presented at the Anglia Ruskin Science and Engineering eighth annual conference (Haine et al., 2019) and also at VSS 2021 (Appendix 10) (Haine et al., 2021).

5.2 Pilot study

5.2.1 Pilot Participants

Recruitment of participants occurred through personal contacts and contact with the "School's Out" summer holiday club, based in Swaffham Prior, Cambridge, United Kingdom. The pilot study was approved by the Anglia Ruskin University Ethics committee (Project number FST/FREP/15/538) and completed in accordance with the Declaration of Helsinki and all information stored in accordance with GDPR guidelines. Twenty-eight visually healthy children (36-137 months) and six visually healthy adult volunteers completed the study. Of these 28 children, nine were aged three to four years old (33%), 12 were five to seven years old (44%), and seven were eight to eleven years old (26%). Habitual refractive correction, if required, was worn throughout. Parents and guardians gave informed written consent, and verbal assent was received from each child prior to participation.

5.2.2 Stimuli

Crowding distance and visual acuity were measured using two different fonts: the "Pelli" font (1, 2, 3, 4, 5, 6, 7, 8, and 9) and Sloan letters of D, H, K, N, O, R, S, V and Z (Sloan, 1959). Five different optotype formats were presented in sequence. First, crowding distance was measured with the trigram arrangement (Figure 5.04), followed by three repeated formats, which were randomly interleaved: a single-line repeated (Figure 5.05), a

single-line with vertical flankers (Figure 5.05) and a full-screen repeated (Figure 5.04). The target to flanker (spacing to width) ratio was set at 1.4x (Pelli et al., 2016; Waugh et al., 2018). Finally, Sloan optotypes were presented in isolated format.

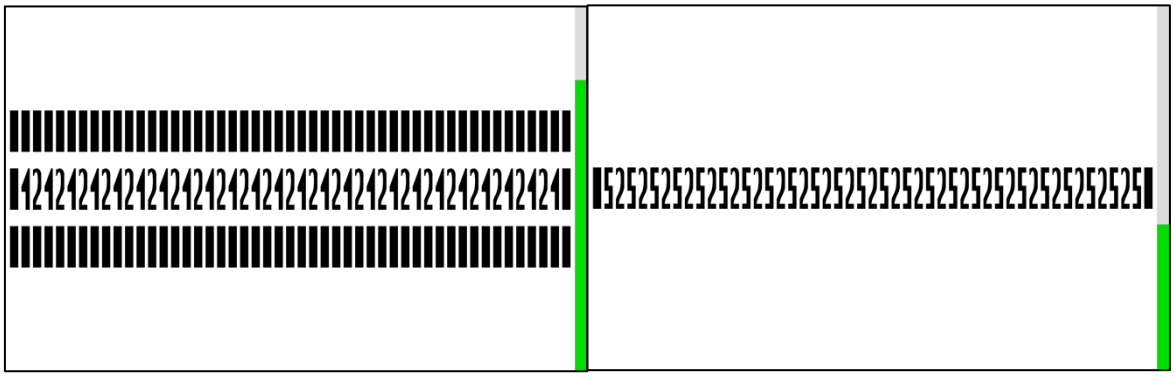


Figure 5.05: *Left - Repeated Pelli optotypes in a single line with vertical flankers arrangement. Right - Repeated Pelli optotypes in a single line arrangement. Reproduced with permission from Haine, Waugh, Formankiewicz and Pelli* (2019).

5.2.3 Procedure

Following autorefraction (See **2.2.1** for details), all participants sat at three meters from the MacBook screen. Three to four-year-old participants were placed at one metre, as larger optotype sizes and spacing are required for this age group (Waugh et al., 2018); therefore, a shorter testing distance allowed for the necessary optotype sizes and spacings to be displayed. Additionally, a reduced testing distance encouraged compliance (Atkinson et al., 1988). All participants were carefully observed throughout to ensure testing distances were maintained. Monocular assessment of visual acuity and crowding distance (deg) were assessed using a QUEST adaptive staircase with 20 trials per arrangement (Pelli and Watson, 1983). Targets were identified using a matching card or named verbally. Participants were asked to identify the central target when the Pelli Trigram was presented, identify both optotypes when the repeated formats were presented, and identify the isolated Sloan optotype.

5.2.4 Analysis

Data analyses were conducted using IBM SPSS Statistics (24.0.0.2), with a repeated-measures analysis of variance (ANOVA) using a Huynh–Feldt correction for the violation of sphericity assumption. A Tukey HSD test was employed for required post hoc analyses.

5.2.5 Pilot Study Results

Threshold visual acuity and foveal crowding distances are displayed in Figure 5.06 and Appendix 11.

Maturity of isolated visual acuity (calculated as 1.5x the adult mean threshold acuity, as per Waugh et al. 2018) occurred in this cohort at approximately 71 months / 5.9 years (Appendix 11). Visual acuity improved by a factor of 1.4 from age three until maturity at six, in line with the results of Waugh et al. (2018).

Maturity of crowding distance, again calculated as 1.5x the adult mean threshold crowding distance, occurred within this cohort at 109 months / 9.08 years for the trigram arrangement, 107 months / 8.92 years for the single line arrangement, 108 months / 9 years for the single line with vertical flankers arrangement and 93 months / 7.75 years) for the full-screen arrangement. The average crowding distance improved by a factor of 4.7 until maturity at 8.8 years (Appendix 11).

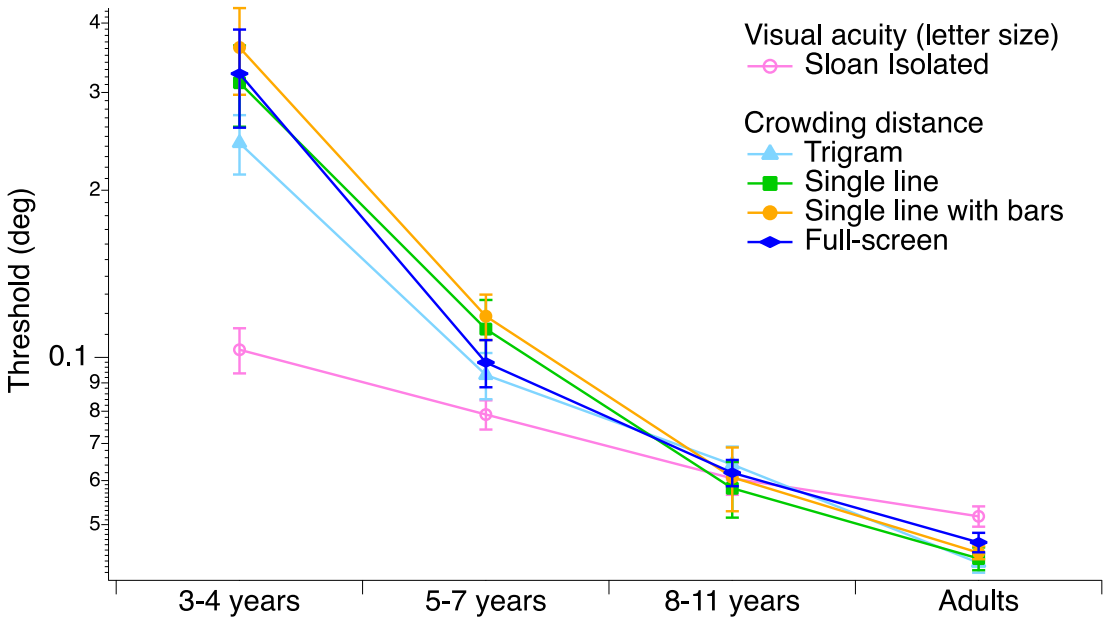


Figure 5.06: *Mean isolated Sloan thresholds and Pelli foveal crowding distances (degrees), with error bars representing the standard error of the mean. Data is grouped into four different age groups and represents the age at the time of examination. Adult indicates an individual aged 18 or over. Graph reproduced from Haine et al., (2021).*

5.2.5.1 Crowding distance analysis

A 4 (age-group) x 4 (test-format) repeated measures ANOVA (featuring one between factor (age-group) and one within factor (test-format) revealed significant decreases in crowding distance with age [$F(3, 30) = 22.632$, **p<.001**], but no significant differences in crowding distance were seen between the four test formats [$F(2.440, 73.192) = 2.243$, p=.103]. The difference in crowding distance seen between age groups, was not significantly affected by test format [$F(7.319, 73.192) = 1.584$, p=.151].

5.2.5.2 Repeated optotype analysis

A further 4 (age-group) x 3 (test-format) repeated measures ANOVA (featuring one between factor (age-group) and one within factor (test-format) comparing the different repeated optotype formats, revealed again significant decreases of crowding distance with increasing

age [*F*(3, 30) = 17.804, **p<.001**], with no other significances were found (Table 5.02). Tukey HSD analysis demonstrated no statistically significant differences in crowding distances were seen between the 5-7 years, 8-11 years, or adult age groups in this cohort (p>.05); however, the 3-4-year-old participants demonstrated significantly larger crowding distances compared with all the other age groups (5-7 years: +0.223±0.040; 8-11 years: +0.272±0.046; Adults: +0.288±0.048, **p<.001** respectively).

Table 5.02*: Repeated measures ANOVA with three test formats (single line, flanked single line and full screen) and four age groups (3-4 years, 5-7 years, 8-11 years, adults).*

	df	**F**	**Sig**	**μ^2**
Test format	2	1.127	0.331	0.036
Error	60			
Age group	**3**	**17.804**	**<0.001**	**0.640**
Error	30			
Test format * age group	6	0.752	0.610	0.070
Error	60			

For repeated formats, the single-line demonstrated the most comparable mean thresholds (+0.163±0.027 deg) to the full-screen format (+0.162±0.030 deg); however, on observation, children were happier to respond to the single line. Quantitative examination of staircase variability demonstrated the highest variability for the full screen repeated format (18.44±3.01%), followed by the single line (17.19±1.39%), and the lowest with the flanked single line (14.97±0.62%) (Figure 5.07).

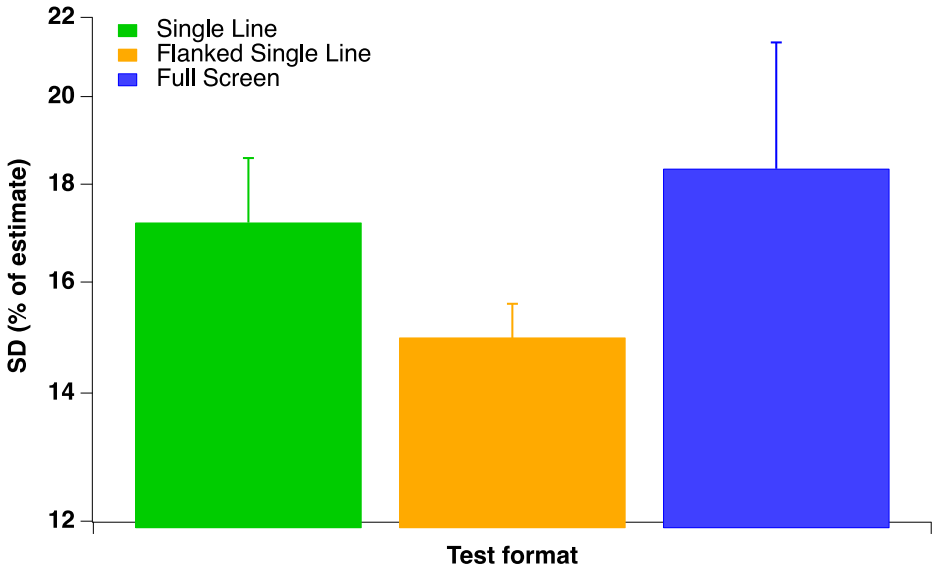


Figure 5.07: *Variability of crowding distance threshold estimates for repeated formats.*

5.2.6 Pilot study summary

The thresholds obtained in this pilot study are comparable to the normative data gathered by Waugh et al. (2018), demonstrating reproducibility of this data for these age groups (Figure 5.08). Of note, larger standard error values were present within the three to four-year-old age group in this pilot study compared with Waugh et al. (2018); however, the combined factors of a smaller sample size and younger participants likely account for these variations within this data.

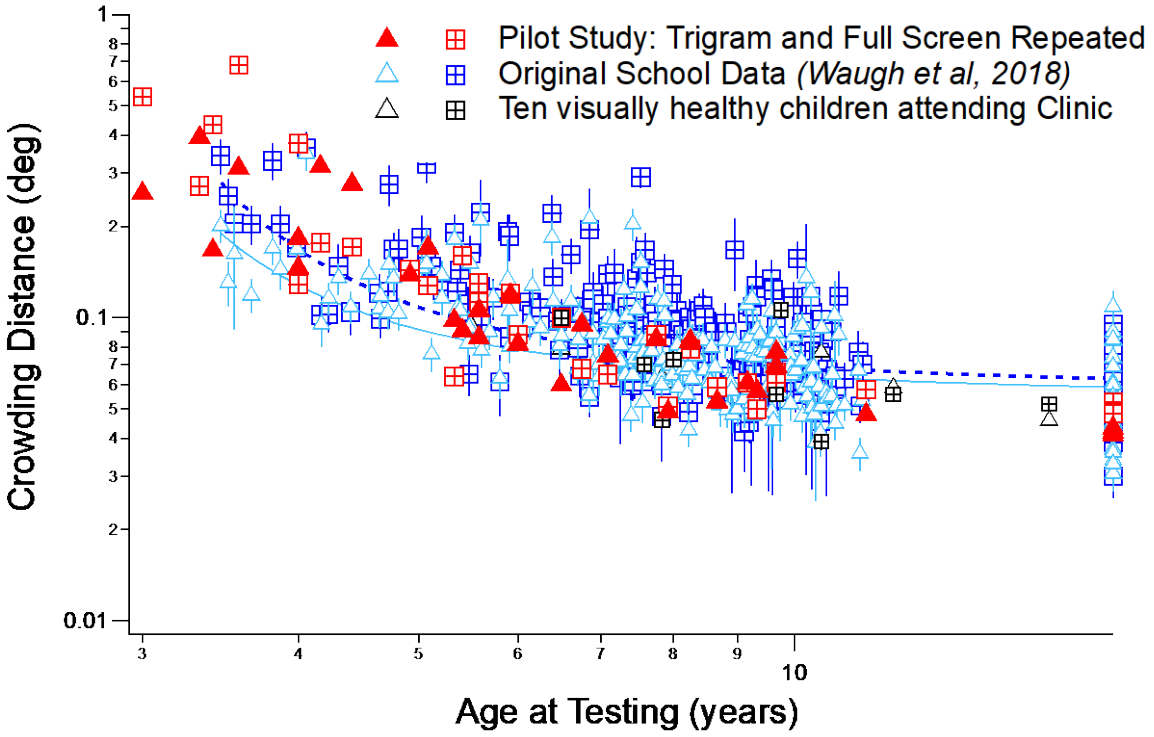


Figure 5.08: *Crowding distance pilot study results overlayed with crowding distance results from the Waugh et al. (2018) study.*

No significant difference was found between the crowding distance results of the three different repeated arrangements, demonstrating that any of these three formats would be appropriate to use for the benefit of individuals with poor central fixation. While all three of the repeated arrangements yielded comparable thresholds, the simplest arrangement, the single line, provided the most similar threshold to the full screen whilst also appearing to be less visually 'overwhelming', requiring less reassurance from the examiner. Additionally, quantitative response variability was lower in the single line than in the full-screen presentation.

Therefore, the single line repeated arrangement was therefore chosen, along with the trigram arrangement, to measure crowding distance in child amblyopes.

5.3 Aims

In the main experiment, the aims are to;

1. Establish the foveal crowding distances of paediatric amblyopes and compare the data to visually healthy children.
2. Determine if there is a difference between the foveal crowding distances of paediatric strabismic/mixed and anisometropic amblyope subtypes.

5.4 Main study

5.4.1 Methodology

Full details of apparatus, stimuli and testing procedure can be found in **2.2.1, 2.2.6 and 2.2.4,** respectively.

5.4.2 Participants

Full participant details can be found in **2.2.3.** One strabismic/mixed participant of this cohort did not successfully complete the crowding distance examination and is therefore excluded from the analysis. In total, sixty-seven participants successfully completed examination (mean age 6.92 years, range 3-11 years). Participant demographics for foveal crowding distance thresholds are summarised in Table 5.03.

5.4.3 Analysis

Full details of planned data analysis can be found in chapter **2.4.** SLT spacing was calculated as the centre-to-centre spacing in degrees, between the target letter and flanking optotype/bar, at the point of acuity threshold. This allowed for a comparison of spacing experienced in current acuity examinations, against crowding distances yielded with the new Pelli crowding distance test. Isolated acuity threshold using Sloan letters (as per the

pilot study) was also examined, so as to allow for a comparison between acuity and crowding distance thresholds.

Table 5.03*: Participant demographics for crowding distance threshold examination.*

	Sex		Age (Years)		Number per age group (years)			Total
	Male	Female	Mean	Range	3 - 4	5 - 7	8 -11	
Controls	**7**	**17**	**7.4**	**4-10**	**6**	**6**	**12**	**24**
Amblyopes	**22**	**21**	**6.6**	**3-10**	**4**	**31**	**8**	**43**
Anisometropes	*10*	*12*	*6.2*	*3-10*	*2*	*19*	*1*	*22*
Strabismic / mixed	*12*	*9*	*7.2*	*4-10*	*2*	*12*	*7*	*21*

5.5 Main study results

5.5.1 Crowding Distance Thresholds

Crowding distance threshold data for the three different tests (Pelli Trigram, Pelli Single Line and SLT) averaged within each eye (RE, LE, Amblyopic eye, Fellow eye) and test group (Controls, Anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Figure 5.09 and Table 5.04. Data are mean±standard error unless stated otherwise. One-way repeated measures ANOVA demonstrated no statistically significant differences in crowding distance/spacing between right and left control eyes for all tests ($p>.05$) (Table 5.05), therefore control RE data were analysed against amblyopic eyes, and control LE data were analysed against fellow eyes. Re-analysis with the control eyes interchanged, did not yield any changes in significance.

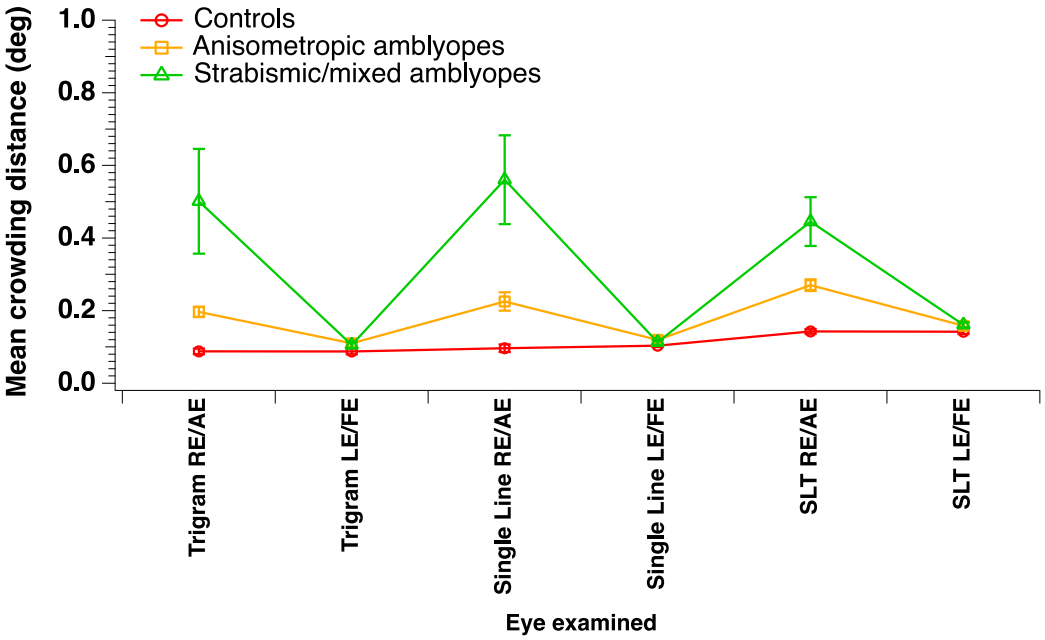


Figure 5.09: *Mean crowding distances (deg) for the Pelli Trigram and Pelli Single Line presentations, and spacing (deg) for the SLT presentations in both eyes (RE-right eye, LE-left eye, AE-amblyopic eye, FE-fellow eye) of controls, anisometropic amblyopes and strabismic/mixed amblyopes. Error bars show ±1SE*

Table 5.04: *Mean crowding distances and spacings (deg) with standard error, for each group, eye, and test.*

	n	Trigram	Single Line	SLT
Controls - Right eye	24	+0.088±0.008	+0.096±0.011	+0.143±0.006
Controls - Left eye	24	+0.088±0.008	+0.103±0.004	+0.142±0.006
Anisometropic amblyopes - amblyopic eye	22	+0.197±0.014	+0.225±0.025	+0.270±0.015
Anisometropic amblyopes - fellow eye	22	+0.110±0.009	+0.120±0.009	+0.158±0.009
Strabismic/mixed amblyopes- amblyopic eye	21	+0.501±0.144	+0.561±0.122	+0.445±0.067
Strabismic/mixed amblyopes - fellow eye	21	+0.106±0.008	+0.113±0.007	+0.161±0.007

Table 5.05: *One-way repeated measures ANOVA examining differences between the crowding distances/spacings of right and left control eyes, for each test presentation (Pelli Trigram, Pelli Single Line and SLT).*

	df	**F**	**Sig**	**μ^2**
Trigram	1	0.011	0.916	0.000
Error	23			
Single Line	1	1.031	0.320	0.043
Error	23			
SLT	1	0.207	0.654	0.009
Error	23			

Strabismic/mixed amblyopes demonstrated larger crowding distances than anisometropic amblyopes or controls. A 3 (group) x 3 (test-format) x 2 (eye) repeated measures ANOVA (featuring one between factor (group) and two within factors (test-format and eye)) revealed that this occurred at a significant level [$F(2, 64) = 8.827$, **p<.001**]**.** Crowding distances seen in amblyopic eyes were significantly larger than those seen in fellow eyes [$F(1, 64) = 16.655$, **p<.001**], an effect that was significantly larger in strabismic/mixed participants [$F(2, 64) = 8.335$, **p<.001**]. Crowding distances were smaller with the Pelli optotype test formats compared with the centre-to-centre spacing of the SLT, with this effect approaching significance [$F(1.669, 106.830) = 3.259$, p=.051]. Crowding distances per group, and per eye, were not significantly affected by the different test formats (p>.05) (Table 5.06). Significant interactions are explored further below.

5.5.1.1 Examination of effects within groups

One-way repeated measures ANOVA revealed that both anisometropic and strabismic/mixed amblyopes showed main effects of eye (**p<.001** respectively), but controls did not (p>.05) (Table 5.07). Pairwise comparisons showed that that amblyopic eye of

anisometropic amblyopes yielded significantly larger crowding distances than the fellow eye for the Trigram format (+0.087±0.013, **p<.001),** the Repeated format (+0.106±0.023, **p<.001**) and significantly larger spacings than the fellow eye for the SLT format (+0.112±0.011, **p<.001**). The same effect occurred for strabismic/mixed amblyopes, with significantly larger crowding distances occurring in the amblyopic eye compared with the fellow eye for the Trigram format (+0.396±0.142, **p=.012),** the Repeated format (+0.448±0.170, **p=.016**) and significantly larger spacings than the fellow eye for the SLT format (+0.284±0.068, **p<.001**) (Figure 5.13).

Table 5.06: *Repeated measures ANOVA with three test formats (Pelli Trigram, Pelli Single Line and SLT), three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) and eyes (Right eye, Left eye, Amblyopic eye and Fellow eye).*

	df	**F**	**Sig**	**μ^2**
Test format	1.669	3.259	0.051	0.048
Error	106.830			
Group	**2**	**8.827**	**<0.001**	**0.216**
Error	64			
Eye	**1**	**16.655**	**<0.001**	**0.206**
Error	64			
Test format * group	3.338	1.490	0.218	0.044
Error	106.830			
Test format * eye	1.692	1.498	0.229	0.023
Error	108.261			
Eye * group	**2**	**8.335**	**<0.001**	**0.207**
Error	64			
Test format * group * eye	3.383	2.029	0.106	0.060
Error	108.261			

Table 5.07: *One-way ANOVA (repeated measures) examining simple main effects of eye (RE, LE, amblyopic eye and fellow eye) for each group (normal, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	1	0.735	0.400	0.031
Error	23			
Anisometropic amblyopes	**1**	**56.809**	**<0.001**	**0.730**
Error	21			
Strabismic/mixed amblyopes	**1**	**9.337**	**0.006**	**0.318**
Error	20			

5.5.1.2 Examination of effects between test-groups

Largest crowding distances were seen in the strabismic/mixed amblyopic group, for all three tests (**p<.05)** (Table 5.08).

Pairwise comparisons revealed that for the Trigram format, strabismic/mixed amblyopic eyes yielded significantly larger crowding distance thresholds than control right eyes and anisometropic amblyopic eyes (+0.413±0.111, **p=.001** and +0.305±0.114, **p=.028)**. Crowding distance thresholds in anisometropic amblyopic eyes were not significantly different from control right eyes (+0.109±0.110, p=.979) (Figure 5.11).

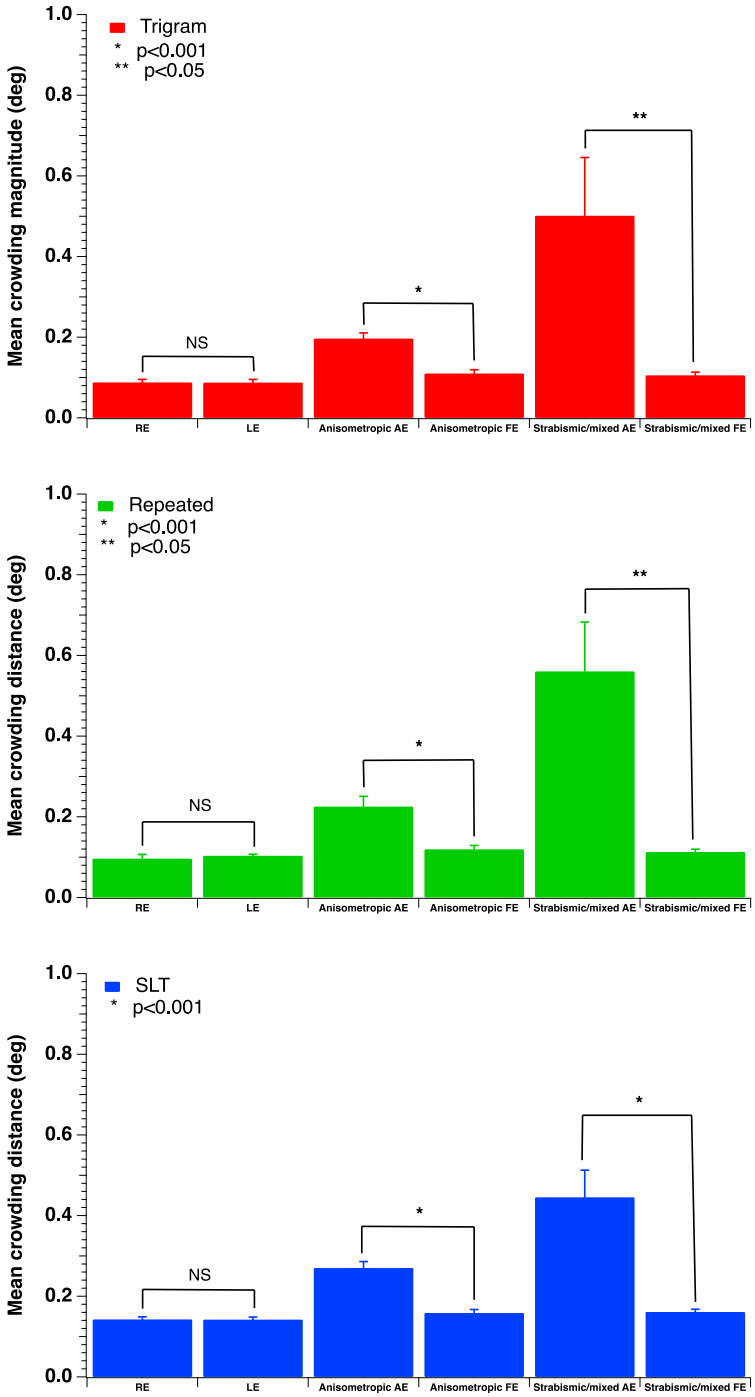


Figure 5.10: *Crowding distance thresholds and centre-to centre spacing (SLT) averaged within each eye category. Top – Pelli Trigram format. Middle – Pelli Single line format. Bottom – SLT format. Error bars show ±1SE.*

For the repeated single line format, strabismic/mixed amblyopic eyes yielded significantly larger crowding distance thresholds than control right eyes (+0.464±0.133, **p=.003**), and anisometropic amblyopic eyes (+0.335±0.135, **p=.048**). Crowding distance thresholds for anisometropic amblyopic eyes were not significantly larger than control right eyes (+0.129±0.131, p=.985) (Figure 5.11).

Table 5.08: *Repeated measures ANOVA examining effects of three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes) upon each test format (Pelli Trigram, Pelli Single Line and SLT).*

	df	**F**	**Sig**	**μ^2**
Trigram	**2**	**7.374**	**0.001**	**0.187**
Error	64			
Repeated single line	**2**	**6.350**	**0.003**	**0.166**
Error	64			
SLT	**2**	**16.591**	**<0.001**	**0.341**
Error	64			

For the SLT format, centre-to-centre spacings experienced by strabismic/mixed amblyopic eyes were significantly larger than control eyes and anisometropic amblyopic eyes (+0.303±0.053, **p<.001** and +0.175±0.054, **p=.006**), while anisometropic amblyopic eyes were not significantly different from control right eyes (+0.127±0.053, p=.055) (Figure 5.11).

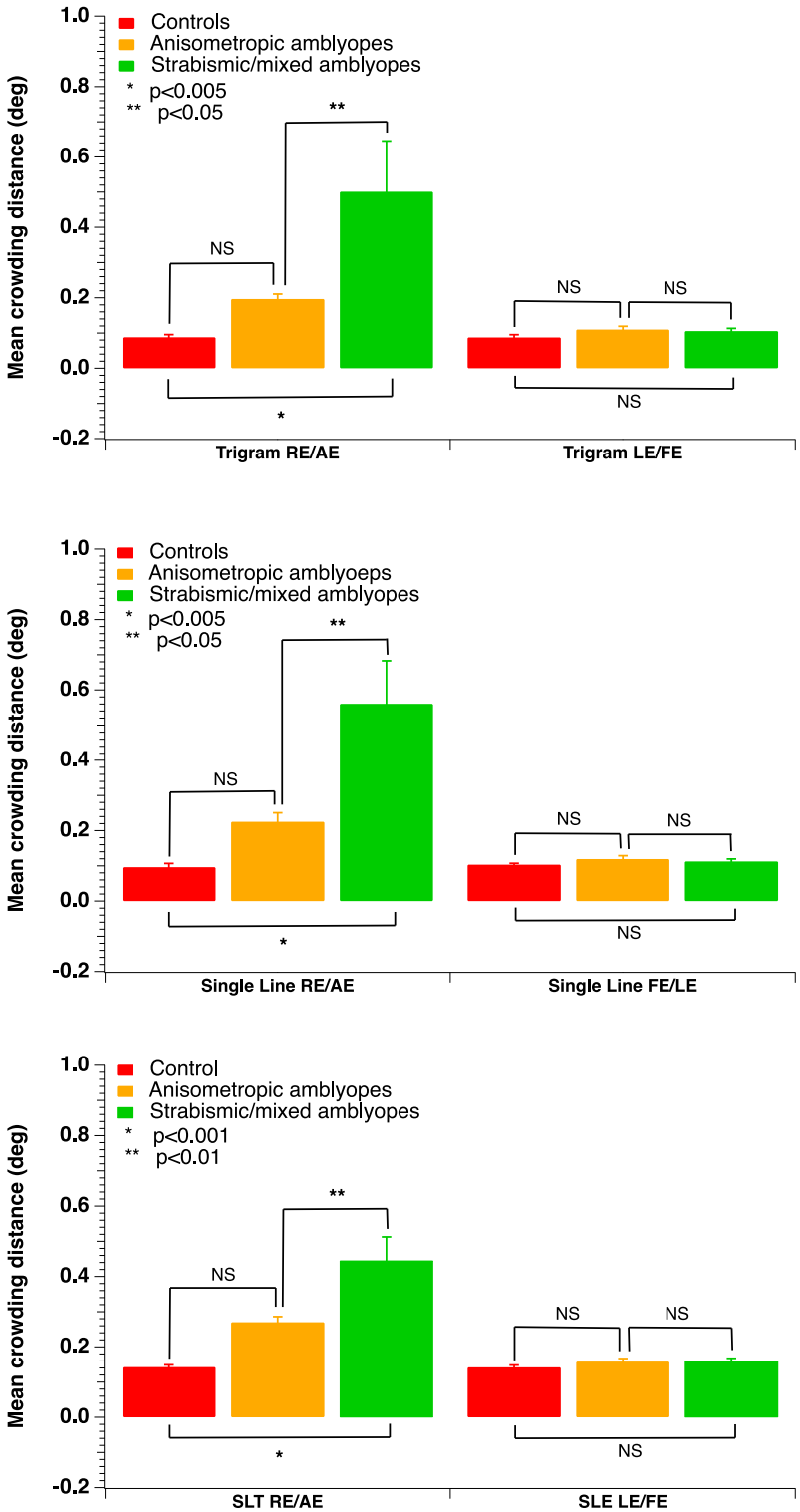


Figure 5.11: *Crowding distance thresholds (Trigram and Repeated single line) and centre-to-centre spacing (SLT) averaged within each eye category, with significance bars. Top – Pelli Trigram format. Middle – Pelli Single Line format. Bottom – SLT format. Error bars show ±1SE.*

Pairwise comparisons examining control left eyes and fellow amblyopic eyes revealed no significant differences in crowding distance threshold between the three groups for all three different test formats (p>.05) (Figure 5.11)

5.5.2 Interocular differences (IOD)

Interocular difference was calculated as the difference (recorded in degrees) between the crowding distances or centre-to-centre spacings, of each eye. Mean IODs for the three different tests (Pelli Trigram, Pelli Single Line and SLT), and test groups (controls, anisometropic amblyopes, strabismic/mixed amblyopes) are shown in Table 5.09. Mean IOD per group for the three visual acuity tests, are shown in Figure 5.12. Data are mean±standard error unless stated otherwise.

Crowding distance IOD was not affected by test format [$F(1.688, 108.037) = 2.168$, p=.127], but statistically significantly larger IODs were confirmed in strabismic/mixed amblyopes [$F(2, 64) = 7.848$, **p<0.001**] with a 3 (group) x 3 (test-format) repeated measures ANOVA (featuring one between factor (group) and one within factor (test-format)). Choice of test format did not affect the crowding distance IOD obtained by each group [$F(3.376, 108.037) = 1.822$, p=.141]. (Table 5.10).

Table 5.09: *Mean crowding distance and centre-to-centre spacing interocular differences (logMAR) with standard error, for each group (controls, anisometropic amblyopes and strabismic/mixed amblyopes) and test format (Pelli Trigram, Pelli Single Line, SLT).*

	n	Trigram IOD	Single line IOD	SLT IOD
Controls	24	+0.014±0.002	+0.023±0.005	+0.004±0.001
Anisometropic amblyopes	22	+0.087±0.018	+0.108±0.023	+0.112±0.011
Strabismic/mixed amblyopes	21	+0.396±0.142	+0.450±0.169	+0.284±0.068

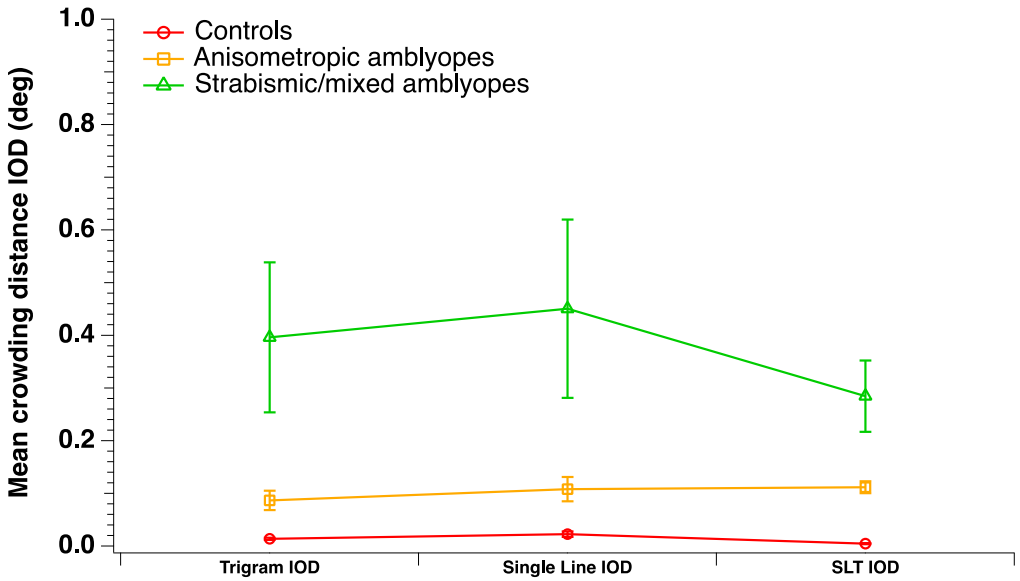


Figure 5.12: *Mean IOD (deg) for each crowding distance (Pelli Trigram, Pelli Single Line) and acuity (SLT) test per group (controls, anisometropic and strabismic/mixed amblyopes). Error bars show ±1SE.*

Table 5.10: *Repeated measures ANOVA examining differences in IOD with three test formats (Pelli Trigram, Pelli Single Line, SLT) for three test groups (controls, anisometropic amblyopes and strabismic/mixed amblyopes).*

	df	**F**	**Sig**	**μ^2**
Test format	1.688	2.168	0.127	0.033
Error	108.037			
Group	**2**	**7.848**	**<0.001**	**0.197**
Error	64			
Test format * group	3.376	1.822	0.170	0.054
Error	108.037			

5.5.2.1 Examination of the effect of group

Posthoc pairwise comparisons of test groups revealed that strabismic/mixed amblyopes demonstrated significantly larger interocular crowding distance / centre-to-centre spacing differences than both controls and amblyopes (+0.363±0.095, **p<.001** and +0.275±0.097,

p=.018 respectively). The crowding distance / centre-to-centre spacing IOD differences of anisometropic amblyopes were larger than controls, but not significantly so (+0.089±0.094, p=1.000).

When examined per test, pairwise comparisons revealed that for the Trigram format, strabismic/mixed amblyopes demonstrated significantly larger interocular crowding distance differences than both controls and anisometropic amblyopes (+0.382±0.109, **p=.003** and +0.309±0.112, **p=.022** respectively). The interocular crowding distance differences of anisometropic amblyopes were larger than controls, but not significantly so (+0.073±0.108, p=1.000).

For the single line repeated format, strabismic/mixed amblyopes demonstrated significantly larger interocular crowding distance differences than both controls and anisometropic amblyopes (+0.428±0.131, **p=.005** and +0.342±0.134, **p=.038** respectively). The interocular crowding distance differences of anisometropic amblyopes were larger than controls, but not significantly so (+0.085±0.129, p=1.000).

For the SLT format, the interocular centre-to-centre spacing differences experienced by strabismic/mixed amblyopes were significantly larger than both controls and anisometropic amblyopes (+0.280±0.053, **p<.001** and +0.173±0.054, **p=.006** respectively). The interocular centre-to-centre spacing differences of anisometropic amblyopes were larger than controls, but not significantly so (+0.107±0.052, p=.129) (Figure 5.13).

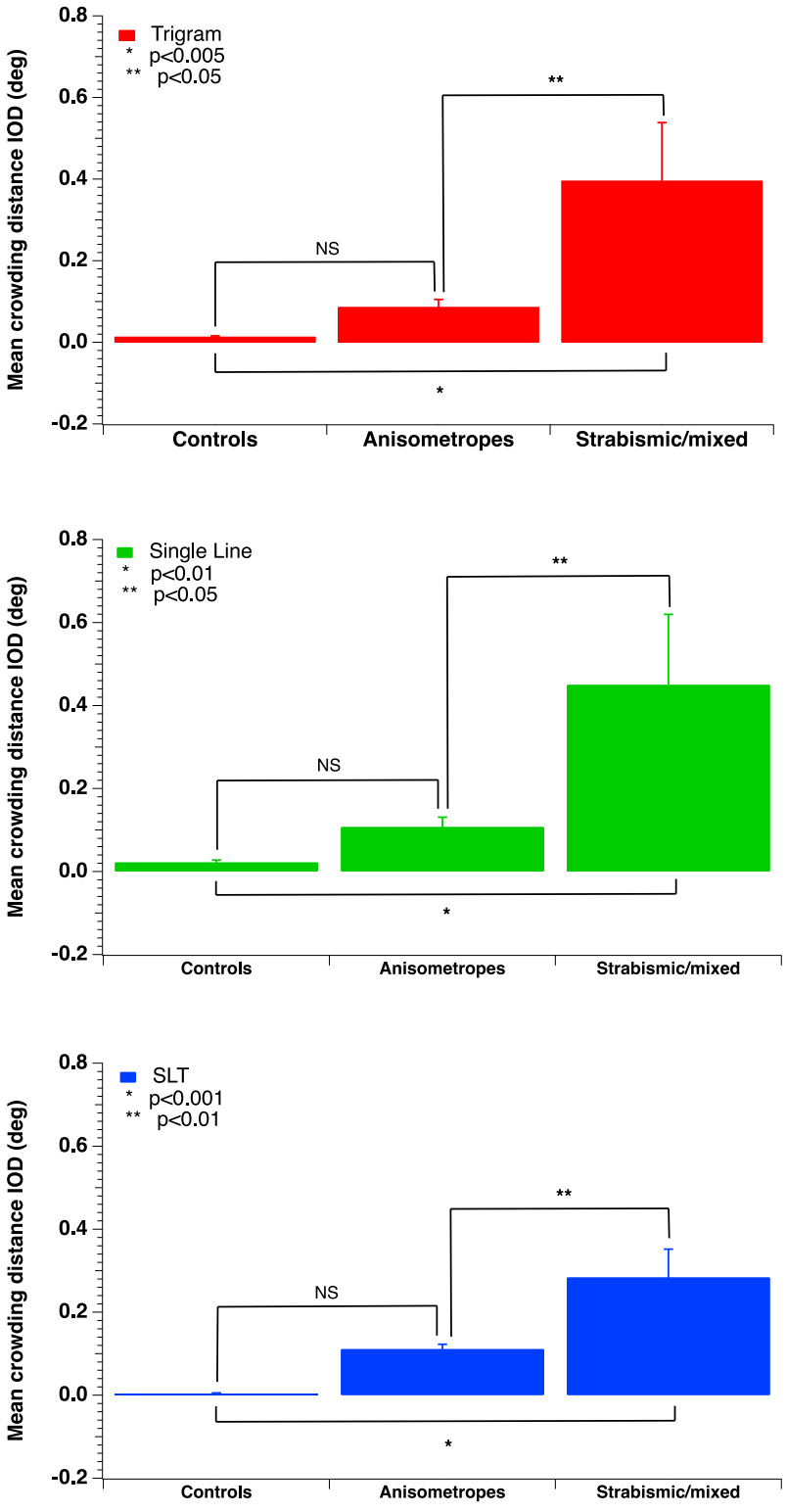


Figure 5.13: *Mean crowding distance IODs (deg) for the Pelli Trigram and single line, and mean centre-to-centre spacing (SLT) per group, with significance bars. Top – Pelli Trigram format, Middle – Pelli Single Line. Bottom – SLT format. Error bars show ±1SE.*

5.5.3 Acuity thresholds vs Crowding thresholds

Examination of threshold differences between controls, anisometropic amblyopes and strabismic/mixed amblyopes, were calculated as ratios (amblyope eye threshold/control eye threshold), to examine for differences in sensitivity. Combined as a single group of amblyopes, amblyopic eye crowding distance ratios (Trigram: 3.9x and Single Line: 3.9x) were larger than isolated letter size threshold ratios (Sloan: 3.1x), although pairwise comparisons revealed these to not be statistically significant (+0.876±0.443, p=.164 and +0.834±0.448, p=.209 respectively). Comparatively, fellow eye crowding distance ratios (Trigram: 1.2x and Single Line: 1.2x) were similar to isolated letter size threshold ratios (Sloan: 1.3x), with isolated Sloan letter size ratios demonstrating statistically significantly larger ratios than single line crowding distance ratios (+0.168±0.051, **p=.006**)(Figure 5.14).

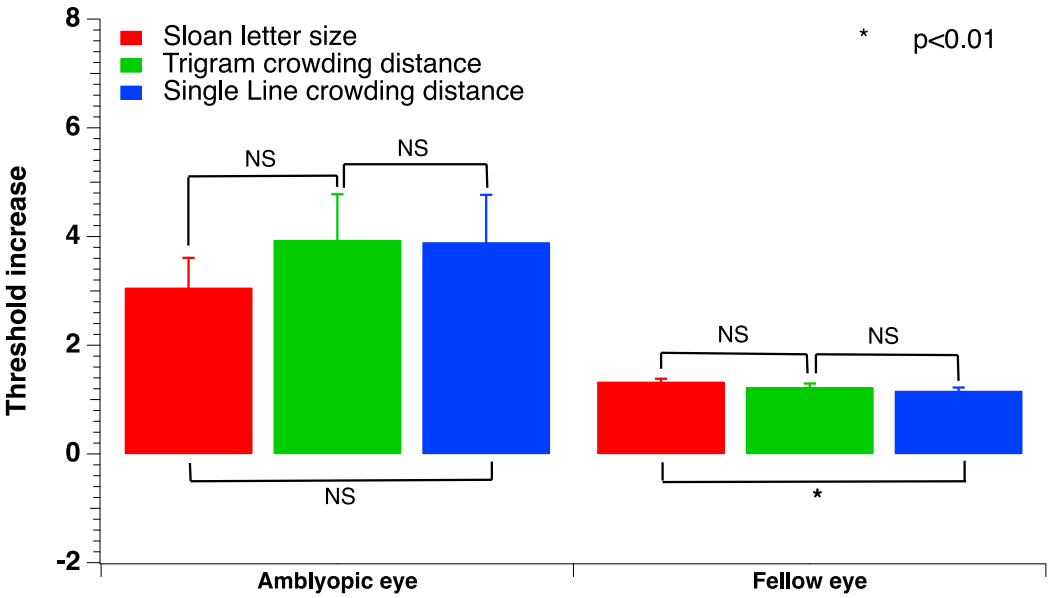


Figure 5.14: *Mean acuity and crowding distance threshold increases for isolated Sloan letters and each crowding distance test (Pelli Trigram, Pelli Single Line) for amblyopes, compared to controls.*

Separated into subgroups, in the amblyopic eye of strabismic/mixed amblyopes, crowding distance ratios (Trigram: 5.7x and Single Line: 5.6x) were significantly larger than isolated

letter size threshold ratios (Sloan: 3.9x, +1.834±0.607, **p=.013** and +1.732±0.619, **p=.023** respectively). Comparatively, fellow eye crowding distance ratios (Trigram: 1.2x and Single Line: 1.1x) were similar to isolated letter size threshold ratios (Sloan: 1.4x) although isolated Sloan letter size ratios demonstrated statistically significantly larger ratio's than single line crowding distance ratio's (+0.231±0.073, **p=.008**) (Figure 5.15).

In anisometropic amblyopes, amblyopic eye crowding distance ratios (Trigram: 2.2x and Single Line: 2.3x) were not significantly larger than isolated letter size threshold ratios (Sloan: 2.3x, +0.038±0.593, p=1.000 and +0.023±0.604, p=1.000 respectively). Fellow eye crowding distance ratios (Trigram: 1.3x and Single Line: 1.2x) were also not significantly larger than isolated letter size threshold ratios (Sloan: 1.3x, +0.050±0.069, p=1.000 and +0.108±0.071, p=.405 respectively) (Figure 5.15).

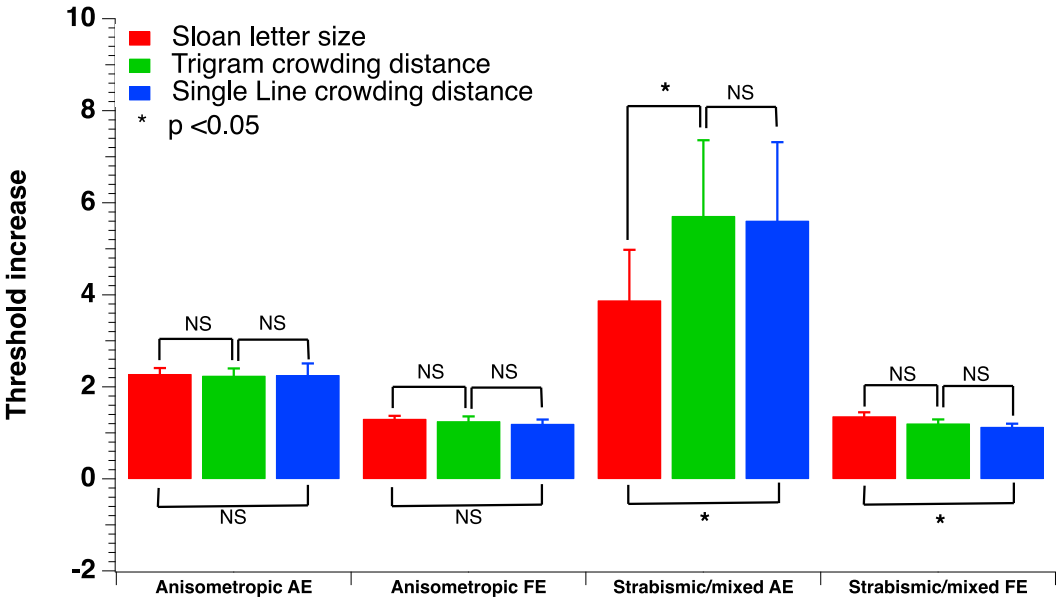


Figure 5.15: *Mean acuity and crowding distance threshold increases for isolated Sloan letters and each crowding distance test (Pelli Trigram, Pelli Single Line) per group (anisometropic and strabismic/mixed amblyopes), compared with controls.*

5.5.4 Acuity IODs vs Crowding IODs

Examination of isolated letter size and crowding distance interocular differences between controls, anisometropic amblyopes and strabismic/mixed amblyopes, were also calculated as ratios (amblyope eye threshold/control eye threshold) (Figure 5.16).

In strabismic/mixed amblyopes crowding distance IOD ratios (Trigram: 28.9x and Single Line: 20.0x) were larger than isolated letter size threshold IOD ratios (Sloan: 15.6x) although pairwise comparisons revealed that only Trigram crowding distance ratios were significantly larger (+13.340±3.917, **p=.004**). However, in anisometropic amblyopes crowding distance IOD ratios (Trigram: 6.3x and Single Line: 4.8x) were not significantly larger than isolated letter size threshold IOD ratios (Sloan: 6.2x, +0.120±3.827, p=1.000 and -1.404±2.396, p=1.000 respectively). When combined as a single group of amblyopes, crowding distance IOD ratios (Trigram: 17.3x and Single Line: 12.2x) were larger than isolated letter size IOD ratios (Sloan: 10.8x), although not significantly so (+6.576±2.890, p=.084 and +1.461±1.753, p=1.000 respectively) (Figure 5.16).

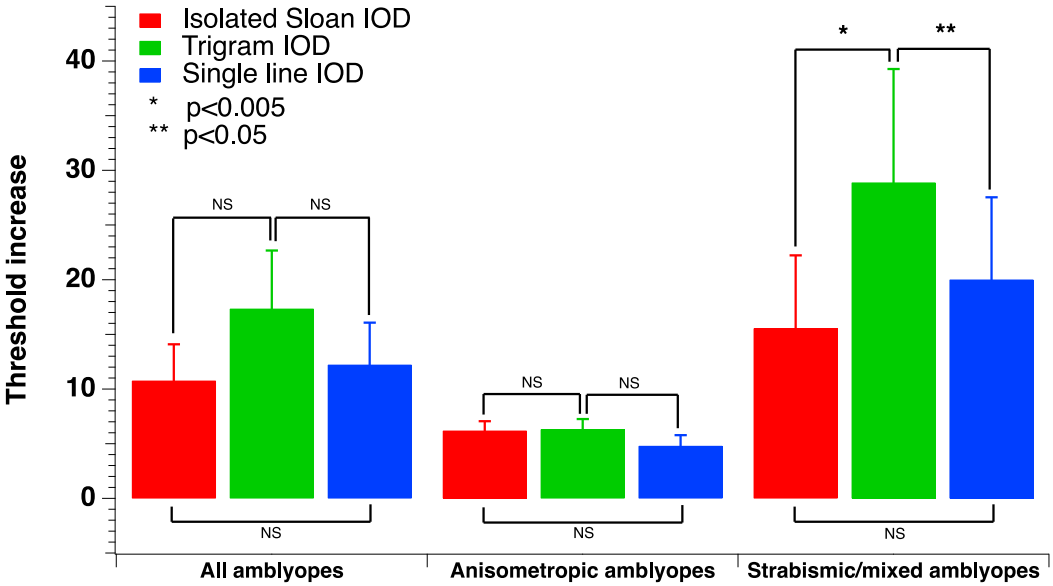


Figure 5.16: *Mean acuity and crowding distance IOD increases for isolated Sloan letters and each crowding distance test (Pelli Trigram, Pelli Single Line) per group (all amblyopes, anisometropic amblyopes and strabismic/mixed amblyopes), compared with controls.*

5.6 Discussion

5.6.1 Isolated acuity maturation

Previous psychophysical research has concluded that maturity of visual acuity occurs between five and nine years of age, depending upon the test choice and methodology used (Mayer and Dobson, 1982; Birch et al., 1983; Atkinson et al., 1988; Ellemberg et al., 1999; Drover et al., 2008). Notwithstanding having a smaller cohort, the results of this study concur with the visual acuity findings of Waugh et al. (2018). Furthermore, this reproducibility adds confidence to this data, further validating the maturation of isolated Sloan visual acuity by age six.

5.6.2 Examining crowding distance in isolation from acuity

While crowded vision tests may be helpful for the detection of crowding limited conditions (such as amblyopia), the ability to independently quantify acuity and crowding distance is of significant clinical importance. Additional independent examination of crowding distance within the fovea would allow clinical differentiation between visual acuity deficits and/or crowding deficits, as poorer function in one area is not intrinsically linked to the other (Song, Levi and Pelli, 2014). Quantifying crowding via comparison of vision tests of varying inter-ocular spacings and flanking features is confusing and may lead to patient fatigue from repeated testing.

To clearly understand an individual's visual perception, acuity and crowding would ideally be assessed separately. Isolated quantification of foveal crowding without interference from other spatially limited phenomena, such as lateral masking, is of considerable importance (Pelli et al., 2016; Waugh et al., 2018); however, previous studies have utilised the term 'crowding' to represent the presence of all deleterious spatial interactions (Atkinson et al., 1988; Vision in Preschoolers (VIP) Study Group, 2003; Saul and Taylor, 2012; Lalor, Formankiewicz and Waugh, 2016; Sailoganathan et al., 2018). The "Pelli" optotype offers

such an ability in central vision due to its narrower horizontal profile (Pelli et al., 2016) and a spacing-to-size ratio of 1.4:1 to prevent additional interference from lateral masking (Song, Levi and Pelli, 2014; Pelli et al., 2016). Clinical use of this optotype would allow for swift establishment of spatial crowding thresholds disentangled from acuity thresholds.

5.6.3 Repeated Optotypes

Repeated optotypes have been shown to be beneficial in eyes where acuity thresholds are limited by gaze control inaccuracies (Kothe and Regan, 1990), a known component of crowding, along with attention (Flom, Weymouth and Kahneman, 1963; Jacobs, 1979). This motor-control mitigating test format allows for greater accuracy of crowding distance threshold assessment without the detrimental effects of poor gaze control (Kothe and Regan, 1990; Regan et al., 1992; Waugh et al., 2018). New clinical acuity tests, such as the recently designed Milan Eye Chart (MEC), have demonstrated the potential for the repeated assessment within visual acuity thresholds (Facchin et al., 2019). A total of 12 eye charts were produced for the MEC, with four different interocular spacings (measured in optotype widths edge-to-edge; 100%, 50%, 25% and 12.5%), and three versions of each spacing were available, utilising different letters respectively. This repeated-testing format allowed for examination of the point at which interocular distance begins to have a deleterious effect on the participants' visual acuity within a single test design. While Facchin et al. (2019) demonstrated the feasibility of the MEC as a clinical assessment tool for the examination of spatial crowding effects, the MEC continues to combine the assessment of the two distinct visual functions of acuity and crowding and has significant disadvantages; namely repeated testing which may become tedious to a younger child, leading to loss of test accuracy (Haine et al., 2021).

Repeated optotype examination of crowding distance (in isolation from acuity) has shown to be beneficial in adults with amblyopia, who demonstrated smaller crowding distances

with a repeated full-screen format compared with a trigram arrangement (Waugh et al., 2018). However, while repeated optotypes showed benefits in amblyopic adults, preliminary examination in visually healthy children with the full-screen repeated presentation demonstrated increased test reluctance evidenced by significantly increased response variability. Simplifying the repeated format from a full screen to a single line improved response variability while maintaining comparable crowding distance thresholds (Haine et al., 2021).

There was no significant difference in crowding distance when the repeated single line was flanked vertically, although response variability was lowest with this format. This can likely be attributed to greater crowding magnitude within the horizontal plane compared with the vertical, which is seen in individuals who are educated to read horizontally, and occurs as a result of increased feature integration and attentional mechanisms pooling horizontal targets and flankers into single units (Feng, Jiang and He, 2007; Kwon, Legge and Dubbels, 2007). However, while this explains the visual behaviour of the majority of this cohort, the younger participants (age three) are likely preliterate. The effect of literacy on crowding distance is currently unknown. In the pilot study, three to four-year-old crowding distances were significantly larger than all other age groups, and it is possible that this is an educational effect. Within the U.K, formal literacy training begins at age four to five years; therefore, all the participants aged five years and older were undergoing formal schooling and learning to read. However, the same cannot be said for all the three to four-year-old pilot participants. This theory can be indicated by studies that show a correlation between decreasing crowding distance and improving reading speeds (Kwon, Legge and Dubbels, 2007; Pelli and Tillman, 2008).

Further examination of literacy has also revealed that younger individuals (four years) demonstrate significant errors in horizontal letter orientation position ($p<.01$), which decreases with age (Cairns and Steward, 1970); and decreasing parafoveal crowding in

horizontal strings of characters (Vejnović and Zdravković, 2015). Interestingly, these changes are not replicated within the vertical plane, adding credence to the theory that this improvement in crowding may occur as part of horizontal literacy education (Vejnović and Zdravković, 2015). Although it is currently unknown whether these effects occur by correlation or causation, Tydgat and Grainger (2009) have hypothesised that throughout reading skill acquisition, decreases in crowding distance may occur due to reductions in the receptive field sizes of letter detectors. Further research with age-matched children is required to determine whether formal reading literacy drives reductions in foveal crowding distances or whether this is a physiological development that coincidentally correlates with formal education.

A future study comparing the crowding distances of age-matched literate and non-literate children would help establish the influence of learned literacy in the improvement and development of foveal crowding distances.

5.6.4 Crowding distance thresholds in amblyopic children

Foveal crowding distances for child amblyopes using the Pelli optotype were established for the first time within this study. Crowding distances established using the trigram and single-line formats were 5.7 and 5.6 times larger (respectively) in the amblyopic eyes of strabismic/mixed amblyopes compared with control eyes, while acuity thresholds were only 3.9 times larger. This finding supports previous work that has suggested that the amblyopic eyes of strabismic/mixed amblyopes are limited by crowding distance, like that experienced in the normal peripheral visual field (Formankiewicz and Waugh, 2013). In comparison, anisometropic amblyopic eyes demonstrated similar threshold increases for both crowding distance and acuity. This supports previous studies that find that anisometropic eyes are not limited by the extent of crowding but can be modelled on the effects of retinal blur (Formankiewicz and Waugh, 2013). Therefore, the assessment of foveal crowding distance

demonstrates no additional benefit to the anisometropic amblyopes in this cohort over isolated acuity assessment. Furthermore, examination of the threshold elevations in fellow eyes of both groups, relative to the control group, demonstrated only minimal increases in thresholds for both isolated acuity and crowding distance (Figure 5.15).

Unlike the adult amblyope findings of Waugh et al. (2018), the repeated single-line format did not improve measures of foveal crowding distance in strabismic/mixed paediatric amblyopes, despite pilot study results demonstrating decreased responses variability (see 5.2.5.2). Poor executive function of attention could explain this finding, as poorer visual attention has been demonstrated both in visually children (Wei and Ma, 2017) and paediatric amblyopes (Black et al., 2021), with the executive function of attention increasing with age (Wei and Ma, 2016).

5.6.5 Crowding distance estimates (centre-to-centre spacing) present within a crowded acuity test (SLT) compared with the Pelli test

Calculations of centre-to-centre spacing experienced at the acuity threshold estimated with the SLT were also compared with crowding distances established with Pelli optotypes (Table 5.11), as it is considered that optotypes such as those in the SLT are too wide to assess foveal crowding distances adequately. The results seen here support that finding and demonstrate that the SLT centre-to-centre spacing experienced by both eyes of controls and anisometropic amblyopes, along with the fellow eyes of strabismic/mixed amblyopes, were larger than the crowding distances measured with the Pelli optotype (trigram and repeated format), and therefore do not provide optimal crowding. For the crowding limited strabismic/mixed amblyopic eyes, the SLT crowding distances were narrower than the Pelli crowding distances (trigram and repeated).

Table 5.11: *Comparison of centre-to-centre spacing experienced by participants at acuity threshold using the SLT and foveal crowding distances with the Pelli optotype.*

	SLT	**Pelli Trigram**	**Pelli Repeated single-line format**
Control RE	0.143±0.006	0.088±0.008	0.096±0.011
Control LE	0.142±0.006	0.088±0.008	0.103±0.004
Anisometropic AE	0.270±0.016	0.197±0.014	0.225±0.119
Anisometropic FE	0.158±0.009	0.110±0.009	0.120±0.009
Strabismic/mixed AE	***0.445±0.067***	***0.501±0.144***	***0.561±0.122***
Strabismic/mixed FE	0.161±0.007	0.106±0.008	0.113±0.007

5.6.6 Crowding distance IODs in amblyopic children

A diagnosis of amblyopia is currently contingent on finding a difference in acuity between the two eyes or interocular difference. For the first time, comparisons of crowding distance IODs in paediatric amblyopes using the Pelli optotypes are presented here. Foveal crowding distance IODs in paediatric amblyopes and controls are larger than isolated letter IODs. Disparities can be seen between anisometropic amblyopes and strabismic/mixed amblyopes. While crowding distance IODs are over six times larger in paediatric anisometropic amblyopes than in visually healthy children, assessment of crowding distance IODs in anisometropic amblyopes was just as effective as isolated letter IODs. In comparison, strabismic/mixed amblyopes demonstrated crowding distance IODs that were approximately 20 to 30x larger than those of visually healthy children, considerably larger than isolated acuity IOD assessment at 15x. When re-calculated as an IOD ratio (AE/FE), anisometropic amblyopes again show no significant difference in IOD between acuity and crowding, while amblyopes with strabismus show significantly larger IOD ratios for crowding compared with acuity [$F(1,64)=20.10$; **$p<.001$**] (Results presented at VSS 2022; Waugh et al, 2022 - in press, Appendix 12). These results suggest that assessment and subsequent

calculation of foveal crowding distance IOD is at least as effective as acuity IOD in the diagnosis and monitoring of amblyopia and, in the case of strabismic/mixed amblyopes, may even be superior.

5.6.7 Spacing/acuity ratios in paediatric amblyopes

Song, Levi and Pelli (2014) calculated crowding distance and acuity ratios (foveal crowding distance threshold (deg)/ isolated acuity threshold (deg)), demonstrating a double dissociation between crowding distance and acuity. Regarding amblyopia, both anisometropic and strabismic adult amblyopes showed reductions in visual acuity (>0.15 degrees letter size at threshold), while comparatively, crowding ratios were disparate between anisometropic amblyopes (<1.84) and strabismic amblyopes (>1.84). Taking the mean letter size (deg) and mean foveal crowding distance thresholds (deg) of each test group (controls, anisometropic amblyopes and strabismic/mixed amblyopes), spacing/acuity ratios were calculated for this paediatric cohort (Table 5.12). The values obtained support the S/A ratio findings of Song, Levi and Pelli (2014) and suggest that this double dissociation effect can also be extended to paediatric amblyopes, despite acuity and crowding distance developmental immaturity.

Table 5.12: *Acuity in degrees compared with spacing/acuity ratios and the categorisation of visual behaviours for paediatric controls and amblyopes. Table adapted from Song, Levi and Pelli (2014).*

		Spacing/acuity ratio	
		SA< 1.84	SA>1.84
Acuity (deg)	<0.15	Visually normal individuals (*A* = 0.067, *SA* = 1.408)	
	>0.15	Anisometropic amblyopia (***A* = 0.152**, *SA* = 1.389)	Strabismic/mixed amblyopia (***A* = 0.258, *SA* = 2.054**)

5.7 Conclusion

Foveal crowding distances for anisometropic and strabismic/mixed paediatric amblyopes using the Pelli optotype are examined and presented here for the first time. The amblyopic eyes of strabismic/mixed amblyopes demonstrate significantly larger crowding distances than control eyes and anisometropic amblyopic eyes, along with increased spacing/acuity ratios. These results support previous evidence of a double dissociation between acuity and crowding distance in adults and now demonstrate the same effects in amblyopic children.

Future studies are required to establish the role of literacy skills in the maturity of foveal crowding distances, as existing foveal crowding distances and maturity rates have been established with British children, for whom school begins the September after their fourth birthday. Children in other countries or those who are home-schooled may begin their literacy education earlier or later, which may influence visual acuity and crowding distance maturity rates, perhaps to different extents.

Chapter Six – Final Conclusions and Future research considerations

Amblyopia is the most common aetiology of paediatric visual loss, second only to refractive error (Robaei et al., 2006; Webber, 2018). In the U.K, vision screening programmes are used to identify paediatric amblyopes between four and five years of age. LogMAR based crowded visual acuity tests are recommended for these vision screening programmes as they are thought to incorporate crowding, to which some amblyopes demonstrate increased sensitivity. More substantial spatial interference effects have been shown to occur in visually healthy adults and children with a Cambridge crowding arrangement and inter-optotype spacing of one stroke width. In both luminance and contrast-modulated presentation, this optimised test format was examined in paediatric amblyopes to establish whether this enhanced test arrangement offered additional benefits for the detection of amblyopia.

6.1 Optimally crowded acuity tests

The L-ECC test arrangement increased measured crowding magnitude compared to the SLT by over 50% in strabismic and mixed strabismic-anisometropic amblyopes, 30% larger than the crowding magnitude increase seen in control eyes (21%). Elevated crowding magnitudes were also seen in anisometropic amblyopes, although this affected the fellow eyes of anisometropes (52%) more than the amblyopic eye (33%). These differences in acuity response to optimally crowded stimuli reflect essential distinctions between these common amblyopic subtypes. The differing influence of crowding magnitude on each eye affects the IOD measured. This is important, as IOD is used to determine the presence of amblyopia, the severity, and the monitoring of treatment progression. While strabismic and mixed amblyopes see an increase in IOD with optimally crowded acuity tests, thereby highlighting the presence of amblyopia, anisometropic amblyopes demonstrate decreased

IODs, owing to the more significant crowding effects measured in the fellow eye. In anisometropic amblyopes, optimally crowded visual acuity tests such as the L-ECC may reduce sensitivity to the detection and monitoring of amblyopia. Therefore, utilising optimally crowded acuity tests for vision screening would increase sensitivity to strabismic and mixed amblyopes but may decrease sensitivity to the presence of anisometropic amblyopia.

Clinically, the interocular difference in crowding magnitude could be used as a differential diagnostic tool to aid the categorisation of amblyopic subtypes. For example, in the presence of an IOD $\geq$0.100 logMAR, a greater crowding magnitude seen in the amblyopic eye / a positive crowding magnitude IOD could indicate the presence of strabismus. In contrast, a greater magnitude seen in the fellow eye / a negative crowding magnitude IOD could indicate anisometropic amblyopia. In a screening clinic where the visual screener may only have access to the results of a vision test, this differential diagnostic test may aid in the streamlining of referrals by directing identified anisometropic amblyopes to an Optometrist of their choice who could potentially perform a refraction and prescribe necessary refractive correction, within days of the original screening test. This would both expedite treatment for these anisometropic amblyopes and reduce the referral load to hospital eye services. In turn, this could benefit identified strabismic/mixed amblyopes due to potentially reduced referral waiting times.

While contrast-modulated stimuli have been shown to demonstrate greater contour interaction and crowding effects than LM stimuli in amblyopic adults (Chung, Li and Levi, 2007; Hairol, Formankiewicz and Waugh, 2013), this effect has not been examined in paediatric amblyopes. Therefore, this study examined the effect of contrast-modulated, optimally crowded optotypes in paediatric amblyopes and discovered that the more substantial crowding effects experienced using the L-ECC (compared with SLT) were not found by presentation using contrast-modulation (CM-ECC). Additionally, threshold

elevations due to CM presentation were smaller for crowded targets (i.e., crowded CM threshold minus crowded L threshold) than for isolated targets (isolated CM threshold minus isolated L threshold). This may happen due to interocular transfer effects (transfer of perceptual effects from one eye to the other (McColl and Mitchell, 1998; Wyatt, Clifford and Wenderoth, 2001)), with CM processing occurring within higher, more binocularly driven, cortical areas such as V2. Consequently, no significant difference in IOD was seen between the existing SLT format, CM-Isolated and the CM-ECC (p=.306). Therefore, as a paediatric visual screening tool, the CM-ECC provides no additional diagnostic benefit in identifying IOD over the existing SLT.

6.2 Crowding distance tests

Crowding distance develops more rapidly than isolated acuity and, therefore, may be a more sensitive measure for detecting and monitoring amblyopes (Waugh et al., 2018). The current study confirmed that a simpler repeated optotype format enhanced cooperation for assessing the critical spacing of crowding in amblyopic children using the Pelli optotypes. Foveal crowding distance IODs were superior to isolated acuity IODs for detecting amblyopia, particularly for strabismic and mixed amblyopes who demonstrated crowding distance IODs of 20 to 30 times larger than those of control children. As per Song, Levi and Pelli (2014), anisometropic amblyopes demonstrated deficits of acuity but not crowding, and acuity thresholds and spacing/acuity ratios established in adults also apply to paediatric patients. This has important implications for clinical use as the Pelli font clinical test replicates the results of lab-based findings of the double dissociation of crowding and acuity, even in younger clinical participants. This quick clinical test could therefore be used not only to help identify strabismic/mixed amblyopes, but also help to quickly identify other crowding limited conditions such as apperceptive agnosia (Song, Levi and Pelli., 2014).

6.3 Study limitations

While these new findings take the understanding of amblyopia and visual crowding further, the limitations of any study must, of course, be considered. One known factor considered during the study design is children's limited attention span and concentration, which could negatively impact the results of tests conducted later in the experimental session and could influence conclusions drawn about that test. In this study, any effects of fatigue or attention were averaged out across tests by randomising the order of test examination (choosing one of 12 numbers out of a bag). A second known factor was that the poorer vision experienced by the amblyopic eye in the amblyopic test groups might also influence attention span and concentration. While some participants may prefer to be examined with their amblyopic eye first to 'get it over with', others may prefer to be examined with their fellow eye first to familiarise themselves with the test. These effects were again minimised by randomising the order of eye examined according to a minimisation procedure.

Sometimes desirable information/data, such as when the patient was first prescribed glasses and their prescription at that time, was missing from the patient files. This occurred, for example, if the patient had been transferred from another hospital or started their management pathway, pre-digitisation of patient records. Examination of filed referral letters, handwritten notes digitisation summaries, and discussions with parents/guardians were used to help complete any gaps in historical data, where possible. Information such as the presence of bifoveal fixation was not always available or had not been clinically examined in some patients. Therefore, small microtropia's may have been miscategorised as anisometropic amblyopes. It would have been helpful to confirm or refute the presence of bifoveal fixation using examination with either a four-dioptre test or a visuoscope prior to examination with the experimental spatial tests (L-ECC, CM-ECC and Pelli optotypes).

While not a study design limitation, it must be noted that at the beginning of 2020, an international outbreak of COVID-19 instigated a series of national lockdowns, which forced the halting of recruitment and testing. Following re-approval and re-commencement of recruitment and testing, fewer new referrals were available for recruitment as vision screening programmes had not been operational, and hospital clinical capacity was temporarily reduced. Consequently, the study utilised more pre-existing amblyopic patients from Addenbrookes' existing caseload. However, it would have been preferable to have examined more amblyopes from the point of referral and initial diagnosis, as this may have resulted in a greater number of moderate and severe amblyopes.

6.4 Future research

Further investigation into the clinical use of interocular crowding magnitude (using isolated and optimally crowded optotypes) for the differential diagnosis of amblyopia subtypes looks promising. Within this study, examination of crowding magnitude IODs and consequent prediction of amblyopic subtype based on whether positive or negative values of crowding magnitude IOD were achieved (as described in chapter three), would have correctly predicted amblyopic categories (as defined in this study) in 18/22 anisometropic amblyopes and 18/22 strabismic/mixed amblyopes (82% respectively). A longitudinal study examining the crowding magnitude IODs of visual screening referrals from their first hospital appointment would help establish the possibility of this test as a clinical diagnostic tool for amblyopic subtypes. Further study into effect of reduced inter-optotype spacing on microtropias specifically would also be advantageous, as microtropias were not specifically identified and examined in this study.

Regarding CM-optotypes, future examination of paediatric amblyopes using LM optotypes will help establish how much the threshold elevation seen using CM-optotypes is due to

internal noise; and whether differences in noise are seen between different amblyopic aetiologies in paediatric patients.

While it has been established that crowding distance has a steeper rate of development compared with isolated acuity (Waugh et al., 2018), it is still uncertain how much of this development is 'nature' (physiological) and how much is 'nurture'. Within this study, the crowding distances of visually healthy 3–4-year-olds were significantly larger than 5–7-year-olds and 8–11-year-olds. Literacy education begins in the first year of U.K. schools (reception class), during the academic year the child turns five. As such, U.K. children are four to five years old when they start school and begin to learn to read. This literacy development may partially account for the significant difference seen between the 3–4-year-olds and 5–7-year-olds; however, this has not yet been examined. Consideration for this arises from the observation of a control participant recruited to this study, who was later excluded when the parent revealed the child had high hypermetropia, which was monitored under an orthoptic clinic but was never diagnosed as amblyopic. The child's SLT visual acuity thresholds were normal for her age, but her crowding distances were more extensive than expected. While this could reflect a now resolved bilateral amblyopia, it was interesting to note that this child was also home-schooled and had not yet begun to learn to read formally. Therefore, how much of our crowding distance development is physiologically driven and how much is experience-driven is still yet to be established.

Finally, the COVID-19 pandemic will likely have negatively impacted referrals of amblyopia. School-based vision screening programmes suspended due to school closures (Solebo and Rahi, 2013; Solebo, Cumberland and Rahi, 2015; BIOS, 2020) combined with reduced outpatient clinics, a subsequent clinical backlog as well as risk-avoidant behaviour reducing clinic attendance (Gardner, Fraser and Peytrignet, 2020) have all contributed to a potential generation of undiagnosed amblyopes. Future research is urgently required to establish

the severity of the impact of the COVID-19 pandemic on amblyopia referral and diagnosis rates.

References

Abrahamsson, M. and Sjostrand, J., 1988. Contrast sensitivity and acuity relationship in strabismic and anisometropic amblyopia. *British Journal of Ophthalmology*, **72**(1), pp.44–49.

Allen, H.F., 1957. A new picture series for preschool vision testing. *American Journal of Ophthalmology*, **44**(1), pp.38–41.

Almoqbel, F.M., Irving, E.L. and Leat, S.J., 2017. Visual acuity and contrast sensitivity development in children: Sweep visually evoked potential and psychophysics. *Optometry and Vision Science*, **94**(8), pp.830–837.

Anstice, N.S., Jacobs, R.J., Simkin, S.K., Thomson, M., Thompson, B. and Collins, A. V., 2017. Do picture-based charts overestimate visual acuity? Comparison of kay pictures, lea symbols, HOTV and Keeler logMAR charts with Sloan letters in adults and children. *PLoS ONE*, **12**(2), pp.1–17.

Anstice, N.S. and Thompson, B., 2014. The measurement of visual acuity in children: An evidence-based update. *Clinical and Experimental Optometry*, **97**(1), pp.3–11.

Armstrong, R.A., 2013. Statistical guidelines for the analysis of data obtained from one or both eyes. *Ophthalmic and Physiological Optics*, **33**(1), pp.7–14.

Ashida, H., Lingnau, A., Wall, M.B. and Smith, A.T., 2007. FMRI adaptation reveals separate mechanisms for first-order and second-order motion. *Journal of neurophysiology*, **97**(2), pp.1319–1325.

Ateiza, A. and Davis, H., 2019. The Effects of Anisometropic Amblyopia on the FNS and TNO Stereotest Thresholds in Four- to Eight-Year-Olds. *The British and Irish orthoptic journal*, **15**(1), pp.72–81.

Atkinson, J., Anker, S., Evans, C., Hall, R. and Pimm-Smith, E., 1988. Visual acuity testing of young children with the Cambridge Crowding Cards at 3 and 6 m. *Acta Ophthalmologica*, **66**(5), pp.505–508.

Atkinson, J. and Braddick, O., 1983. Assessment of Visual Acuity in Infancy and Early Childhood. *Acta Ophthalmologica*, **61**(157 S), pp.18–26.

Atkinson, J., Pimm-Smith, E., Evans, C., Harding, G. and Braddick, O., 1986. Visual Crowding in Young Children. In: *Documenta Ophthalmologica Proceedings Series*. Dordrecht: Springer, pp.201–213.

Attebo, K., Mitchell, P., Cumming, R., Smith, W., Jolly, N. and Sparkes, R., 1998. Prevalence and causes of amblyopia in an adult population. *Ophthalmology*, **105**(1), pp.154–159.

Awan, M., Proudlock, F.A. and Gottlob, I., 2005. A randomized controlled trial of unilateral strabismic and mixed amblyopia using occlusion dose monitors to record compliance. *Investigative Ophthalmology and Visual Science*, **46**(4), pp.1435–1439.

Bach, M., 1996. The Freiburg Visual Acuity Test - Automatic Measurement of Visual Acuity. *Optometry and Vision Science*, **73**(1), pp.49–53.

Bailey, I.L. and Lovie, J.E., 1976. New design principles for visual acuity letter charts. *Optometry and Vision Science*, **53**(11), pp.740–745.

Baker, C. and Mareschal, I., 2001. Processing of second-order stimuli in the visual cortex. *Progress in brain research*, **134**, pp.171–191.

Baker, C.L., 1999. Central neural mechanisms for detecting second-order motion. *Current Opinion in Neurobiology*, **9**(4), pp.461–466.

Baker, C.L., Boulton, J.C. and Mullen, K.T., 1998. A nonlinear chromatic motion mechanism. *Vision Research*, **38**(2), pp.291–302.

Banks, M.S., 1980. The Development of Visual Accommodation during Early Infancy. *Child development*, **51**(3), pp.646–666.

Barnes, G.R., Hess, R.F., Dumoulin, S.O., Achtman, R.L. and Pike, G.B., 2001. The cortical deficit in humans with strabismic amblyopia. *Journal of Physiology*, **533**(1), pp.281–297.

Barrett, B.T., Bradley, A. and Candy, T.R., 2013. The relationship between anisometropia and amblyopia. *Progress in Retinal and Eye Research*, **36**, pp.120–158.

Beardsell, R., Clarke, S. and Hill, M., 1999. Outcome of Occlusion Treatment for Amblyopia. *Journal of Pediatric Ophthalmology and Strabismus*, **36**(1), pp.19–24.

Bedell, H.E., Siderov, J., Waugh, S.J., Zemanová, R., Pluháček, F. and Musilová, L., 2013. Contour interaction for foveal acuity targets at different luminances. *Vision Research*, **89**, pp.90–95.

Beh, S.C., Muthusamy, B., Calabresi, P., Hart, J., Zee, D., Patel, V. and Frohman, E., 2015. Hiding in plain sight: A closer look at posterior cortical atrophy. *Practical Neurology*, **15**(1), pp.5–13.

Bennett, A.G., 1965. Ophthalmic test types. A review of previous work and discussions on some controversial questions. *The British journal of physiological optics*, **22**(4), pp.238–271.

Benton, C.P., Johnston, A. and McOwan, P.W., 2000. Computational modelling of interleaved first- and second-order motion sequences and translating 3f+4f beat patterns. *Vision Research*, **40**(9), pp.1135–1142.

Berardi, N., Pizzorusso, T. and Maffei, L., 2000. Critical periods during sensory development. *Current Opinion in Neurobiology*, **10**(1), pp.138–145.

Bernard, J.B. and Chung, S.T.L., 2011. The dependence of crowding on flanker complexity and target-flanker similarity. *Journal of Vision*, **11**(8), pp.1–1.

Bertone, A., Hanck, J., Guy, J. and Cornish, K.M., 2010. The development of luminance- and texture-defined form perception during the school-aged years. *Neuropsychologia*, **48**(10), pp.3080–3085.

Bi, T., Cai, P., Zhou, T. and Fang, F., 2009. The effect of crowding on orientation-selective adaptation in human early visual cortex. *Journal of Vision*, **9**(11), pp.13–13.

BIOS, 2020. Vision screening provision in children aged 4-5 years in England. Findings from a Freedom of Information Request 2019.

Birch, E.E., 2013. Amblyopia and binocular vision. *Progress in Retinal and Eye Research*, **33**(1), pp.67–84.

Birch, E.E., Castañeda, Y.S., Cheng-Patel, C.S., Morale, S.E., Kelly, K.R., Beauchamp,

C.L. and Webber, A., 2019. Self-perception in Children Aged 3 to 7 Years with Amblyopia and Its Association with Deficits in Vision and Fine Motor Skills. *JAMA Ophthalmology*, **137**(5), pp.499–506.

Birch, E.E., Gwiazda, J., Bauer, J.A., Naegele, J. and Held, R., 1983. Visual acuity and its meridional variations in children aged 7-60 months. *Vision Research*, **23**(10), pp.1019–1024.

Birch, E.E. and Holmes, J.M., 2010. The clinical profile of amblyopia in children younger than 3 years of age. *Journal of AAPOS*, **14**(6), pp.494–497.

Birch, E.E., Jost, R.M., Gilmore, J.F., De La Cruz, A. and Kelly, K.R., 2015. Reading rate and Scantron completion time in children with amblyopia. *Journal of American Association for Pediatric Ophthalmology and Strabismus*, **19**(4), p.e10.

Birch, E.E. and Kelly, K.R., 2017. Pediatric ophthalmology and childhood reading difficulties. *Journal of AAPOS*, **21**, pp.442–444.

Birch, E.E. and Swanson, W.H., 2000. Hyperacuity deficits in anisometropic and strabismic amblyopes with known ages of onset. *Vision Research*, **40**(9), pp.1035–1040.

Black, A.A., Wood, J.M., Hoang, S., Thomas, E. and Webber, A.L., 2021. Impact of Amblyopia on Visual Attention and Visual Search in Children. *Investigative Ophthalmology & Visual Science*, **62**(4), pp.15–15.

Blakemore, C. and Vital-Durand, F., 1992. Abstracts: Contrasts in Vision 1991. *Ophthalmic and Physiological Optics*, **12**(1), pp.81–102.

Bolger, P.G., Stewart-Brown, S.L., Newcombe, E. and Starbuck, A., 1991. Vision screening in preschool children: Comparison of orthoptists and clinical medical officers as primary screeners. *British Medical Journal*, **303**(6813), pp.1291–1294.

Bondarko, V.M. and Semenov, L.A., 2005. Visual acuity and the crowding effect in 8- to 17-year-old schoolchildren. *Human Physiology*, **31**(5), pp.532–538.

Bonneh, Y.S., Sagi, D. and Polat, U., 2004. Local and non-local deficits in amblyopia: Acuity and spatial interactions. *Vision Research*, **44**(27), pp.3099–3110.

Bonneh, Y.S., Sagi, D. and Polat, U., 2007. Spatial and temporal crowding in amblyopia.

Vision Research, **47**(14), pp.1950–1962.

Bouma, H., 1970. Interaction effects in parafoveal letter recognition. *Nature*, **226**(5241), pp.177–8.

Bradfield, Y.S., 2013. Identification and treatment of amblyopia. *American Family Physician*, **87**(5), pp.348–352.

Bradley, A. and Freeman, R.D., 1981. Conrast sensitivity in anisometropic amblyopia. *Investigative Ophthalmology & Visual Science*, **21**(3), pp.467–476.

British Standards Institution, 2003. BS 4274-1:2003. Visual acuity test types - Part 1: Test charts for clinical determination of distance visual acuity - Specification.

Bruce, A., Fairley, L., Chambers, B., Wright, J. and Sheldon, T.A., 2016. Impact of visual acuity on developing literacy at age 4-5 years: A cohort-nested cross-sectional study. *BMJ Open*, **6**(2), pp.1–8.

Bulakowski, P.F., Post, R.B. and Whitney, D., 2011. Reexamining the possible benefits of visual crowding: dissociating crowding from ensemble percepts. *Attention, perception & psychophysics*, **73**(4), pp.1003–9.

Bullier, J., 2001. Integrated model of visual processing. *Brain Research Reviews*, **36**(2–3), pp.96–107.

Burton, R.G., 1959. What Is Visual Acuity? *The Australian Journal of Optometry*, **42**(10), pp.430–432.

Cairns, N.U. and Steward, M.S., 1970. Young Children's Orientation of Letters as a Function of Axis of Symmetry and Stimulus Alignment. *Child development*, **41**(4), pp.993–1002.

Calvert, J., Manahilov, V., Simpson, W.A. and Parker, D.M., 2005. Human cortical responses to contrast modulations of visual noise. *Vision Research*, **45**(17), pp.2218–2230.

Campbell, F.W. and Green, D.G., 1965. Optical and retinal factors affecting visual resolution. *The Journal of Physiology*, **181**(3), pp.576–593.

Candy, R.T., Mishoulam, S.R., Nosofsky, R.M. and Dobson, V., 2011. Adult discrimination performance for pediatric acuity test optotypes. *Investigative Ophthalmology and*

Visual Science, **52**(7), pp.4307–4313.

Carkeet, A., Levi, D.M. and Manny, R.E., 1997. Development of Vernier acuity in childhood. *Optometry and Vision Science*, **74**(9), pp.741–750.

Carlton, J., Karnon, J., Czoski-Murray, C., Smith, K. and Marr, J., 2008. The clinical effectiveness and cost-effectiveness of screening programmes for amblyopia and strabismus in children up to the age of 4-5 years: a systematic review and economic evaluation. *Health Technology Assessment*, **12**(25), pp.iii, xi–194.

Catford, G. V and Oliver, A., 1973. Development of visual acuity. *Archives of disease in childhood*, **48**(1), pp.47–50.

Cavanagh, P. and Mather, G., 1989. Motion: The long and short of it. *Spatial Vision*, **4**(2–3), pp.103–129.

Cem Mocan, M., Najera-Covarrubias, M. and Wright, K.W., 2005. Comparison of visual acuity levels in pediatric patients with amblyopia using Wright figures©, Allen optotypes, and Snellen letters. *Journal of AAPOS*, **9**(1), pp.48–52.

Chakravarthi, R. and Cavanagh, P., 2007. Temporal properties of the polarity advantage effect in crowding. *Journal of Vision*, **7**(2), p.11.

Chakravarthi, R. and Cavanagh, P., 2009. Recovery of a crowded object by masking the flankers: determining the locus of feature integration. *Journal of vision*, **9**(10), pp.4.1-9.

Chakravarthi, R. and Herbert, A., 2019. Two's company, three's a crowd: Individuation is necessary for object recognition. *Cognition*, **184**, pp.69–82.

Chambers, L. and Wolford, G., 1983. Lateral masking vertically and horizontally. *Bulletin of the Psychonomic Society*, **21**(6), pp.459–461.

Chanceaux, M., Mathôt, S. and Grainger, J., 2014. Effects of number, complexity, and familiarity of flankers on crowded letter identification. *Journal of Vision*, **14**(6), pp.1–12.

Chandna, A., Pennefather, P.M., Kovács, I. and Norcia, A.M., 2001. Contour integration deficits in anisometropic amblyopia. *Investigative Ophthalmology and Visual Science*, **42**(3), pp.875–878.

Chia, A., Dirani, M., Chan, Y.H., Gazzard, G., Au Eong, K.G., Selvaraj, P., Ling, Y., Quah, B.L., Young, T.L., Mitchell, P., Varma, R., Wong, T.Y. and Saw, S.M., 2010. Prevalence of amblyopia and strabismus in young singaporean chinese children. *Investigative Ophthalmology and Visual Science*, **51**(7), pp.3411–3417.

Chima, A.S., Formankiewicz, M.A. and Waugh, S.J., 2015. Investigation of interocular blur suppression using luminance-modulated and contrast-modulated noise stimuli. *Journal of Vision*, **15**(3), p.22.

Chou, R., Dana, T. and Bougatsos, C., 2011. Screening for visual impairment in children ages 1-5 years: Update for the USPSTF. *Pediatrics*, **127**(2), pp.e442–e479.

Chubb, C., Olzak, L. and Derrington, A., 2001. Second-order processes in vision: introduction. *Journal of the Optical Society of America A*, **18**(9), p.2175.

Chubb, C. and Sperling, G., 1988. Drift-balanced random stimuli: a general basis for studying non-Fourier motion perception. *Journal of the Optical Society of America A*, **5**(11), p.1986.

Chung, S.T.L., 2002. The effect of letter spacing on reading speed in central and peripheral vision. *Investigative Ophthalmology and Visual Science*, **43**, pp.1270–1276.

Chung, S.T.L., 2012. Dependence of reading speed on letter spacing in central vision loss. *Optometry and Vision Science*, **89**(9), pp.1288–1298.

Chung, S.T.L., 2016. Spatio-temporal properties of letter crowding. *Journal of Vision*, **16**(6), pp.1–20.

Chung, S.T.L., Li, R.W. and Levi, D.M., 2007. Crowding between first- and second-order letter stimuli in normal foveal and peripheral vision. *Journal of Vision*, **7**(2), pp.1–13.

Chung, S.T.L., Li, R.W. and Levi, D.M., 2008a. Crowding between first- and second-order letters in amblyopia. *Vision Research*, **48**(6), pp.788–798.

Chung, S.T.L., Li, R.W. and Levi, D.M., 2008b. Learning to identify near-threshold luminance-defined and contrast-defined letters in observers with amblyopia. *Vision research*, **48**(27), pp.2739–50.

Chung, S.T.L.L., Kumar, G., Li, R.W. and Levi, D.M., 2015. Characteristics of fixational eye

movements in amblyopia: Limitations on fixation stability and acuity? *Vision research*, **114**, pp.87–99.

Chung, S.T.L.L., Levi, D.M. and Legge, G.E., 2001. Spatial-frequency and contrast properties of crowding. *Vision Research*, **41**(14), pp.1833–1850.

Ciner, E., Cyert, L., Dobson, V., Kulp, M.T., Maguire, M., Moore, B., Orel-Bixler, D., Peskin, E., Quinn, G., Redford, M., Schmidt, P. and Schultz, J., 2003. Threshold visual acuity testing of preschool children using the crowded HOTV and Lea Symbols acuity tests. *Journal of AAPOS*, **7**(6), pp.396–399.

Ciuffreda, K.J., Levi, D.M. and Selenow, A., 1991. Amblyopia : basic and clinical aspects. Boston: Butterworth-Heinemann, p.507.

Clarke, M.P., Wright, C.M., Hrisos, S., Anderson, J.D., Henderson, J. and Richardson, S.R., 2003. Randomised controlled trial of treatment of unilateral visual impairment detected at preschool vision screening. *British Medical Journal*, **327**(7426), pp.1251–1254.

Clausen, M.M. and Arnold, R.W., 2007. Pediatric eye/vision screening: Referral criteria for the PediaVision PlusOptix S04 photoscreener compared to visual acuity & digital photoscreening: 'Kindergarten computer photoscreening'. *Binocular Vision and Strabismus Quarterly*, **22**(2), pp.83–89.

Cleary, M. and Reinecke, R.D., 2001. Efficacy of occlusion for strabismic amblyopia: Can an optimal duration be identified? *Evidence-Based Eye Care*, **2**(1), pp.46–47.

Coates, D.R., Chin, J.M. and Chung, S.T.L., 2013. Factors Affecting Crowded Acuity. *Optometry and Vision Science*, **90**(7), pp.628–638.

Coates, D.R., Levi, D.M., Touch, P. and Sabesan, R., 2018. Foveal Crowding Resolved. *Scientific Reports*, **8**(1), p.9177.

Consilium Ophthalmologicum Universale, 1984. Visual Acuity Measurement Standard. *Italian Journal of Ophthalmology*, **2**, pp.1–18.

Cornsweet, T.N., 1962. The Staircase-Method in Psychophysics. *The American Journal of Psychology*, **75**(3), p.485.

Corwin, T.R., Kintz, R.T. and Beaty, W.J., 1979. Computer-aided estimation of

psychophysical thresholds by Wetherill tracking. *Behavior Research Methods & Instrumentation*, **11**(5), pp.526–528.

Cotter, S.A., 2006. Treatment of Anisometropic Amblyopia in Children with Refractive Correction. *Ophthalmology*, **113**(6), pp.895–903.

Cotter, S.A., Cyert, L.A., Miller, J.M., Quinn, G.E., Russ, S.A., Block, S.S., Walker, M., Bergren, M.D., Bunner, R.T., Grason, H.A., Hartmann, E.E., Hughes, K.F., Hutchinson, A.K., Kemper, A.R., Leonard, S., Lyons, S.A., Marsh-Tootle, W.L., Mika, R., Moore, B.D., Pratt, N., Ramsey, J.E., Repka, M.X. and Wallace, D.K., 2015. Vision screening for children 36 to G72 Months: Recommended practices. *Optometry and Vision Science*, **92**(1), pp.6–16.

Crutch, S.J., 2014. Seeing why they cannot see: Understanding the syndrome and causes of posterior cortical atrophy. *Journal of Neuropsychology*, **8**(2), pp.157–170.

Crutch, S.J. and Warrington, E.K., 2007. Foveal crowding in posterior cortical atrophy: A specific early-visual-processing deficit affecting word reading. *Cognitive Neuropsychology*, **24**(8), pp.843–866.

Curcio, C.A., Sloan, K.R., Kalina, R.E. and Hendrickson, A.E., 1990. Human photoreceptor topography. *Journal of Comparative Neurology*, **292**(4), pp.497–523.

Cyert, L., 2004. Preschool visual acuity screening with HOTV and Lea symbols: Testability and between-test agreement. *Optometry and Vision Science*, **81**(9), pp.678–683.

Dakin, S.C., Bex, P.J., Cass, J.R. and Watt, R.J., 2009. Dissociable effects of attention and crowding on orientation averaging. *Journal of Vision*, **9**(11), pp.28–28.

Dakin, S.C., Cass, J., Greenwood, J.A. and Bex, P.J., 2010. Probabilistic, positional averaging predicts object-level crowding effects with letter-like stimuli. *Journal of Vision*, **10**(10), pp.14–14.

Danilova, M. V and Bondarko, V.M., 2007. Foveal contour interactions and crowding effects at the resolution limit of the visual system. *Journal of Vision*, **7**(2), p.25.

Daugman, J.G. and Downing, C.J., 1995. Demodulation, predictive coding, and spatial vision. *Journal of the Optical Society of America A*, **12**(4), p.641.

Davage, R.H. and Sumner, F.C., 1950. Isolation as a Factor in Lowering the Threshold of Visual Perception. *The Journal of Psychology*, **30**(1), pp.191–194.

Davidson, H.P., 1934. A Study of Reversals in Young Children. *Pedagogical Seminary and Journal of Genetic Psychology*, **45**(2), pp.452–465.

Davidson, H.P., 1935. A Study of the Confusing Letters B, D, P, and Q. *Pedagogical Seminary and Journal of Genetic Psychology*, **47**(2), pp.458–468.

Daw, N.W., 1998. Critical periods and amblyopia. *Archives of ophthalmology*, **116**(4), pp.502–505.

Daw, N.W., 2014. Visual development. *Visual Development*, Boston, MA: Springer US.

Derrington, A.M., Badcock, D.R. and Henning, G.B., 1993. Discriminating the direction of second-order motion at short stimulus durations. *Vision Research*, **33**(13), pp.1785–1794.

Dobson, V., Clifford-Donaldson, C.E., Miller, J.M., Garvey, K.A. and Harvey, E.M., 2009. A comparison of Lea Symbol vs ETDRS letter distance visual acuity in a population of young children with a high prevalence of astigmatism. *Journal of AAPOS*, **13**(3), pp.253–257.

Donaghy, C.L. and Larson, S.A., 2015. Vision Screening for Amblyopia. American Academy of Ophthalmology <https://www.aao.org/disease-review/vision-screening-amblyopia>.

Doron, R., Spierer, A. and Polat, U., 2015. How crowding, masking, and contour interactions are related: A developmental approach. *Journal of Vision*, **15**(8), p.5.

Droste, P.J., Archer, S.M. and Helveston, E.M., 1991. Measurement of Low Vision in Children and Infants. *Ophthalmology*, **98**(10), pp.1513–1518.

Drover, J.R., Felius, J., Cheng, C.S., Morale, S.E., Wyatt, L. and Birch, E.E., 2008. Normative pediatric visual acuity using single surrounded HOTV optotypes on the Electronic Visual Acuity Tester following the Amblyopia Treatment Study protocol. *Journal of AAPOS*, **12**(2), pp.145–149.

Drover, J.R., Wyatt, L.M., Stager, D.R. and Birch, E.E., 2009. The teller acuity cards are effective in detecting amblyopia. *Optometry and Vision Science*, **86**(6), pp.755–759.

Dubowitz, D., 1980. Portable version of the Fantz box for assessment of visual function. *The Lancet*, **316**(8207), pp.1279–1280.

Dubowitz, L.M., Dubowitz, V. and Morante, A., 1980. Visual function in the newborn: A study of preterm and full-term infants. *Brain and Development*, **2**(1), pp.15–27.

Ehlers, H., 1953. Clinical testing of visual acuity. *A.M.A. Archives of Ophthalmology*, **49**(4), pp.431–434.

El-Shamayleh, Y., Kiorpes, L., Kohn, A. and Movshon, J.A., 2010. Visual motion processing by neurons in area MT of macaque monkeys with experimental amblyopia. *Journal of Neuroscience*, **30**(36), pp.12198–12209.

Elder, M.J., 1994. Occlusion therapy for strabismic amblyopia. *Australian and New Zealand Journal of Ophthalmology*, **22**(3), pp.187–191.

Ellemberg, D., Lewis, T.L., Defina, N., Maurer, D., Brent, H.P., Guillemot, J.P. and Lepore, F., 2005. Greater losses in sensitivity to second-order local motion than to first-order local motion after early visual deprivation in humans. *Vision Research*, **45**(22), pp.2877–2884.

Ellemberg, D., Lewis, T.L., Hong Liu, C. and Maurer, D., 1999. Development of spatial and temporal vision during childhood. *Vision Research*, **39**(14), pp.2325–2333.

Ellemberg, D., Lewis, T.L., Maurer, D., Brar, S. and Brent, H.P., 2002. Better perception of global motion after monocular than after binocular deprivation. *Vision Research*, **42**(2), pp.169–179.

Ellemberg, D., Lewis, T.L., Meghji, K.S., Maurer, D., Guillemot, J.P. and Lepore, F., 2003. Comparison of sensitivity to first- and second-order local motion in 5-year-olds and adults. *Spatial Vision*, **16**(5), pp.419–428.

Elliott, M.C. and Firth, A.Y., 2009. The logMAR Kay picture test and the logMAR acuity test: A comparative study. *Eye*, **23**(1), pp.85–88.

Facchin, A., Maffioletti, S., Martelli, M. and Daini, R., 2019. Different trajectories in the development of visual acuity with different levels of crowding: The Milan Eye Chart (MEC). *Vision Research*, **156**, pp.10–16.

Farzin, F., Rivera, S.M. and Whitney, D., 2009. Holistic crowding of Mooney faces. *Journal of Vision*, **9**(6), pp.1–15.

Faubert, J., 2002. Visual perception and aging. *Canadian Journal of Experimental Psychology*, **56**(3), pp.164–176.

Feng, C., Jiang, Y. and He, S., 2007. Horizontal and vertical asymmetry in visual spatial crowding effects. *Journal of Vision*, **7**(2), pp.13–13.

Fern, K.D., Manny, R.E., Davis, J.R. and Gibson, R.R., 1986. Contour interaction in the preschool child. *Optometry and Vision Science*, **63**(5), pp.313–318.

Ffooks, O., 1965. Vision Test for Children: Use of Symbols. *The British journal of ophthalmology*, **49**(6), pp.312–4.

Fink, W.H., 1945. An evaluation of visual-acuity symbols. *American Journal of Ophthalmology*, **28**(7), pp.701–711.

Fletcher, K. and Sutherland, S., 2009. Identification of Text and Symbols on a Liquid Crystal Display Part I: Characterisation of the Luminance, Temporal and Spectral Characteristics. Australian Government: Defence Science and Technology Organisation.

Flom, M.C., 1991. Contour interaction and the crowding effect. *Problems in Optometry*, **3**(2), pp.237–257.

Flom, M.C., Heath, G.G. and Takahashi, E., 1963. Contour Interaction and Visual Resolution: Contralateral Effects. *American Association for the Advancement of Science*, **142**(3594), pp.979–980.

Flom, M.C. and Neumaier, R.W., 1966. Prevalence of amblyopia. *Public health reports*, **81**(4), pp.329–341.

Flom, M.C., Weymouth, F.W. and Kahneman, D., 1963. Visual Resolution and Contour Interaction. *Journal of the Optical Society of America*, **53**(9), p.1026.

Flynn, J.T. and Cassady, J.C., 1978. Current Trends in Amblyopia Therapy. *Ophthalmology*, **85**(5), pp.428–450.

Flynn, J.T., Schiffman, J., Feuer, W. and Corona, A., 1998. The therapy of amblyopia: an

analysis of the results of amblyopia therapy utilizing the pooled data of published studies. *Transactions of the American Ophthalmological Society*, **96**, pp.431–453.

Flynn, J.T., Woodruff, G., Thompson, J.R., Hiscox, F., Feuer, W., Schiffman, J., Corona, A., Smith, L.K., Mitchell, P.R., Apt, L., Stamper, R.L. and Helveston, E.M., 1999. The therapy of amblyopia: An analysis comparing the results of amblyopia therapy utilizing two pooled data sets. *Transactions of the American Ophthalmological Society*, **97**, pp.373–395.

Formankiewicz, M.A. and Waugh, S.J., 2013. The effects of blur and eccentric viewing on adult acuity for pediatric tests: implications for amblyopia detection. *Investigative Ophthalmology and Visual Science*, **54**(10), pp.6934–6943.

Freeman, J., Chakravarthi, R. and Pelli, D.G., 2012. Substitution and pooling in crowding. *Attention, Perception, and Psychophysics*, **74**(2), pp.379–396.

Freeman, J. and Pelli, D.G., 2007. An escape from crowding. *Journal of Vision*, **7**(2), pp.22–23.

Friedman, D.S., Repka, M.X., Katz, J., Giordano, L., Hawse, P. and Tielsch, J.M., 2009. Prevalence of Amblyopia and Strabismus in White and African- American Children. *Ophthalmology*, **116**(11), pp.2128–34.

Fronius, M., Cirina, L., Ackermann, H., Kohnen, T. and Diehl, C.M., 2014. Efficiency of electronically monitored amblyopia treatment between 5 and 16 years of age: New insight into declining susceptibility of the visual system. *Vision Research*, **103**, pp.11–19.

Gao, Y., Reynaud, A., Tang, Y., Feng, L., Zhou, Y. and Hess, R.F., 2015. The amblyopic deficit for 2nd order processing: Generality and laterality. *Vision Research*, **114**, pp.111–121.

Gardner, T., Fraser, C. and Peytrignet, S., 2020. Elective care in England Assessing the impact of COVID-19 and where next. *The Health Foundation <https://www.health.org.uk/publications/long-reads/elective-care-in-england-assessing-the-impact-of-covid-19-and-where-next>*, .

Gestalder, R.J. and Green, D.G., 1971. Laser Interferometric Acuity In Amblyopia. *Journal of Pediatric Ophthalmology & Strabismus*, **8**(4), pp.251–256.

Giaschi, D.E., Regan, D., Kraft, S.P. and Kothe, A.C., 1993. Crowding and contrast in amblyopia. *Optometry and Vision Science*, **70**(3), pp.192–197.

Glen, J.C. and Dakin, S.C., 2013. Orientation-crowding within contours. *Journal of Vision*, **13**(8), pp.14–14.

Goodwin, R.T. and Romano, P.E., 1985. Stereoacuity degradation by experimental and real monocular and binocular amblyopia. *Investigative ophthalmology & visual science*, **26**(7), pp.917–23.

Van De Graaf, E.S., Despriet, D.D.G., Klaver, C.C.W. and Simonsz, H.J., 2016. Patient-reported utilities in bilateral visual impairment from amblyopia and age-related macular degeneration. *BMC Ophthalmology*, **16**(1), p.56.

Graham, N., 1980. Spatial-frequency channels in human vision: Detecting edges without edge detectors. In: *C. Harris (Ed.), Visual coding and adaptability*. Hillsdale, NJ: Erlbaum, pp.215–252.

Grainger, J., Tydgat, I. and Isselé, J., 2010. Crowding Affects Letters and Symbols Differently. *Journal of Experimental Psychology: Human Perception and Performance*, **36**(3), pp.673–688.

Green, D.G., 1970. Regional Variations in the Visual Acuity for Interference Fringes on the Retina. *Journal of Physiology*, **207**, pp.351–356.

Greenwood, J.A., Tailor, V.K., Sloper, J.J., Simmers, A.J., Bex, P.J. and Dakin, S.C., 2012. Visual acuity, crowding, and stereo-vision are linked in children with and without amblyopia. *Investigative Ophthalmology and Visual Science*, **53**(12), pp.7655–7665.

Gunton, K.B., 2013. Advances in Amblyopia: What Have We Learned From PEDIG Trials? *Pediatrics*, **131**, pp.540–547.

Guo, X., Fu, M., Lü, J., Chen, Q., Zeng, Y., Ding, X., Morgan, I.G. and He, M., 2015. Normative Distribution of Visual Acuity in 3- to 6-Year-Old Chinese Preschoolers: The Shenzhen Kindergarten Eye Study. *Investigative Opthalmology & Visual Science*,

56(3), p.1985.

Gurnsey, R., Roddy, G. and Chanab, W., 2011. Crowding is size and eccentricity dependent. *Journal of vision*, **11**(7), p.15.

Habak, C. and Faubert, J., 2000. Larger effect of aging on the perception of higher-order stimuli. *Vision Research*, **40**(8), pp.943–950.

Haine, L., Waugh, S., Formankiewicz, M. and Pelli, D., 2021. Simplifying the repeated crowding-distance test for normal and amblyopic children. *Journal of Vision*, **21**(9), p.1982.

Haine, L.A., Waugh, S.J., Formankiewicz, M.A. and Pelli, D.G., 2019. A single line of repeated letters is repeated enough for assessment of crowding distance in young healthy children. In: *Anglia Ruskin University Faculty of Science & Engineering 8th Annual Research Conference*. Cambridge.

Hairol, M.I., Abd-Latif, N., Woi, P.J., Ahmad-Rashaidi, N.H., Kaur, S. and Waugh, S.J., 2014. Visual acuity and spatial interaction zones: investigating the periphery in anisometropic amblyopia. *Journal of Vision*, **14**(10), pp.772–772.

Hairol, M.I., Formankiewicz, M.A. and Waugh, S.J., 2013. Foveal visual acuity is worse and shows stronger contour interaction effects for contrast-modulated than luminance-modulated Cs. *Visual Neuroscience*, **30**(3), pp.105–120.

Hairol, M.I., Omair, Q.M. and Kaur, S., 2016. Effects of flanker type and position on foveal letter recognition. *F1000Research*, **5**, p.1013.

Hairol, M.I. and Waugh, S.J., 2010a. Lateral facilitation revealed dichoptically for luminance-modulated and contrast-modulated stimuli. *Vision Research*, **50**(23), pp.2530–2542.

Hairol, M.I. and Waugh, S.J., 2010b. Lateral interactions across space reveal links between processing streams for luminance-modulated and contrast-modulated stimuli. *Vision research*, **50**(9), pp.889–903.

Hamm, L.M., Black, J., Dai, S. and Thompson, B., 2014. Global processing in amblyopia: a review. *Frontiers in psychology*, **5**, p.583.

Hamm, L.M., Yeoman, J.P., Anstice, N. and Dakin, S.C., 2018. The Auckland Optotypes:

An open-access pictogram set for measuring recognition acuity. *Journal of Vision*, **18**(3), pp.1–15.

Hansen, M.H., Munch, I.C., Li, X.Q., Skovgaard, A.M., Olsen, E.M., Larsen, M. and Kessel, L., 2019. Visual acuity and amblyopia prevalence in 11- to 12-year-old Danish children from the Copenhagen Child Cohort 2000. *Acta Ophthalmologica*, **97**(1), pp.29–35.

Hariharan, S., Levi, D.M. and Klein, S.A., 2005. 'Crowding' in normal and amblyopic vision assessed with Gaussian and Gabor C's. *Vision Research*, **45**(5), pp.617–633.

Harrison, W.J. and Bex, P.J., 2015. A Unifying Model of Orientation Crowding in Peripheral Vision. *Current Biology*, **25**(24), pp.3213–3219.

He, D., Wang, Y. and Fang, F., 2019. The Critical Role of V2 Population Receptive Fields in Visual Orientation Crowding. *Current Biology*, **29**(13), pp.2229-2236.e3.

He, S., Cavanagh, P. and Intriligator, J., 1996. Attentional resolution and the locus of visual awareness. *Nature*, **383**(6598), pp.334–337.

Heinrich, S.P. and Bach, M., 2013. Resolution acuity versus recognition acuity with Landolt-style optotypes. *Graefe's Archive for Clinical and Experimental Ophthalmology*, **251**(9), pp.2235–2241.

Herzog, M.H. and Manassi, M., 2015. Uncorking the bottleneck of crowding: a fresh look at object recognition. *Current Opinion in Behavioral Sciences*, **1**, pp.86–93.

Hess, R.F. and Bradley, A., 1980. Contrast perception above threshold is only minimally impaired in human amblyopia. *Nature*, **287**(5781), pp.463–464.

Hess, R.F., Dakin, S.C., Tewfik, M. and Brown, B., 2001. Contour interaction in amblyopia: Scale selection. *Vision Research*, **41**(17), pp.2285–2296.

Hess, R.F. and Howell, E.R., 1977. The threshold contrast sensitivity function in strabismic amblyopia: Evidence for a two type classification. *Vision Research*, **17**(9), pp.1049–1055.

Hess, R.F. and Jacobs, R.J., 1979. A preliminary report of acuity and contour interactions across the amblyope's visual field. *Vision Research*, **19**(12), pp.1403–1408.

Hess, R.F. and Thompson, B., 2015. Amblyopia and the binocular approach to its therapy.

Vision Research, **114**, pp.4–16.

Von Der Heydt, R., Peterhans, E. and Baumgartner, G., 1984. Illusory contours and cortical neuron responses. *Science*, **224**(4654), pp.1260–1262.

Hillis, A., Flynn, J.T. and Hawkins, B.S., 1983. The evolving concept of amblyopia: A challenge to epidemiologists. *American Journal of Epidemiology*, **118**(2), pp.192–205.

Hilton, A.F. and Stanley, J.C., 1972. Pitfalls in testing children's vision by the Sheridan Gardiner single optotype method. *British Journal of Ophthalmology*, **56**(2), pp.135–139.

Holmes, J.M. and Archer, S.M., 1993. Vernier acuity cards: A practical method for measuring vernier acuity in infants. *Journal of Pediatric Ophthalmology and Strabismus*, **30**(5), pp.312–314.

Holmes, J.M. and Clarke, M.P., 2006. Amblyopia. *The Lancet*, **367**(9519), pp.1343–1351.

Holmes, J.M., Lazar, E.L., Melia, B.M., Astle, W.F., Dagi, L.R., Donahue, S.P., Frazier, Marcela, G., Hertle, R.W., Repka, M.X., Quinn, Graham, E., Weise, K.K., Frazier, M.G., Hertle, R.W., Repka, M.X., Quinn, G.E. and Weise, K.K., 2011. Effect of age on response to amblyopia treatment in children. *Archives of Ophthalmology*, **129**(11), pp.1451–1457.

Holmes, J.M., Repka, M.X., Kraker, R.T. and Clarke, M.P., 2006. The Treatment of Amblyopia. *Strabismus*, **14**(1), pp.37–42.

Howard, C. and Firth, A.Y., 2006. Is the Cardiff acuity test effective in detecting refractive errors in children? *Optometry and Vision Science*, **83**(8).

Howell, E.R., Mitchell, D.E. and Keith, C.G., 1983. Contrast thresholds for sine gratings of children with amblyopia. *Investigative ophthalmology & visual science*, **24**(6), pp.782–787.

Hu, F., Hu, Y., Ma, Z. and Rosenberger, W.F., 2014. Adaptive randomization for balancing over covariates. *WIREs Computational Statistics*, **6**(4), pp.288–303.

Huang, C., Tao, L., Zhou, Y. and Lu, Z.-L., 2007. Treated amblyopes remain deficient in spatial vision: A contrast sensitivity and external noise study. *Vision Research*, **47**(1),

pp.22–34.

Hubel, D.H. and Livingstone, M.S., 1987. Segregation of form, color, and stereopsis in primate area 18. *Journal of Neuroscience*, **7**(11), pp.3378–3415.

Hubel, D.H. and Wiesel, T.N., 1962. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *Journal of Physiology*, **160**(1), pp.106-154.2.

Huynh, S.C., Wang, X.Y., Ip, J., Robaei, D., Kifley, A., Rose, K.A. and Mitchell, P., 2006. Prevalence and associations of anisometropia and aniso-astigmatism in a population based sample of 6year old children. *British Journal of Ophthalmology*, **90**(5), pp.597–601.

Hyvärinen, L., Näsänen, R. and Laurinen, P., 1980. New visual acuity test for pre-school children. *Acta ophthalmologica*, **58**(4), pp.507–11.

Ingram, R.M., 1979. Refraction of 1-year-old children after atropine cycloplegia. *British Journal of Ophthalmology*, **63**(5), pp.343–347.

Jacobs, R.J., 1979. Visual resolution and contour interaction in the fovea and periphery. *Vision Research*, **19**(11), pp.1187–1195.

Jeon, S.T., Hamid, J., Maurer, D. and Lewis, T.L., 2010. Developmental changes during childhood in single-letter acuity and its crowding by surrounding contours. *Journal of Experimental Child Psychology*, **107**(4), pp.423–437.

Karakosta, A., Vassilaki, M., Plainis, S., Elfadl, N.H., Tsilimbaris, M. and Moschandreas, J., 2012. Choice of analytic approach for eye-specific outcomes: one eye or two? *American journal of ophthalmology*, **153**(3), pp.571-579.e1.

Kay, H., 1983. New method of assessing visual acuity with pictures. *British Journal of Ophthalmology*, **67**(2), pp.131–133.

Keith, C.G., Diamond, Z. and Stansfield, A., 1972. Visual acuity testing in young children. *British Journal of Ophthalmology*, **56**(11), pp.827–832.

Kelly, K.R., Cheng-Patel, C.S., Jost, R.M., Wang, Y.-Z. and Birch, E.E., 2019. Fixation instability during binocular viewing in anisometropic and strabismic children. *Experimental Eye Research*, **183**, pp.29–37.

Kelly, K.R., Jost, R.M., De La Cruz, A., Dao, L., Beauchamp, C.L., Stager, D. and Birch, E.E., 2017. Slow reading in children with anisometropic amblyopia is associated with fixation instability and increased saccades. *Journal of AAPOS*, **21**(6), pp.447-451.e1.

Kernan, W.N., Viscoli, C.M., Makuch, R.W., Brass, L.M. and Horwitz, R.I., 1999. Stratified Randomization for Clinical Trials. *Journal of Clinical Epidemiology*, **52**(1), pp.19–26.

Kiorpes, L. and McKee, S.P., 1999. Neural mechanisms underlying amblyopia. *Current Opinion in Neurobiology*, **9**(4), pp.480–486.

Kiorpes, L. and Movshon, J.A., 1989. Differential development of two visual functions in primates (visual development/grating acuity/vernier acuity/strabismus). *Proc. Nati. Acad. Sci. USA*, **86**, pp.8998–9001.

Kirk, V.G., Clausen, M.M., Armitage, M.D. and Arnold, R.W., 2008. Preverbal Photoscreening for Amblyogenic Factors and Outcomes in Amblyopia Treatment: Early Objective Screening and Visual Acuities. *Archives of Ophthalmology*, **126**(4), pp.489–492.

Kooi, F.L., Levi, D.M., Tripathy, S.P. and Toet, A., 1994. The effect of similarity and duration on spatial interaction in peripheral vision. *Spatial Vision*, **8**(2), pp.255–279.

Korte, W., 1923. Uber die Gestaltauffassung im indirekten Sehen. *Zeitschrift fur Psychologie*, **93**, pp.17–82.

Kothe, A.C. and Regan, D., 1990. The component of gaze selection/control in the development of visual acuity in children. *Optometry and Vision Science*, **67**(10), pp.770–778.

Kovács, I., Polat, U. and Norcia, A.M., 1996. Breakdown of binding mechanisms in amblyopia. *Investigative Ophthalmology and Visual Science*, **37**(3), p.3078.

Kraehenmann, R., Vollenweider, F.X., Seifritz, E. and Kometer, M., 2012. Crowding deficits in the visual periphery of schizophrenia patients. *PLoS ONE*, **7**(9), p.e45884.

Kushner, B.J., Lucchese, N.J. and Morton, G. V, 1995. Grating visual acuity with Teller cards compared with Snellen visual acuity in literate patients. *Archives of ophthalmology*, **113**(4), pp.485–93.

Kvarnström, G. and Jakobsson, P., 2005. Is vision screening in 3-year-old children feasible? Comparison between the Lea Symbol chart and the HVOT (LM) chart. *Acta Ophthalmologica Scandinavica*, **83**(1), pp.76–80.

Kwon, M., Legge, G.E. and Dubbels, B.R., 2007. Developmental Changes in the Visual Span for Reading MiYoung. *Vision Research*, **47**(22), pp.2889–2900.

Kwon, M.Y. and Legge, G.E., 2013. Higher-contrast requirements for recognizing low-pass-filtered letters. *Journal of Vision*, **13**(1), pp.13–13.

Lai, X.J., Alexander, J., He, M., Yang, Z. and Suttle, C., 2011. Visual functions and interocular interactions in anisometropic children with and without amblyopia. *Investigative Ophthalmology and Visual Science*, **52**(9), pp.6849–6859.

Lai, Y.H., Wang, H.Z. and Hsu, H.T., 2011. Development of visual acuity in preschool children as measured with Landolt C and Tumbling e charts. *Journal of AAPOS*, **15**(3), pp.251–255.

Lalor, S.J.H., Formankiewicz, M.A. and Waugh, S.J., 2016. Crowding and visual acuity measured in adults using paediatric test letters, pictures and symbols. *Vision Research*, **121**, pp.31–38.

Lalor, S.J.H.H., 2018. Measures of Visual Acuity, Contour Interaction and Crowding with Contrast-Modulated Optotypes in Adults and Children. Anglia Ruskin University.

Langaas, T., 2011. Visual acuity in children: The development of crowded and single letter acuities. *Scandinavian Journal of Optometry and Visual Science*, **4**(2), pp.20–26.

Larsson, J., Landy, M.S. and Heeger, D.J., 2006. Orientation-selective adaptation to first- and second-order patterns in human visual cortex. *Journal of Neurophysiology*, **95**(2), pp.862–881.

Leat, S.J., Li, W. and Epp, K., 1999. Crowding in central and eccentric vision: the effects of contour interaction and attention. *Investigative ophthalmology & visual science*, **40**(2), pp.504–12.

Ledgeway, T. and Smith, A.T., 1994. Evidence for separate motion-detecting mechanisms for first- and second-order motion in human vision. *Vision Research*, **34**(20), pp.2727–

2740.

van Leeuwen, R., Eijkemans, M.J.C., Vingerling, J.R., Hofman, A., de Jong, P.T.V.M. and Simonsz, H.J., 2007. Risk of bilateral visual impairment in individuals with amblyopia: the Rotterdam study. *The British Journal of Ophthalmology*, **91**(11), pp.1450–1451.

Lev, M., Yehezkel, O. and Polat, U., 2014. Uncovering foveal crowding? *Scientific Reports*, **4**(1), p.4067.

Leventhal, A.G., Wang, Y., Schmolesky, M.T. and Zhou, Y., 1998. Neural correlates of boundary perception. *Visual Neuroscience*, **15**(6), pp.1107–1118.

Levi, D.M., 2008. Crowding-An essential bottleneck for object recognition: A mini-review. *Vision Research*, **48**(5), pp.635–654.

Levi, D.M., 2011. Visual crowding. *Current Biology*, **21**(18), pp.R678–R679.

Levi, D.M., 2020. Rethinking amblyopia 2020. *Vision Research*, **176**, pp.118–129.

Levi, D.M. and Carney, T., 2009. Report Crowding in Peripheral Vision : Why Bigger Is Better. *Current Biology*, **19**(23), pp.1988–1993.

Levi, D.M. and Carney, T., 2011. The effect of flankers on three tasks in central, peripheral, and amblyopic vision. *Journal of vision*, **11**(1), pp.10–10.

Levi, D.M., Hariharan, S. and Klein, S. a, 2002a. Suppressive and facilitatory spatial interactions in peripheral vision: peripheral crowding is neither size invariant nor simple contrast masking. *Journal of vision*, **2**, pp.167–177.

Levi, D.M., Hariharan, S. and Klein, S.A., 2002b. Suppressive and facilitatory spatial interactions in amblyopic vision. *Vision Research*, **42**(11), pp.1379–1394.

Levi, D.M. and Harwerth, R.S., 1977. Spatio-temporal interactions in anisometropic and strabismic amblyopia. *Investigative Ophthalmology & Visual Science*, **16**(1), pp.90–95.

Levi, D.M. and Klein, S., 1982a. Differences in vernier discrimination for gratings between strabismic and anisometropic amblyopes. *Investigative Ophthalmology and Visual Science*, **23**(3), pp.398–407.

Levi, D.M. and Klein, S., 1982b. Hyperacuity and amblyopia. *Nature*, **298**(5871), pp.268–270.

Levi, D.M. and Klein, S., 1985. Venier acuity, crowding and amblyopia. *Vision Research*, **25**(7), pp.979–991.

Levi, D.M. and Klein, S.A., 1983. Spatial localization in normal and amblyopic vision. *Vision Research*, **23**(10), pp.1005–1017.

Levi, D.M. and Klein, S.A., 1990. Equivalent intrinsic blur in amblyopia. *Vision Research*, **30**(12), pp.1995–2022.

Levi, D.M., Klein, S.A. and Yen Lee Yap, 1987. Positional uncertainty in peripheral and amblyopic vision. *Vision Research*, **27**(4), pp.581–597.

Levi, D.M. and Li, R.W., 2009. Perceptual learning as a potential treatment for amblyopia : A mini-review. *Vision Research*, **49**(21), pp.2535–2549.

Levi, D.M., Song, S. and Pelli, D.G., 2007. Amblyopic reading is crowded. *Journal of Vision*, **7**(2), p.21.

Li, G., Yao, Z., Wang, Z., Yuan, N., Talebi, V., Tan, J., Wang, Y., Zhou, Y. and Baker, C.L., 2014. Form-cue invariant second-order neuronal responses to contrast modulation in primate area V2. *Journal of Neuroscience*, **34**(36), pp.12081–12092.

Li, X., Dumoulin, S.O., Mansouri, B. and Hess, R.F., 2007. Cortical deficits in human amblyopia: Their regional distribution and their relationship to the contrast detection deficit. *Investigative Ophthalmology and Visual Science*, **48**(4), pp.1575–1591.

Li, Y.P., Zhou, M.W., Forster, S.H., Chen, S.Y., Qi, X., Zhang, H.M. and Luo, J., 2019. Prevalence of amblyopia among preschool children in central south China. *International Journal of Ophthalmology*, **12**(5), pp.820–825.

Lippmann, O., 1971. Vision screening of young children. *American Journal of Public Health*, **61**, pp.1586–1601.

Little, J.A., Molloy, J. and Saunders, K.J., 2012. The differing impact of induced astigmatic blur on crowded and uncrowded paediatric visual acuity chart results. *Ophthalmic and Physiological Optics*, **32**(6), pp.492–500.

Liu, T., Jiang, Y., Sun, X. and He, S., 2009. Reduction of the Crowding Effect in Spatially Adjacent but Cortically Remote Visual Stimuli. *Current Biology*, **19**(2), pp.127–132.

Livne, T. and Sagi, D., 2007. Configuration influence on crowding. *Journal of Vision*, **7**(2), p.4.

Livne, T. and Sagi, D., 2010. How do flankers' relations affect crowding? *Journal of Vision*, **10**(3), pp.1–14.

Loomis, J.M., 1978. Lateral masking in foveal and eccentric vision. *Vision Research*, **18**(3), pp.335–338.

Loudon, S.E., Polling, J.R. and Simonsz, H.J., 2003. Electronically measured compliance with occlusion therapy for amblyopia is related to visual acuity increase. *Graefe's Archive for Clinical and Experimental Ophthalmology*, **241**(3), pp.176–180.

Louie, E.G., Bressler, D.W. and Whitney, D., 2007. Holistic crowding: Selective interference between configural representations of faces in crowded scenes. *Journal of Vision*, **7**(2), pp.1–11.

Maia da Silva, M.N., Millington, R.S., Bridge, H., James-Galton, M. and Plant, G.T., 2017. Visual Dysfunction in Posterior Cortical Atrophy. *Frontiers in Neurology*, **8**, pp.1–11.

Manassi, M., Sayim, B. and Herzog, M.H., 2012. Grouping, pooling, and when bigger is better in visual crowding. *Journal of Vision*, **1213**(10), pp.1–14.

Manny, R.E. and Klein, S.A., 1984. The development of Vernier acuity in infants. *Current Eye Research*, **3**(3), pp.453–462.

Mansouri, B., Allen, H.A. and Hess, R.F., 2005. Detection, discrimination and integration of second-order orientation information in strabismic and anisometropic amblyopia. *Vision Research*, **45**(18), pp.2449–2460.

Mareschal, I. and Baker, C.L., 1998. Temporal and spatial response to second-order stimuli in cat area 18. *Journal of Neurophysiology*, **80**(6), pp.2811–2823.

Marsden, J., Stevens, S. and Ebri, A., 2019. Visual Acuity | AOA. *Community eye health*, **32**(107), p.16.

Martelli, M., Di Filippo, G., Spinelli, D. and Zoccolotti, P., 2009. Crowding, reading, and developmental dyslexia. *Journal of Vision*, **9**(4), pp.14–14.

Martelli, M., Majaj, N.J. and Pelli, D.G., 2005. Are faces processed like words? A diagnostic

test for recognition by parts. *Journal of Vision*, **5**(1), pp.58–70.

Mathers, M., Keyes, M. and Wright, M., 2010. A review of the evidence on the effectiveness of children's vision screening. *Child: Care, Health and Development*, **36**(6), pp.756–780.

Maunsell, J.H.R. and van Essen, D.C., 1987. Topographic organization of the middle temporal visual area in the macaque monkey: Representational biases and the relationship to callosal connections and myeloarchitectonic boundaries. *Journal of Comparative Neurology*, **266**(4), pp.535–555.

Maunsell, J.H.R. and Van Essen, D.C., 1983a. Functional properties of neurons in middle temporal visual area of the macaque monkey. I. Selectivity for stimulus direction, speed, and orientation. *Journal of neurophysiology*, **49**(5), pp.1127–1147.

Maunsell, J.H.R. and Van Essen, D.C., 1983b. The connections of the middle temporal visual area (MT) and their relationship to a cortical hierarchy in the macaque monkey. *Journal of Neuroscience*, **3**(12), pp.2563–2586.

Mayer, D.L. and Dobson, V., 1982. Visual acuity development in infants and young children, as assessed by operant preferential looking. *Vision Research*, **22**(9), pp.1141–1151.

Mayer, D.L., Fulton, A.B. and Rodier, D., 1984. Grating and Recognition Acuities of Pediatric Patients. *Ophthalmology*, **91**(8), pp.947–953.

Mayer, D.L. and Gross, R.D., 1990. Modified Allen Pictures to Assess Amblyopia in Young Children. *Ophthalmology*, **97**(6), pp.827–832.

McColl, S.L. and Mitchell, D.E., 1998. Stereodeficient subjects show substantial differences in interocular transfer of two motion adaptation aftereffects. *Vision Research*, **38**(12), pp.1889–1900.

McCullough, S. and Saunders, K., 2019. Visual Profile of Children who Passed or Failed the UK School Vision Screening Protocol. *British and Irish Orthoptic Journal*, **15**(1), p.36.

McDonald, M.A., 1986. Assessment of visual acuity in toddlers. *Survey of Ophthalmology*, **31**(3), pp.189–210.

McDonald, M.A., Dobson, V. and Sebris, S.L., 1985. The acuity card procedure: A rapid test of infant acuity. *Investigative Ophthalmology and Visual Science*, **26**(8), pp.1158–1162.

McGraw, P. V. and Winn, B., 1993. Glasgow Acuity Cards: a new test for the measurement of letter acuity in children. *Ophthalmic and Physiological Optics*, **13**(4), pp.400–404.

McGraw, P. V., Winn, B., Gray, L.S. and Elliott, D.B., 2000. Improving the reliability of visual acuity measures in young children. *Ophthalmic and Physiological Optics*, **20**(3), pp.173–184.

McGraw, P., Winn, B. and Whitaker, D., 1995. Reliability of the Snellen chart. *BMJ*, **310**(6993), p.1481.

McKee, S.P., Levi, D.M. and Movshon, J.A., 2003. The pattern of visual deficits in amblyopia. *Journal of vision*, **3**(5), pp.380–405.

McKee, S.P., Levi, D.M., Schor, C.M. and Movshon, J.A., 2016. Saccadic latency in amblyopia. *Journal of Vision*, **16**(5), pp.1–15.

McMonagle, P., Deering, F., Berliner, Y. and Kertesz, A., 2006. The cognitive profile of posterior cortical atrophy. *Neurology*, **66**(3), pp.331–338.

Meng, Z., Fu, J., Chen, W., Li, L., Su, H., Dai, W. and Yao, Y., 2021. Prevalence of Amblyopia and Associated Risk Factors in Tibetan Grade One Children. *Ophthalmic Research*, **64**(2), pp.280–289.

Merigan, W.H., Nealey, T.A. and Maunsell, J.H.R., 1993. Visual effects of lesions of cortical area V2 in macaques. *Journal of Neuroscience*, **13**(7), pp.3180–3191.

Milling, A., Newsham, D., Tidbury, L., O'Connor, A.R. and Kay, H., 2015. The redevelopment of the Kay picture test of visual acuity. *British and Irish Orthoptic Journal*, **13**, p.14.

Mohindra, I., Held, R., Gwiazda, J. and Brill, S., 1978. Astigmatism in infants. *Science*, **202**(4365), pp.329–331.

Morad, Y., Werker, E. and Nemet, P., 1999. Visual acuity tests using chart, line, and single optotype in healthy and amblyopic children. *Journal of American Association for*

Pediatric Ophthalmology and Strabismus, **3**(2), pp.94–97.

Motoyoshi, I., Nishida, S., Sharan, L. and Adelson, E.H., 2007. Image statistics and the perception of surface qualities. *Nature*, **447**(7141), pp.206–209.

Motter, B.C., 2006. Modulation of transient and sustained response components of V4 neurons by temporal crowding in flashed stimulus sequences. *Journal of Neuroscience*, **26**(38), pp.9683–9694.

Nandy, A.S. and Tjan, B.S., 2012. Saccade-confounded image statistics explain visual crowding. *Nature Neuroscience*, **15**(3), pp.463–469.

Neri, P. and Levi, D.M., 2006. Spatial Resolution for Feature Binding Is Impaired in Peripheral and Amblyopic Vision. *J Neuro-physiol*, **96**, pp.142–153.

Newman, D.K. and East, M.M., 1999. Preschool vision screening: negative predictive value for amblyopia. *British Journal of Ophthalmology*, **83**(6), pp.676–679.

Newman, D.K. and East, M.M., 2000. Prevalence of amblyopia among defaulters of preschool vision screening. *Ophthalmic Epidemiology*, **7**(1), pp.67–71.

Newsham, D., 2000. Parental non-concordance with occlusion therapy. *The British journal of ophthalmology*, **84**(9), pp.957–62.

Niechwiej-Szwedo, E., Colpa, L. and Wong, A.M.F., 2019. Visuomotor Behaviour in Amblyopia: Deficits and Compensatory Adaptations. *Neural Plasticity*, **2019**, pp.1–18.

Noda, S., Hayasaka, S. and Setogawa, T., 1993. Occlusion therapy of Japanese children with anisometropic amblyopia without strabismus. *Annals of ophthalmology*, **25**(4), pp.145–7.

Von Noorden, G.K., 1974. Factors involved in the production of amblyopia. *British Journal of Ophthalmology*, **58**, pp.158–164.

Norcia, A.M. and Tyler, C.W., 1985. Spatial frequency sweep VEP: Visual acuity during the first year of life. *Vision Research*, **25**(10), pp.1399–1408.

Norgett, Y. and Siderov, J., 2011. Crowding in Children's Visual Acuity Tests—Effect of Test Design and Age. *Optometry and Vision Science*, **88**(8), pp.920–927.

Norgett, Y. and Siderov, J., 2014. Foveal crowding differs in children and adults. *Journal of*

vision, **14**(2014).

Norgett, Y. and Siderov, J., 2017. Effect of stimulus configuration on crowding in strabismic amblyopia. *Journal of Vision*, **17**(13), p.5.

Nucci, P., Alfarano, R., Piantanida, A. and Brancato, R., 1992. Compliance in antiamblyopia occlusion therapy. *Acta Ophthalmologica*, **70**(1), pp.128–131.

O'Boyle, C., Chen, S.I. and Little, J.-A.A., 2017. Crowded letter and crowded picture logMAR acuity in children with amblyopia: A quantitative comparison. *British Journal of Ophthalmology*, **101**(4), pp.457–461.

O'Connor, A.R., Knox, P.C., Bridson, J. and Tompkin, R., 2007. Visual function measurement using a laptop computer: does the screen angle matter? *British and Irish Orthoptic Journal*, **4**(0), p.65.

O'Connor, A.R. and Milling, A., 2020. Normative data for the redesigned Kay Pictures visual acuity test. *Journal of AAPOS*, **24**(4), pp.242–244.

O'Keefe, L.P. and Movshon, J.A., 1998. Processing of first- and second-order motion signals by neurons in area MT of the macaque monkey. *Visual Neuroscience*, **15**(2), pp.305–317.

Ogle, K.N., 1960. Blurring of the retinal image and contrast thresholds in the fovea. *Journal of the Optical Society of America*, **50**(4), pp.307–315.

Oliver, M., Neumann, R., Chaimovitch, Y., Gotesman, N. and Shimshoni, M., 1986. Compliance and Results of Treatment for Amblyopia in Children More Than 8 Years Old. *American Journal of Ophthalmology*, **102**(3), pp.340–345.

Østerberg, G., 1936. A Sight-test Chart for Children. *Acta Ophthalmologica*, **14**(3–4), pp.397–405.

Pan, Y., Tarczy-Hornoch, K., Cotter, S., Wen, G., Borchert, M.S., Azen, S.P., Varma, R., Abbott, L., Ayala, G., Barak, T., Borchert, M., Chang, J., Chen, F.K., Chon, R., Deneen, J., Diaz, J., DiLauro, A., Donofrio, J., Dozal, C., Foong, A.W., Gardner, J., Garriott, R., Lau, J., Lin, J., Martinez, G., McKean-Cowdin, R., Milo, K., Moya, C., Paz, S., Penate, A., Reiner, A., Salazar, C., Song, E., Torres, M., Uribe, N., Verrico, I., Wang, Y., Zhao,

P. and Zhu, A., 2009. Visual acuity norms in pre-school children: The multi-ethnic pediatric eye disease study. *Optometry and Vision Science*, **86**(6), pp.607–612.

Paradiso, M.A., Shimojo, S. and Nakayama, K., 1989. Subjective contours, tilt aftereffects, and visual cortical organization. *Vision Research*, **29**(9), pp.1205–1213.

Pardhan, S., 1997. Crowding in visually impaired patients: Contour interaction and/or gaze-selection defects? *Neuro-Ophthalmology*, **18**(2), pp.59–65.

Parkes, L., Lund, J., Angelucci, A., Solomon, J.A. and Morgan, M., 2001. Compulsory averaging of crowded orientation signals in human vision. *Nature Neuroscience*, **4**(7), pp.739–744.

PEDIG, 2010. A Randomized Trial Comparing Bangerter Filters and Patching for the Treatment of Moderate Amblyopia in Children. *Ophthalmology*, **117**(5), pp.998-1004.e6.

Peh, K.K., Agelis, L. and Chen, F.K., 2012. Are ETDRS Scores at 1m and 4m interchangeable? *ARVO Meeting Abstracts*, **53**(6), p.4786.

Pelli, D.G., 1997. The VideoToolbox software for visual psychophysics: Transforming numbers into movies. *Spatial Vision*, **10**(4), pp.437–442.

Pelli, D.G., 2008. Crowding: a cortical constraint on object recognition. *Current Opinion in Neurobiology*, **18**(4), pp.445–451.

Pelli, D.G., Burns, C.W., Farell, B. and Moore-Page, D.C., 2006. Feature detection and letter identification. *Vision Research*, **46**(28), pp.4646–4674.

Pelli, D.G. and Farell, B., 1999. Why use noise? *Journal of the Optical Society of America A*, **16**(3), p.647.

Pelli, D.G., Levin, D.M. and Chung, S.T.L., 2004. Using visual noise to characterize amblyopic letter identification. *Journal of Vision*, **4**(10), pp.904–920.

Pelli, D.G., Palomares, M. and Majaj, N.J., 2004. Crowding is unlike ordinary masking: Distinguishing feature integration from detection. *Journal of Vision*, **4**(12), p.12.

Pelli, D.G. and Tillman, K.A., 2008. The uncrowded window of object recognition. *Nature Neuroscience*, **11**(10), pp.1129–1135.

Pelli, D.G. and Watson, A.., 1983. QUEST: A Bayesian adaptive psychometric method. *Perception & Psychophysics*, **33**(2), pp.113–120.

Pelli, D.G., Waugh, S.J., Martelli, M., Crutch, S.J., Primativo, S., Yong, K.X., Rhodes, M., Yee, K., Wu, X., Famira, H.F. and Yiltiz, H., 2016. A clinical test for visual crowding. *F1000Research*, **5**, p.81.

Pointer, J.S., 2008. Recognition versus resolution: A comparison of visual acuity results using two alternative test chart optotype. *Journal of Optometry*, **1**(2), pp.65–70.

Pointer, J.S., Gilmartin, G. and Larke, J.R., 1980. The evolution of the broken ring visual acuity test figure. *Journal of the American Optometric Association*, **51**(8), pp.741–745.

Polat, U., Mizobe, K., Pettet, M.W., Kasamatsu, T. and Norcia, A.M., 1998. Collinear stimuli regulate visual responses depending on cell's contrast threshold. *Nature 1998 391:6667*, **391**(6667), pp.580–584.

Polat, U. and Norcia, A.M., 1996. Neurophysiological evidence for contrast dependent long-range facilitation and suppression in the human visual cortex. *Vision Research*, **36**(14), pp.2099–2109.

Polat, U. and Sagi, D., 1993. Lateral interactions between spatial channels: Suppression and facilitation revealed by lateral masking experiments. *Vision Research*, **33**(7), pp.993–999.

Polat, U. and Sagi, D., 1994a. Spatial interactions in human vision: From near to far via experience- dependent cascades of connections. *Proceedings of the National Academy of Sciences of the United States of America*, **91**(4), pp.1206–1209.

Polat, U. and Sagi, D., 1994b. The architecture of perceptual spatial interactions. *Vision Research*, **34**(1), pp.73–78.

Polat, U., Sagi, D. and Norcia, A.M., 1997. Abnormal long-range spatial interactions in amblyopia. *Vision Research*, **37**(6), pp.737–744.

Poth, C.H. and Horstmann, G., 2017. Assessing the monitor warm-up time required before a psychological experiment can begin. *The Quantitative Methods for Psychology*, **13**(3), pp.166–173.

Powell, C. and Hatt, S.R., 2009. Vision screening for amblyopia in childhood. *The Cochrane database of systematic reviews*, (3), p.CD005020.

Rahi, J.S., Cumberland, P.M. and Peckham, C.S., 2006. Does amblyopia affect educational, health, and social outcomes? Findings from 1958 British birth cohort. *British Medical Journal*, **332**(7545), pp.820–824.

Rahi, J.S. and Dezateux, C., 1997. The future of preschool vision screening services in Britain. *BMJ*, **315**(7118), pp.1247–1248.

Rahi, J.S., Logan, S., Timms, C., Russell-Eggitt, I. and Taylor, D., 2002. Risk, causes, and outcomes of visual impairment after loss of vision in the non-amblyopic eye: a population-based study. *The Lancet*, **360**(9333), pp.597–602.

Raymond, J.E., 1993. Complete interocular transfer of motion adaptation effects on motion coherence thresholds. *Vision Research*, **33**(13), pp.1865–1870.

Regan, D., Giaschi, D.E., Kraft, S.P. and Kothe, A.C., 1992. Method for identifying amblyopes whose reduced line acuity is caused by defective selection and/or control of gaze. *Ophthalmic and Physiological Optics*, **12**(4), pp.425–432.

Repka, M.X., Beck, R.W., Kraker, R.T., Cole, S.R., Holmes, J.M., Birch, E.E., Tien, D.R., Astle, W.F. and Cotter, S.A., 2002. The clinical profile of moderate amblyopia in children younger than 7 years. *Archives of Ophthalmology*, **120**(3), pp.281–287.

Rivest, J. and Cavanagh, P., 1996. Localizing contours defined by more than one attribute. *Vision research*, **36**(1), pp.53–66.

Robaei, D., Rose, K.A., Kifley, A., Cosstick, M., Ip, J.M. and Mitchell, P., 2006. Factors Associated with Childhood Strabismus. Findings from a Population-Based Study. *Ophthalmology*, **113**(7), pp.1146–1153.

Robol, V., Tibber, M.S., Anderson, E.J., Bobin, T., Carlin, P., Shergill, S.S. and Dakin, S.C., 2013. Reduced Crowding and Poor Contour Detection in Schizophrenia Are Consistent with Weak Surround Inhibition. *PLoS ONE*, **8**(4), p.e60951.

Rodier, D.W., Mayer, D.L. and Fulton, A.B., 1985. Assessment of Young Amblyopes: Array vs. Single Picture Acuities. *Ophthalmology*, **92**(9), pp.1197–1202.

Ronconi, L., Bertoni, S. and Bellacosa Marotti, R., 2016. The neural origins of visual crowding as revealed by event-related potentials and oscillatory dynamics. *Cortex*, **79**, pp.87–98.

Roper-Hall, G., 2007. Current Concepts of Amblyopia: A Neuro-Ophthalmology Perspective. *American Orthoptic Journal*, **57**(1), pp.2–12.

Rosen, S., Chakravarthi, R. and Pelli, D.G., 2014. The Bouma law of crowding, revised: critical spacing is equal across parts, not objects. *Journal of vision*, **14**(6), p.10.

Rydberg, A., Ericson, B., Lennerstrand, G., Jacobson, L. and Lindstedt, E., 1999. Assessment of visual acuity in children aged 1 1/2 -6 years, with normal and subnormal vision. *Strabismus*, **7**(1), pp.1–24.

Saarela, T.P., Westheimer, G. and Herzog, M.H., 2010. The effect of spacing regularity on visual crowding. *Journal of vision*, **10**(10), p.17.

Sailoganathan, A., Rou, L.X., Buja, K.A. and Siderov, J., 2018. Assessment of Visual Acuity in Children Using Crowded Lea Symbol Charts. *Optometry and Vision Science*, **95**(8), pp.643–647.

Salt, A.T., Wade, A.M., Proffitt, R., Heavens, S. and Sonksen, P.M., 2007. The Sonksen logMAR Test of Visual Acuity: I. Testability and reliability. *Journal of AAPOS*, **11**(6), pp.589–596.

Saul, T. and Taylor, K., 2012. Normative data for the crowded logMAR Kay's pictures vision test in children. *British and Irish Orthoptic Journal*, **9**(0), p.36.

Schaller, M.J. and Harris, L.J., 1975. 'Upright' orientations of forms change with subject age and with features of form. *Perception & Psychophysics*, **17**(2), pp.179–188.

Schira, M.M., Tyler, C.W., Breakspear, M. and Spehar, B., 2009. The foveal confluence in human visual cortex. *Journal of Neuroscience*, **29**(28), pp.9050–9058.

Schlenker, M.B., Christakis, T.J. and Braga-Mele, R.M., 2010. Comparing a traditional single optotype visual acuity test with a computer-based visual acuity test for childhood amblyopia vision screening: A pilot study. *Canadian Journal of Ophthalmology*, **45**(4), pp.368–374.

Schmucker, C., Grosselfinger, R., Riemsma, R., Antes, G., Lange, S., Lagrèze, W. and Kleijnen, J., 2009. Diagnostic accuracy of vision screening tests for the detection of amblyopia and its risk factors: A systematic review. *Graefe's Archive for Clinical and Experimental Ophthalmology*, **247**(11), pp.1441–1454.

Schofield, A.J. and Georgeson, M.A., 1999. Sensitivity to modulations of luminance and contrast in visual white noise: separate mechanisms with similar behaviour. *Vision research*, **39**(16), pp.2697–2716.

Schor, C., Terrell, M. and Peterson, D., 1976. Contour interaction and temporal masking in strabismus and amblyopia. *Optometry and Vision Science*, **53**(5), pp.217–223.

Semenov, L.A., Chernova, N.D. and Bondarko, V.M., 2000. Measurement of visual acuity and crowding effect in 3–9-year-old children. *Human Physiology*, **26**(1), pp.16–20.

Semenov, L.A., Chernova, N.D. and Bondarko, V.M., 2002. Age-Related Dynamics of Discrimination of the Orientation of Rectangular Gratings at the Resolution Limit of the Visual System. *Human Physiology*, **28**(4), pp.383–390.

Sengpiel, F., 2013. Amblyopia : Out of the Dark , Into the Light. *Current Biology*, **23**(5), pp.R195–R196.

Shah, N., Dakin, S.C. and Anderson, R.S., 2012. Effect of optical defocus on detection and recognition of vanishing optotype letters in the fovea and periphery. *Investigative Ophthalmology and Visual Science*, **53**(11), pp.7063–7070.

Shah, N., Laidlaw, D.A.H., Rashid, S. and Hysi, P., 2012. Validation of printed and computerised crowded Kay picture logMAR tests against gold standard ETDRS acuity test chart measurements in adult and amblyopic paediatric subjects. *Eye*, **26**(4), pp.593–600.

Sharma, V., Levi, D.M. and Klein, S.A., 2000. Undercounting features and missing features: Evidence for a high-level deficit in strabismic amblyopia. *Nature Neuroscience*, **3**(5), pp.496–501.

Shaw, D.E., Fielder, A.R., Minshull, C. and Rosenthal, A.R., 1988. Amblyopia-factors influencing age of presentation. *Lancet*, **2**(8604), pp.207–9.

Sheridan, M.D. and Gardiner, P.A., 1970. Sheridan-Gardiner Test for Visual Acuity. *British Medical Journal*, **2**(5701), pp.108–109.

Sheth, B.R., Sharma, J., Rao, S.C. and Sur, M., 1996. Orientation maps of subjective contours in visual cortex. *Science*, **274**(5295), pp.2110–2115.

Siderov, J., Waugh, S.J. and Bedell, H.E., 2013. Foveal contour interaction for low contrast acuity targets. *Vision Research*, **77**, pp.10–13.

Simmers, A.J., Gray, L.S., McGraw, P. V and Winn, B., 1999. Contour interaction for high and low contrast optotypes in normal and amblyopic observers. *Ophthalmic & physiological optics : the journal of the British College of Ophthalmic Opticians (Optometrists)*, **19**(3), pp.253–60.

Simmers, A.J., Gray, L.S. and Spowart, K., 1997. Screening for amblyopia: a comparison of paediatric letter tests. *The British journal of ophthalmology*, **81**(6), pp.465–9.

Simmers, A.J., Gray, L.S. and Winn, B., 2000. Visual function thresholds in children. *Current Eye Research*, **21**(2), pp.616–626.

Simmers, A.J., Ledgeway, T. and Hess, R.F., 2005. The influences of visibility and anomalous integration processes on the perception of global spatial form versus motion in human amblyopia. *Vision Research*, **45**(4), pp.449–460.

Simmers, A.J., Ledgeway, T., Hess, R.F. and McGraw, P. V, 2003. Deficits to global motion processing in human amblyopia. *Vision Research*, **43**(6), pp.729–738.

Simmers, A.J., Ledgeway, T., Hutchinson, C.V. and Knox, P.J., 2011. Visual deficits in amblyopia constrain normal models of second-order motion processing. *Vision Research*, **51**(18), pp.2008–2020.

Simmers, A.J., Ledgeway, T., Mansouri, B., Hutchinson, C.V. and Hess, R.F., 2006. The extent of the dorsal extra-striate deficit in amblyopia. *Vision Research*, **46**(16), pp.2571–2580.

Simons, K., 1983. Visual acuity norms in young children. *Survey of Ophthalmology*, **28**(2), pp.84–92.

Simons, K., 1996. Preschool vision screening: Rationale, methodology and outcome.

Survey of Ophthalmology, **41**(1), pp.3–30.

Simons, K., 2005. Amblyopia Characterization, Treatment, and Prophylaxis. *Survey of Ophthalmology*, **50**(2), pp.123–166.

Simpson, T.L., Barbeito, R. and Bedell, H.E., 1986. The effect of optical blur on visual acuity for targets of different luminances. *Ophthalmic and Physiological Optics*, **6**(3), pp.279–281.

Sireteanu, R. and Fronius, M., 1981. Naso-temporal asymmetries in human amblyopia: Consequence of long-term interocular suppression. *Vision Research*, **21**(7), pp.1055–1063.

Siu, C.R. and Murphy, K.M., 2018. The development of human visual cortex and clinical implications. *Eye and Brain*, **10**, pp.25–36.

Skoczenski, A.M. and Norcia, A.M., 2002. Late maturation of visual hyperacuity. *Psychological Science*, **13**(6), pp.537–541.

Sloan, L.L., 1959. New test charts for the measurement of visual acuity at far and near distances. *American Journal of Ophthalmology*, **48**(6), pp.807–813.

Smith, A. and Ledgeway, T., 1997. Separate detection of moving luminance and contrast modulation: fact or artifact? *Vision Research*, **37**(1), pp.45–62.

Smith, A.T., Singh, K.D., Williams, A.L. and Greenlee, M.W., 2001. Estimating Receptive Field Size from fMRI Data in Human Striate and Extrastriate Visual Cortex. *Cerebral Cortex*, **11**(12), pp.1182–1190.

Snellen, H., 1965. Letterproeven tot Bepaling der Gezigtsscherpte (PW van der Weijer 1862). *Br J Physiol Opt*, **22**, pp.238–271.

Snowdon, S.K. and Stewart-Brown, S.L., 1997. Preschool vision screening: results of a systematic review. University of York. York.

Snowling, M. and Davidoff, J., 1992. Visual deficits in dyslexia? *Current Biology*, **2**(4), pp.196–197.

Solebo, A.L., Cumberland, P.M. and Rahi, J.S., 2015. Whole-population vision screening in children aged 4-5 years to detect amblyopia. *The Lancet*, **385**(9984), pp.2308–2319.

Solebo, A.L. and Rahi, J.S., 2013. Vision screening in children aged 4-5 years. UK National Screening Committee.

Song, S., Levi, D.M. and Pelli, D.G., 2014. A double dissociation of the acuity and crowding limits to letter identification, and the promise of improved visual screening. *Journal of Vision*, **14**(5), p.3.

Sonksen, P.M., Wade, A.M., Proffitt, R., Heavens, S. and Salt, A.T., 2008. The Sonksen logMAR test of visual acuity: II. Age norms from 2 years 9 months to 8 years. *Journal of AAPOS*, **12**(1), pp.18–22.

Spekreijse, H., 1983. Comparison of acuity tests and pattern evoked potential criteria: Two mechanisms underly acuity maturation in man. *Behavioural Brain Research*, **10**(1), pp.107–117.

Spowart, K.M., Simmers, A. and Tappin, D.M., 1998. Vision testing in schools: An evaluation of personnel, tests, and premises. *Journal of Medical Screening*, **5**(3), pp.131–132.

Srebro, R., 1983. Fixation of Normal and Amblyopic Eyes. *Archives of Ophthalmology*, **101**(2), pp.214–217.

Sternberg, S., 2003. Process decomposition from double dissociation of subprocesses. *Cortex*, **39**, pp.180–182.

Stevens, S., 2007. Test distance vision using a Snellen chart. *Community Eye Health Journal*, **20**(63), p.52.

Stewart, C.E., Fielder, A.R., Stephens, D.A. and Moseley, M.J., 2002. Design of the Monitored Occlusion Treatment of Amblyopia Study (MOTAS). *British Journal of Ophthalmology*, **86**(8), pp.915–919.

Stewart, C.E., Fielder, A.R., Stephens, D.A. and Moseley, M.J., 2005. Treatment of Unilateral Amblyopia: Factors Influencing Visual Outcome. *Investigative Opthalmology & Visual Science*, **46**(9), p.3152.

Stewart, C.E., Moseley, M.J., Stephens, D.A. and Fielder, A.R., 2004. Treatment dose-response in amblyopia therapy: The Monitored Occlusion Treatment of Amblyopia

Study (MOTAS). *Investigative Ophthalmology and Visual Science*, **45**(9), pp.3048–3054.

Stiers, P., Vanderkelen, R. and Vandenbussche, E., 2003. Optotype and grating visual acuity in preschool children. *Investigative Ophthalmology and Visual Science*, **44**(9), pp.4123–4130.

Strappini, F., Pelli, D.G., Di Pace, E. and Martelli, M., 2017. Agnosic vision is like peripheral vision, which is limited by crowding. *Cortex*, **89**, pp.135–155.

Strasburger, H., 2005. Unfocussed spatial attention underlies the crowding effect in indirect form vision. *Journal of Vision*, **5**(11), pp.1024–1037.

Strasburger, H. and Malania, M., 2013. Source confusion is a major cause of crowding. *Journal of Vision*, **13**(1), pp.24–24.

Stuart, J.A. and Burian, H.M., 1962. A Study of Separation Difficulty*. *American Journal of Ophthalmology*, **53**(3), pp.471–477.

Sukumar, S. and Waugh, S.J., 2007. Separate first- and second-order processing is supported by spatial summation estimates at the fovea and eccentrically. *Vision Research*, **47**(5), pp.581–596.

Sutter, A., Sperling, G. and Chubb, C., 1995. Measuring the spatial frequency selectivity of second-order texture mechanisms. *Vision Research*, **35**(7), pp.915–924.

Tailor, V., Bossi, M., Greenwood, J.A. and Dahlmann-Noor, A., 2016. Childhood amblyopia: Current management and new trends. *British Medical Bulletin*, **119**(1), pp.75–86.

Tang, Y. and Zhou, Y., 2009. Age-related decline of contrast sensitivity for second-order stimuli: Earlier onset, but slower progression, than for first-order stimuli. *Journal of Vision*, **9**(7), pp.1–15.

Teller, D.Y., 1979. The forced-choice preferential looking procedure: A psychophysical technique for use with human infants. *Infant Behavior and Development*, **2**(1), pp.135–153.

Teller, D.Y., McDonald, M.A., Preston, K., Sebris, S.L. and Dobson, V., 1986. Assessment of Visual Acuity in Infants and Children; the Acuity Card Procedure. *Developmental*

Medicine & Child Neurology, **28**(6), pp.779–789.

Thibault, D., Brosseau-Lachaine, O., Faubert, J. and Vital-Durand, F., 2007. Maturation of the sensitivity for luminance and contrast modulated patterns during development of normal and pathological human children. *Vision Research*, **47**(12), pp.1561–1569.

Thompson, B., Chung, S.T.L., Kiorpes, L., Ledgeway, T. and McGraw, P. V., 2015. A window into visual cortex development and recovery of vision: Introduction to the Vision Research special issue on Amblyopia. *Vision Research*, **114**, pp.1–3.

Thompson, B., Villeneuve, M.Y., Casanova, C. and Hess, R.F., 2012. Abnormal cortical processing of pattern motion in amblyopia: Evidence from fMRI. *NeuroImage*, **60**(2), pp.1307–1315.

Thorn, F. and Schwartz, F., 1990. Effects of dioptric blur on snellen and grating acuity. *Optometry and Vision Science*, **67**(1), pp.3–7.

Toet, A. and Levi, D.M., 1992. The two-dimensional shape of spatial interaction zones in the parafovea. *Vision Research*, **32**(7), pp.1349–1357.

Tripathy, S.P. and Cavanagh, P., 2002. The extent of crowding in peripheral vision does not scale with target size. *Vision Research*, **42**(20), pp.2357–2369.

Tydgat, I. and Grainger, J., 2009. Serial Position Effects in the Identification of Letters, Digits, and Symbols. *Journal of Experimental Psychology: Human Perception and Performance*, **35**(2), pp.480–498.

Vaegan and Taylor, D., 1979. Critical period for deprivation amblyopia in children. *Transactions of the ophthalmological societies of the United Kingdom*, **99**(3), pp.432–9.

Vagge, A. and Nelson, L.B., 2017. Compliance with the prescribed occlusion treatment for amblyopia. *Current Opinion in Ophthalmology*, **28**(5), pp.454–459.

Vaina, L.M., Cowey, A. and Kennedy, D., 1999. Perception of first- and second-order motion: Separable neurological mechanisms? *Human Brain Mapping*, **7**(1), pp.67–77.

Vejnović, D. and Zdravković, S., 2015. Side flankers produce less crowding, but only for letters. *Cognition*, **143**, pp.217–227.

Vision in Preschoolers (VIP) Study Group, 2003. Threshold visual acuity testing of preschool children using the crowded HOTV and Lea Symbols acuity tests. *Journal of AAPOS*, **7**(6), pp.396–399.

Vlaskamp, B.N.S. and Hooge, I.T.C., 2006. Crowding degrades saccadic search performance. *Vision Research*, **46**(3), pp.417–425.

Wallis, T.S.A. and Bex, P.J., 2011. Visual crowding is correlated with awareness. *Current Biology*, **21**(3), pp.254–258.

Wang, J., 2015. Compliance and patching and atropine amblyopia treatments. *Vision Research*, **114**, pp.31–40.

Wang, P. and Nikolić, D., 2011. An LCD Monitor with Sufficiently Precise Timing for Research in Vision. *Frontiers in human neuroscience*, **5**, p.85.

Waugh, S., Pelli, D., Álvaro, L. and Formankiewicz, M., 2018. Crowding distance in healthy children. *Journal of Vision*, **18**(10), p.855.

Waugh, S.J. and Formankiewicz, M.A., 2019. Grouping of flankers is similar in children to adults and does not break crowding. *Journal of Vision*, **19**(10), p.119a.

Waugh, S.J., Lalor, S.J.H. and Hairol, M.I., 2010. Binocular summation for luminance- and contrast-modulated noise stimuli. *Journal of Vision*, **9**(8), pp.1012–1012.

Webber, A.L., 2018. The functional impact of amblyopia. *Clinical and Experimental Optometry*, **101**(4), pp.443–450.

Webber, A.L., Wood, J.M., Gole, G.A. and Brown, B., 2008a. Effect of amblyopia on self-esteem in children. *Optometry and Vision Science*, **85**(11), pp.1074–1081.

Webber, A.L., Wood, J.M., Gole, G.A. and Brown, B., 2008b. The effect of amblyopia on fine motor skills in children. *Investigative Ophthalmology and Visual Science*, **49**(2), pp.594–603.

Webber, A.L., Wood, J.M. and Thompson, B., 2016. Fine motor skills of children with amblyopia improve following binocular treatment. *Investigative Ophthalmology and Visual Science*, **57**(11), pp.4713–4720.

Wei, C.C. and Ma, M.Y., 2017. Influences of Visual Attention and Reading Time on Children

and Adults. *Reading and Writing Quarterly*, **33**(2), pp.97–108.

Westheimer, G., 1975. Visual acuity and hyperacuity. *Investigative Ophthalmology*, **14**(8), pp.570–572.

Westheimer, G., 1987. Visual acuity and hyperacuity: resolution, localization, form. *American journal of optometry and physiological optics*, **64**(8), pp.567–574.

Westheimer, G., 2012. Optical superresolution and visual hyperacuity. *Progress in Retinal and Eye Research*, **31**(5), pp.467–480.

Whitney, D. and Levi, D.M., 2011. Visual crowding: A fundamental limit on conscious perception and object recognition. *Trends in Cognitive Sciences*, **15**(4), pp.160–168.

Wiesel, T.N. and Hubel, D.H., 1963. Single-Cell Responses In Striate Cortex Of Kitten Deprived of Vision in One Eye. *Journal of Neurophysiology*, **26**(6), pp.1003–17.

Williams, C., 2009. Amblyopia. *BMJ Clinical Evidence*, **09**.

Williams, C., Harrad, R.A., Harvey, I. and Sparrow, J.M., 2001. Screening for amblyopia in preschool children: Results of a population-based, randomised controlled trial. *Ophthalmic Epidemiology*, **8**(5), pp.279–295.

Williams, C., Harrad, R.A., Sparrow, J.M., Harvey, I., Golding, J., Lee, J., Adams, G., Sloper, J., McIntyre, A., Fielder, A.R., Aylward, G.W., Rahi, J. and Dezateux, C., 1998. Future of preschool vision screening. *BMJ*, **316**(7135), pp.937–937.

Williamson, T.H., Andrews, R., Dutton, G.N., Murray, G. and Graham, N., 1995. Assessment of an inner city visual screening programme for preschool children. *British Journal of Ophthalmology*, **79**(12), pp.1068–1073.

Witton, C., Talcott, J.B. and Henning, G.B., 2017. Psychophysical measurements in children: challenges, pitfalls, and considerations. *PeerJ*, **5**, p.e3231.

Woi, P.J., Kaur, S., Waugh, S.J. and Hairol, M.I., 2016. Visual acuity measured with luminance-modulated and contrast-modulated noise letter stimuli in young adults and adults above 50 years old. *F1000Research*, **5**, p.1961.

Woi, P.J., Sharanjeet-Kaur and Hairol, M.I., 2019. Binocular and monocular resolution thresholds throughout adulthood for luminance-modulated and contrast-modulated

noise letters. *Sains Malaysiana*, **48**(10), pp.2185–2190.

Wolford, G. and Chambers, L., 1983. Lateral masking as a function of spacing. *Perception & Psychophysics*, **33**(2), pp.129–138.

Wong, E.H. and Levi, D.M., 2005. Second-order spatial summation in amblyopia. *Vision Research*, **45**(21), pp.2799–2809.

Wong, E.H., Levi, D.M. and McGraw, P. V., 2001. Is second-order spatial loss in amblyopia explained by the loss of first-order spatial input? *Vision Research*, **41**(23), pp.2951–2960.

Wong, E.H., Levi, D.M. and McGraw, P. V., 2005. Spatial interactions reveal inhibitory cortical networks in human amblyopia. *Vision Research*, **45**(21), pp.2810–2819.

Woodhouse, J.M., Adoh, T.O., Oduwaiye, K.A., Batchelor, B.G., Megji, S., Unwin, N. and Jones, N., 1992. New acuity test for toddlers. *Ophthalmic and Physiological Optics*, **12**(2), pp.249–251.

Woodruff, G., Hiscox, F., Thompson, J.R. and Smith, L.K., 1994. Factors affecting the outcome of children treated for amblyopia. *Eye*, **8**(6), pp.627–631.

Woodruff, M.E., 1972. Observations on the visual acuity of children during the first five years of life. *Optometry and Vision Science*, **49**(3), pp.205–214.

Wu, C. and Hunter, D.G., 2006. Amblyopia: Diagnostic and therapeutic options. *American Journal of Ophthalmology*, **141**(1).

Wyatt, A.M., Clifford, C.W.G. and Wenderoth, P., 2001. Hierarchy of spatial interactions in the processing of contrast-defined contours. *JOSA A, Vol. 18, Issue 9, pp. 2190-2196*, **18**(9), pp.2190–2196.

Yeshurun, Y. and Rashal, E., 2010. Precueing attention to the target location diminishes crowding and reduces the critical distance. *Journal of Vision*, **10**(10), pp.1–12.

Yildirim, F., Meyer, V. and Cornelissen, F., 2015. Eyes on crowding: Crowding is preserved when responding by eye and similarly affects identity and position accuracy. *Journal of Vision*, **15**(2015).

Yu, D., Cheung, S.H., Legge, G.E. and Chung, S.T.L., 2007. Effect of letter spacing on

visual span and reading speed. *Journal of Vision*, **7**(2), pp.2–2.

Yuodelis, C. and Hendrickson, A., 1986. A qualitative and quantitative analysis of the human fovea during development. *Vision Research*, **26**(6), pp.847–855.

Zenger, B. and Sagi, D., 1996. Isolating Excitatory and Inhibitory Nonlinear Spatial Interactions Involved in Contrast Detection. *Vision Research*, **36**(16), pp.2497–2513.

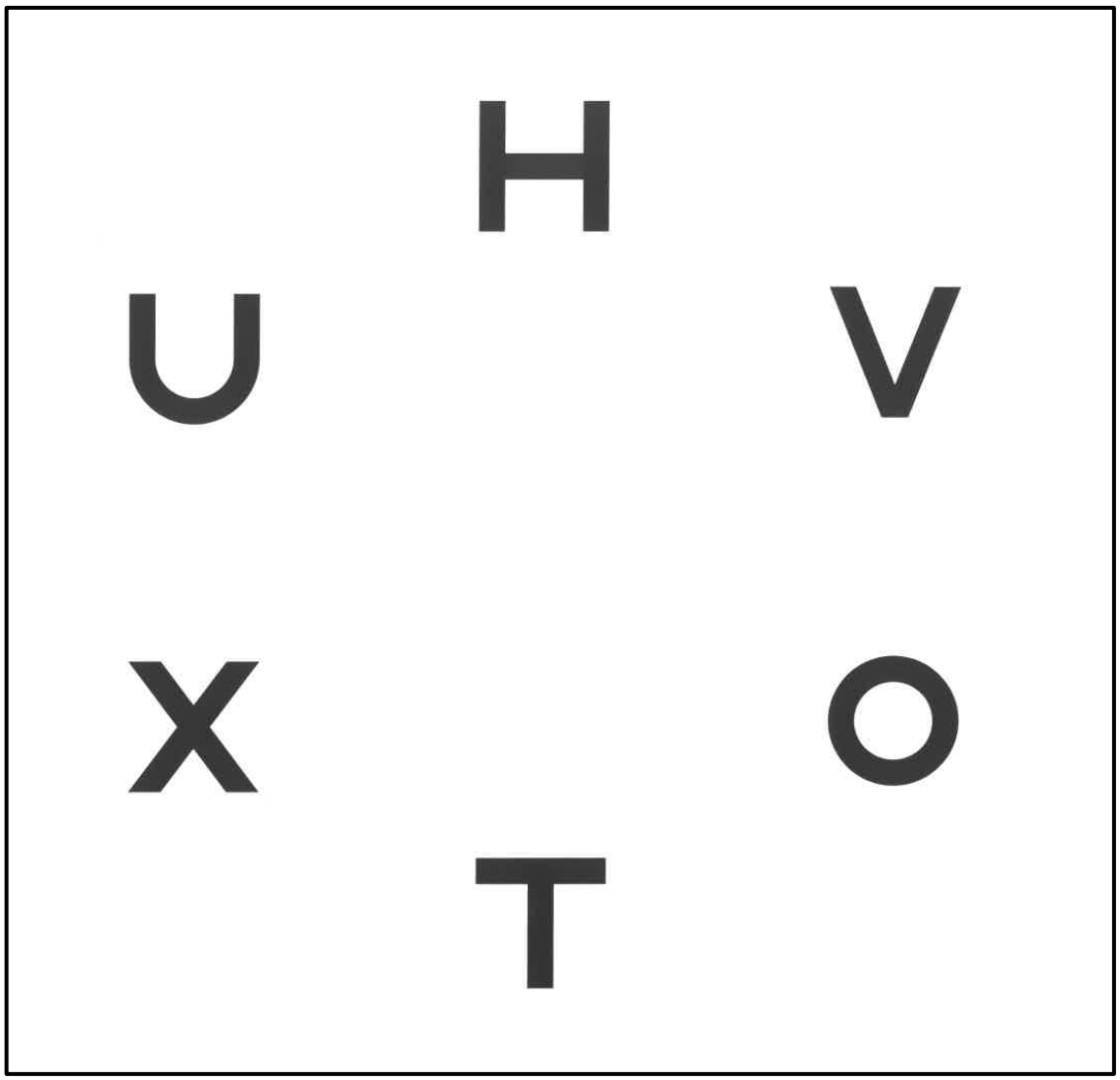
Zhang, J.Y., Zhang, T., Xue, F., Liu, L. and Yu, C., 2009. Legibility of Chinese characters in peripheral vision and the top-down influences on crowding. *Vision Research*, **49**(1), pp.44–53.

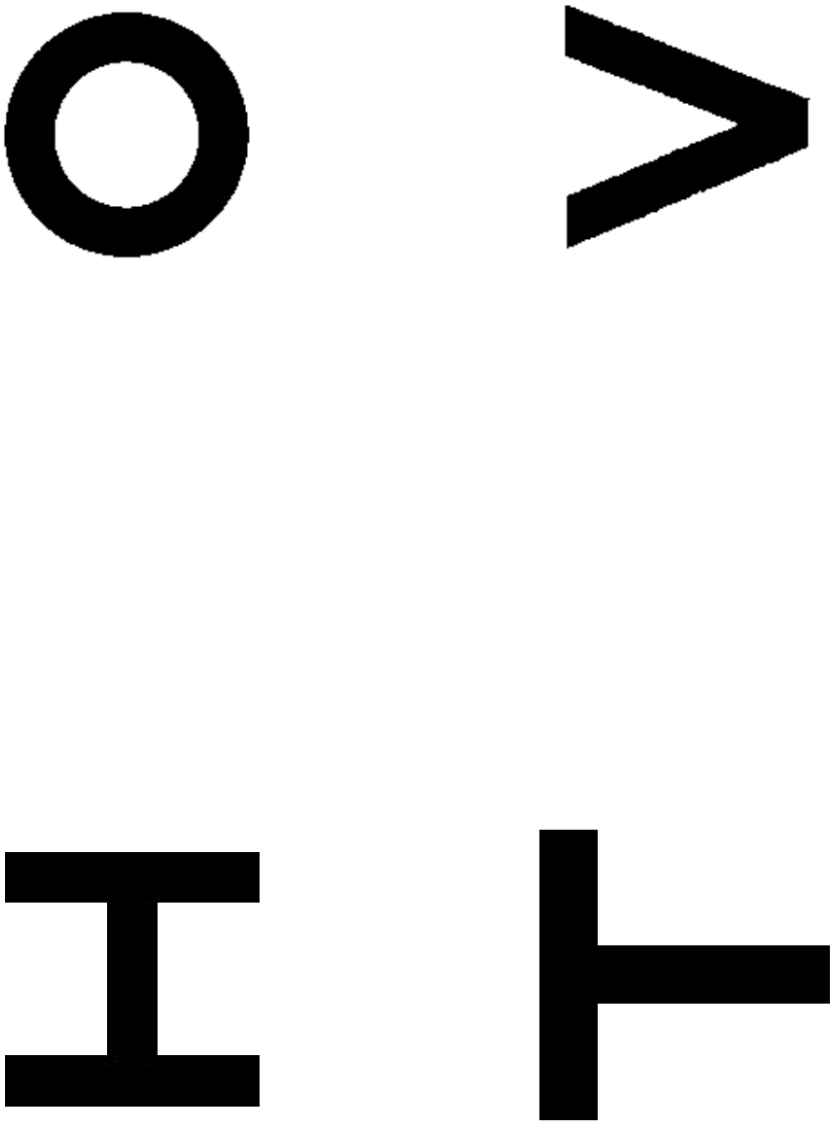
Zhou, Y.X. and Baker, C.L., 1994. Envelope-responsive neurons in areas 17 and 18 of cat. *Journal of Neurophysiology*, **72**(5), pp.2134–2150.

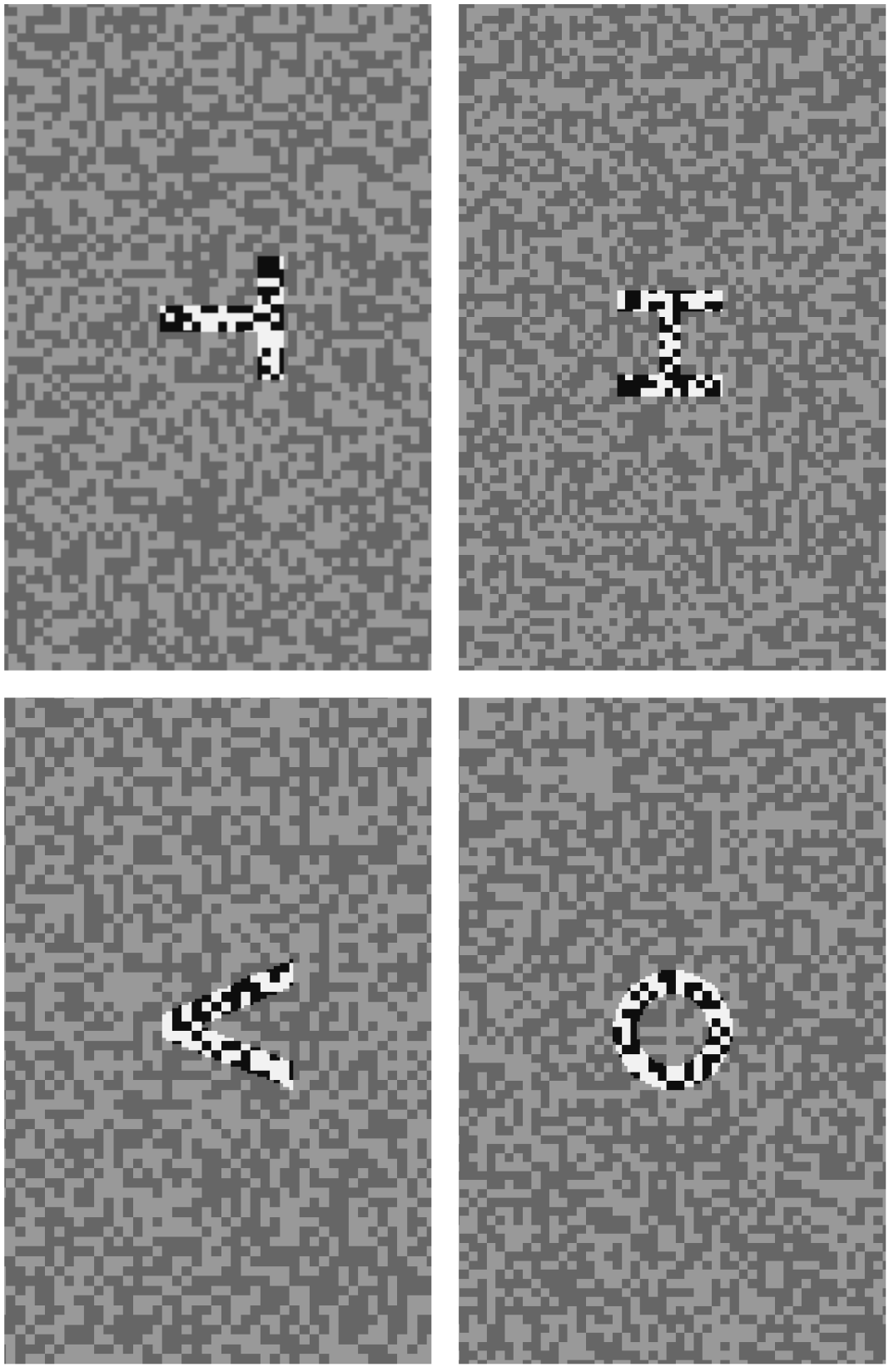
Appendices

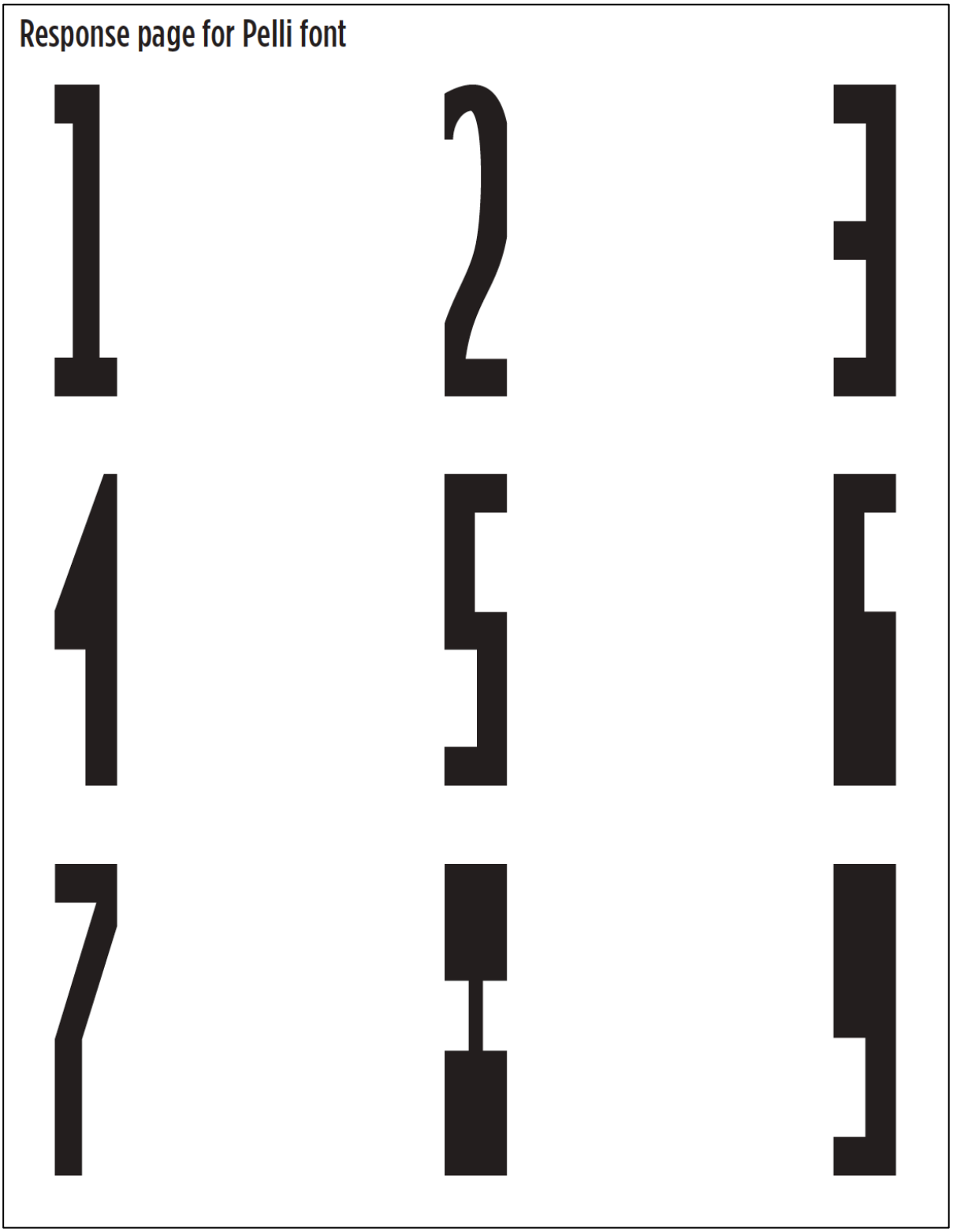
Appendix One: Matching Cards

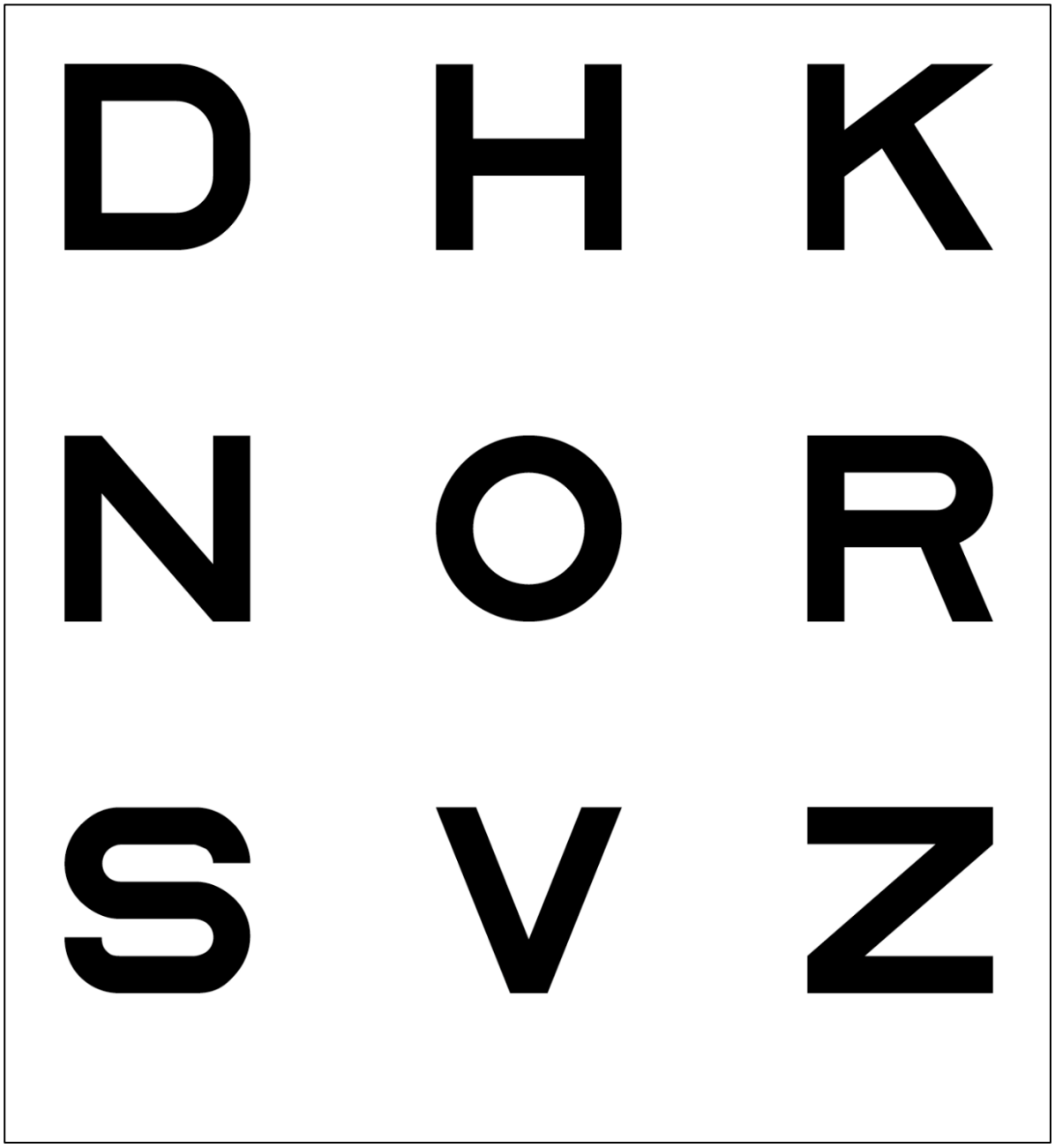
- Sonksen logMAR test matching card
- L-ECC matching card
- CM-ECC matching card
- Pelli crowding distance test matching card
- Sloan letter matching card


H
U
V
X
O
T






Response page for Pelli font


D H K
N O R
S V Z

Appendix Two– Guardian information sheet

Cambridge University Hospitals NHS
NHS Foundation Trust

a.r.u. | Anglia Ruskin University

INFORMATION FOR PARENTS / GUARDIANS

Title of Project: New visual acuity and crowding tests for better detection of amblyopia.

Secondary title: Examining new vision tests for better detection of childhood visual loss.

IRAS project ID number: 238449

We would like to invite your child to take part in our research study. Before you as a parent / guardian decide, we would like you to understand why the research is being done and what it would involve for you and your child.

One of our team will go through the information sheet with you and answer any questions you have.

Please talk to others about the study if you wish, and decide whether or not you and your child would like to take part. Ask us if there is anything that is not clear.

What is the purpose of the study?

This study aims to investigate whether new changes to vision tests will offer better sensitivity for detecting amblyopia (or “lazy eye”) compared to what is currently used.
Your child has been invited to participate because they have been diagnosed with amblyopia or have no visual concerns, and they are the right age for this study.

Who is doing the research?

This research is being conducted by Mrs Louisa Haine under the supervision of Dr Sarah J Waugh within the discipline of Vision and Hearing Sciences at Anglia Ruskin University. Mrs Haine is a qualified orthoptist and Dr Sarah Waugh is a consultant optometrist and the Anglia Ruskin University Academic Lead for ACPOS (Addenbrooke’s Community Paediatric Ophthalmology Service) at the University Eye Clinic.

Does my child have to take part?

No, it is entirely up to your child and you, whether or not you decide to participate. If you do decide to take part, both you and your child will be given time to discuss the study with a research member of staff and to ask any questions. As a parent / guardian, you will be asked to provide written consent, and your child will be asked if they want to participate, before we commence with the research project.

What if myself or my child change our minds?

Your child can withdraw from the study at any time, without having to give a reason. There are no negative consequences to yourself or your child should you wish to withdraw. Any data already collected will be retained by the researchers, however it will be anonymised so that no-one would be able to identify information about any particular individual.

What do we have to do?

You and your child would be required to visit the Anglia Ruskin University Eye Clinic, ideally following their routine NHS eye exam, if required, to participate in the research. The research only requires one single visit. It would be valuable to us if we could repeat the research after additional routine future follow up clinical appointments, if required, however this is completely optional, and identical to the first visit. If your child does not attend the hospital eye clinic, your child will have their vision and 3D vision measured by a qualified orthoptist prior to commencing the research.

During the research session, your child will participate in a number of computer generated vision tests. They will be asked to identify the letters / numbers either verbally or by matching what they see on a card. Each eye will be tested. The tests are designed to be engaging and fun, and we use stickers, stamps and praise to encourage your child.

The results of these computer vision tests will be compared to the results of the vision test that your child performed during their routine clinical eye appointment. We ask that you consent to us as researchers to ask your child's clinician about these, and access their clinic notes. If during research any 'incidental findings' are found with these new 'un-validated' tests, then these will be reported to your child's medical team (with your consent) who can re-examine your child's clinically validated results where needed. Please ask the researcher if you would like further clarification regarding this.

What are the possible disadvantages and risks / side effects of taking part?

Please advise the researcher if you are concerned that you or your child are at high (clinically vulnerable) or very high risk (clinically extremely vulnerable) of contracting COVID-19, so that we can consider excluding you from this research study. If you have appointments in ACPOS this is unlikely, but we are happy to discuss this with you. We take the same precautions to minimise risks for all of our research participants as are taken in ACPOS,.

The examination would require you and your child to remain at the Anglia Ruskin University Eye Clinic, for 30 minutes to 1 hour after the conclusion of your NHS appointment, or we could organise a separate visit if you would like us to. The computer safety manual lists several medical conditions, which could possibly be "affected by viewing the computer screen", however these are equivalent to viewing a television or using a computer at home. One of the vision tests uses a moving

background which presents as a flickering / twinkling image, like static on a television. Therefore as a precaution, individuals with known epilepsy are excluded from this study.

Due to current COVID-19 regulations, hand washing and face masks are required prior to, and during assessment (although young children are encouraged, rather than required to wear a mask). Social distancing between the researcher and participants will also be adhered to, wherever possible.

If you are concerned about any potential risks, please ask and we can provide you and your child with more information.

What are the possible benefits of taking part?

This study will not directly benefit your child's vision, but your child will be contributing to our research, which is aimed at understanding and better detecting amblyopia ('lazy eye') for the future. We won't know the results of our tests until the conclusion of the project, however if we feel that any of our findings might benefit your child's individual clinical care, with your consent, we will inform your child's clinical team.

What if new information becomes available?

Sometimes in the course of a research project new information about the topic under investigation becomes available. If this occurs, we will be happy to discuss it with you, so please ask if you have questions at any time. You are always free to continue participating in our study, or you may wish to withdraw without any negative consequence.

What will happen when the research study is completed?

If you would like us to, we will send written information about our study's overall findings to you once it is completed, in clear, understandable language.

What if something goes wrong?

There are minimal risks involved in your child taking part, similar to those experienced during a normal eye test, or in watching television. If someone is harmed during the study, there are no special arrangements for compensation. If you have any concerns about this study please e-mail Mrs Louisa Haine at louisa.haine@pgr.anglia.ac.uk or phone her on 01223 698584. Alternatively email her Supervisor Dr Sarah J Waugh at sarah.waugh@anglia.ac.uk or phone her on 01223 698386. We will do our best to answer your questions.

Will data collected in this study be kept confidential?

Your contact details will be recorded by the researcher in compliance with ARU Privacy information guidelines and the government track-and-trace system for management of COVID-19 and your welfare. More information about this can be found here: https://aru.ac.uk/privacy-and-cookies/COVID-19-management

All personal information collected about your child for the purposes of the research project will be treated in strictest confidence. It will be stored securely during the project, accessible only to members of the research team, after which physical copies will be destroyed (6 months after the conclusion of the study). Digital anonymised copies will be encrypted, stored on University protected space for up to 20-years, and only accessible to the research team.

If your child joins the study, some parts of their records and data collected will be looked at only by authorised persons (the researcher and members of your child's clinical team). The hospital eye care team and your child's GP will be notified that your child is taking part in the research study, but they will not know about the specific results generated.

What will happen to the results of the research study?

The data measured on your child will be stored using an unidentifiable code from the outset, so that no-one outside the research team will know from which person the data comes from. Results of this study may be reported at scientific meetings or appear in scientific publications, but your child's data will never be identifiable to them personally. It will be averaged with data from others, and will only use an anonymous code as an identifier. Anonymised data only will be stored digitally, where it can be shared for the benefit of the future researchers and may be used for future analysis and additional research.

Who is organising and funding the research?

The project is organised and funded by Anglia Ruskin University, Cambridge. Some funding is available to offer you a £10 Amazon voucher as compensation for your time each time you participate.

Who has reviewed the study?

Ethical approval for this study has been given by an independent group of people, called a Research Ethics Committee, to protect you and your child's safety, rights, wellbeing and dignity. This study has been reviewed by Anglia Ruskin University's Faculty of Science and Technology Research Ethics Panel, and also by an NHS REC panel. If you have any complaints or concerns about your childs treatment before, during or after this study then you should contact the Chair of the Faculty of Science and Technology Research Ethics Committee, Professor Peter Bright (peter.bright@aru.ac.uk).

Contact for further information:

If you or your child have any questions regarding any aspects of this study, please e-mail Mrs Louisa Haine at lah222@pgr.aru.ac.uk or phone her on 01223 698584. Alternatively, please email her Supervisor Dr Sarah J Waugh at sarah.waugh@aru.ac.uk or phone her on 01223 698386.

Thank you for taking time to read this information

YOU WILL BE GIVEN A COPY OF THIS FORM TO KEEP, TOGETHER WITH A COPY OF YOUR CONSENT FORM.

The conduct of this study has been approved by the Faculty of Science and Technology Research Ethics Panel (FREP), Anglia Ruskin University, and by an NHS Research Ethics Committee (REC).

Appendix Three – Participant information sheet

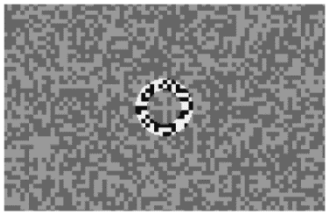
Participant Information Sheet

Title: 'New visual acuity and crowding tests for better detection of amblyopia.'

IRAS project ID: 238449

Hello,

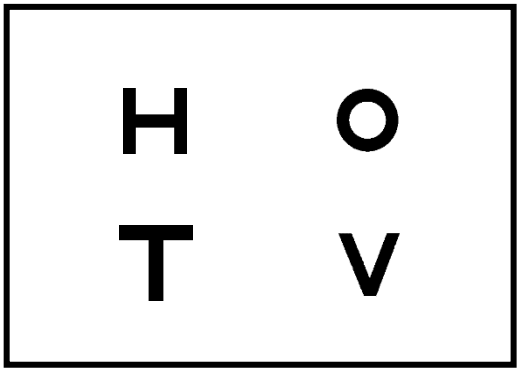
I am learning about how some children's eyes see different letters and numbers. I want to try to help improve the eye tests we use and would like you to help me.



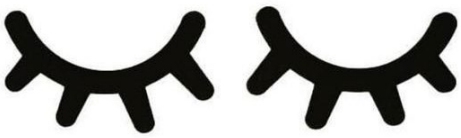
I would like you to look at some of these letters and numbers on a computer screen with one eye covered and tell me what you think they are. You may also look at some letters on a chart and some 3D pictures.

If you normally wear glasses, you should keep them on.

Do not worry if you cannot remember the name of the number or letter, because you can point to it on a card instead. If you need to guess, that is ok too.



All of these tests are safe and should not hurt your eyes, but we will have lots of rests between your games to make sure you do not get tired.



Have a talk with your parent / guardian about whether you would like to join in and if you have any other questions, do please ask.

Thank you for your help.

Louisa Haine: LAH222@pgr.aru.ac.uk
Tel: 01223 698584
My Teacher:
Dr Sarah J Waugh: sarah.waugh@aru.ac.uk
Tel: 01223 698 685

Appendix Four – Participant Assent form

Child's Assent form

(To be read aloud to the child)

Title: 'New visual acuity and crowding tests for better detection of amblyopia.'

IRAS project ID: 238449

Hi. My name is **Louisa.** I am a scientist who is trying to learn about how we see. I am interested in how children like you see letters and numbers on a vision chart.

If you agree to be in my study, you will play a game looking at pictures of some letters and numbers on a computer screen. You may also look at letters on a chart and some 3D shapes. Sometimes they will be easy to see and sometimes hard to see.

I will ask you to tell me what you see, or you can just point to find a similar picture on my card. You will only be asked to do this for a few minutes at a time, and we will have lots of rests in between.

There are no wrong answers. Just tell me what you think you see.

By being in our study, you will be helping me to understand how children who have eyes like yours, see.

Please have a think, or talk to your parent/carer to help you decide if you want to help us. I will also ask your parent if it is OK for you to take part, but even if they say "yes," you can still say "no" if you don't want to. No one will be upset if you don't.

Even if you want to start, and then decide to stop, that is okay too. Also, remember that no one else outside the room will know what you have said.

Do you have any questions that you would like to ask me?

If you have a question later that you did not think of now, you can call me or my teacher, Dr Waugh, at (01223) 698 386 or email her at sarah.waugh@aru.ac.uk .

Would you like to be a part of my study and look at some pictures now?

☐ Participant gives clear verbal assent (and can tick the box if they want to) in presence of Guardian. Only a definite yes will be taken as consent to participate.

Date .....

The conduct of this research project has been reviewed and approved by the Faculty of Science and Technology Research Ethics Panel (FREP), Anglia Ruskin University, and a NHS ethics panel

Appendix Five– Parent/Guardian consent form

Cambridge University Hospitals NHS
NHS Foundation Trust

a.r.u. | Anglia Ruskin University

CONSENT FORM FOR PARENT/GUARDIAN

Project Title: New visual acuity and crowding tests for better detection of amblyopia.
IRAS project ID number: 238449 **Name of child:** _____

Main investigator and contact details: Mrs Louisa Haine, Postgraduate Researcher, Anglia Vision Research, Vision and Hearing Sciences, Tel: 01223 698 584, Email:lah222@pgr.aru.ac.uk

Research Supervisor: Dr Sarah J Waugh, Tel: 01223 698 386, Email:sarah.waugh@aru.ac.uk

1. I agree for my child/children to take part in the above research. I have read the information sheets provided (Version 5.0 – 10/10/20). I understand what my child's role will be, and all my questions and those of my child have been answered to our satisfaction.

2. I understand that I am free to withdraw my child from the research at any time, for any reason and without prejudice.

3. I understand that I am, and my child is, free to ask questions at any time during this study.

4. I have been provided with a copy of this form, the Parent's Information Sheet and the Child's Participant Information Sheet.

5. For the purposes of this research, I consent to the researchers contacting my child's ophthalmology clinical team and reviewing the medical notes, to find out information that relates to my child's visual status, spectacle prescription and binocular vision status; and for my child's GP to be informed of their participation.

6. I have been informed that the confidentiality of the information provided on behalf of my child will be safeguarded. I understand that data collected from this study will be anonymised and used for publication and sharing only in anonymised format.

7. I consent to my contact details being passed to 'track and trace', for the purposes of COVID-19 monitoring, if required.

Data Protection: I agree to the University processing personal data which I have supplied. I agree to the processing of such data for any purposes connected with the Research Project as outlined to me.

Name of parent or guardian (print)…….. ……...

Signed……..…… Date……

PLEASE SIGN AND RETURN ONE COPY AND KEEP THE OTHER

If you wish to withdraw your child from this study, please speak to the researcher or email her at lah222@pgr.aru.ac.uk or her research supervisor at sarah.waugh@aru.ac.uk stating the title of the research. You do not have to give a reason for why you would like to withdraw.

The conduct of this study has been approved by the Faculty of Science and Technology Research Ethics Panel (FREP), Anglia Ruskin University, and by an NHS Research Ethics Committee (REC).

Appendix Six - Amblyope participant background data

Participant number	Age (Years)	Gender	Ethnicity	Recruited group	Refraction prescribed	Age at start of refractive adaptation	Patching/occlusion treatment prescribed	Atropine occlusion treatment prescribed	Optical penalisation treatment prescribed	Age at start of amblyopia treatment	Strabismus surgery?	Type of strabismus surgery	Age at strabismus surgery
1	3	M	White british	Anisometropic	Y	2	Y	N	N	3	N		
4	9	M	White british	Strabismic	N		Y	Y	N	9	N		
9	6	M	White british	Anisometropic	Y	5	N	N	N		N		
10	8	M	White british	Strabismic (mixed)	Y	4	Y	Y	N	5	N		
11	5	F	White british	Anisometropic	Y	5	Y	N	N	5	N		
13	5	F	White british	Anisometropic	Y	1	Y	N	N	5	N		
14	6	M	White british	Strabismic (mixed)	Y	2	Y	N	N	3	N		
20	6	F	White Other	Anisometropic	Y	5	Y	N	N	5	N		
21	6	F	White british	Strabismic (mixed)	Y	2	Y	N	N	2	Y	Bilateral recession of medial recti muscles	3

Participant number	Age (Years)	Gender	Ethnicity	Recruited group	Refraction prescribed	Age at start of refractive adaptation	Patching/occlusion treatment prescribed	Atropine occlusion treatment prescribed	Optical penalisation treatment prescribed	Age at start of amblyopia treatment	Strabismus surgery?	Type of strabismus surgery	Age at strabismus surgery
22	5	F	White british	Anisometropic	Y	4	Y	N	N	5	N		
25	8	M	White british	Strabismic (mixed)	Y	not recorded	Y	N	N	not recorded	N		
26	6	M	White british	Anisometropic	Y	5	Y	N	N	6	N		
27	6	F	White british	Anisometropic	Y	5	Y	N (prescribed but not used)	N	5	N		
29	7	M	White british	Strabismic	Y	3	Y	N	N	3	N		
30	8	F	White british	Strabismic	Y	3	Y	N	N	3	N		
31	5	F	White british	Anisometropic	Y	5	N	N	N		N		
32	6	M	White british	Anisometropic	Y	4	Y	N	N	5	N		
34	6	M	White british	Anisometropic	Y	5	N	N	N		N		

Participant number	Age (Years)	Gender	Ethnicity	Recruited group	Refraction prescribed	Age at start of refractive adaptation	Patching/occlusion treatment prescribed	Atropine occlusion treatment prescribed	Optical penalisation treatment prescribed	Age at start of amblyopia treatment	Strabismus surgery?	Type of strabismus surgery	Age at strabismus surgery
35	5	M	White british	Anisometropic	Y	5	N	N	N		N		
39	5	M	White british	Strabismic (mixed)	Y	4	Y	N	N	4	N		
40	6	F	White british	Strabismic (mixed)	Y	1	Y	N	N	3	N		
42	8	F	White british	Strabismic	Y	1	Y	Y for refractive adaptation, but N for amblyopia treatment	N	3	N		
43	6	F	White british	Anisometropic	Y	3	Y	N	N	5	N		
52	7	M	White british	Strabismic (mixed)	Y	2	Y	N	N	2	Y	Recession of medial rectus muscle and resection of lateral rectus muscle of L eye	4
53	6	M	White british	Strabismic (mixed)	Y	5	Y	N	N	5	N		
54	5	F	Chinese	Anisometropic	Y	4	Y	N	N	6	N		
55	6	M	White british	Strabismic (mixed)	Y	4	Y	N	N	5	Y	R inferior oblique transposition	4

Participant number	Age (Years)	Gender	Ethnicity	Recruited group	Refraction prescribed	Age at start of refractive adaptation	Patching/occlusion treatment prescribed	Atropine occlusion treatment prescribed	Optical penalisation treatment prescribed	Age at start of amblyopia treatment	Strabismus surgery?	Type of strabismus surgery	Age at strabismus surgery
56	6	M	Not stated	Anisometropic	Y	6	N	N	N		N		
57	7	M	White british	Strabismic (mixed)	Y	2	Y	Y	Y	3	N		
58	5	F	White british	Anisometropic	Y	5	N	N	N		N		
60	7	F	White british	Anisometropic	Y	5	Y	N	N	6	N		
61	4	F	White british	Strabismic (mixed)	Y	3	Y	N	N	3	N		
62	6	M	White british	Anisometropic	Y	2	Y	N	N	4	N		
63	4	M	White british	Anisometropic	Y	4	Y	Y	N	4	N		
65	6	M	Not stated in notes	Anisometropic	Y	5	Y	N	N	6	N		
66	7	F	White british	Strabismic	Y	3	Y	N	N	5	N		

Participant number	Age (Years)	Gender	Ethnicity	Recruited group	Refraction prescribed	Age at start of refractive adaptation	Patching/occlusion treatment prescribed	Atropine occlusion treatment prescribed	Optical penalisation treatment prescribed	Age at start of amblyopia treatment	Strabismus surgery?	Type of strabismus surgery	Age at strabismus surgery
67	10	F	White british	Anisometropic	Y	9	Y	N	N	9	N		
68	5	F	White british	Anisometropic	Y	5	Y	N	N	5	N		
69	5	M	White british	Strabismic (mixed)	Y	2	Y	Y	N	2	N		
70	10	M	White british	Strabismic	Y	Unclear but definitely by age 6	Y	N	N	Unclear but definitely by age 6	N		
72	5	F	White british	Strabismic	Y	1	Y	N	N	2	N		
73	7	F	White british	Strabismic (mixed)	Y	5	Y	N	N	5	N		
74	4	F	White british	Strabismic (mixed)	Y	3	Y	Y	Y	3	N		
76	8	M	White british	Strabismic (mixed)	Y	0·2 (notes prior to epic)	Y	N	N	0·2 (notes prior to epic)	Y	Bimedial recessions	4

Appendix Seven - Control vs Amblyope L-ECC crowding magnitude data

- Effect of group
- Effect of eye

Main effect of group

Planned analysis examining the main effects of group (controls vs amblyopes) showed no statistically significant differences in crowding magnitudes for amblyopic eyes compared with right eyes ($p>.05$ for all test formats) or fellow eyes compared with control left eyes ($p>.05$ for all test formats)(Table A7-1). Amblyopic eyes and fellow eyes did not demonstrate significantly larger crowding magnitude than control eyes.

Table A7-1: *Contrast test examining the crowding magnitude effects of two test groups (controls and amblyopes) on each test format (Isolated, SLT and L-ECC) per eye (Right eye and amblyopic eye, left eye and fellow eye)*

	df	**t**	**Sig**
SLT format RE/AE	65	0.758	0.451
SLT format LE/FE	65	1.499	0.139
L-ECC format RE/AE	65	-1.891	0.063
L-ECC format LE/FE	65	-1.294	0.200

Main effect of eye

Planned analysis examining the main effects of eye within two groups (controls vs amblyopes), showed no statistically significant differences in crowding magnitudes for right eyes compared with left eyes ($p>.05$ for all test formats) or amblyopic eyes compared with fellow eyes ($p>.05$ for all test formats) (Table A7-2).

Table A7-2: *Repeated measures ANOVA examining simple main effects of eye (RE, LE, amblyopic eye and fellow eye) for each group (controls and amblyopes).*

	df	**F**	**Sig**	**μ^2**
Controls	1	0.001	0.974	0.000
Error	23			
Amblyopes	1	0.626	0.433	0.014
Error	43			

Appendix Eight - Control vs Amblyope L-ECC acuity threshold data

- Effect of group

Main effect of group

Planned analysis examining the main effects of group (controls vs amblyopes) showed that significantly larger acuity thresholds occurred for amblyopic eyes compared with right eyes for all test formats (**p<.001** for all formats) and significantly larger thresholds for fellow eyes compared with control left eyes (Isolated format, **p=.002**; SLT format, **p=.025**; L-ECC format, **p<.001**) (Table A8-1). Post hoc analysis demonstrated significantly poorer thresholds for amblyopic eyes compared with control right eyes for all test formats (Isolated: +0.374±0.051, SLT: +0.352±0.043, L-ECC: +0.431±0.056, **p<.001 respectively**), and significantly poorer thresholds were seen for fellow eyes compared with control left eyes (Isolated: +0.092±0.031, **p=.004**; SLT: +0.054±0.022, **p=.020**; L-ECC:+0.127±0.030, **p<.001**) (Figure A8-1).

Table A8-1: *Contrast test examining effects of two test groups (controls and amblyopes) on each test format (Isolated, SLT and L-ECC) per eye (Right eye and amblyopic eye, left eye and fellow eye)*

	df	**t**	**Sig**
Isolated format RE/AE	**51.160**	**-8.699**	**<0.001**
Isolated format LE/FE	**56.406**	**-3.201**	**0.002**
SLT format RE/AE	**51.039**	**-10.507**	**<0.001**
SLT format LE/FE	**43.734**	**-2.315**	**0.025**
L-ECC format RE/AE	**47.032**	**-10.130**	**<0.001**
L-ECC format LE/FE	**49.242**	**-4.383**	**<0.001**

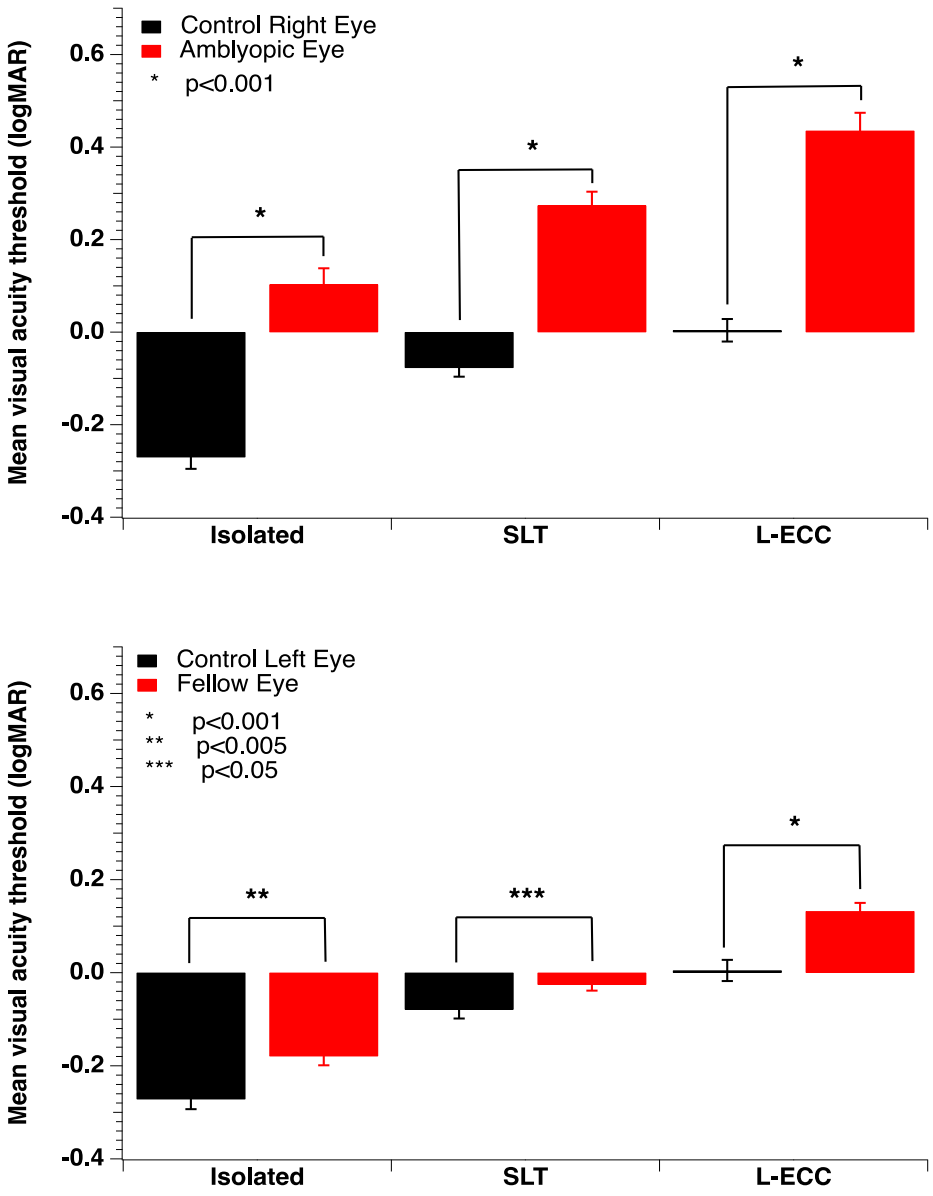


Figure A8-1: *Mean visual acuity thresholds for control amblyopic eyes, with significance bars. Top – Mean visual acuity thresholds per test for control right eyes and amblyopic eyes. Middle – Mean visual acuity thresholds per test for control left eyes and fellow eyes.*

Appendix Nine - Control vs Amblyope L-ECC IOD data

- Two-way mixed ANOVA

- Effect of group

Two-way mixed ANOVA

A 2 (group) x 3 (test format) repeated measures ANOVA revealed no two-way interaction between group (controls and amblyopes) and test-format (isolated, SLT, and L-ECC) [*F*(1.6, 105.614) = 3.102, p=.083) (Table A9-1 and Figure A9-1), and no significant main effect of test (isolated, SLT and L-ECC) was seen [*F*(1.6, 105.614) = 1.557, p=.218]. Main effect of group is explored below.

Table A9-1: *Repeated measures ANOVA examining interaction and main effects of three test formats (Isolated, SLT, and L-ECC) and two test groups (controls and amblyopes) on IOD.*

	df	**F**	**Sig**	**μ^2**
Test format	1.6	1.557	0.218	0.023
Error	105.614			
Group	**1**	**33.874**	**<0.001**	**0.339**
Error	66			
Test format * group	1.6	3.102	0.083	0.045
Error	105.614			

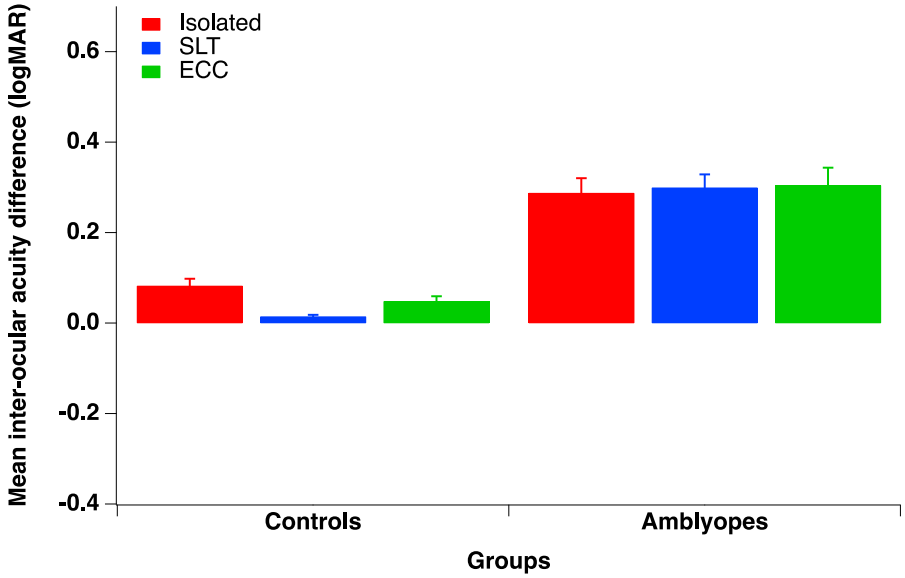


Fig A9-1*: Mean interocular acuity differences averaged format (isolated format, SLT format and L-ECC format) for each group (controls and amblyopes). Error bars show ±1SE.*

Main effect of group

Planned analysis examining the main effect of group (controls vs amblyopes) showed significant differences in IODs occurring for all test formats (**p<.001** for all formats) (Table A9-2). Overall, amblyopic participants were associated with a mean IOD of +0.249±0.043 logMAR (95% CI, 0.163 to 0.334) higher than control participants, at a statistically significant level, ***p*<.001.** Post hoc analysis demonstrated significantly larger IODs for amblyopic eyes compared with control eyes for all test formats (Isolated: +0.205±0.046, SLT: +0.285±0.040, L-ECC: +0.257±0.053, **p<.001 respectively**) (Figure A9-2).

Table A9-2: *Contrast test examining effects of two test groups (controls and amblyopes) within each test format (Isolated, SLT and L-ECC) on IOD.*

	df	t	Sig
Isolated format	**49.485**	**-5.625**	**<0.001**
SLT format	**27.289**	**-10.317**	**<0.001**
L-ECC format	**33.694**	**-7.243**	**<0.001**

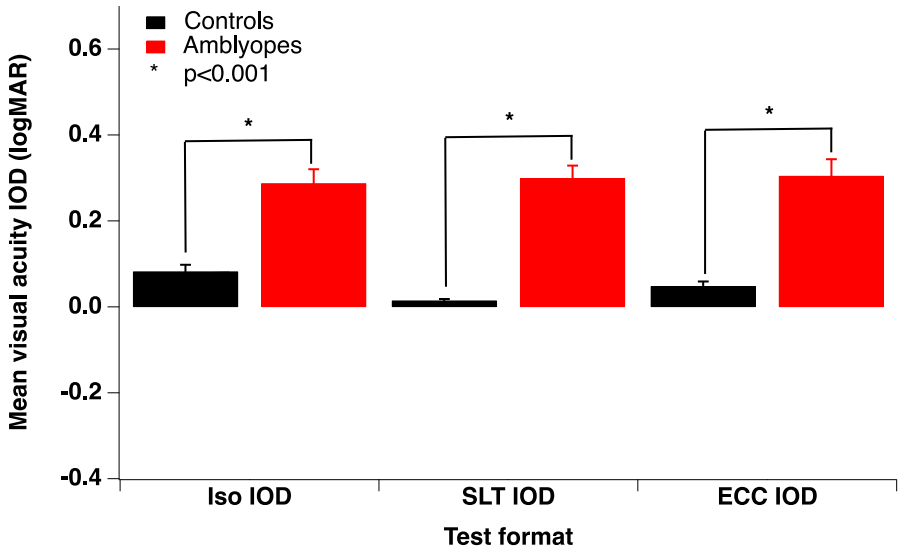


Figure A9-2: *Mean IOD for each acuity test (Isolated, SLT and L-ECC) per group (controls and amblyopes), with significance bars. Error bars show ±1SE*

Appendix 10 – VSS 2021 Poster

Simplifying the repeated-letters crowding test for normal and amblyopic children

Louisa A Haine[1], Sarah J Waugh[1, 2], Monika A Formankiewicz[1], and Denis G Pelli[3]

[1] Anglia Vision Research, Anglia Ruskin University, [2] Department of Optometry and Vision Sciences, University of Huddersfield, [3] Psychology Department, NYU

***SUMMARY**: Crowding is potentially more sensitive than acuity in screening children for amblyopia. Children, especially amblyopic children, have trouble fixating. Repeating the target allows the crowding test to tolerate fixation errors. However, full-screen repetition alarms some children and increases variance. Happily, repetition along one line achieves both fixation-error tolerance and minimum variance.*

INTRODUCTION. We want to track development of crowding in normal and amblyopic children. Crowding distance increases linearly with eccentricity, making accurate fixation essential, yet young and especially amblyopic children, find fixation challenging. For acuity measurement, Regan *et al*, (1992) suggested repeating letters over a large area so one letter would always be viewed foveally, regardless of fixation ability.

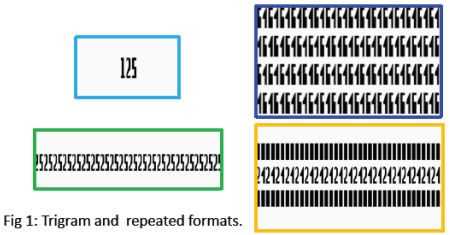


Fig 1: Trigram and repeated formats.

Pelli *et al* (2016) applied the same idea to crowding by presenting repeating lines of alternating optotypes across the screen (Fig. 1). Waugh *et al* (2018) measured visual acuity (with isolated Sloan letters) and crowding distance (using top two Fig. 1 formats) in 201 visually healthy children aged 3-11 yrs. In that study, 8% of children were noted to be alarmed when confronted with a screen full of optotypes, even though they were repetitions of just two. Staircase variability (or Standard Deviation, SD, in %) of crowding distance was greater for the full-screen repeated, than for the trigram format (p=.020) or for acuity estimates (p=.000025) (Fig. 2).

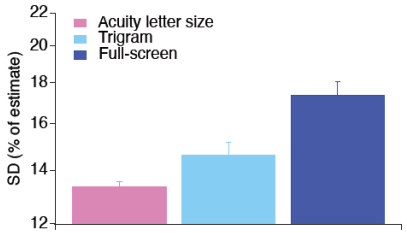


Fig 2: Variability of acuity and crowding distance estimates.

METHODS. Crowding distances (deg) with Pelli optotypes were measured with trigrams and randomly interleaved presentations of "repeated" formats (single-line, single-line-with-bars, full-screen) (Fig. 1). Size covaried with spacing to maintain a spacing-to-width ratio of 1.4:1. Visual acuity (deg) was measured with isolated Sloan letters. 20 trials of 9AFC optotype identification, were guided by QUEST to estimate thresholds for size and centre-to-centre spacing. Staircase variability SD (%) was also monitored where:

$$SD(\%) = \frac{SD\ (\text{deg})}{\textit{Crowding or Letter size threshold}\ (\text{deg})} \times 100$$

PARTICIPANTS AND PROCEDURE. After autorefraction to rule out undiagnosed refractive error, visual acuity and crowding distance were measured on 28 visually healthy children (aged 3 to 11 years) and 6 adults. Targets were named verbally or identified on a matching card. Written consent (adults and parents/guardians) and verbal assent (children) was obtained before testing.

RESULTS. In line with Waugh, *et al* (2018), acuity is adult-like at age ~6 yrs, developing less steeply than crowding distance, which matures at 8-9 yrs. No significant difference was found in crowding distance thresholds for the 3 different repeated formats (p=0.331) (Fig. 3).

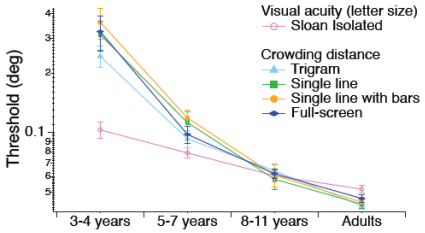


Fig 3: Acuity letter size and crowding distance across age.

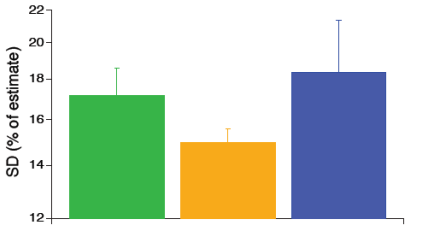


Fig 4: Variability of crowding distance estimates for repeated formats.

For repeated formats, crowding distance (threshold±SE) was most similar for single-line (0.163±0.027 deg) and full-screen (0.162±0.030 deg) formats, however children were happier to respond to the single line. Even with interleaving of repeated formats, variability of estimates (Fig. 5) was still highest for the full-screen format. It was lowest for the trigram (12.97±0.26% - not shown).

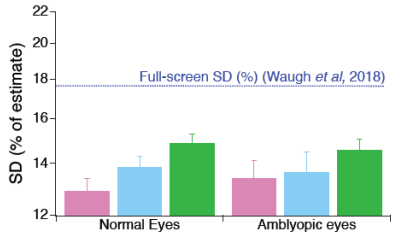


Fig 5: Variability of letter acuity and crowding distance measures.

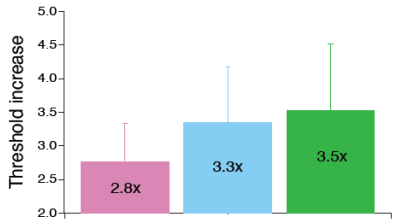


Fig 6: Ratios of acuity and crowding (amblyopic/normal eyes).

100% testability of crowding distance was possible with the single line repeated format in 41 amblyopic children (and 23 controls). Variability of estimates was similar for all in Fig. 5. (p=.305). Crowding distances are 3.4x larger in amblyopic eyes, than in control eyes (0.319±0.101 vs 0.093±0.019 deg) (see Fig. 6). Acuity ratios are smaller at 2.8x.

ACKNOWLEDGEMENTS. Support from ARU QR Fund, a VC PhD Studentship to Waugh for Haine and NIH Grant 1RO1EY027964 to Pelli.

REFERENCES

Pelli, DG, Waugh, SJ, Martelli, M, et al. (2016). A clinical test for visual crowding. F1000Research:5,1–11
Regan D, Giaschi DE, Kraft SP, et al. (1992) Method for identifying amblyopes whose reduced line acuity is caused by defective selection and/or control of gaze. Ophthal Physiol Opt, 12, 425–432.
Waugh, SJ, Pelli, DG, Álvaro, L & Formankiewicz, MA. (VSS2018) Crowding distance in healthy children.

CITATION.: Haine, L.A., Waugh, S.J, Formankiewicz, M.A & Pelli, D.G (2021) **Simplifying the repeated-letters crowding test for normal and amblyopic children.** Vision Sciences Society, V-VSS 2021. May 21-26, online.

APPENDICES

Appendix 11 – Pilot study results

- Isolated visual acuity and foveal crowding distance data
- Isolated acuity maturation rates
- Pelli optotype crowding distance maturation rates

Table A10-1: *Mean isolated Sloan thresholds and Pelli foveal crowding distances measured in degrees, with the standard error. Data is grouped into four different age groups and represents the age at the point of examination. Adult indicates an individual aged 18 or over.*

	Trigram	**Single Line**	**Flanked Single Line**	**Full Screen**	**Isolated Sloan acuity**
3-4 years	+0.243±0.030	+0.312±0.052	+0.361±0.064	+0.324±0.065	+0.103±0.010
5-7 years	+0.093±0.009	+0.113±0.014	+0.119±0.011	+0.098±0.010	+0.079±0.005
8-11 years	+0.064±0.005	+0.058±0.007	+0.061±0.008	+0.062±0.003	+0.061±0.004
Adults	+0.043±0.002	+0.043±0.002	+0.045±0.001	+0.046±0.002	+0.051±0.001

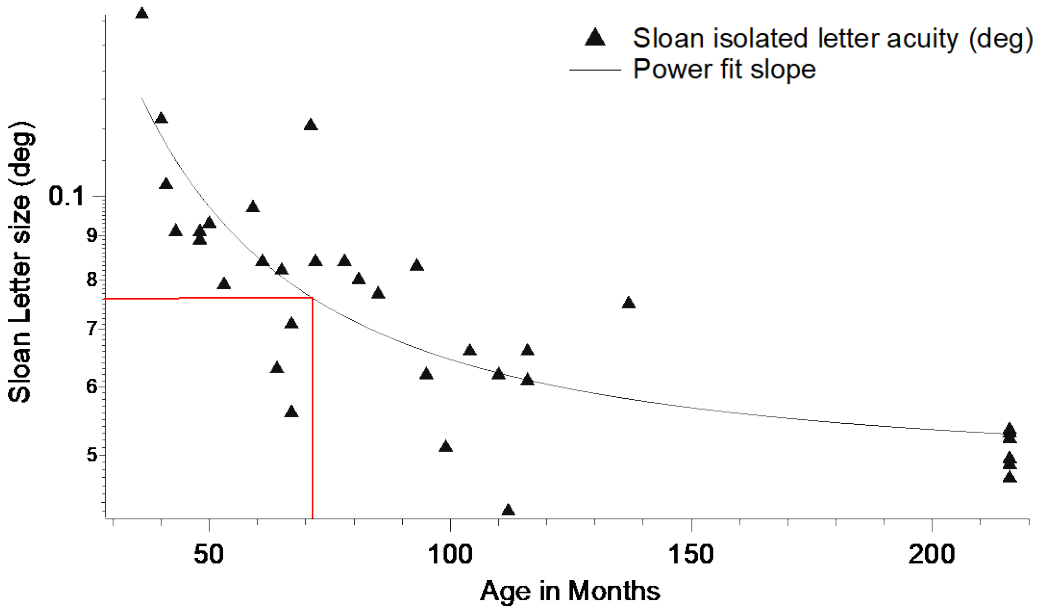


Figure A10-1: *Isolated Sloan visual acuity thresholds (deg). The red line identifies the age of visual maturity.*

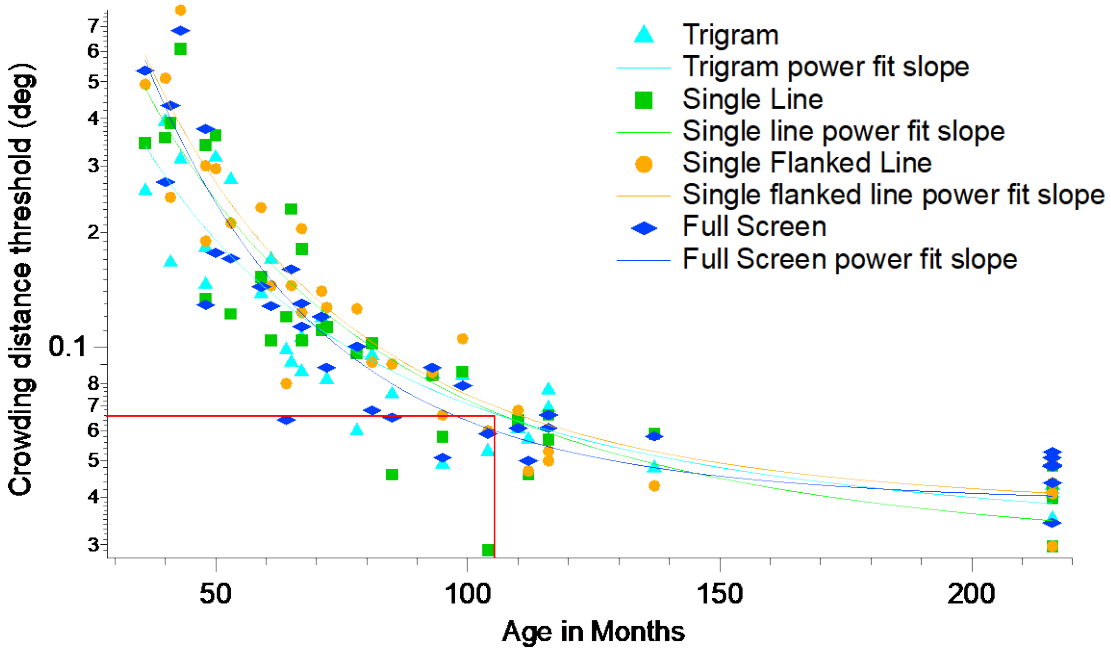


Figure 10-2: *Pelli crowding distance thresholds (deg), for four different test arrangements, measured in degrees. The red line indicates the age of crowding distance visual maturity (averaged across all four crowding distance tests).*

Appendix 12 – VSS 2022 Poster

Crowding distance beats acuity and crowded acuity in detecting strabismic amblyopia.

Sarah J Waugh[1,2], Louisa A Haine[2], Monika A Formankiewicz[2], and Denis G Pelli[3]

[1] Centre for Vision across the Life Span, University of Huddersfield, [2] Anglia Vision Research, Anglia Ruskin University, [3] Psychology Department, NYU

Introduction

Amblyopia ("lazy eye") is a developmental condition that if untreated, leads to permanent vision loss. It is due to uncorrected differences in refractive error (anisometropia) and/or misalignment (strabismus) between the eyes. Amblyopes have poor visual acuity, a size limit, in the affected eye. Strabismic amblyopes also have increased crowding, a spacing limit, making a target harder to recognize in nearby clutter.[1,2] Vision screening programmes assess acuity under "crowded" conditions. However, acuity and crowding have different developmental time courses[1] so they should be tested separately. Child vision screenings aim to detect degraded acuity and interocular differences (IOD), hallmarks of amblyopia. We ask: could measures of crowding distance during screening improve detection of strabismic amblyopia?

Methods

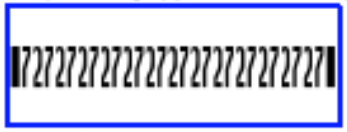
Standard letter construction (5:1 optotype-width:stroke-width) means they cannot get close enough together to measure crowding distance in central vision.[4] Pelli optotypes[4] (2:1 optotype-width:stroke-width) are narrower, but still resolvable. Thresholds for 4 tests (2 acuity, 2 crowding distance, see **Fig 1**) were measured in amblyopic children. The Sonsken logMAR test[5] is the gold standard "crowded" acuity test for Cambridge University Hospital Trust. The booklet has pages with lines of letters that differ in size. The other tests are presented onto a MacBook Pro screen.[4] They use QUEST to obtain size and spacing thresholds in 20 trials. The child indicated their response verbally or by using a matching card with all options. Data are all shown as mean±1SE.

Fig 1: Examples of 2 Acuity (left) and 2 Crowding Distance (right) stimuli.

1) Sonsken LogMAR Test (SLT)
Read all (options: O, X, H, T, U, V)[3]

2) Isolated Sloan Letter Acuity
(options: D H K N O R A V Z)[4]

3) Pelli trigram (spacing to size ratio of 1.4x).
Identify middle target (options: 1 2 3 4 5 6 7 8 9)[4]



4) Pelli line (spacing to size ratio of 1.4x).
Identify both targets (options: 1 2 3 4 5 6 7 8 9)[4]

Conclusion

Crowding distance tests magnify interocular differences in strabismic amblyopia. They could be more effective than acuity tests at detecting strabismic amblyopia in children.

Three groups of children participated: visually healthy controls (n=24; age=7.4±2.0 years), anisometropic amblyopes (n=22; age=6.2±1.3 years) and strabismic amblyopes (n=21; age=7.2±1.6 years). Each eye (RE and LE, amblyopic AE and fellow FE) was tested. Amblyopic children were recruited from Addenbrookes Clinical Paediatric Ophthalmology Service (ACPOS).

Fig 2: Acuity letter size for normal and amblyopic children

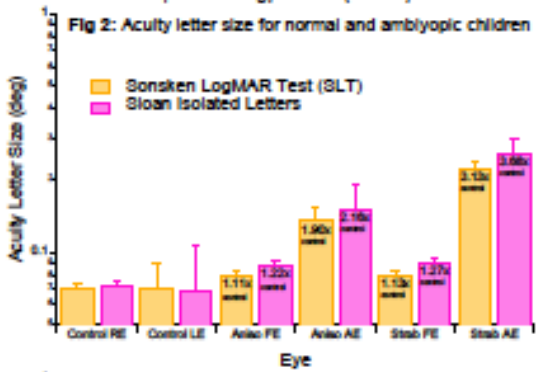
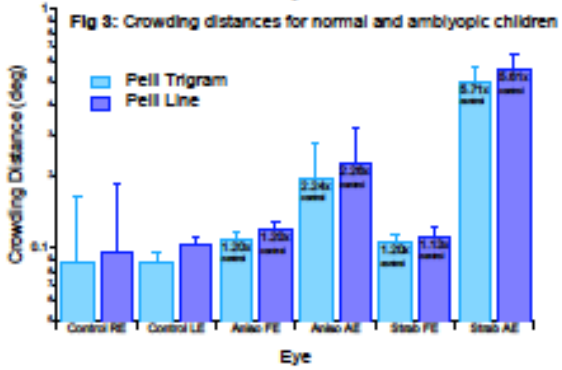


Fig 3: Crowding distances for normal and amblyopic children



ACKNOWLEDGEMENTS Support from UoH URF Fund, ARU QR Fund, a VC PhD Studentship to Waugh for Haine and NIH Grant 1RO1EY027964 to Pelli.

Results

Visual acuity (**Fig 2**) for control eyes is 0.071±0.014deg (-0.070±0.086 logMAR). Test used (SLT vs Sloan) made no significant difference [$F(1,64)=2.01$; $p=0.16$] but acuity depended on participant group and eye [$F(2,64)=7.88$; $p=0.0009$]. Tukey posthoc testing revealed that acuity in control eyes was different from amblyopic eyes (all $p<0.001$) but not from fellow eyes (all $p>0.8000$).

Crowding distance (**Fig 3**) for control eyes is 0.094±0.042deg. Test used (Trigram vs Line) made no significant difference [$F(1,64)=3.42$; $p=0.069$] but distance depended on group and eye [$F(2,64)=7.01$; $p=0.0018$]. Tukey posthoc testing revealed that distance in control eyes was different from amblyopic eyes (all $p<0.002$) but not from fellow eyes (all $p>0.5000$).

Fig 4: Interocular differences for normal and amblyopic children

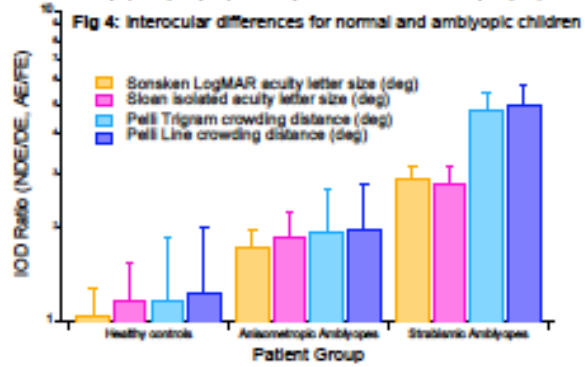


Fig 4 shows IOD ratios (AE/FE in amblyopes: NDE (non-dominant eye)/DE(dominant eye) in controls) for 4 tests. In control eyes they are 1.15±0.301. For anisometropic amblyopes, they are significantly higher [$F(1,44)=38.91$; $p=0.0000$] but not different between acuity and crowding measures. For strabismic amblyopes, IOD ratios are also higher than in controls [$F(1,43)=9.17$; $p=0.0042$] but depend on test [$F(3,129)=4.67$; $p=0.0039$]. A planned comparison of IOD ratios between the 2 acuity tests and 2 crowding tests for the strabismic group found a highly significant difference [$F(1,64)=20.10$; $p=0.000031$].

REFERENCES

1. Waugh, SJ., Pelli, DG, Alvaro, L & Formankiewicz, MA, 2018. Crowding distance in healthy children. F1000 Research
2. Formankiewicz MA, Waugh SJ. (2018) The effects of blur and eccentric viewing on adult acuity for pediatric tests: implications for amblyopia detection. IOVS, 59, 4998-4987
3. Song S, Levi DM, Pelli DG. (2014) A double dissociation of the acuity and crowding limits to letter identification, and the promise of improved visual screening. J Vis, 14, 3-57
4. Pelli, DG, Waugh, SJ, Martelli, M, et al. (2016). A clinical test for visual crowding. F1000Research,5,1-11
5. Sell AT, Wade AM, Proffitt R, Hennem L, Sonksen PM. (2017). The Sonksen logMAR Test of Visual Acuity: I. Testability and reliability. J AAPOS 21,389–394
6. Haine, LA, Waugh, SJ, Formankiewicz, MA & Pelli, DG (2021) Simplifying the repeated letters crowding test for normal and amblyopic children. Vision Science Society, V-VSS 2021.

APPENDICES