Form Deprivation Myopia in the Guinea Pig and Chick: Influence of Ambient Lighting Parameters

Andrew Vicary Collins

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Abstract

Aims: To determine the effects of periodic, white or quasi-monochromatic, high intensity ambient illumination on the development of form-deprivation myopia (FDM) in the guinea pig and chick.

Methods: Experiment (i): FDM was induced in one week old guinea pigs by monocular diffuser wear. Experimental animals (n = 10) were raised for 14 days under a periodic lighting regime where 15 minutes of 300 lux white fluorescent lighting was alternated with 15 minutes 10,000 lux halogen lighting (50% duty cycle) in a 12:12 hour light:dark cycle. Control animals (n = 10) were raised under 300 lux fluorescent ambient lighting in a 12:12 hour light:dark cycle. Cycloplegic refraction was measured with an IR optometer, corneal curvature with an IR videokeratometer, and axial length with high-frequency A-scan ultrasound. Retinal tissue samples were collected for dopamine concentration (DAC) analysis by high performance liquid chromatography - mass spectrometry (HPLC - MS).
Experiment (ii): FDM was induced in 4 day old chicks by monocular diffuser wear. Experimental animals were raised for 3 days under a periodic lighting regime where 15 minutes of 300 lux white LED lighting was alternated with 15 minutes of 10,000 lux white (n = 11), or equivalent irradiance blue (n = 12), green (n = 10) or red (n = 13) LED lighting (50% duty cycle) in a 12:12 hour light:dark cycle. Control animals (n = 10) were raised under 300 lux LED ambient lighting on a 12:12 hour light:dark cycle. Non-cycloplegic refraction and biometric measurements were made as for experiment (i).

Results: Experiment (i): Guinea pigs raised under the periodic high intensity halogen lighting paradigm exhibited a significant reduction in the degree of relative myopia induced (-2.38 ± 0.41 D) when compared to the 300 lux condition (-4.01 ± 0.61 D; $p = 0.038$). While this difference in refractive outcome was not significantly correlated with either vitreous chamber depth (VCD) or axial length (AXL), the absolute anterior chamber depth (ACD) of the deprived eyes exposed to high intensity lighting exhibited significantly smaller ACD ($p = 0.012$). No significant difference was found in retinal DAC by lighting condition. Experiment (ii): No significant difference in relative refraction was found for any of the periodic high intensity white, blue, green or red LED ambient lighting conditions when compared to the 300 lux white LED condition. However, when compared to a previously published control group of chicks (Backhouse, Collins et al. 2013) raised under 300 lux fluorescent lighting (-9.73 ± 0.96 D), significantly less myopia (-5.21 ± 1.47 D, $p = 0.017$) was induced in the chicks raised under white LED-based 300 lux illumination in this study. The degree of FDM induced was strongly correlated with an increase in VCD under all lighting conditions ($R^2 = 0.594$, $p < 0.0001$).

Conclusions: (i) Daily exposure to periodic high intensity white halogen light (10,000 lux) suppresses development of FDM in pigmented guinea pigs. (ii) Conversely, no significant difference was found in the degree of FDM induced in chicks under the white or quasi-monochromatic high intensity LED lighting conditions. However significantly less FDM (54%) was induced under the 300 lux white LED ambient light condition, when compared to an equivalent control group previously raised under 300 lux fluorescent light in the same laboratory (Backhouse, Collins et al. 2013). Analysis showed that the differing spectral irradiance distributions of white LED and fluorescent lights may explain this outcome.

For Melinda and Victoria

and

In memory of

Rex and June Collins

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ACD	anterior chamber depth
ANOVA	analysis of variance
AXL	axial length
D	dioptre (m^{-1})
DAC	dopamine concentration
DOPAC	3,4-Dihydroxyphenylacetic acid concentration
ERG	electroretinogram
FD	form deprivation
FDM	form-deprivation myopia
HPLC	high performance liquid chromatography
HPLC - MS	high performance liquid chromatography – mass spectrometry
IP	intraperitoneal
IR	infrared
LCA	longitudinal chromatic aberration
LED	light emitting diode
LIH	lens-induced hyperopia
LIM	lens-induced myopia
LT	lens thickness
MGLM	multivariate general linear model
RCT	randomised clinical trial
SEM	standard error of the mean
TCA	transverse chromatic aberration
UGLM	univariate general linear model
UV	ultraviolet
VCD	vitreous chamber depth

1 Introduction

1.1 Thesis Overview

The primary theme explored in this thesis is the relationship between aspects of ambient illumination including intensity, timing and wavelength and the development of experimentally-induced myopia using two animal models; the guinea pig and chick. Form-deprivation was used to induce myopia in the experiments reported in this thesis, as it is a reliable and well-characterised method of producing axial myopia in both chicks (Wallman and Adams 1987; Wallman and Winawer 2004) and guinea pigs (Howlett and McFadden 2002; Howlett and McFadden 2006). High intensity ambient illumination, approximating outdoor light levels, has been shown previously to suppress form-deprivation myopia (FDM) in the chick (Ashby, Ohlendorf et al. 2009) in a dose-dependent manner (Karouta and Ashby 2015), while it has also been shown to slow the development of, or partially suppress, lens-induced myopia (LIM) in the chick (Ashby and Schaeffel 2010) and guinea pig (Li, Lan et al. 2014).

The suppression of FDM in chicks also exhibits a time-of day effect, with two hour periods of high intensity illumination (10,000 lux) in the middle of the day proving to be more effective at suppressing myopia development than two hours in the evening (Backhouse, Collins et al. 2013). While high intensity illumination in the order of 10,000 to 15,000 lux has generally been used to suppress FDM in animal models of myopia, 12 hours of continuous ambient illumination at 2000 lux has been found to be more effective at suppressing FDM than two hours of high intensity illumination either in the morning or evening (Backhouse, Collins et al. 2013).

A pilot study (Backhouse 2011) demonstrated that the ambient illumination levels experienced by school-age children vary greatly between periods of high and relatively low intensity during the day. Therefore, the experiments in this thesis were intended to investigate the hypothesis that periodic high intensity illumination would be effective at inhibiting the development of FDM in two well-established animal models of myopia, the guinea pig and the chick.

Chapter 1 of this thesis reviews the literature regarding the increasing prevalence of myopia in humans worldwide, but particularly in East Asia, and discusses the possible aetiologies of myopia. The relationship between the environment and myopia development is addressed, particularly with regard to the epidemiological evidence for the ameliorating effect of outdoor activity on myopia development in adolescents (Jones, Sinnott et al. 2007; Rose, Morgan et al. 2008; Rose, Morgan et al. 2008; Dirani,

Tong et al. 2009). The experimental evidence for the high ambient light levels experienced outdoors being the mediator of the antimyopiagenic effect of outdoor activity is examined for both human and animal studies. Furthermore, the evidence for a relationship between ambient light, dopaminergic pathways within the retina, and suppression of axial growth of the eye is considered. Chapter 1 concludes with the specification of the hypothesis and aims of this study.

Chapter 2 describes the general methods employed in this study for both the guinea pig and chick experiments, including the induction of FDM, anaesthesia, and measurements of refractive and biometric outcomes.

In Chapter 3, periodic high intensity illumination is shown to suppress the development of FDM in the guinea pig, which is a novel finding.

Chapter 4 investigates the effect of high intensity lighting of 4 different spectral irradiance distributions (apparent colours) on FDM in chicks. Periodic high intensity illumination did not produce a significant suppression of FDM relative to chicks raised under standard room illumination condition. However, a comparison of the degree of FDM induced under the white LED 300 lux lighting condition in this experiment with a previously published control group raised under white fluorescent 300 lux lighting (Backhouse, Collins et al. 2013) in the same laboratory reveals that the use of a white LED lighting source appears to have partially suppressed, or slowed, the development of FDM in chicks. In order to investigate this finding further, measurement and analysis of the spectral irradiance distributions of the both the LED-based light sources used in this experiment, and the fluorescent-based white light source used previously (Backhouse, Collins et al. 2013) was undertaken. This analysis leads to an extensive discussion on the spectral energy distribution of fluorescent versus LED sources of white light, and how lighting stimuli should be measured and defined in animal experiments. In particular, consideration is given to whether the measurement of lighting stimuli should take into account both the spectral sensitivity of cone-mediated photopic vision, as represented by the relative spectral luminous efficiency function V(λ), and also the spectral sensitivity of the additional photopigment melanopsin, found in the intrinsically-photosensitive retinal ganglion cells (ipRGCs) of many vertebrates (Lucas, Peirson et al. 2014).

Finally, Chapter 5 discusses the overall conclusions regarding light and myopia developed from these two sets of experiments.

1.2 Background

1.2.1 Myopia: Prevalence and Aetiology

Myopia is considered to be reaching epidemic levels in Asia due to the rapid increase in prevalence in some populations over one to two generations (Morgan, Ohno-Matsui et al. 2012; Pan, Ramamurthy et al. 2012; Dolgin 2015). The prevalence of myopia has reached 80% or higher in school-age children in the urban areas of countries and regions of east and south-east Asia such as; China (He, Zeng et al. 2004; Guo, Yang et al. 2016), Taiwan (Lin, Shih et al. 2004), Hong Kong (Goh and Lam 1994), Singapore (Seet, Wong et al. 2001) and Japan (Matsumura and Hirai 1999). One of the highest myopia prevalence statistics reported is for Seoul, Korea, where 96.5% of 19 year old males exhibited a myopic refractive error of greater than -0.50 D (Jung, Lee et al. 2012). The prevalence of high myopia (< -6 D) in this cohort was 21.61%, which is of concern due to the increased risk of ocular pathology associated with higher degrees of myopia even in the relatively young (Saw, Gazzard et al. 2005; Cheng, Lam et al. 2013). On a more positive note, a follow-up study in Hong Kong by Lam *et al* (Lam, Lam et al. 2012) established that prevalence of myopia (<-0.50 D) in school children of Chinese ethnicity, although high at 61.5%, had not increased further over a period of 20 years. This suggests that prevalence of myopia in a population may reach a plateau depending on the relationships within that population between environment and genetic predisposition (Chen, Scurrah et al. 2007; Ip, Rose et al. 2008; Fan, Wojciechowski et al. 2014).

Regions outside Asia are also experiencing an increase in prevalence of myopia including the United States (Vitale, Sperduto et al. 2009), Europe (Logan, Davies et al. 2005; Logan, Shah et al. 2011; Williams, Hysi et al. 2013; Williams, Bertelsen et al. 2015) and most likely, Australia (Rose, Smith et al. 2001; French, Morgan et al. 2013a). However, in these regions the prevalence of myopia is currently lower than in the urban areas of Asia at generally less than 50% of the population. In comparison, in a semi-rural region of China, similar or lower myopia prevalence values were found in 11 - 12 year old children in an ethnically-diverse (Han, Hui and Uyghur) population (Chin, Siong et al. 2015). Furthermore, a number of populations still exhibit relatively low levels for the prevalence of myopia, including those of north-eastern Iran (4.3% of 6 - 17 year old children)(Rezvan, Khabazkhoob et al. 2012), South Africa (4% of 5 – 12 year old children)(Naidoo, Raghunandan et al. 2003) and rural Nepal (1.2% of 5 – 15 year old children)(Pokharel, Negrel et al. 2000). This variation in prevalence of myopia by country, region, and ethnicity (Wu, Seet et al. 2001; Logan, Shah et al. 2011; Pan, Zheng et al. 2013) has led to much debate and investigation into the relative roles of genetics and environment in

the aetiology of myopia (Wojciechowski 2011; Pan, Ramamurthy et al. 2012; Goldschmidt and Jacobsen 2014).

Myopia, Definition and Classifications

Before considering the demographics and consequences of myopia further, it is useful to review the classification and definitions of myopia. Myopia, colloquially known as short-sightedness as distant objects cannot be seen clearly, has been defined in a number of ways; including the location of the far point at a real distance in front of the eye by Helmholtz (Edwards 1998), to the focus of light rays in front of the retina as by Duke-Elder (Duke-Elder and Abrams 1970). Current definitions include the requirement that accommodation is relaxed such that parallel rays of light entering the eye from a distant object are focused anterior to the retina, and conversely, the conjugate point of the retina (i.e. far point) is located at a finite distance in front of the eye (Millodot 1993; Cline, Hofstetter et al. 1997; Edwards 1998). Helmholtz noted that given the position of the far point in myopia, light entering the eye must be divergent in order to focus on the retina, hence optical correction of myopia is by means of concave (negatively-powered) lenses (Edwards 1998).

Myopia has been classified in a number of ways which have been reviewed in detail by Grosvenor (Grosvenor 1987), Edwards (Edwards 1998) and Wildsoet (Wildsoet 1998). These classification schemes can be broadly divided into; aetiological (Emsley 1952; Sorsby, Leary et al. 1962), physiological vs. pathological (Curtin 1979), age of onset (Grosvenor 1987) and degree of myopia (Wildsoet 1998; Saw 2006).

As the final refractive state of the eye is dependent upon both the dioptric power of the optical components and the axial length of the eye (Rabbetts 1998), ametropia may be divided into two broad aetiological categories (Emsley 1952; Sorsby, Leary et al. 1962); axial and refractive ametropia. In axial myopia, the overall axial length is too long relative to the refractive power of the eye; whereas in refractive myopia, the overall dioptric power is too high relative to the axial length. In both forms, the image plane of a distant object falls in front of the retina (Edwards 1998).

The seminal studies by Sorsby and collaborators (Sorsby, Benjamin et al. 1957) resulted in the proposition that all forms of ametropia (including myopia) can be separated into one of two groups; correlation ametropia or component ametropia (Sorsby, Leary et al. 1962). In correlation ametropia the individual variables of refractive state, such as corneal power, lens power and axial length, fall within the "normal" ranges as found in emmetropic eyes, however they are poorly correlated (or coordinated)

such that ametropia arises (Sorsby, Leary et al. 1962). This form of ametropia was claimed to lie within the range of refractions of ±4.00 D (and possibly up to +6.00 D of hyperopia), which accounted for some 97% of the population studied (Sorsby, Leary et al. 1962). In contrast, for refractions greater than ±4.00D, one of the individual variables, or components, of the refractive state was found to lie outside the normal range; usually an abnormal axial length (Sorsby, Leary et al. 1962). This was therefore termed component ametropia (Sorsby, Leary et al. 1962). A similar approach was adopted by Borish (Grosvenor 1987) who classified myopia as correlative (or simple, benign) and component, where one variable of refractive was pathologically abnormal, possibly due to congenital or acquired disease (Grosvenor 1987).

Curtin (Curtin 1979) defined pathological myopia as excessive axial elongation of the globe that primarily involves the posterior pole resulting in peripheral fundus changes and posterior staphyloma formation. However, it is now recognised that pathological retinal changes may occur in relatively young myopic people in the absence of overt changes such as stapyloma (Cheng, Lam et al. 2013).

Grosvenor (Grosvenor 1987) proposed a classification system for myopia based on the age-of-onset. His classification included four categories; congenital, youth-onset, early adult-onset, and late adult onset. One benefit of such a system was the removal of the implication of an aetiology as is inherent in the term “school myopia”, where youth-onset myopia would be the equivalent form, arising from around 6 to 20 years of age. Early adult-onset myopia occurs between 20 – 30 years, while late adult-onset arises after 40 years of age (Grosvenor 1987). Therefore, school myopia, common myopia and youth onset myopia refer to effectively the same condition.

Classifications based on the degree of myopia are arbitrary and non-standardised, although a refraction of -6 D or less is commonly used as a threshold for high myopia (Wildsoet 1998; Flitcroft 2012). Similarly, the limit for low myopia is often defined between -2 to -3 D, with moderate myopia lying between this limit and high myopia. While these definitions may have some clinical use, they represent a proxy for the risk of developing myopic retinal degeneration, and so an axial length threshold been suggested as a more appropriate classification measurement (Cheng, Lam et al. 2013).

Myopia and Emmetropisation

Emmetropisation is the cooperation of the various components determining the refractive state of the eye to produce a higher than expected prevalence of emmetropia within the population than would be produced by a random process (Siegwart and Norton 2011). Active emmetropisation mechanisms

have been shown to exist in a range of animals including the chick, guinea pig, tree shrew and monkey, particularly through the demonstration of active axial growth compensation for lens-induced retinal defocus (Wildsoet 1997; Wallman and Winawer 2004; Howlett and McFadden 2009). In humans, indirect evidence for the presence of an active emmetropisation mechanism is taken from the leptokurtic refractive distributions found in adults where there are more emmetropic or low hyperopic eyes than would be predicted by a simple random selection of refractive components (Sorsby, Sheridan et al. 1960; Siegwart and Norton 2011).

If emmetropisation is the active process by which the refractive and biometric components of the eye are coordinated so that the adult refractive state is emmetropic, then refractive errors such as common myopia can be considered a failure of emmetropisation (Wallman and Winawer 2004). The finding that the interruption of vision through eyelid closure during development resulted in the induction of axial myopia in the macaque monkey (Wiesel and Raviola 1977; Raviola and Wiesel 1985; Smith, Harwerth et al. 1987) demonstrated that the visual environment clearly influences eye growth and refractive outcome. This type of experimentally-induced refractive error is termed form-deprivation myopia (FDM) as it results from the degradation of spatial contrast information in the retinal image, while retaining a degree of retinal illumination (Wallman and Winawer 2004; Rucker 2013). For this reason, translucent occluders are often used to induce FDM in animal models of myopia as in this study. FDM has been induced in a wide range of animals including the chick (Wallman, Turkel et al. 1978), tree shrew (Sherman, Norton et al. 1977; McBrien and Norton 1992), guinea pig (Howlett and McFadden 2002; Howlett and McFadden 2006), marmoset (Troilo and Judge 1993) and mouse (Schaeffel, Burkhardt et al. 2004). FDM also occurs in humans as evidenced by infantile myopia associated with congenital cataract and ptosis (O'Leary and Millodot 1979; von Noorden and Lewis 1987).

Myopia and Public Health

The increasing prevalence of myopia has been identified as an important public health issue particularly in regions of Asia (Saw, Wu et al. 2001). The World Health Organisation recognizes uncorrected refractive error as a major cause of visual impairment worldwide, and that the correction of myopia with spectacles imposes a relatively high financial burden on many families (Vitale, Cotch et al. 2006; Resnikoff, Pascolini et al. 2008). In addition to the socio-economic burden of providing optical corrections, common myopia can limit career choice, and increases the risk of various ocular diseases (Flitcroft 2012) including open-angle glaucoma (Mitchell, Hourihan et al. 1999; Marcus, de Vries et al. 2011) and cataract (Younan, Mitchell et al. 2002). Myopia also increases the risk of retinal

detachment, chorioretinal degeneration and subsequent visual impairment (Chen, Wen et al. 2012)(Saw, Gazzard et al. 2005)(Hyman 2007; Flitcroft 2012; Morgan, Ohno-Matsui et al. 2012). In high myopia, retinal changes such as optic nerve crescents, white-without-pressure, and lattice degeneration can be found in adolescents by 18 years of age (Cheng, Lam et al. 2013). While high myopia is often defined as a spherical equivalent refraction (SER) of at least -6 D, this limit is arbitrary and a range of SER from -5 D to -12 D have been used as the threshold for high myopia in previous studies (Saw 2006). However, Cheng *et al* (Cheng, Lam et al. 2013) established that an axial length measurement of greater than 26.5 mm was an independent risk factor for both the peripheral retinal changes and the presence of optic nerve head crescents associated with myopia. This suggests that when monitoring myopia progression in a clinical setting, axial length measurement should be an essential component of clinical practice, rather than just monitoring the change in refractive error.

Myopia, Ethnicity and Genetics

While some studies have shown that the prevalence of myopia varies within a population by ethnicity, for example in Singapore (Pan, Zheng et al. 2013), others have reported little variation in the prevalence of refractive error by ethnicity within a common environment (Pan, Ramamurthy et al. 2012). In a mixed ethnicity U.K. university student population (mean age = 19.6 years), a comparison of prevalence rates between British European (white) and British Asian groups, found no significant difference in myopia (50% vs. 53.4%) or hyperopia (18.8% vs. 17.3%) prevalence between the groups. Furthermore, when Rose *et al* (Rose, Morgan et al. 2008) compared refractive status of young children (6 -7 years of age) of Chinese ethnicity located in Sydney or Singapore, the prevalence of myopia was found to be significantly lower in Sydney (3.3%) than in Singapore (29.1%), suggesting that environmental differences between the sites had a far greater influence than ethnicity on myopia development.

Of particular interest, and the subject of further discussion later, is the finding by Rose et al (Rose, Morgan et al. 2008) that the most significant factor associated with the difference in prevalence of myopia described above was that children in Sydney spent on average 10 hours more time in outdoor activities when compared with children in Singapore (13.75 vs. 3.05 hours per week) (Rose, Morgan et al. 2008). Evidence of a similar protective effect of outdoor activity on the incidence of myopia has been published in a longitudinal study of children of both East Asian and European Caucasian ethnicities in Sydney (French, Morgan et al. 2013b). Conversely, extensive periods of study have been associated with the development of higher degrees of myopia within a Jewish population in Israel

(Zylbermann, Landau et al. 1993). The refractive status of teenage children attending Orthodox religious schools were compared to those attending secular schools. The male students who attended the Orthodox school were on average -2.4 D more myopic than those who attended the secular school, while no difference was found in refraction between the female students of the two schools. The higher degree of myopia developed by the boys attending the Orthodox school was postulated as being due to the extensive and prolonged near work associated with the study of religious texts by the boys (Zylbermann, Landau et al. 1993). Such comparisons suggest that environment may have a primary role in the development of low or common myopia, with ethnicity or genetic effects as secondary factors (Flitcroft 2012; Pan, Ramamurthy et al. 2012; Goldschmidt and Jacobsen 2014).

While environment may indeed prove to be paramount in determining whether common myopia develops in an individual, genetic factors have long been implicated in the aetiology of myopia (Sorsby 1962). For example, children with myopic parents have a higher than normal risk of developing myopia (Mutti, Mitchell et al. 2002; Jones, Sinnott et al. 2007) and twin studies show a higher level of concordance of common myopia in monozygotic compared to dizygotic twins (Dirani, Chamberlain et al. 2006; Dirani, Shekar et al. 2008). However, while genetic studies have identified at least 25 genes and some 20 loci clearly associated with myopia development and refractive traits, these appear to have low or modest effect sizes, apart from in the particular case of high myopia (Hewitt, Kearns et al. 2007; Tang, Yip et al. 2007; Baird, Schache et al. 2010; Wojciechowski 2011).

Myopia and Near Work

Near work has long been associated with the development of myopia (Goldschmidt and Jacobsen 2014). In fact, the relationship between education, near work, lighting and the development of myopia was recognised by the ophthalmologist, Hermann Cohn, in Germany in middle of the 19th century (Schaeffel 2016). The writings of the eminent ophthalmologist Duke-Elder on the aetiology and management of myopia have also received renewed recognition (Polling, Verhoeven et al. 2016). In his text on refraction (Duke-Elder 1963) he noted that:

> "the most important accessory risk factors in the aetiology of the condition [myopia] are excessive near work, bad ocular hygiene and physical debility in the early years of growth".

However, it is now apparent that it is not necessarily near work *per se*, but higher educational achievement that is most likely to be the risk factor for the development of myopia (Wu, Seet et al. 2001; Mutti and Zadnik 2009; Jung, Lee et al. 2012; Morgan and Rose 2013; Fan, Wojciechowski et al.

2014; Fan, Verhoeven et al. 2016). Nevertheless, aspects of accommodative function may be different in myopic people including higher accommodative lag (Gwiazda, Thorn et al. 1993; Rosenfield and Gilmartin 1998) and difficulty in interpreting small changes in defocus (Strang, Day et al. 2011). However, the main argument against the act of accommodation itself during near work having a primary role in the development of myopia is from animal studies in chicks, where accommodation is mediated by nicotinic receptors within the intraocular muscles of the chick eye (McBrien, Moghaddam et al. 1993). Atropine, which had previously been shown to prevent the progression of myopia in adolescent humans (Bedrossian 1979) and monkeys (Raviola and Wiesel 1985), was also shown to reduce the degree of FDM induced in chicks by a non-accommodative mechanism, as atropine did not abolish nicotinic agonist-induced accommodation (McBrien, Moghaddam et al. 1993).

The question then remains, if it is not the activation of accommodation itself, associated with near work and higher educational achievement, that is the trigger for the development of myopia what other aspects of near work and the indoor (school) environment may be implicated?

Myopia and Peripheral Refraction

Flitcroft (Flitcroft 2012) has extensively reviewed the relationship between retinal, optical and environmental factors in the aetiology of myopia. The demonstration in monkeys that an intact fovea is not essential for emmetropisation to occur (Smith, Ramamirtham et al. 2007) revealed that the control of eye growth by the retinal image (Wallman and Winawer 2004) is not limited to foveal defocus, but also guided by the position of the peripheral image relative to the retina (Flitcroft 2012). The finding that myopic eyes often exhibit relative peripheral hyperopia (RPH), where the image plane lies beyond the peripheral retina, led to the proposal that the development of axial myopia may, at least in part, be driven by the presence of RPH (Mutti, Sholtz et al. 2000; Atchison, Jones et al. 2004; Mutti, Hayes et al. 2007; Ehsaei, Mallen et al. 2011). If the development of myopia is driven by RPH, then interventions aimed at producing relative peripheral myopia (RPM) should slow the progression of axial myopia (Smith, Campbell et al. 2013). However, there has been much debate as to whether RPH is a cause of myopia or simply an effect of axial elongation resulting in an oblate ocular shape (Flitcroft 2012). The apparent ability of orthokeratology (OK) to slow the progression of myopia has been argued as evidence of the role of RPH in myopia development, where the corneal remodelling associated with OK results not only in correction of the foveal focal plane, but the induction of RPM, which is held to provide the antimyopiagenic treatment effect (Cho, Cheung et al. 2005; Cho and Cheung 2012; Kang and Swarbrick 2013; Kang and Swarbrick 2016). Similarly, multifocal soft contact

lenses have been reported to produce a relative myopic shift in peripheral refraction when compared with standard single vision soft contact lenses (Kang, Fan et al. 2013) which again advocates that the reported ability for specially-designed dual focus (Anstice and Phillips 2011) or multi-focal contact lenses (Sankaridurg, Holden et al. 2011) to slow myopia progression may be due to changes in the peripheral refractive state (Flitcroft 2012). Conversely, the correction of moderate to high myopia with single vision soft contact lenses has also been reported to produce RPM (Backhouse, Fox et al. 2012; Kwok, Patel et al. 2012), which would therefore be expected to slow the progression of myopia. However this effect that has not been found in practice (Horner, Soni et al. 1999; Walline, Jones et al. 2008). Furthermore, while the peripheral retinal image position may be sufficient to guide an emmetropisation mechanism, the initial relative peripheral refraction in children does not appear to have predictive value regarding future myopia development (Lee and Cho 2013). In order to understand the relationship between the shape of the retina and retinal image shell and the development of myopia, Flitcroft (Flitcroft 2012) contends that the 3-dimensional nature of the environment must be considered.

1.2.2 Myopia and Outdoor Activity

The Optical Environment

Flitcroft (Flitcroft 2012) argues that the local environment, in the form of the dioptric distance across the whole visual field, can influence refractive development. Evidence comes from the observation of lower field myopia in ground feeding birds such as the pigeon, which has been proposed to be an optical adaptation to the near distance of the inferior visual field, keeping it in focus for feeding (Fitzke, Hayes et al. 1985; Hodos and Erichsen 1990). Similarly, raising monkeys in a restricted near visual environment leads to the development of myopia (Young 1963), while a low ceiling environment can produce superior field myopia in chicks (Miles and Wallman 1990). Furthermore, in military personnel, service in submarines or underground missile installations is correlated with increased rates of myopia when compared to more typical forms of service (Kinney, Luria et al. 1980). The prevalence of myopia in a populations has been shown to be directly correlated with increasing urbanisation (Saw, Hong et al. 2001; Pan, Ramamurthy et al. 2012; Guo, Liu et al. 2013a) and education level (Saw, Wu et al. 2001; Morgan and Rose 2013). Conversely time spent outdoors appears to be protective against the progression of myopia (Jones, Sinnott et al. 2007; Rose, Morgan et al. 2008; French, Ashby et al. 2013), although whether it is some aspect of outdoor illumination such as intensity, or the greater

dioptric distances experienced outdoors, is the subject of debate (Ngo, Saw et al. 2013). Flitcroft (Flitcroft 2012) presents a compelling argument that the role of 3-dimensional dioptric structure of the environment may play a significant role in the aetiology of myopia. Using computer-generated dioptric distance maps of the environment, Flitcroft demonstrates that the outdoor environment is far less complex dioptrically than an indoor environment such as being seated at a standard office desk. While the outdoor environment can be considered to be dioptrically flat, the indoor desk environment is not only dioptrically closer (e.g. 40 cm working distance = 2.5D accommodation stimulus), but also much more variable, even across a computer screen (Flitcroft 2012). When this variation in the dioptric distance of the near environment is convolved with the effects of eye shape and optical aberrations such as curvature of field, an estimation of the 3-D pattern of defocus across the whole retina can be derived. Flitcroft proposes that the near environment would produce areas of hyperopic defocus superiorly and inferiorly, which would be expected to promote myopic compensatory growth of the eye resulting in inferior and superior field myopia, similar to that seen in the pigeon (Flitcroft 2012). However at this stage the human data available for refraction in the vertical meridian is limited although there is some evidence for inferior field myopia (Seidemann, Schaeffel et al. 2002; Atchison, Pritchard et al. 2006; Berntsen, Mutti et al. 2010). At present, the role of dioptric distance across the 3-D visual field on the development of axial myopia is still up for debate, but it may have a useful role in explaining the antimyopiagenic effects of dual focus and multi-focal contact lenses (Anstice and Phillips 2011; Sankaridurg, Holden et al. 2011) and progressive-addition lenses (Berntsen, Barr et al. 2013).

The Ambient Light Environment

Fuchs, an eminent Austrian ophthalmologist, in his Textbook of Ophthalmology first published in 1889 stated that:

> *'The following means are advised to put a stop to the extension of myopia in schools…..more time should be allotted to bodily exercise, especially in the open air, than has hitherto been the case…..illumination of sufficient strength and falling upon the work in the proper direction''* (Duane 1919).

The case in favour of outdoor activity as a protective factor against the development of myopia was renewed in the mid to late 2000s following the publication of a number of epidemiological articles that linked increased time spent on outdoor activities with a lower prevalence of myopia in several young study populations (Jones, Sinnott et al. 2007; Rose, Morgan et al. 2008; Rose, Morgan et al. 2008; Dirani, Tong et al. 2009). In an extensive review of the association between outdoor activity and

myopia, French *et al* (French, Ashby et al. 2013) noted that similar associations between time spent outdoors and rate of myopia progression (Parssinen and Lyyra 1993), or risk of development (Mutti, Mitchell et al. 2002; Saw, Zhang et al. 2002), in children had been reported earlier. Furthermore, the protective benefit of sports and outdoors activity was shown to not be a simple matter of substitution for near work, as measured by reading hours per week (Jones, Sinnott et al. 2007). Similarly Rose *et al* (Rose, Morgan et al. 2008a) found that children who reported high levels of outdoor activity and high levels of near work, did not have significantly different refractive errors when compared to those who reported low levels of near work. Conversely children who reported low levels of outdoor activity combined with high levels of near work had a nearly 3-times greater odds ratio for myopia when compared to the group having the opposite trends of high levels of outdoor activity combined with low levels of near work (Rose, Morgan et al. 2008a). Furthermore, in a comparison of 6 - 7 year old children of Chinese ethnicity resident in Sydney or Singapore, the significant difference in the prevalence of myopia found between the two locations (3.3% vs. 29.1% respectively) was found to be significantly associated with time spent outdoors (13.75 vs. 3.05 hours per week) (Rose, Morgan et al. 2008). Interestingly, small but significant differences were found in the amounts of near work carried out between the two locations, but these were perhaps counter-intuitive as it was the Sydney children, with the lower prevalence of myopia, who spent more hours in total near work activity (29.93 vs. 23.54 hours) including reading, writing and using computers. However, the Singapore children did spend significantly more time in after-school coaching classes (1.74 vs. 1.21 hours), which has been identified as a potential risk factor for myopia development (Rose, Morgan et al. 2008b; Morgan and Rose 2013).

Subsequently, studies world-wide have confirmed the association between reduced prevalence or risk of myopia and increased time spent in outdoor activity (French, Ashby et al. 2013). In the United Kingdom, the Avon Longitudinal Study of Parents and Children (ALSPAC) followed the refractive development of a cohort of students from 7 to 15 years of age and assessed their reported time spent outdoors between 8 – 9 years by parental questionnaire and physical activity at 11 years with a data-logging accelerometer (Guggenheim, Northstone et al. 2012). Both higher levels of time spent outdoors (odds ratio = 0.65) and increased levels of physical activity (OR = 0.87) were independently associated with lower risk for incident myopia at 15 years, which led the authors to conclude that the time spent outdoors is a more important factor in the prevention of myopia than physical activity (Guggenheim, Northstone et al. 2012).

In China, a series of studies on school children from both rural and urban locations in greater Beijing have also confirmed the relationship between increased time spent outdoors and decreased

prevalence of myopia (Guo, Liu et al. 2013a; Guo, Liu et al. 2013b; Wu, You et al. 2015). The presence of myopia in primary school children was also associated with urban living and greater time spent indoors studying (Guo, Liu et al. 2013a). Furthermore, in high school students from the Beijing region, an increased prevalence of myopia was associated with longer periods of near work (OR = 1.43) and shorter near working distances (OR = 1.87) (Wu, You et al. 2015).

In Finland, Parssinen *et al* (Parssinen, Kauppinen et al. 2014) reported a 23-year follow-up study based on a group of myopic school children recruited previously (Parssinen and Lyyra 1993). Progression of myopia was associated with less time spent on outdoor activities and sports in childhood. However, parental myopia status was also associated with myopia progression in the school children, while time spent on near work was only related to progression during the first 3 years of the study (Parssinen, Kauppinen et al. 2014).

In Australia, a series of articles have reported on prevalence, incidence and risk factors for myopia in a 5 - 6 year follow-up of children originally recruited for the Sydney Myopia Study (French, Morgan et al. 2013a; French, Morgan et al. 2013b; French, Morgan et al. 2013c). The prevalence of myopia in both the 12 and 17 year old cohorts had increased between baseline measurements and follow-up to 14.4% and 29.6% respectively, while the annual incidence of myopia in each age cohort was 2.2% and 4.1% respectively (French, Morgan et al. 2013a). The incidence of myopia was associated with less time spent outdoors in both the younger and older cohorts, while more hours of near work was significantly associated with increased incidence of myopia in the younger cohort only (French, Morgan et al. 2013b). Furthermore, it was found that the time spent outdoors reduced with age while the time spent on near work increased, suggesting that their environment became more myopiagenic with age (French, Morgan et al. 2013c), which is most likely associated with increased time spent on educational activities including after school tutorials (Morgan and Rose 2013).

In 2011, Jones-Jordan *et al* (Jones-Jordan, Mitchell et al. 2011) reported results from the Collaborative Longitudinal Evaluation of Ethnicity and Refractive Error (CLEERE) study which indicated that children who became myopic spent significantly fewer hours per week (1.1 – 1.8 hours) in outdoor or sports activities both before and after myopia onset. In a follow-up article form the CLEERE study (Jones-Jordan, Sinnott et al. 2012), time spent outdoors or on sports activity was not significantly associated with myopia progression. Furthermore, a report from the Correction of Myopia Evaluation Trial (COMET) found no association between the diary records of outdoor activity and stabilisation of myopia (reduction of progression) by 15 years of age (Scheiman, Zhang et al. 2014). Therefore, the

protective effect of outdoor activity appears to be more effective at reducing incidence, rather than progression, particularly at a younger age (French, Ashby et al. 2013).

However, not all studies have found an association between time outdoors and myopia development. A study of 15 year old children in rural China which found no association between either time spent outdoors, or time spent on near activities and myopia (Lu, Congdon et al. 2009). Furthermore, In Singapore, in a large cross-sectional study of preschool children, no association between myopia and outdoor activity or near work was found (Low, Dirani et al. 2010), however only a relatively small proportion of the children were myopic (11.4%) and the children were reported to be spending less than 1 hour per day on total outdoor activities, which may have been insufficient to produce a measurable effect.

One aspect of research into the effect of outdoor activity and light on the development of myopia that may confound such studies is the reliability of self-reported outdoor activity data, produced from diaries or questionnaires. The use of small portable data-logging light meters (Backhouse 2011) has revealed that relatively poor agreement is found between quantitative measures of light exposure and the data obtained from diaries (Dharani, Lee et al. 2012) or questionnaires (Alvarez and Wildsoet 2013). Alternatively, the biomarker, Conjunctival Ultraviolet Autofluorescence (CUVAF), has been shown to be positively correlated with time spent outdoors, and may have the benefit of providing a long-term record of outdoor light exposure (Kearney, O'Donoghue et al. 2016).

As discussed above, some debate still exists regarding which aspect of outdoor activity (physical, illuminance or dioptric distance) is responsible for reducing the prevalence or risk of developing myopia in children (Ngo, Saw et al. 2013). In a study investigating myopia development in university medical students Jacobsen *et al* (Jacobsen, Jensen et al. 2008), concluded that 1 hour per day of physical activity had an antimyopiagenic effect countering 3 hours per day of near work. Therefore, the question arose as to whether it was physical activity associated with being outdoors that was actually the protective factor. While Jacobsen *et al* found an inverse association between time spent in physical activity and myopic refractive change over time, the index of physical fitness ($V_O{}^2{}_{max}$), was not significantly different between the myopic and non-myopic students (Jacobsen, Jensen et al. 2008). This suggests that fitness itself did not have a direct influence on refractive status in these students. Furthermore, Rose *et al* (Rose, Morgan et al. 2008a) were able to separate out the effects of indoor sport and outdoor activity on the prevalence of myopia in their Sydney study cohorts. They found no association between indoor sport and myopia, and concluded that total time spent outdoors, including

hours in non-active endeavours rather than outdoor physical activity, was the relevant factor in predicting the prevalence of myopia in the study populations (Rose, Morgan et al. 2008a).

Myopia and Light at Night

The evidence for a protective effect of outdoor activity, most likely mediated by the higher light intensity levels found outdoors (Phillips, Backhouse et al. 2012; French, Ashby et al. 2013; Ngo, Saw et al. 2013) provides an interesting counterpoint to the controversy from the early 2000s regarding whether the use of night-time ambient lighting before the age of 2 years was strongly associated with the prevalence of myopia in later childhood (Quinn, Shin et al. 1999; Czepita, Goslawski et al. 2004) or not (Gwiazda, Ong et al. 2000; Zadnik, Jones et al. 2000; Saw, Zhang et al. 2002; Guggenheim, Hill et al. 2003). The proposal that ambient light levels at night can influence refractive status receives additional support from the finding that in a cohort of law students (mean age 27 years), those who reported less than 5.6 darkness hours per day were more likely to report myopia progression during the course of their degree study (Loman, Quinn et al. 2002). Therefore, less daily exposure to darkness, most likely the result of studying at night under artificial light, was associated with increased risk of myopia progression.

That ambient lighting at night might promote myopia while outdoor ambient lighting during the day might protect against myopia suggests a role for the modification of retinal circadian rhythms by light in the control of eye growth (Phillips, Backhouse et al. 2012; Feldkaemper and Schaeffel 2013; French, Ashby et al. 2013). Several initial hypotheses were proposed to explain this association, including that outdoor activity produces a “stop” signal for axial elongation through mechanisms as diverse as increased choroidal blood flow (Jones, Sinnott et al. 2007) to an increase in retinal dopamine levels which inhibit eye growth (Rose, Morgan et al. 2008a).

Myopia, Light and Dopamine

The role of the retinal neurotransmitter dopamine as a possible mediator of the effect of outdoor activity on myopia is supported by experiments in the chick which have shown that light exposure increased retinal dopaminergic activity as measured by vitreal DOPAC levels (Megaw, Morgan et al. 1997; Megaw, Morgan et al. 2001; Megaw, Boelen et al. 2006) and that dopamine synthesis is related to increased light intensity in an approximately log-linear relationship (Cohen, Peleg et al. 2012). Furthermore, dopaminergic activity is reduced, and the response to light exposure is also attenuated by form-deprivation (Megaw, Morgan et al. 1997). Conversely, the administration of exogenous

dopaminergic agonists reduces axial elongation due to form-deprivation in monkeys (Iuvone, Tigges et al. 1991) and chicks (Stone, Lin et al. 1989), thereby reducing the degree of myopia induced. Therefore, the relationship between FDM, light and retinal dopamine levels has been clearly established, supporting the previously proposed hypothesis of a dopamine-dependent "retinal light-dark switch" in the control of ocular growth (Morgan and Boelen 1996). The role of retinal dopamine in the control of eye growth is discussed further in Section 1.2.5.

The role of high outdoor ambient light levels as the protective aspect of outdoor activity on the development of myopia has been supported by experiments which have investigated the effect of high ambient lighting on FDM and LIM in a range of animal models. Previously, high intensity ambient illumination, approximating outdoor light levels, has been shown to suppress form-deprivation myopia (FDM) in the chick (Ashby, Ohlendorf et al. 2009; Lan, Feldkaemper et al. 2014) in a dose-dependent manner (Karouta and Ashby 2015), in the monkey (Smith, Hung et al. 2012) and in the tree shrew (Siegwart, Ward et al. 2012). Bright light has also been shown to slow the development of lens-induced myopia (LIM) in the chick (Ashby and Schaeffel 2010) and guinea pig (Li, Lan et al. 2014), but proven to have minimal effect on LIM in the monkey (Smith, Hung et al. 2013). Therefore, the high ambient illumination level experienced during outdoor activities is the primary candidate as the stimulus responsible for the reduction in prevalence of myopia found in human epidemiological studies. The role of animal experiments in elucidating the potential mechanisms underlying the effect of light on refractive development is discussed further in Section 1.2.4.

Myopia and Diurnal, Seasonal and Annual Effects

Both human and animal studies have provided clear evidence for a relationship between myopia development and ambient light exposure. These effects are not only related to the diurnal photoperiod and intensity, but there is also evidence for seasonal and annual effects on the refractive status of humans (Phillips, Backhouse et al. 2012) and chicks (Sivak, Barrie et al. 1989; Schmid and Wildsoet 1996).

Animal models of myopia have demonstrated that a "normal" diurnal light:dark photoperiod is essential for normal refractive development in chicks (Li, Troilo et al. 1995; Stone, Lin et al. 1995), guinea pigs (Di, Lu et al. 2014), tree shrews (Norton, Amedo et al. 2006) and rhesus monkeys (Smith, Bradley et al. 2001). Under a normal diurnal light:dark cycle, chick eyes exhibit a diurnal growth rhythm in which greatest eye elongation occurs during the day with reduced growth rates at night (Weiss and Schaeffel 1993; Nickla, Wildsoet et al. 1998a; Nickla 2013). The chick choroid also exhibits a similar diurnal

rhythm in which the choroid thins during the day and thickens at night (Papastergiou, Schmid et al. 1998; Nickla, Wildsoet et al. 2001; Nickla and Wallman 2009). In chick eyes developing FDM, a phase shift occurs in the relationship between the axial length and choroidal rhythms such that peak axial growth occurs earlier than in the control eyes. Furthermore, the amplitude of the rhythmic variation in choroidal thickness is significantly greater than in normal eyes, with peak axial length coinciding with minimum choroidal thickness in the afternoon (Nickla, Wildsoet et al. 1998a). Conversely, in chick eyes exposed to myopic defocus (either induced by previous exposure to form-deprivation or negative lens wear, or with the use of positive lens wear) the diurnal rhythms of axial growth and choroidal thickness shift into phase and axial growth slows as expected (Nickla 2006).

While light during the daytime phase of a diurnal cycle is beneficial to normal refractive development, light at night can be disruptive (Phillips, Backhouse et al. 2012). Li and Howland (Li, Howland et al. 2000) demonstrated that at least a 4 hour period of darkness per diurnal cycle was necessary to produce normal emmetropisation in the chick, and that the dark period was most effective when presented as a single block rather than in multiple periods of darkness of 4 hours in total. However, total darkness may not be necessary for normal emmetropisation as chicks raised under light:dim (1500:500 $\mu W/cm^2$) or light:dark diurnal cycles had comparable ocular refractions and dimensions (Liu, Pendrak et al. 2004). Conversely, two hours of light in the middle of the night was recently reported to disrupt the normal sinusoidal diurnal rhythms of variation in axial length and choroidal thickness in chicks (Nickla and Totonelly 2016). Of particular interest is the finding that the ocular growth rate increased acutely on exposure to light for the following 6 hour period from midnight to 6 am. Nickla and Totonelly (Nickla and Totonelly 2016) postulated that this increased growth may have been responsible for the more myopic refractive error that developed in these chicks. They also noted that these findings in chicks had parallels to the reports of an increase in prevalence of myopia in children who slept with night-lights in childhood, as discussed previously (Quinn, Shin et al. 1999).

There is some published evidence for a seasonal effect on susceptibility to FDM in chicks (Sivak, Barrie et al. 1989; Schmid and Wildsoet 1996). In an investigation into influences of chick breed (strain) and gender on induction and recovery from FDM, Schmid and Wildsoet (Schmid and Wildsoet 1996) concluded that both breed and gender-dependent differences existed between White Leghorn and Broiler-Cross cross chicks in their response to both FD and normal ocular growth. These results were consistent with a similar investigation by Sivak (Sivak, Barrie et al. 1989), and Wildsoet and Schmid concluded that evidence of a variation in growth rates and development of FDM within breeds

may be evidence of a seasonal variation in susceptibility (Schmid and Wildsoet 1996). A seasonal variation in susceptibility to FDM induction in White Leghorn chicks, with a reduction in induced myopia in winter, has also been observed by other researchers (Ashby, 2017; *personal communication*).

The length of the daylight phase of the diurnal light cycle associated with the season of birth may also influence refractive error late in life in humans. The longer photoperiod associated with birth during summer months has been identified as a risk factor for the development of myopia in a study of 276,911 adolescents born in Israel (Mandel, Grotto et al. 2008). Prevalence of both moderate and severe degrees of myopia varied by birth month, with the summer months (June/July) associated with a higher prevalence than the winter months (December/January). Furthermore, increasing photoperiod was associated with increasing myopia prevalence in a dose-dependent pattern. Severe myopia associated with the longest birth photoperiod category had the highest OR of 1.24 relative to the shortest photoperiod. Conversely, mild myopia was not associated with either season of birth or photoperiod (Mandel, Grotto et al. 2008). Season of birth was also found to be significantly associated with adult refractive status in a UK study, where being born in summer (OR = 1.17) or autumn (OR = 1.16) was significantly associated with the presence of high myopia (< -6.00 D) (McMahon, Zayats et al. 2009). However as both summer and autumn birth were associated with a higher risk of myopia, postnatal photoperiod was not directly associated with prevalence of myopia in adulthood.

In a retrospective study of the refractive data of 1 - 3 month old children, collected over a 32 year period, Deng and Gwiazda (Deng and Gwiazda 2011), found that the photoperiod length 30 days after birth was significantly associated with refractive error. Children born in the season with the highest daylight hours (i.e. summer) were found to have a lower (more myopic) refraction on average when compared to those born in the period with the lowest daylight hours (i.e. winter).

More recently, a study of children 3 year of age or younger conducted in eastern China found that children born in winter had on average a significantly more myopic refraction by 0.12 D when compared to those born in summer (Ma, Xu et al. 2014). This finding is the opposite of the previous studies of Mandel *et al* (Mandel, Grotto et al. 2008) and Deng and Gwiazda (Deng and Gwiazda 2011). Therefore, the exact association between season of birth, perinatal photoperiod and refractive status is unclear, with potentially many confounding environmental factors affecting refraction, particularly later in life.

Seasonal variations in photoperiod between summer and winter become more extreme at higher latitudes and therefore seasonal effects on refractive error might be expected to be more pronounced

in populations living at these latitudes. However, in a study of Finnish military conscripts (Vannas, Ying et al. 2003), no associations were found between myopia prevalence and birth month, global irradiance at birth month, or daily hours of darkness during the birth month. Nevertheless, there was a trend towards higher prevalence of myopia among conscripts living above the Arctic Circle where a 24 daylight photoperiod is present during the summer months, and conversely a 24 period of near darkness exists during winter.

While the indications for an effect of season of birth on refractive status are conflicting, there is published evidence of an annual variation in the rate of myopia progression, axial length elongation and corneal power change in Danish children (8 - 14 years) with myopia (Cui, Trier et al. 2013). As Denmark is located relatively northern latitudes of Europe, the day length varies from 7 hours in winter to 17.5 hours in summer. Increased axial growth, myopia progression and increase in corneal power were all significantly associated with the lower hours of daylight in winter, while the opposite relationships were associated with the longer daylight hours of summer (Cui, Trier et al. 2013). This annual variation in myopia progression associated with hours of daylight, is consistent with the association between increased levels of outdoor activity (and exposure to outdoor light levels) and the lower prevalence and incidence of myopia reported in the literature (French, Ashby et al. 2013).

Interestingly, season of birth has also been identified as influencing adult personality traits (Natale, Adan et al. 2002; Natale, Adan et al. 2007) that may be related to central nervous system (CNS) dopamine turnover rates which are greater in adults who were born during November/December (Northern winter), and lowest for those born in May/June (Northern summer)(Chotai and Adolfsson 2002). Therefore, a link between peri-natal photoperiod and adult dopamine turnover provides a possible mechanism for the interaction with myopia development and photoperiod via dopaminergic pathways.

1.2.3 Myopia: Interventions

Optical and pharmacological interventions are aimed primarily at slowing the progression of myopia, while environmental interventions may be effective at both decreasing the incidence and slowing the progression of myopia. Optical and pharmacological interventions have been comprehensively reviewed in both a Cochrane Review (Walline, Lindsley et al. 2011) and more recently in a meta-analysis of 16 interventions including low dose atropine, orthokeratology, defocus-modifying contact

lenses and progressive addition spectacles (Huang, Wen et al. 2016). Therefore, this section will briefly review optical and pharmacological interventions before discussing some encouraging ambient light environmental interventions that are aimed at preventing myopia development or progression in populations rather than individuals.

Optical Interventions

Optical interventions for myopia were initially based on the concept of managing accommodative demand, which was held to be a factor in the development of myopia based on its association with near work (Gwiazda 2009; Morgan, Ohno-Matsui et al. 2012; Schaeffel 2016). Under-correction of myopia with single vision lenses (SVL) was promoted by clinicians as it would reduce accommodative effort and therefore the supposed stimulus for myopia development. However, when this approach was assessed in randomised clinical trials (RCT) the results did not support the clinicians' beliefs (Chung, Mohidin et al. 2002; Adler and Millodot 2006). For example, in the RCT of Chung *et al* (Chung, Mohidin et al. 2002) the under-corrected groups actually exhibited significantly more progression (-1.0 D) than the fully-corrected control group (-0.75 D; $p < 0.01$).

Clinicians also traditionally prescribed bifocal lenses for myopia management on the basis of reducing accommodative demand. Bifocal lens correction of children with near-point esophoria was shown in a RCT to reduce the progression of myopia over 30 months (-0.99 D) when compared to SVL correction (-1.24 D; $p = 0.046$), however the effect was small and the authors did not consider that the evidence was strong enough to unequivocally recommend the intervention (Fulk, Cyert et al. 2000).

Correction of myopia with progressive addition lenses (PAL) has been shown to reduce myopia progression in a number of studies including the Correction of Myopia Evaluation Trails (COMET)(Gwiazda, Hyman et al. 2003) (COMET 2 Study Group 2011). Leung and Brown (Leung and Brown 1999) found that wearing PALs did reduce the progression of myopia by about half over two years, with +2.00 D addition lenses slowing progression (-0.66 D) more than +1.50 D lenses (-0.76 D). The COMET studies also found a statistically significant reduction in myopia progression, although it was considered to not be clinically significant (Gwiazda, Hyman et al. 2003; COMET 2 Study Group 2011). Conversely a 2-year longitudinal study of PAL wear in Hong Kong children found no statistically significant reduction in myopia progression when compared to SVL wear (Edwards, Li et al. 2002). These results indicate little effective role for PAL wear as an evidence-based myopia intervention.

The finding that myopic eyes often display relative peripheral hyperopic refractions (Mutti, Sholtz et al. 2000) has led to a number of optical solutions aimed at controlling the position of the peripheral image shell, including novel spectacle lens designs (Sankaridurg, Donovan et al. 2010). A one-year trail of peripheral defocus-controlling spectacle lenses showed no effect overall on myopia progression when compared to SVL wearers. However, a specific group of 6 - 12 year old children with a parental history of myopia displayed significantly less progression (0.29 ± 11 D less) with one of the trial lens designs (Sankaridurg, Donovan et al. 2010). A trial of similarly designed contact lenses (Sankaridurg, Holden et al. 2011), which eliminated the optical issues associated with eye movement behind a fixed spectacle lens, showed a reduction in myopia progression of 34%, and 33% less axial elongation when compared to SVL wear, which suggests that this approach is worthy of further investigation.

Further evidence for the potential of optical control of myopia progression with a novel contact lens design has been provided by Anstice *et al* (Anstice and Phillips 2011) who trialled a dual-focus soft contact lens design where the central distance correction zone was surrounded by a concentric addition zone to produce simultaneous myopic retinal defocus for myopia control. In a paired-eye cross-over experimental design, myopia progression was reduced by at least 30% in the eye wearing the dual-focus treatment lens during the first 10 months of the study when compared to the fellow eye wearing a single vision distance lens (Anstice and Phillips 2011). This lens design has been successfully commercialised as the CooperVision MiSight™ lens (CooperVision, NY; http://coopervision.com.my/contact-lenses/misight) and has proven to be efficacious in controlling myopia progression in practice (Turnbull and Phillips 2015; Turnbull, Munro et al. 2016).

Orthokeratology (OK), where rigid contact lenses are worn while sleeping, is typically utilised to correct myopia by flattening the corneal epithelium to reduce the effective corneal power during waking hours. A secondary effect has been identified in several longitudinal studies where OK has been shown to significantly reduce myopia progression by approximately 45% (Cho and Cheung 2012; Sun, Xu et al. 2015). Similar results were obtained in a study comparing axial growth progression in myopic children who wore an OK lens overnight in one eye, with a conventional rigid gas-permeable (RGP) contact lens worn in the other eye during the day. The RGP wearing eye exhibited a significant increase in axial elongation at 12 months while the OK eye showed no change from baseline axial length eye (Swarbrick, Alharbi et al. 2015).

A recent study by Kang and Swarbrick (Kang and Swarbrick 2016) investigated the role of peripheral refraction as a potential mechanism for the antimyopiagenic effect of OK. They demonstrated that OK

lenses produced significant refractive changes along both the horizontal and vertical meridians resulting in more myopic peripheral defocus, which may be the mechanism by which OK slows the progression of myopia (Kang and Swarbrick 2016). OK has also proven to be effective in controlling myopia progression in a retrospective case-series from a public myopia control clinic (Turnbull, Munro et al. 2016). Turnbull *et al* conclude that clinicians, on the basis of current evidence, should be offering contact lens-based methods of myopia control to patients at-risk of progression.

Pharmacological Interventions

Atropine is a non-specific muscarinic receptor antagonist and its ability to reduce the progression of axial myopia has been recognised for nearly 150 years (Schaeffel 2016). However, in the standard commercially available topical 1% concentration, the side-effects of mydriasis, photophobia and cycloplegia have limited its use as a myopia intervention, except in East Asia (McBrien, Stell et al. 2013). Nevertheless, the use of atropine as a myopia intervention has received renewed attention with the publication of a range of studies from small trials (Bedrossian 1979; Lee, Sun et al. 2016; Loughman and Flitcroft 2016) through to major RCT studies (Chua, Balakrishnan et al. 2006; Chia, Chua et al. 2012; Chia, Chua et al. 2014; Chia, Lu et al. 2016). Evidence for the differential efficacy of atropine treatment of myopia in Asian versus European (white) children has also been reported (Li, Wu et al. 2014; Polling, Kok et al. 2016). Meta-analysis of RCT studies has convincingly demonstrated that atropine eye drops in high dose (1% and 0.5%), moderate-dose (0.1%) and low dose (0.01%) can slow the progression of myopia in children (Huang, Wen et al. 2016). The finding that low dose 0.01% atropine had comparable clinical efficacy, but minimal side effects, when compared to the high and moderate doses, provided evidence for the use of the lower dose forms as a myopia intervention which would be better tolerated by children (Chia, Chua et al. 2012). A follow-up study revealed that at 12 months following cessation of atropine treatment, a degree of myopic rebound occurred, which was greatest (-1.15 D) in the high dose (1% atropine) group and lowest (-0.72 D) in the low dose (0.05%) group (Chia, Chua et al. 2014). The authors suggested that this differential dose-related rebound effect could indicate that the lower dose atropine acts at a more anterior site within the eye, rather than at the retina.

While the mechanism for the antimyopiagenic action of atropine is uncertain, evidence from animal studies suggest a role for the M_4 and M_1 receptor sub-types in the retina (McBrien, Arumugam et al. 2011; Arumugam and McBrien 2012; McBrien, Stell et al. 2013). Furthermore, evidence from chick studies strongly suggests that the antimyopiagenic action of atropine works through a non-

accommodative mechanism (McBrien, Moghaddam et al. 1993) and may even involve a non-receptor based mechanism in the sclera (Lind, Chew et al. 1998; McBrien, Stell et al. 2013).

Interestingly, there is evidence that atropine slows myopia progression more effectively in Asian than European (white) children. Although the mechanism that might explain a difference based on ethnicity is still to be explored, it may simply be related to the initially higher degrees of myopia and rates of progression in the studies of Asian children analysed (Li, Wu et al. 2014).

Environmental Interventions: Outdoor Activity and Ambient Light

Although the current epidemic of myopia is supposedly a modern phenomenon, a similar increase in myopia was well-recognised in Europe some 150 years ago with prevalence figures as high as 81% recorded for theology students in Tübingen, Germany by the ophthalmologist Hermann Cohn (Schaeffel 2016). At the time, Cohn identified extended periods of near work with poor illumination as a potential cause of myopia development, and he recommended a minimum luminance in work-places of “10 metre candles”, which is equivalent to 10 lux of illumination, as an important intervention (Schaeffel 2016). Although 10 lux may have represented a large improvement in lighting at the time (presumably in comparison to 1 candle or gas-light), it would be considered a very low illumination level in a modern work environment where task illumination of at least 500 lux is recommended. In turn, a task illumination of 500 lux is of low magnitude compared to the light levels of up to 100,000 lux that can be experienced outdoors on a sunny day (van Bommel and Rouhana 2011).

The role of high outdoor ambient light levels as the protective aspect of outdoor activity on the development of myopia is supported by experiments which have investigated the effect of high ambient lighting on the suppression of FDM or slowing of LIM in a range of animal models including; the chick (Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010; Lan, Feldkaemper et al. 2014; Karouta and Ashby 2015), the guinea pig (Li, Lan et al. 2014), the monkey (Smith, Hung et al. 2012) and the tree shrew (Siegwart, Ward et al. 2012). Therefore, in humans the high ambient illumination levels experienced during outdoor activities are likely to provide the stimulus responsible for the protective effect of outdoor activity on myopia. However, other aspects of light such as wavelength distribution (including UV content) should also be considered (Sherwin, Reacher et al. 2012).

While many animal experiments have investigated the influence artificial high intensity illumination on the development of both FDM and LIM, a recent study has examined the development of FDM in White Leghorn chicks in a natural outdoor environment (Stone, Cohen et al. 2016). The chicks were raised

during daylight hours in an outdoor rural environment, where the outdoor light intensity varied from >20,000 lux (assumed to be as high as 100,000 lux) on a sunny day to between 1500 – 3300 lux at chick eye level on a cloudy day. An interesting effect of duration of exposure to the outdoor environment was found where FDM was reduced by some 44% (4.1 D) at 4 days post-FD, but by 11 days post-FD the protective effect was either absent (chicks FD at 5 days post-hatch) or reversed (chicks FD at 9 days post-hatch) with chicks raised outdoors having approximately 80% more relative myopia than those chicks raised indoors (Stone, Cohen et al. 2016). In all cases the changes in refraction were strongly correlated with changes in both VCD and AXL. Only the outdoor-raised chicks that were occluded at 9 days post-hatch and measured at 11 days showed an increase in ACD which may have resulted from corneal steepening, but this was not measured. No statistically significant differences were found between levels of retinal dopamine by either outdoor versus indoor raising status, or by age at deprivation. However, form-deprived eyes consistently showed reduced retinal and vitreous DOPAC levels when compared to contralateral non-deprived eyes (Iuvone, Tigges et al. 1991). Therefore, unlike previous indoor experiments with consistent and well-controlled levels of high intensity illumination, outdoor rearing of chicks produced only an incomplete and transient decrease in FDM. It is likely that this difference in outcome is related to the variability of the outdoor light environment, including diurnal intensity profiles and spectral composition. Stone *et al* (Stone, Cohen et al. 2016) argue that this actually a strength of the study as it is of greater relevance to the experience of children outdoors than the standard laboratory-based artificial light experiments reported previously.

A meta-analysis of the epidemiological evidence for outdoor activity as an effective myopia intervention found that every additional hour per week of outdoor activity reduced the odds of myopia by 2% (Sherwin, Reacher et al. 2012). Moreover, evidence was found that incidence, prevalence and progression of myopia were all reduced by increasing time spent outdoors (Sherwin, Reacher et al. 2012). In the period after the publication of Sherwin's meta-analysis a number of interventional trials have been conducted involving either increasing the time children spend outdoors (Morgan, Xiang et al. 2012; Wu, Tsai et al. 2013; Ngo, Pan et al. 2014; He, Xiang et al. 2015) or by increasing the light levels within schoolrooms (Hua, Jin et al. 2015).

The Guangzhou Outdoor Activity Longitudinal Study (GOALS) is an interventional trial involving increased outdoor activity periods for schoolchildren attending primary schools in Guangzhou, China (Morgan, Xiang et al. 2012). The children were in grade 1 (6 -7 years) at the beginning of the trial, and schools were assigned randomly to the control and intervention arms. In the intervention schools, children undertook additional structured outdoor activities after school. After one year of intervention

the prevalence of myopia increased by 8.6% in the control arm and by only 5.5% in the intervention arm (Morgan, Xiang et al. 2012). While the differences were statistically significant, the authors noted that the effects were very small, and that longer periods of outdoor activity might be required to reach clinical significance.

In Taiwan, another school-based outdoor activity intervention trial required children in the intervention arm to spent recess periods (total of 80 minutes per day) outside the classroom in outdoor activities (Wu, Tsai et al. 2013). No special programmes or activities took place during recess in the control school. After one year, significant differences in onset of myopia (8.41% vs. 17.65%; $p < 0.001$) and magnitude of myopic shift (-0.25 D vs. -0.38D; $p = 0.029$) were found for the intervention and control groups respectively. However, multivariate analysis showed that these effects were evidence of a protective effect for non-myopic children only, but not for myopic children (Wu, Tsai et al. 2013). Therefore, the primary effect of this trial appears to have been a decrease in incidence of myopia, rather than progression.

In Singapore, an incentive-based RCT of increased outdoor activity called the Family Incentive Trial (FIT) was undertaken (Ngo, Pan et al. 2014). Children between 6 - 12 years of age were randomly allocated to the intervention or control arms of the study. In intervention arm, children received education regarding myopia and good eye care habits, structured weekend activities and pedometers to increase their overall daily activity. Outdoor activity time was measured via a questionnaire and diary. At 6 months, outdoor time was significantly higher in the intervention group by approximately 2.4 hours per week, however by 9 months no significant difference remained in time spent outdoors (Ngo, Pan et al. 2014). While this study did not assess refractive outcomes, it did demonstrate the limitations of an activity-based intervention programme where participation is likely to decrease over time.

One answer to the issue of decreased compliance with outdoor activity programmes over time is to change the indoor environment to make it less myopiagenic. Hue e*t al* (Hua, Jin et al. 2015) investigated the effect of increasing the indoor illumination on myopia development in four schools in North-east China. Two schools (one primary and one secondary) were assigned to the intervention arm while another two were assigned to the control arm. In the intervention schools, the classroom lighting systems were redesigned with the aim of attaining a minimum illuminance of 300 lux on the desks. In fact, following the installation of the new lighting systems, the median illuminance at desk level was 558 lux. In the control schools the median illumination of desks was measured to be only 98 lux. At the one year follow-up, the intervention arm had a statistically significantly lower incidence of

myopia (4% vs. 10%) when compared to the control arm. Furthermore, for non-myopic children, both the decrease in refractive error (-0.25 D vs. -0.47 D) and increase in axial length (0.13 vs. 0.18mm) were significantly less in the intervention arm than in the control arm (Hua, Jin et al. 2015). Therefore, this study demonstrated the effectiveness of relatively small increases in indoor illumination levels on reducing the incidence of myopia in schoolchildren. While the targeted indoor illumination level at the desk was only 300 lux, several orders of magnitude lower than a child might be exposed to outdoors for short periods, the results are supported by the experimental findings of Backhouse *et al* (Backhouse, Collins et al. 2013) who demonstrated that exposure of chicks undergoing form-deprivation to 2000 lux illumination for 12 hours per day was more effective at suppressing FDM than 10,000 lux presented for only 2 hours per day. As outdoor activity-based programmes may suffer from decreased compliance over time, intervening by changing the lighting conditions of the indoor school environment may prove to be a more effective public health measure against myopia in the long term.

1.2.4 Animal Models of Myopia

Animal models of myopia have proven invaluable in the exploration of the underlying mechanisms of emmetropisation and myopia development including: the discovery that growth of the eye is visually guided and locally controlled through experiments using form-deprivation or defocussing lenses; the expansion of our understanding of some of the signalling cascade molecules that trigger eye growth; the elucidation of diurnal and circadian rhythms within the eye of neurotransmitters such as dopamine; the visually-induced changes in choroidal thickness; the activation of scleral remodelling and growth; and of particular relevance to this study, the demonstration that high ambient illumination levels can suppress form-deprivation myopia (FDM) (Wallman and Winawer 2004; Ashby, Ohlendorf et al. 2009; Feldkaemper and Schaeffel 2013; Morgan, Ashby et al. 2013; Rucker 2013; Schaeffel and Feldkaemper 2015).

The majority of animal models of myopia represent a form of experimental myopia, where the refractive error is induced by an intervention that alters some aspect of the retinal image, such as the reduction of spatial contrast using translucent diffusers in FDM, or the production of hyperopic retinal defocus, as in lens-induced myopia (LIM). Although both FDM and LIM generally induce axial myopia with associated choroidal thinning, FDM is an open-loop process with no visual feedback to control the end point of axial growth, while in LIM the eye is a closed loop process with the eye effectively emmetropising to the imposed defocus which provides an end-point to the growth signal (Wallman and

Winawer 2004; Morgan, Ashby et al. 2013). Furthermore, several differences have been reported between FDM and LIM such as the effect of constant light (Bartmann, Schaeffel et al. 1994), and the role of parasympathetic input (Nickla and Schroedl 2012) on myopia development. Also in chicks, while the progression of FDM could be fully suppressed by sufficiently bright ambient illumination (Karouta and Ashby 2015), high ambient illumination only tended to slow the development of LIM, with the final end point of refraction unchanged (Ashby and Schaeffel 2010).

The results of Bartmann *et al* (Bartmann, Schaeffel et al. 1994) are of particular relevance to this study as they demonstrated that raising chicks under constant light fully suppressed the development of FDM after 13 days, while LIM was unaffected after 11 days. As both retinal dopamine and vitreal DOPAC levels were reduced by FDM and constant light, but not by LIM alone, the authors suggested that the two forms of experimental myopia may not share a common dopaminergic signalling pathway (Bartmann, Schaeffel et al. 1994). Conversely, Morgan and Ashby, in a point-counterpoint article with Nickla (Morgan, Ashby et al. 2013), argued that it is likely that FDM and LIM share a common dopaminergic signalling pathway as dopamine agonists have been shown to block the axial elongation associated with either FDM or LIM in chicks (Stone, Lin et al. 1989; Schmid and Wildsoet 2004; Feldkaemper and Schaeffel 2013) and that the retinal amacrine cell synthesis of the intermediate-early gene product ZENK/Egr-1 is suppressed in both conditions (Fischer, McGuire et al. 1999).

While FDM can be been induced in a wide range of animals including the chick (Wallman, Turkel et al. 1978), tree shrew (Sherman, Norton et al. 1977; McBrien and Norton 1992), macaque monkey (Wiesel and Raviola 1977; Raviola and Wiesel 1985; Smith, Harwerth et al. 1987), guinea pig (Howlett and McFadden 2002; Howlett and McFadden 2006), marmoset (Troilo and Judge 1993) and mouse (Schaeffel, Burkhardt et al. 2004), naturally-occurring myopia in animals is rare. Several breeds of dog including the German Shepherd, Rottweiler, and Miniature Schnauzer have been reported as exhibiting naturally-occurring myopia, with prevalence levels as high as 53% of German Shepherd dogs in one veterinary practice population, although this may have been associated with the development of cataract in older dogs (Murphy, Zadnik et al. 1992). In the Labrador Retriever, reported prevalence estimates for myopia ≤ -0.50 D ranged from 14.7% (Mutti, Zadnik et al. 1999) to 31% (Black, Browning et al. 2008). Furthermore, the myopia was found to be due to an increase in vitreous chamber depth (Mutti, Zadnik et al. 1999) and heritable, with approximately equal genetic and environmental variance (Black, Browning et al. 2008). Axial myopia has also been reported to develop in selected strains of guinea pigs, both in pigmented (Jiang, Schaeffel et al. 2009), but more predominantly in albino guinea pigs where some 70% were reported to be myopic at 2 weeks of age (Jiang, Long et al. 2011).

As this study was primarily aimed at investigating the relationship between aspects of ambient illumination and the development of myopia in animal models, form-deprivation was used to induce myopia in these experiments, as it is a reliable and well-characterised method of producing axial myopia in both chicks (Wallman and Adams 1987; Wallman and Winawer 2004) and guinea pigs (Howlett and McFadden 2002; Howlett and McFadden 2006).

The Guinea Pig

In Chapter 3, the guinea pig (*Cavia porcellus*) is used as an animal model of myopia to investigate the effects of regular, periodic high intensity illumination on FDM and retinal dopamine concentration. The guinea pig has the advantage of being a mammalian model of myopia, with a typical simple mammalian scleral structure (Backhouse 2009) unlike the chick (Schaeffel and Feldkaemper 2015). The guinea pig is a precocial species with newborn pups able to walk and eat solid food immediately after birth, they can be weaned as early as one week of age and are easy to handle and maintain (Sluckin and Fullerton 1969; Wagner and Manning 1976). The guinea pig also has been widely used as a model of human metabolism including the effects of exercise on endurance training and plasma lipoprotein levels (Hoppeler, Altpeter et al. 1995; Turner, Hoppeler et al. 1995; Fernandez 2001; Ensign, McNamara et al. 2002; West and Fernandez 2004).

Although guinea pigs are members of the order Rodentia, they are active during daylight hours (Harper 1976), have vision in the same order as the tree shrew (Schaeffel and Feldkaemper 2015) and show OKN responses (Hayes and Ireland 1969). The eye is similar in size to the chick's, with a predicted axial length of 8.4 mm at 21 days of age (Howlett and McFadden 2007). Emmetropisation occurs from birth where an average refraction of +4.40 ± 0.40 D two days after birth reduces at a rate of approximately -0.17 D/day, reaching a refraction of +0.70D at 30 days of age (Howlett and McFadden 2007). While the retina of the guinea pig is rod-dominated (λ_{max} ~500 nm), it also possesses two types of cones: the short-wavelength S-cone (λ_{max} ~400 nm) and medium-wavelength M-cone (λ_{max} ~530 nm) which indicates that the guinea pig is likely to have dichromatic colour vision (Parry and Bowmaker 2002). However, the S-cones are predominantly found in the lower half of the retina while the M-cones are predominantly found in the upper half of the retina, while in the central transition zone, approximately 10% of cones express both S and M opsins although their exact function is not yet determined (Parry and Bowmaker 2002).

The guinea pig reliably develops both form-deprivation and lens-induced myopia (Howlett and McFadden 2002; McFadden, Howlett et al. 2004; Howlett and McFadden 2006; Lu, Zhou et al. 2006;

Zhou, Qu et al. 2006; Howlett and McFadden 2007; Howlett and McFadden 2009). Like the chick, the development of experimentally-induced myopia is primarily associated with an increase in vitreous chamber depth (VCD), although changes in the anterior segment including steepening of the cornea, increasing anterior chamber depth and thickening of the crystalline line following form-deprivation have been reported (Howlett and McFadden 2006; Howlett and McFadden 2009). Significantly, Howlett and McFadden (Howlett and McFadden 2006) found that the power of the cornea was well-correlated with vitreous chamber depth in the deprived eye, and concluded that these two components of refractive error may exhibit coordinated growth. Furthermore, they postulated that the growth of the crystalline lens may also be regulated by visual input. Overall, FDM develops more slowly in the guinea pig than in the chick with -4.8 D of relative myopia induced on average after 6 days of form-deprivation, and -6.6 D after 16 days (Howlett and McFadden 2006).

The Chick

In Chapter 4, the domestic chick (*Gallus gallus domesticus*) is used as an animal model of myopia to investigate whether periodic high intensity white and quasi-monochromatic (coloured) light suppresses FDM development in an avian model (Wallman, Turkel et al. 1978; Wallman and Adams 1987). First described some 38 years ago, the chick is a very widely used model of experimentally-induced myopia (Schaeffel and Feldkaemper 2015) with a major role in the investigation of many aspects of myopia from the demonstration of the local control of ocular growth (Troilo, Gottlieb et al. 1987); the investigation of growth-related retinal signalling molecules including dopamine (Stone, Lin et al. 1989), retinoic acid (Mertz and Wallman 2000) and nitric oxide (Nickla and Wildsoet 2004); scleral modification in FDM (Gottlieb, Joshi et al. 1990); to the inhibition of both FDM and LIM by high ambient illumination (Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010).

Chicks are also precocial and easy to maintain (Bell and Weaver 2002), have well described visual optics (Schaeffel, Glasser et al. 1988; Schaeffel and Howland 1988), good visual acuity (6-7 cycles/degree) (Demello, Foster et al. 1992), and tetrachromatic colour vision (Osorio, Vorobyev et al. 1999; Hart 2001). While chicks have active accommodation of up to 20 D (Troilo and Wallman 1987; Schaeffel, Glasser et al. 1988), the ciliary muscle contains nicotinic receptors (rather than the muscarinic receptors found in humans)(McBrien, Moghaddam et al. 1993). While this means that accommodation cannot be paralysed with the muscarinic antagonist atropine, it did allow for the pivotal

demonstration that atropine exerts its antimyopiagenic effects via a non-accommodative mechanism (McBrien, Moghaddam et al. 1993).

While chicks are a widely used animal model for the study of myopia there is evidence of variation in susceptibility to the induction of experimental myopia both between and within breeds (strains) and between genders (Stone, Lin et al. 1995; Troilo, Li et al. 1995; Schmid and Wildsoet 1996; Guggenheim, Erichsen et al. 2002). For example, White Leghorn chicks have been shown to be both more susceptible to the induction of myopia by FD than a Broiler cross breed (Schmid and Wildsoet 1996), and less susceptible than another Broiler strain (Sivak, Barrie et al. 1989). Furthermore, Sivak *et al* (Sivak, Barrie et al. 1989) found that two groups of White Leghorn chicks displayed a difference in the degree of FDM induced that was of the same order of magnitude as the differences between breeds. Such a difference in susceptibility to FDM has been selectively bred for in White Leghorn chicks (Chen, Hocking et al. 2011; Chen, Prashar et al. 2011). After selectively breeding high-susceptibility and low-susceptibility lines for 3 generations, the high-susceptibility line developed approximately twice as much FDM after 10 days of form-deprivation (Chen, Hocking et al. 2011). Up to 50% of the susceptibility effect was explained by an additive genetic effect, however the effect was not symmetrical, with the low susceptibility line exhibiting 49% less myopia, while the high susceptibility line exhibited 14% more myopia (Chen, Hocking et al. 2011; Chen, Prashar et al. 2011). Therefore, when making comparisons between experiments carried out on the same breeds at different times, or between different breeds, consideration must be given to differential susceptibility to the induction of experimental myopia as potential source of any difference in outcomes.

1.2.5 Myopia, Light and Dopaminergic Pathways

A wide range of signal molecules and receptors have been proposed as being involved in the local control of ocular growth including: dopamine (Iuvone, Tigges et al. 1991; Rohrer, Spira et al. 1993; Weiss and Schaeffel 1993; Guo, Sivak et al. 1995; Luft, Iuvone et al. 2004; Mao, Liu et al. 2010; Dong, Zhi et al. 2011; Cohen, Peleg et al. 2012; Lan, Yang et al. 2016), melatonin (Hoffmann and Schaeffel 1996; Rada and Wiechmann 2006; Wang, Zhou et al. 2011), nitric oxide (Nickla and Wildsoet 2004; Wu, Liu et al. 2007; Nickla, Lee et al. 2013), retinoic acid (Mertz and Wallman 2000; McFadden, Howlett et al. 2004; Troilo, Nickla et al. 2006; Mao, Liu et al. 2012), serotonin (George, Schmid et al. 2005; Kumari, Sreetama et al. 2007), ZENK/Egr-1 (Fischer, McGuire et al. 1999; Ashby, McCarthy et al. 2007; Ashby, Zeng et al. 2014), and muscarinic acetylcholine antagonists and receptors (Stone, Lin et al. 1991; Cottriall and McBrien 1996; Ashby, McCarthy et al. 2007; Liu, Wu et al. 2007; Arumugam and McBrien 2012).

In particular, the role of the retinal neurotransmitter dopamine and associated dopaminergic pathways (Witkovsky 2004) in the control of ocular growth has been investigated extensively (Feldkaemper and Schaeffel 2013). This interest followed the demonstration that raising chicks under high ambient illumination conditions (up to 10,000 lux) significantly increased vitreal DOPAC, a metabolite of dopamine, in a logarithmic relationship, which in turn was correlated with refractive status (Cohen, Peleg et al. 2012). The link between light, dopamine and experimental myopia is further evidenced by the findings that: retinal dopamine levels are reduced in FDM in chicks (Stone, Lin et al. 1989); dopamine agonists or promoters slow the development of FDM in chicks (Stone, Lin et al. 1989; Ashby, McCarthy et al. 2007), monkeys (Iuvone, Tigges et al. 1991) and guinea pigs (Mao, Liu et al. 2010; Mao, Liu et al. 2016); high ambient illumination levels can suppress FDM and slow LIM in chicks (Schmid and Wildsoet 2004; Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010), suppress FDM in the monkey (Smith, Hung et al. 2012; Smith, Hung et al. 2013) and reduce LIM in the guinea pig (Li, Lan et al. 2014); and the intravitreal injection of the dopaminergic D_2 receptor antagonist spiperone can abolish the protective effect of high ambient illumination on FDM in the chick (Ashby and Schaeffel 2010).

However, the relationship between a putative retinal dopaminergic growth-control pathway and LIM is less clear as in the guinea pig the subconjunctival injection of the non-selective dopaminergic agonist apomorphine (APO) did not significantly inhibit LIM, while it showed a dose-dependent inhibitory effect on FDM (Dong, Zhi et al. 2011). Furthermore, in the albino guinea pig a complex relationship has been

demonstrated between the effects of various dopaminergic agonists, and the development of myopia which occurs naturally in these animals (Jiang, Long et al. 2014). The non-specific agonist APO inhibited the development of myopia in relatively high doses (250 and 750 ng), but unexpectedly promoted myopia development at a lower dose (25 ng). The D_1 agonist SKF38393 (100 ng) and the D_2 antagonist sulphride (2500 ng) also inhibited, while the D_2 agonist quinpirole (100 ng) and D_1 antagonist SCH23390 (2500 ng) promoted myopia development in this strain of albino guinea pigs (Jiang, Long et al. 2014). Therefore, the overall results suggest that activation of D_1-like receptors inhibits the development of myopia, while the activation of D_2-like receptors promotes myopia, but that the dose-response is biphasic, a more complex situation than had been described previously in the chick (McCarthy, Megaw et al. 2007; Nickla, Totonelly et al. 2010).

Rohrer *et al* (Rohrer, Spira et al. 1993) using FDM in the chick demonstrated that the protective effects of the non-specific agonist APO were blocked by the D_2 specific antagonist spiperone, but not by the D_1 specific antagonist SCH-23390. As APO has a 22 times higher affinity for D_2 than D_1 receptors (Feldkaemper and Schaeffel 2013) this suggests that the antimyopiagenic effect of dopamine agonists is primarily mediated by D_2 receptors. Moreover, in LIM in the chick, both APO (non-selective) and quinpirole (D_2 specific) agonists prevented development of myopia associated with hyperopic defocus (Nickla, Totonelly et al. 2010). Conversely the D_1 specific antagonist SKF-38393 did not significantly reduce the development of LIM, however the resulting refractions were not as myopic as the lens-wearing control eyes which received a sham saline injection. The authors concluded that while their results suggested the D_2 receptor mechanism predominated in the control of FDM where there was no visual feedback, a secondary D_1 receptor mechanism may be active in LIM where visual feedback was present (Nickla, Totonelly et al. 2010; Nickla and Totonelly 2011).

Although atropine, a muscarinic acetylcholine receptor antagonist, has proven to be a viable pharmacological intervention against myopia in humans (Chua, Balakrishnan et al. 2006; Chia, Chua et al. 2012; Chia, Chua et al. 2014), the standard higher dose (1%) preparations have multiple side-effects associated with their mydriatic and cycloplegic receptor-based actions in the anterior eye (Chia, Chua et al. 2012). Therefore, some effort has been directed towards finding a safe and effective dopaminergic agent for the treatment of progressive myopia. One candidate is cytidine 5'-diphosphocholine (CDP-choline), or Citicoline which is available as dietary supplement in the USA (Cognizin; http://cognizin.com/what-is-citicoline). It has been shown to stimulate dopaminergic systems in the CNS, and has been used to treat conditions such as Parkinson's disease, head trauma and amblyopia (Mao, Liu et al. 2016). Intraperitoneal (IP) administration of citicoline has also been shown

to increase retinal dopamine concentrations in rabbits (Mao, Liu et al. 2016). Therefore, Mao *et al* (Mao, Liu et al. 2016) investigated the effect of IP citicoline on FDM in guinea pigs. Administration of citicoline during 10 days of form-deprivation significantly reduced the degree of induced myopia and significantly raised retinal dopamine levels (0.55 ± 0.21 ng to 0.81 ng ± 0.24 ng, $p < 0.01$), demonstrating that systemic administration of citicoline was potentially an effective antimyopiagenic therapeutic agent and worthy of further investigation (Mao, Liu et al. 2016).

Interestingly, both cholinergic (e.g. pharmacological control of myopia with atropine) and dopaminergic (e.g. environmental control of myopia with outdoor activity/ambient light) signals affecting eye growth may act through a common pathway (Schmid and Wildsoet 2004; Ashby, McCarthy et al. 2007; Ashby, Kozulin et al. 2010). For example, intravitreal injection of the muscarinic antagonist atropine has been shown to increase retinal dopamine levels (Schwahn, Kaymak et al. 2000). Furthermore, both muscarinic antagonists and dopamine agonists have been shown to increase expression of the *ZENK/Egr-1* gene and decrease the resulting difference in axial length between the eyes in both FDM and LIM in chicks (Ashby, McCarthy et al. 2007; Ashby, Kozulin et al. 2010). The authors concluded that both muscarinic antagonists and dopamine agonists were likely to exert their antimyopiagenic effects early in the signal cascade that controls eye growth (Ashby, McCarthy et al. 2007; Ashby, Kozulin et al. 2010). More recently Ashby *et al* (Ashby, Zeng et al. 2014) demonstrated in the guinea pig that the expression of the *ZENK/Egr-1* gene, as measured by mRNA analysis, was down-regulated during the induction of LIM and then subsequently up-regulated during recovery from LIM. Consequently, ZENK/Egr-1 was proposed as a possible bidirectional growth signal for ocular growth in both avian (*ZENK*) and mammalian (*Egr-1*) species (Ashby, Zeng et al. 2014).

1.2.6 Myopia and Chromaticity of Ambient Light

The wavelength distribution of the source of illumination should also be considered in any investigation of the role of ambient light as the likely mediator of the effect of outdoor activity on the development of myopia in children. For example, sunlight contains a considerably greater proportion of short wavelength components, including blue and UV light, when compared to indoor sources such as incandescent and fluorescent lamps (van Bommel and Rouhana 2011). However, LED sources, particularly white LEDs which combine an underlying blue LED source with a white phosphor coating, may produce relatively more of their spectral energy output at the blue end of the visible spectrum (see Sections 3.2.1 and 4.2.1)

Sunlight also contains a significant UV component that is generally absent or minimised in indoor lighting systems (van Bommel and Rouhana 2011) and so UV has been suggested as a possible mediator of the inhibitory effect of outdoor activity on myopia development (Prepas 2008). However animal experiments which have successfully demonstrated the protective effect of high ambient illumination levels have been conducted with UV-free light (Ashby, Ohlendorf et al. 2009; Smith, Hung et al. 2012; Backhouse, Collins et al. 2013; Karouta and Ashby 2015) indicating that UV wavelengths are not a necessary factor for the inhibition of experimentally-induced myopia. Furthermore, the demonstration of a reduction in the incidence of myopia in schoolchildren in China over one year by increasing the ambient light levels in the classroom to the relatively moderate level of 500 lux achieved at desk level using a redesigned fluorescent lighting system, also demonstrates that UV wavelengths are not a necessary requirement for the inhibition of naturally-occurring myopia in humans (Hua, Jin et al. 2015).

Experiments using animal models have established that the vertebrate eye can use both luminance and chromatic cues to guide the emmetropisation process (Rucker 2013). Under monochromatic conditions the eye can utilise luminance contrast to guide emmetropisation, although the end point of the process is dependent on the degree of chromatic defocus associated with the stimulus wavelength. Chromatic defocus arises due to the variation in refractive index of the ocular media with wavelength, which under white light conditions produces longitudinal chromatic aberration (LCA) of the retinal image (Rucker 2013). In the chick, the degree of LCA has been calculated as being 1.24 D (470 – 680 nm)(Mandelman and Sivak 1983) although values as high as 3 D have been found experimentally (Schmid and Wildsoet 1997). As a result, chicks raised under red (long wavelength) light develop a more myopic refraction when compared to those raised under blue (short wavelength) light (Seidemann and Schaeffel 2002; Foulds, Barathi et al. 2013). Similar outcomes have been demonstrated in the guinea pig (Long, Chen et al. 2009; Liu, Qian et al. 2011; Qian, Dai et al. 2013), rhesus monkeys (Liu, Hu et al. 2014), cichlid fish (Kroger and Wagner 1996) and squid (Turnbull, Backhouse et al. 2015).

Under white light however, the magnitude of luminance contrast (or blur) is insufficient to explain how emmetropisation determines the sign of defocus when blur is produced by either positive or negative lenses (Rucker 2013). For example, chick eyes wearing diffusers and positive lenses simultaneously were able to emmetropise to the lens power alone and develop a hyperopic refraction (Park, Winawer et al. 2003). Under white light, chromatic cues produced by the LCA of the eye may be sufficient to guide emmetropisation (Rucker and Wallman 2009). In an eye that exhibits LCA, a defocussed black

and white sinusoidal grating will be imaged on the retina with coloured fringes. In hyperopic defocus, the blue component will be more in-focus and the image will exhibit red fringes. Conversely in myopic defocus, the image will have blue coloured fringes. These variations in the colour distribution of the retinal image produced from a monochromatic (black and white) object by the LCA of the eye have been shown to provide a colour signal sufficient for the detection the sign of defocus via a relative cone contrast mechanism which measures the differential stimulation of short and longer wavelength cones (Rucker and Wallman 2012; Rucker 2013).

Another aspect of the chromaticity of ambient illumination that may affect the emmetropisation process is the relative stimulation not only of the retinal cones, but also of the intrinsically-sensitive retinal ganglion cells (ipRGCs) of the inner retina (Berson, Dunn et al. 2002; Hattar, Liao et al. 2002; Bailes and Lucas 2010; Guido, Valdez et al. 2010; Lok 2011; Phillips, Backhouse et al. 2012; Lucas, Peirson et al. 2014). ipRGCs are ganglion cells which contain the photopigment melanopsin, which is most sensitive to short wavelength blue light around 480 nm (Berson, Dunn et al. 2002; Hattar, Liao et al. 2002; Torii, Kojima et al. 2007). These photosensitive ganglion cells project to the suprachiasmal nucleus and other visual centres, can entrain circadian rhythms (Berson, Dunn et al. 2002; Berson 2003), and have a role in control of the pupillary light reflex (Valdez, Nieto et al. 2009; Bailes and Lucas 2010; Lucas, Peirson et al. 2014). Furthermore, in the mouse, ipRGCs may be encoding overall irradiance levels in the environment (Brown, Gias et al. 2010).

In chicks, melanopsin-expressing ipRGCs have been identified in the inner retina and *in vitro* cultures of retinal ganglion cells (Guido, Valdez et al. 2010). As form-deprivation depresses levels of retinal dopamine in chicks (Stone, Lin et al. 1989), the suppression of FDM by high light levels may be due to an increase in dopamine synthesis where the ipRGCs act as the photoreceptor (Norton and Siegwart 2013). This hypothesis is supported by interconnectivity between ipRGCs and amacrine cells in the inner plexiform layer of the retina (Bailes and Lucas 2010). As direct signalling from ipRGCs to dopaminergic amacrine cells has been established in the mouse (Zhang, Wong et al. 2008) then a putative pathway exists for light exposure to increase dopamine production in the form-deprived eye, and therefore suppress the development of axial myopia.

1.3 Experimental Hypothesis and Aims

The primary hypothesis investigated by this thesis is that periodic high intensity illumination is as effective at inhibiting the development of FDM as continuous high intensity illumination in animal models of myopia.

The experiments were designed using a paired-eye comparison model with the primary outcome measurements presented as the relative or difference value between the treatment (deprived) and contralateral control (non-deprived) eyes for each lighting condition. A paired-eye comparison has the advantage of accounting for individual variation between animals in terms of eye size due to factors such as differential growth rates (Wallman and Adams 1987). As such it tends to increase statistical power of small sample sizes by decreasing the variability between individual outcome measures allowing for the use of smaller sample sizes (Anderson and Vingrys 2001). However, it does have the limitation of not including age-matched normal animals as a baseline comparison for outcome effects. Conversely, the additional power of the paired-eye design achievable with relatively small sample sizes is consistent with the ethical principal of reduction of the number of animals used in an experiment to the minimum required to obtain useful results as promoted by the 3Rs of animal research, Replace, Refine and Reduce (NC3Rs 2017).

The specific aims of this thesis were:

Aim 1. To investigate whether periodic, low frequency, high intensity ambient illumination can suppress the development of form-deprivation myopia in a mammalian model of myopia, the guinea pig.

Aim 2. To measure the retinal dopamine concentration of guinea pigs exposed to periodic high intensity ambient illumination during the induction of form-deprivation myopia, and to determine whether the illumination paradigm affects the dopamine concentration.

Aim 3. To investigate whether periodic, low frequency, high intensity ambient illumination can suppress the development of form-deprivation myopia in an avian model of myopia, the chick.

Aim 4. To investigate the effect of periodic, quasi-monochromatic, high intensity ambient illumination on the development of form-deprivation myopia in an avian model of myopia, the chick.

Aims 1 and 2 are addressed in Chapter 3, where the periodic high intensity illumination paradigm was shown to produce a statistically significant reduction in relative form-deprivation myopia in the guinea pig. This is a novel finding, as previously, high intensity ambient illumination has only been shown to suppress lens-induced myopia in the guinea pig (Li, Lan et al. 2014). Retinal dopamine concentrations were successfully measured by high performance liquid chromatography (HPLC), however no significant difference in relative concentrations by illumination condition was found.

Aims 3 and 4 are addressed in Chapter 4. While no significant differences in relative refraction by intensity or quasi-monochromatic nature of the ambient illumination were found, a difference in the degree of FDM induced under the standard LED-based 300 lux illumination condition was revealed when the outcome of this experiment was compared with the refractive outcome of form-deprived chicks raised under 300 lux provided by triphosphor fluorescent lamps in the same laboratory, as previously reported by Backhouse *et al* (Backhouse, Collins et al. 2013). This finding resulted in an extensive investigation, analysis and discussion of the methods for quantifying illumination in animal experiments, and the potential role of the intrinsically-photosensitive retinal ganglion cells (ipRGCs) in inhibition of experimental myopia by high intensity ambient illumination.

2 Methods: General

In order to investigate the effects of exposure to periodic high intensity light on induction of myopia by form-deprivation in animal models, two distinct sets of experiments were carried out in this study. The first experiment used the domestic guinea pig (*Cavia porcellus*) to investigate whether periodic high intensity white light suppressed form-deprivation myopia (FDM) development in a mammalian model of myopia (Howlett and McFadden 2006), and to determine whether retinal dopamine levels were affected by the high intensity illumination paradigm used during FDM induction.

The guinea pig has the advantage of being a mammalian model and therefore perhaps more relevant to human myopia (Schaeffel and Feldkaemper 2015). It also had been used successfully to investigate the effects of FDM on scleral compliance (Backhouse, Phillips et al. 2006) in the same laboratory. However, pilot investigations conducted prior to the experiments described in Chapter 3 identified that a high proportion of guinea pigs supplied by the institutional breeding colony had congenital cataract which interfered with both outcome measurements and potentially refractive development by introducing a secondary form-deprivation source in the non-deprived eye. While an initial trial using a 10% high intensity duty cycle was commenced, the inability to complete outcome measurements satisfactorily meant that further experiments were suspended until the animal unit could supply non-cataractous animals. This required the introduction of new bloodlines to the colony through the addition of two new smooth-haired sires, and a selective breeding programme using non-cataractous dams selected by routine ophthalmoscopic observation by the investigator. While the initial intention was to investigate a duty cycle dose-response effect (i.e. 10%, 20%, 50%), once a sustainable supply of guinea pigs with clear media was established, the periodic high intensity lighting experiments were re-commenced with a 50% duty cycle to maximise the likelihood of observing an inhibitory effect on the development of FDM. Due to ongoing limitations on the supply of guinea pigs with clear media the decision was made to change to the chick model for further experiments.

The second experiment used the domestic chick (*Gallus gallus domesticus*) to investigate whether periodic high intensity white and quasi-monochromatic (coloured) light suppressed FDM development in an avian model of myopia (Wallman, Turkel et al. 1978). The chick was used in the second experiment due to the previously demonstrated suppression of FDM by high intensity white light (Ashby, Ohlendorf et al. 2009) in this animal model of myopia. Also, as a more practical consideration, the use of readily-supplied chicks from a commercial breeder overcame issues experienced with

sourcing visually-competent guinea pigs in the first experiment (see Section 3.3.2). As the ability of high intensity illumination to inhibit the development of FDM in chicks was well-established by the commencement of the chick experiments in 2014 (Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010) the decision was made to expand the experiments to include an investigation of the effect of wavelength on the inhibition of FDM in the chick while maintaining the 50% duty-cycle periodic high intensity illumination paradigm as successfully used to demonstrate the inhibition of FDM in the guinea pig (Chapter 3). The investigation of wavelength effects was made possible by the adoption of an LED-based lighting system (Section 4.2.1) which had the additional benefits of having a minimal heat output and could be controlled with commercially available software.

2.1 Experimental Animals and Form Deprivation

All experiment procedures were approved by the Animal Ethics Committee of The University of Auckland, and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experiments were conducted within the facilities of the central animal care unit, The University of Auckland, as used previously for experiments on form-deprivation myopia in both the guinea pig (Backhouse and Phillips 2010) and chick (Backhouse, Collins et al. 2013).

2.1.1 Animal Provision and Care

Guinea Pigs

Pigmented guinea pigs (*Cavia porcellus*) were obtained from the breeding colony of the animal care unit at The University of Auckland. As it was found that this colony displayed a moderate incidence of central cataract in new-born guinea pig pups, animals were screened using standard direct ophthalmoscopy techniques, and by autorefraction, to ensure the presence of clear media before they were accepted into the experiment. Successful attempts were made in conjunction with the animal care unit to reduce the incidence of cataract by selective breeding of non-cataractous animals, as guinea pigs are known to have a high prevalence (38%) of lens abnormalities (Williams and Sullivan 2010) including both inherited congenital nuclear cataracts (Bettelheim, Churchill et al. 1997) and acquired cataract due to vitamin C deficiency (Mody Jr, Kakar et al. 2005). While initially maternally-raised, as guinea pigs are precocial animals, they were weaned at one week of age. Animals that passed the ocular media screening were transferred to the open-topped plastic pens (86 mm L x 55 cm

W x 50 cm H) used for the experiments (see Section 3.2.2), where they were kept with at least one other littermate to acclimatise to the new environment, and supplied with water, food pellets (commercial guinea pig feed fortified with vitamin C) and hay *ad libitum*. The guinea pigs' diet was regularly supplemented with fresh apple and carrot pieces. The floor of the pens was covered with pine wood shavings, which were replaced regularly as required by the Standard Operating Procedures (SOP) of the animal unit. The guinea pigs were weighted daily and their behaviour assessed both prior to and during the experimental phase to ensure that the animals were growing normally. Failure to thrive, according to a predetermined set of criteria under the ethics approval, resulted in early termination of the experiment for that animal. Monocular translucent diffusers were attached following one day of acclimatisation to the experimental pens, and the experimental lighting cycle started (see Section 3.2.1).

Chicks

One day old Cobb chicks (*Gallus gallus domesticus*) were obtained from a local commercial hatchery. No chicks were found to have cataracts or similar ocular media pathologies. The chicks were transferred to the open topped plastic pens used for the experiment (see Section 4.2). Typically, six chicks were kept together in a pen for the duration of the experiment. The chicks were supplied with water and chick starter crumbles (commercial chick food) *ad libitum*. The floor of the pens was covered with newspaper which was changed daily. A heating pad was located under the newspaper floor covering. The chicks were weighed daily and behaviour assessed both prior to and during the experimental phase to ensure that the animals were growing normally. Failure to thrive, according to a predetermined set of criteria under the ethics approval, resulted in early termination of the experiment for that animal. Monocular translucent diffusers were attached on day 4 post-hatch, following 3 days of acclimatisation to the experimental pens, and the experimental lighting cycle commenced (see Section 4.2.1). Outcome measurements were made on the 4th day after 3 complete days of exposure to the experimental lighting cycle (day 8 post-hatch).

2.1.2 Form Deprivation with Translucent Diffusers

Translucent goggle-style diffusers were used to induce monocular form-deprivation in both the guinea pig (Chapter 3) and chick (Chapter 4) experiments. The same diffusers were used in both sets of experiments with minor modification to the mounting methods as detailed below. The diffusers were constructed from thermoplastic polyester sheeting that was surface abraded to render it translucent and then shaped using a custom mould in a heated water bath to produce hemispherical diffusers approximately 22 mm in diameter with a central dome-shaped zone (~19 mm diameter) which vaulted the eye of the animal when attached. The diffusers were found to transmit ~78% of incident light when measured with a non-directional illuminance (lux) meter (LX-105, Lutron, http://www.lutron.com.tw).

Diffuser Mounting and Maintenance – Guinea Pigs

The diffusers were attached while the guinea pigs were gently held by hand. No anaesthesia was required as the animals relaxed quickly in this situation. In order to achieve a secure attachment of the plastic diffuser in front of one eye, an intermediate layer of duct tape was used between the fur of the guinea pig and the Velcro™ mount of the diffuser. An oval piece of duct tape (~3 x 4 cm) was prepared with a central circular hole, approximately the same diameter of the dome of the diffuser. A self-adhesive Velcro™ ring was attached around the central hole in the duct tape patch. A small amount of cyanoacrylate glue was spread over the edges of the duct tape patch to provide a more secure adhesion of the tape to the fur. The guinea pig's fur was trimmed initially to remove any unevenness due to the texture of the animal's coat, and the duct tape patch was placed on the fur around one eye so that the hole was centred on the eye. A diffuser, with matching Velcro™ ring attached, was then carefully placed on the duct tape/Velcro™ mount. The diffusers were mounted over right or left eyes in an alternating manner to achieve approximately equal number of animals with right or left eyes under form-deprivation conditions (Figure 2.1). Following attachment of the diffuser, the guinea pigs were monitored for at least 30 minutes to ensure that the diffuser remained attached and the animals were showing normal behaviour such as eating and drinking. The diffusers were applied after 16:00 in the afternoon, prior to the centrally controlled room "lights-out" at 18:00. The experimental lighting system was set to commence cycling from 06:00 the following morning (see Section 3.2.1). The guinea pigs were checked at least twice a day (morning and late afternoon) to check that the diffusers were still securely attached. The diffusers were worn continuously for two weeks, except for brief periods where they were removed for cleaning or replacement due to partial loss of adhesion of the duct tape mount to the fur. This was usually undertaken during the morning

check, and the diffuser was removed for less than 5 minutes during the replacement procedure. Generally, the tape mount was replaced once per animal at the end of one week of diffuser wear during the two week experimental period.

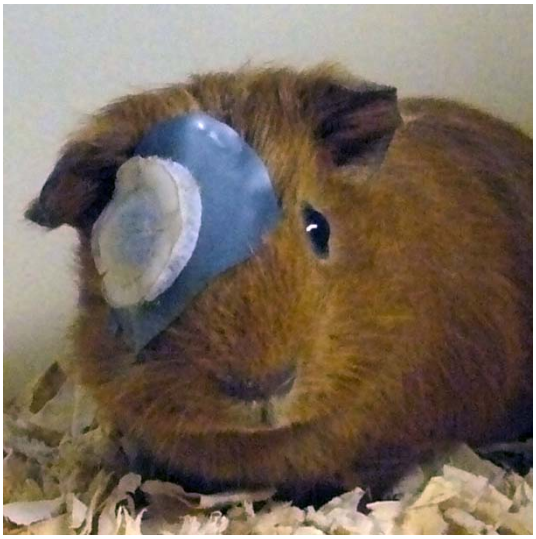

Figure 2.1. Image of a guinea pig wearing a duct tape mount with associated Velcro™ ring attachment and translucent occluder over the right eye. In the chick experiments the Velcro™ ring attachment was mounted directly onto the feathers surrounding the eye.

Diffuser Mounting and Maintenance – Chicks

The diffusers were attached while the chicks were gently held by hand. No anaesthesia was required as the animals could be suitably restrained in this manner. A self-adhesive Velcro™ ring was attached to the feathers surround one eye of the chick. Supplementary adhesion was again provided by the addition of cyanoacrylate glue on the adhesive side of the ring. The Velcro™ ring was placed around one eye of the chick, with the side of attachment alternated between the right and left eyes of the subsequent chicks. A diffuser, with matching Velcro™ ring attached, was then carefully placed on the duct tape/Velcro™ mount. Following attachment of the diffuser, the chicks were monitored for at least 30 minutes to ensure that the diffuser remained attached and the animals were showing normal behaviour such as eating and drinking. The diffusers were applied after 16:00 in the afternoon, prior to the centrally controlled room “lights-out” at 18:00. The experimental lighting system was set to commence cycling from the later time of 08:00 the following morning as the room lights were not utilised to provide the experimental illumination conditions in the chick experiments (see Section 4.2.1). The chicks were checked at least twice a day (morning and late afternoon) to check that the diffusers were still securely attached. The diffusers were worn continuously for three days, except for brief periods where they were removed for cleaning or replacement due to partial loss of adhesion of the feathers around the eye. This procedure was usually undertaken during the morning check, and the

diffuser was removed and replaced as quickly as possible. The diffusers were left in place on the fourth day until after the chick was readied for ocular measurements following anaesthesia (see Section 2.3.2).

2.2 Lighting Systems and Protocols

Animals were exposed to periodic high intensity illumination while undergoing monocular form-deprivation in order to assess the effect of the high intensity light on refractive development. In the guinea pig experiments (Chapter 3), animals were exposed daily to a total of 6 hours of high intensity halogen-based white light with background lighting provided by standard room fluorescent lamps in a 15:15 minute cycle over a 12 hour period. In the chick experiments (Chapter 4), animals were exposed daily to a total of 6 hours of high intensity LED-based white or coloured light in a 15:15 minute cycle, with background, or standard white lighting, provided by the same LED-based luminaires over a 12 hour period.

Details of the lighting systems and lighting protocols used in this study are described in detail in Section 3.2.1 for the guinea pig and Section 4.2.1 for the chick experiments respectively.

2.3 Anaesthesia

The anaesthetic protocols were approved as Institutional Drug Administration Orders (IDAO) by the Animal Welfare Officer (resident prescribing veterinarian) of The University of Auckland.

2.3.1 Guinea Pig Anaesthesia

While refraction (Section 2.4) and videokeratometry (Section 2.5) could be carried out in awake guinea pigs using only gentle hand-held restraint, ultrasound biometric measurements (Section 2.6) required anaesthesia. The anaesthetic protocol for the three week old guinea pigs used in this study followed the methods used by Backhouse (Backhouse 2009), which were developed from the Melbourne University guidelines for guinea pig anaesthesia and the guidelines of Chaib *et al* (Chaib, Charrueau et al. 2004).

Following 14 days of form-deprivation, Guinea pigs were given a single pre-medication dose of diazepam (5 mg/kg body weight)(Parnell Technologies NZ Ltd, New Zealand) delivered as an intraperitoneal (IP) injection using a 23 gauge hypodermic needle on a 1 mL syringe approximately 10 minutes prior to administration of the main anaesthetic. The aim of this pre-medication dose was to relax the guinea pig and help maintain respiration during anaesthesia (Backhouse 2009).

Anaesthesia was induced by a combined dose of ketamine hydrochloride (44 mg/kg body weight)(Parnell Technologies NZ Ltd, New Zealand) and xylazine hydrochloride (5 mg/kg body weight)(Phoenix Pharma Distributors Ltd, New Zealand) delivered as an intraperitoneal (IP) injection using a 23 gauge hypodermic needle on a 1 mL syringe. The depth of anaesthesia was assessed by the loss of the withdrawal reflex to a rear paw pinch and by the loss of the eye-blink reflex to touching the medial canthus. If the anaesthesia was assessed as being too light an additional quarter dose of the ketamine/xylazine anaesthetic was given by IP injection. Post-experiment euthansia was achieved with the administration of a IP dose of sodium pentobarbitone (90 mg/kg body weight)(Provet NZ Pty Ltd, NZ).

2.3.2 Chick Anaesthesia

All refractive and biometric measurements were performed under anaesthesia as awake measurement of refraction could not be made due to lid closure by the chicks. The anaesthetic protocol used in this study followed the previously published method used by Phillips *et al* (Phillips, Khalaj et al. 2000). Chicks were anaesthetised on day 4 of form-deprivation with a combination dose of ketamine hydrochloride (50 mg/kg body weight)(Parnell Technologies NZ Ltd, New Zealand) and xylazine hydrochloride (3.5 mg/kg body weight)(Phoenix Pharma Distributors Ltd, New Zealand) delivered as an intramuscular (IM) injection to the gastrocnemius leg muscle using a 23 gauge hypodermic needle on a 1 mL syringe. The depth of anaesthesia was assessed by the loss of the withdrawal reflex to a foot pinch and by the loss of the eye-blink reflex to touching the medial canthus. If the anaesthesia was assessed as being too light an additional quarter dose of the ketamine/xylazine anaesthetic was given by IM injection. Post-experiment euthansia was achieved with the administration of a IP dose of sodium pentobarbitone (100 mg/kg body weight)(Provet NZ Pty Ltd, NZ).

2.4 Refraction

A range of different methods have been used to measure the refractive status of animals in myopia development experiments including: retinoscopy (Wallman, Turkel et al. 1978; Murphy, Zadnik et al. 1992; Troilo and Judge 1993; Wildsoet, Howland et al. 1993; McBrien, Gentle et al. 1999; Phillips, Khalaj et al. 2000; Smith, Bradley et al. 2001; Howlett and McFadden 2006); optometers (Gottlieb, Fugate-Wentzek et al. 1987; Troilo, Gottlieb et al. 1987; McBrien and Norton 1992; Backhouse, Collins et al. 2013); and photorefractors (Schaeffel and Howland 1988; Schaeffel and Howland 1991; Seidemann and Schaeffel 2002; Ashby, Ohlendorf et al. 2009; Karouta and Ashby 2015; Turnbull, Backhouse et al. 2015). This study utilised two methods of refraction, both with the aim of obtaining more objective measures of refractive status than might be provided by retinoscopy. For the guinea pig experiments, trials demonstrated that an autorefractor (Humphrey Autorefractor HARK-599, Carl Zeiss Meditec, Germany), previously used for human myopia experiments (Anstice and Phillips 2011), could measure refraction successfully following cycloplegia. However, in the chick experiments, the autorefractor was found to be unable to take measurements, and so an infrared (IR) optometer (Topcon RM100 Refractometer, Japan) was utilised, which we have used previously in our laboratory for animal refractive measurements (Backhouse and Phillips 2010; Backhouse, Collins et al. 2013).

2.4.1 Guinea Pig Autorefraction

Refraction was measured under cycloplegia in awake hand-held guinea pigs using the autorefractor (Figure 2.2). Cycloplegia was induced with 2 drops of cyclopentolate hydrochloride 1% (Alcon Laboratories Ltd, Australia) instilled 10 minutes apart in each eye. Measurements were made 10 minutes after instillation of the second drop. The autorefractor was used in automatic measurement mode apart from the requirement to initially locate and centre the view on the eye of the guinea pig. The autorefractor was then able to track the eye and measure refraction automatically. Four sphero-cylindrical refraction measurements were taken in succession of the right eye followed by the left eye. As the eye that was subject to form-deprivation was alternated between the right and left eyes, this protocol meant that the first measurement eye was also alternated between deprived and non-deprived eyes for consecutive animals. Average mean sphere refraction was calculated for each eye and no correction was made for small eye artefact, which is reported to not be present in adult guinea pigs (Howlett and McFadden 2007). The primary refractive outcome measurement, relative refraction, was

calculated by subtracting the mean sphere of the contralateral non-deprived eye from the mean sphere of the deprived eye. Therefore, a myopic relative refraction outcome for an individual animal implies that the deprived eye developed a more myopic (or less hyperopic) refraction than the non-deprived eye.

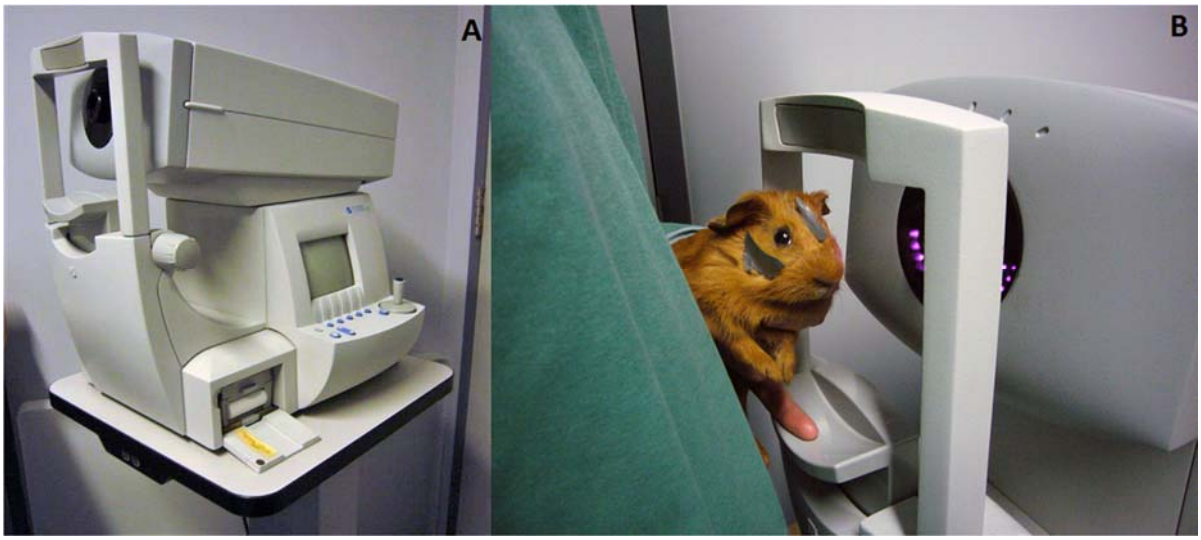

Figure 2.2. Refractive status of the guinea pigs was measured objectively with a Humphrey Autorefractor HARK-599 (A). Measurements could be made on awake hand-held guinea pigs (B). If necessary, the head was stabilised by gently holding the muzzle of the guinea pig with the other hand (not shown).

2.4.2 Chick Refraction - Infra-Red Optometer

Refraction was measured in chicks using the IR optometer (Figure 2.3) approximately 10 minutes after induction of anaesthesia (Section 2.3.2). Prior to the measurement of an eye the upper and lower lid was removed as the lids assumed a closed position under anaesthesia. Chicks were monitored carefully for signs of arousal during the measurement procedures and additional aesthetic doses supplied as required (Section 2.3.2). The use of the IR refractometer to measure refractive status is considered to provide a degree of objectivity to the measurement as the operator's task was to align vernier targets on the optometer screen, rather than making a subjective judgement of the movement of a retinal reflex as in retinoscopy (Backhouse, Collins et al. 2013). Refractions were measured four times consecutively at 0^{o} and 90^{o} meridians. The mean sphere refraction was calculated as the average of each pair of 0^{o} and 90^{o} measurements. No correction for small eye artefact was made as is common in chick myopia experiments (Wallman and Adams 1987; Backhouse, Collins et al. 2013). The primary refractive outcome measurement, relative refraction, was calculated by subtracting the mean sphere of the contralateral non-deprived eye from the mean sphere of the deprived eye.

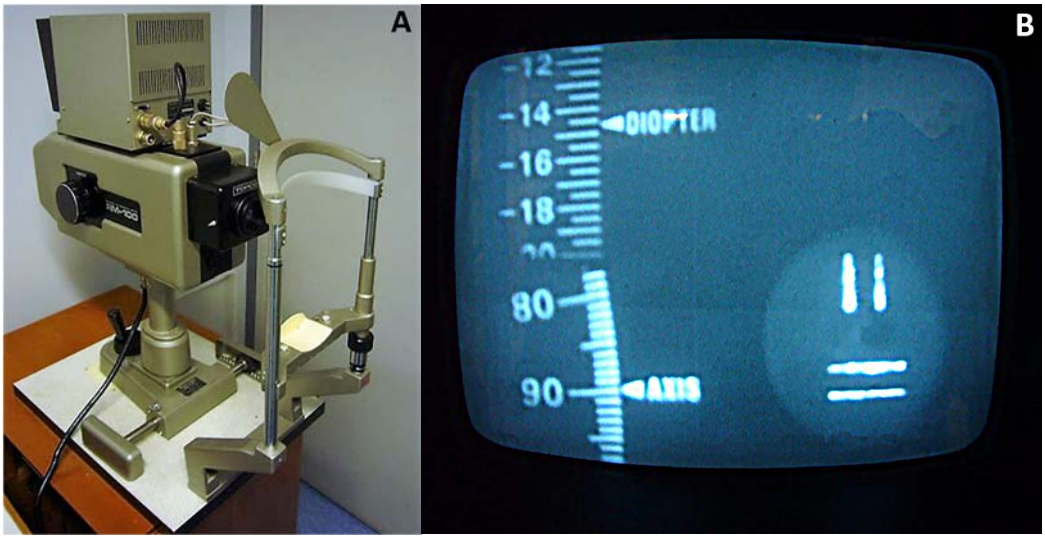

Figure 2.3. Refractive status of the chicks was measured with an IR optometer (Topcon Refractometer RM-100) (A). This method provided a semi-objective measurement as the operator's task was to align vernier targets on a display screen (B). Measurements were made on anaesthetised hand-held chicks, while the head was stabilised by gently holding the beak with the other hand (not shown).

2.5 Corneal Biometry - Infra-Red Videokeratometry

An infra-red videokeratometer, constructed for this study by the author, was used to measure corneal curvature of both awake guinea pigs and anaesthetised chicks. The infra-red videokeratometer was constructed with the aim of developing a device with which multiple images of the corneal reflection could be collected rapidly, minimising the time required to collect data in awake animals. The device consisted of an infrared webcam (Webcam C600, Logitech Inc., USA), mounted at the centre of a placido disc cone, with two concentric rings of infra-red LEDs (3 mm ZD1946, Jaycar, http://www.jaycar.co.nz) at 36 mm and 57 mm radial distances from the centre of the lens within the first and second black rings of the placido disc (Figure 2.4). As the infra-red LEDs were arranged radially at a 45^{o} spacing interval, corneal curvatures at 0^{o}, 45^{o}, 90^{o}, and 135^{o} could be measured simultaneously. An auxiliary +20 D lens was used to set the focal length of the device at 5 cm, which was coincident with the edge of the placido disc cone, allowing for visual judgement of the plane of focus (working distance) when making corneal measurements.

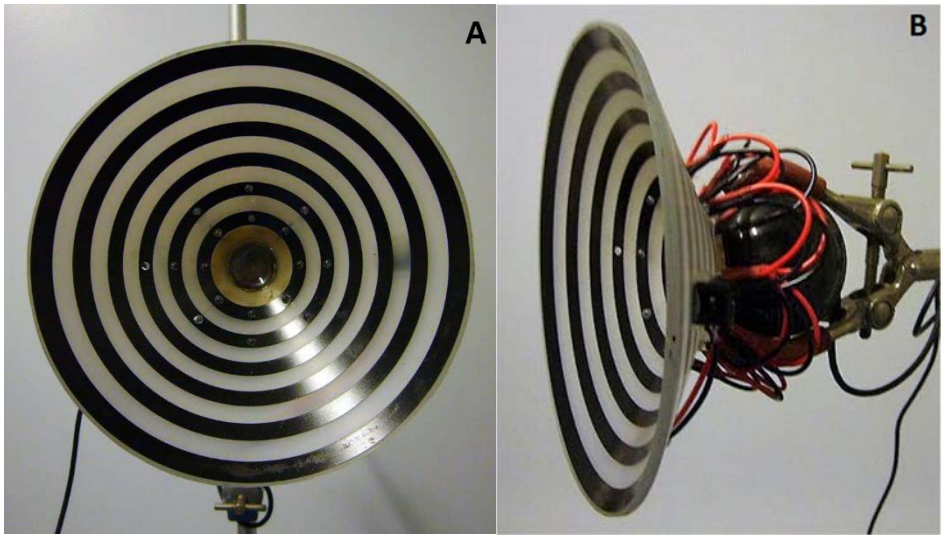

Figure 2.4. Corneal radius of curvature was measured with a bespoke IR videokeratometer. The device was constructed from a conic placido disc with IR LEDs set in two concentric rings at a 45º radial spacing (A). Images were captured using infra-red sensitive webcam with its focal plane coincident with the edge of the cone (B).

Webcam images of the IR LED reflections were captured at a resolution of 1600 x 1200 pixels and analysed using the line measure function of ImageJ (version 1.50b, Wayne Rasband, National Institute of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/). The distance between pairs of corneal IR LED purkinje images in the same meridian was measured in pixels. The pixel value was then converted to corneal radius of curvature using a calibration curve which had been determined by measuring a range of steel ball bearings of known radii with the same technique. Four images were analysed for each eye in both the guinea pig and chick experiments. The average of the radii from the 0º and 90º meridians were used to determine the corneal radius of curvature of each eye.

In practice, only the first Purkinje images of the inner ring of IR LEDs (~36º eccentricity) were used in corneal curvature analysis as the images of the outer ring tended to be obscured by the margins of the palpebral aperture in both guinea pigs and chicks. The primary outcome measurement, relative corneal radius, was calculated by subtracting the average corneal radius of the contralateral non-deprived eye from the average corneal radius of the deprived eye. Therefore, a positive value for relative corneal radius implies that the deprived eye developed a flatter corneal curvature (longer radius) relative to the non-deprived eye, conversely a negative value implies that the deprived eye developed a steeper corneal curvature (shorter radius).

2.6 Axial Biometry - Ultrasound

Myopia induced by form-deprivation has been shown to be primarily due to excessive axial elongation in the chick (Wallman and Adams 1987; Schaeffel and Howland 1988), the guinea pig (Howlett and McFadden 2006), the tree shrew (McBrien and Norton 1992), the macaque monkey Smith (Smith, Harwerth et al. 1987) and in humans (Sorsby, Leary et al. 1962). Therefore, A-scan ultrasound measurements were made of anterior chamber depth (ACD), lens thickness (LT) and vitreous chamber depth (VCD) for both the guinea pig and chick experiments in this study to investigate their relation to the refractive outcome of the animals.

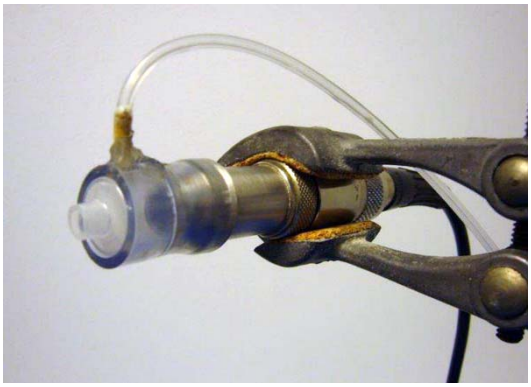

Figure 2.5. Image of the ultrasound probe with plastic stand-off attached. The inlet and silicon tube through which saline was pumped to act as a coupling fluid is shown on the superior aspect of the stand-off. The ultrasound probe was hand-held during its operation.

Ultrasound measurements were made with a 15 MHz ultrasound probe with a 1 inch (25.45 mm) focal length (Panametrics NDT V313, Olympus NDT Inc., USA). To account for the focal length of the probe, and the relatively small available contact area of the guinea pig or chick cornea, a 17 mm plastic stand-off was attached to the end of the probe which also contained a small diameter inlet (Figure 2.5). Saline, which acted as a coupling fluid between the probe and the cornea, was fed into the stand-off inlet from a syringe pump (Razel A-99.EHZ, Razel Scientific, USA).

For the guinea pig measurements, the head of the anaesthetised animal was restrained with a non-traumatic head-holder (Narishige, Japan)(Figure 2.6), and the lids were held open with a custom-made eyelid retractor (Backhouse 2009). The eyelid retractor was placed to minimise the contact with the cornea of the animal during measurements to avoid either distorting the cornea or reducing the anterior chamber depth. The head of the animal was held in a relatively normal position during measurements, with small axial rotations made to raise the position of the eye being measured for probe alignment purposes.

For chick experiments, a similar procedure was followed, where the head of the anaesthetised chick was held using a beak mount custom-made from dental wax (Figure 2.6). The open beak was placed over the beak mount and held in place with small strips of PVC tape. The head was held in a relatively normal position and a gap was maintained around end of the mount and beak to ensure that the chick had an unimpeded airway. As the eyelids had been previously removed under anaesthesia, an eyelid retractor was not required. Ocular lubricant drops were applied to the corneas regularly to avoid corneal desiccation during the procedure.

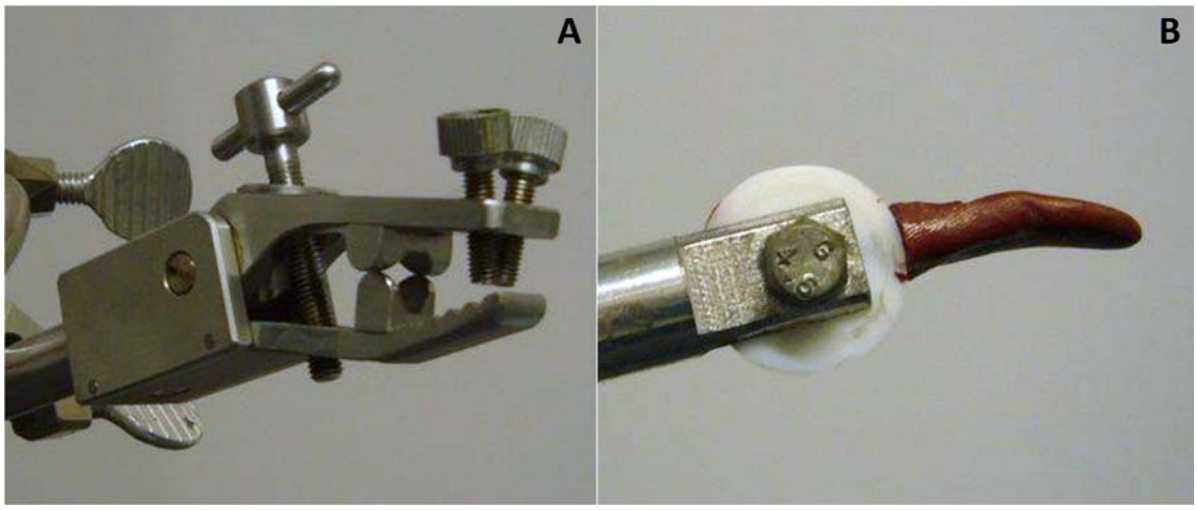

Figure 2.6. Images of the guinea pig non-traumatic head holder (A) and the dental wax moulded beak mount used for chicks (B). In both guinea pig and chick experiments the holder was orientated so that the animal's head was in a relatively normal position with an open airway. Both holders could be rotated axially to allow the best positioning of the eye for ultrasound measurements.

The ultrasound probe was hand-held during the measurements, which allowed the operator to make small movements relative to the eye in order to achieve the optimal positioning for the probe. The operator could watch the real-time output of the probe displayed on a computer screen to gain feedback as to the appropriate positioning of the probe. In particular, the posterior lens reflection (Figure 2.7) was found to be most sensitive to alignment in both guinea pigs and chicks and its presence and relative amplitude was used to judge when the probe was coincident with the geometric axis of the eye.

The ultrasound probe was driven via a Panametrics 5073 pulser/receiver control unit (Olympus NDT Inc., USA). The analogue output of the ultrasound control unit was sampled by a Gage Cobra 21G8 1 GS/s digital acquisition card (DynamicSignals LLC, USA). Data capture was undertaken using custom-made MyopiaScope 2009 software (Kerry King, University of Auckland, NZ) written in LabVIEW 8.6.1 (National Instruments Corp., USA), while data visualisation and timing measurements were made using

MyopiaAnalysis 2011 software (Kerry King, University of Auckland, NZ) written in LabVIEW 2009 (National Instruments Corp., USA)(Figure 2.7).

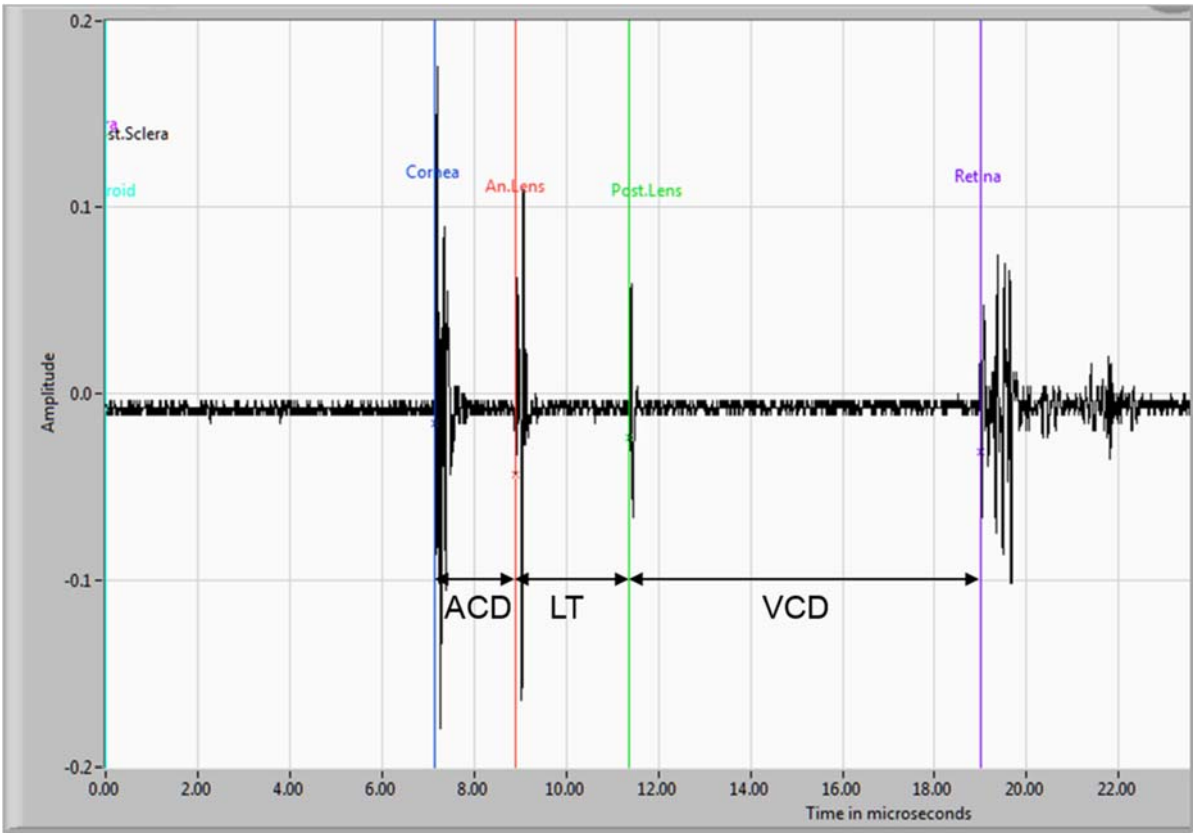

Figure 2.7. Typical chick eye ultrasound trace displayed in the MyopiaAnalysis 2011 software. The x-axis displays the time for the return of the signal pulse from the probe. The coloured cursors were moved to coincide with the initial peak for each echo; cornea, anterior lens, posterior lens and retina. The software measured and outputted the timing of each cursor point in microseconds. The distances corresponding to the anterior chamber depth (ACD), lens thickness (LT), and vitreous chamber depth (VCD) were then calculated from the times, given the appropriate velocity of sound for each medium.

At least 10 measurements were acquired for each eye and stored as data files. Using the MyopiaAnalysis software, each measurement was then analysed in terms of the timing of the ultrasound echo received from each surface interface of interest within the eye. A cursor was moved and placed at the initial peak for the echo corresponding to the anterior cornea, anterior lens, posterior lens and inner limiting membrane of the retina (Figure 2.7). Given these timings and the appropriate velocities of sound within each medium (Table 2.1), the distances between surfaces corresponding to the anterior chamber depth (ACD), lens thickness (LT), and vitreous chamber depth (VCD) could be calculated. The sum of these three dimensions gave overall axial length (AXL) of the eye.

Velocity of Sound by medium	Aqueous humour (ms^{-1})	Lens (ms^{-1})	Vitreous humour (ms^{-1})
Guinea Pig[1]	1534	1774	1534
Chick[2]	1534	1608	1534

Table 2.1 Velocities of sound used in calculation of ocular dimensions based on echo timings from ultrasound measurements. (1) Guinea pig media velocities after Howlett and McFadden (Howlett and McFadden 2006). (2) Chick media velocities after Wallman and Adams (Wallman and Adams 1987).

2.7 Statistical Analysis

SPSS version 22 (IBM, USA) was used to conduct statistical analyses, including one-way ANOVA, General Linear Model (GLM) and regression analysis. GraphPad Prism version 7 (GraphPad Software Inc., USA, http://graphpad.com/) was used to conduct two-way ANOVA analysis. G*Power version 3.1.9.2 (University of Dusseldorf, Germany, http://www.gpower.hhu.de/en.html) was used for post-hoc statistical power and sample size calculations (Faul, Erdfelder et al. 2007). GraphPad QuickCalcs (GraphPad Software Inc., USA, http://graphpad.com/quickcalcs) online statistical calculators were used to conduct Grubbs' outlier tests, and for t-test comparisons where only descriptive statistics were available from publications. All statistical testing assumed a significance level of $p = 0.05$.

3 The Guinea Pig: Periodic High Intensity Illumination and Form Deprivation Myopia

3.1 Introduction

In the experiment described in this chapter, the guinea pig (*Cavia porcellus*) is used to investigate the effects of regular, periodic high intensity illumination on the development of FDM and retinal dopamine concentration. The guinea pig has the advantage of being a mammalian model of myopia, with a typical simple mammalian scleral structure (Backhouse 2009). Guinea pigs reliably develop FDM, however it develops more slowly than in the chick (Howlett and McFadden 2002; McFadden, Howlett et al. 2004; Howlett and McFadden 2006; Lu, Zhou et al. 2006; Zhou, Qu et al. 2006; Howlett and McFadden 2007; Howlett and McFadden 2009).

The overall aim of the experiment is to investigate the underlying mechanisms by which exposure to the high ambient light levels might mediate the protective effect of outdoor activity on myopia development in children (Rose, Morgan et al. 2008a).

3.2 Methods: Guinea Pig Experiment Specific

3.2.1 Lighting System – Halogen/Fluorescent

The guinea pig experiments utilised a combination lighting system consisting of an array of incandescent halogen lamps mounted across the top of the pen to provide high intensity 10,000 lux illumination lighting, while standard 300 lux illumination lighting was provided by the laboratory's existing ceiling-mounted fluorescent luminaire.

The halogen lamp lighting array was planned using Philips CalcuLuX Indoor lighting design software (version 5.0b, Philips Lighting B.V.) with the aim of producing an illumination of 10,000 lux at 40 cm distance from the lamps, as evenly distributed as practicable across the floor of the pen. The 40 cm distance was estimated to be the location of guinea pig eye level relative to the lighting rig with the woodchip bedding in place. Solux™ halogen lamps (Tailored Lighting Inc., USA, http://www.solux.net) with a colour temperature of 4700 K were selected to provide daylight-like illumination under the high intensity lighting condition. The Solux™ halogen lamp specifications were: MR16-type 12 VDC, 50 W, 36^{o} beam, with a colour-rendering index (CRI) of 99. The lamps were manufactured with dichroic

reflectors which the manufacturer claimed to reduce IR radiation within the output beam to 20% of a standard MR16 50 W lamp (Tailored-Lighting-Inc. 2014). While the manufacturer also stated that the lamps filter out UV radiation, radiometric measurements at the dimming setting required to produce 10,000 lux illuminance at chick eye level, did show the presence of a relatively small UV component at 380 nm with an irradiance of 16 µW/cm2 (Figure 3.3).

The design process resulted in a lighting rig consisting of a 4 x 3 array of lamps mounted on brass rails and attached to a PVC pipe frame, which could be pivoted up at one end to provide access to the pen for animal care and maintenance purposes (Figure 3.1). Each row of 3 lamps was supplied by a single 240 VAC to 12 VDC dimming electronic transformer (Tridonic TE0105U, Tridonic GmbH & Co KG., Austria, http://www.tridonic.com). The dimming range of 1-100% could be controlled via a 1-10 VDC input signal. A custom-made control system was developed in-house (Kerry King, University of Auckland, NZ) which consisted of lighting control software which interfaced with a digital to analogue converter (DAC; Figure 3.1)(NI USB-6211, National Instruments Inc., USA, http://www.ni.com). The analogue output of the DAC was used to control the dimming circuit (Dimmer; Figure 3.1), while the digital output was used to switch the 240 VAC power supply (Power; Figure 3.1) to the electronic transformers supplying the halogen lamps. The lighting state (on / off / illumination level) could be controlled in 1 minute intervals over a 24 hour period.

The halogen lighting rig design process proved to be successful as, following construction, an average illumination value of 10264 ± 393 lux was measured with an LX-105 lux meter (Lutron, http://www.lutron.com.tw) across the floor of the pen with the lighting control output set to maximum.

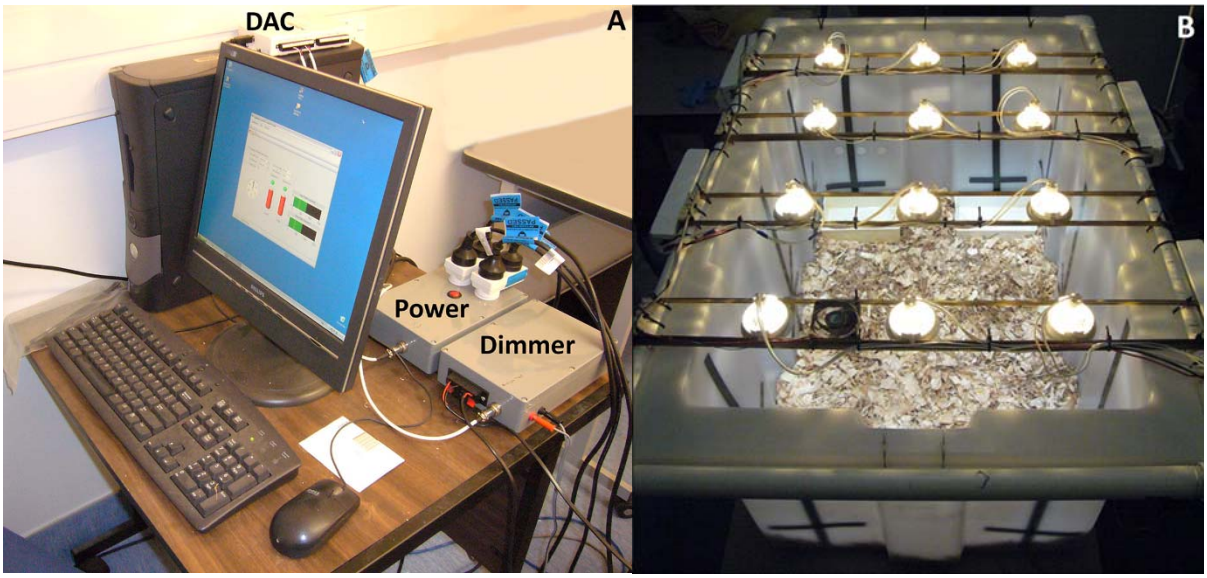

Figure 3.1. (A) The halogen lighting system was controlled by custom software driving a digital-analogue converter (DAC) which sent DC control signals to the 240 VAC power relays (Power) and the 0-10 VDC dimmer control (Dimmer). (B) View of the experimental pen illuminated by the 4 x 3 array of Solux™ halogen lamps at the 10,000 lux dimmer setting. Each row of 3 lamps was connected to one electronic dimmable transformer mounted at the edge of the lighting rig. A computer power supply fan was mounted on the rails in the lower left hand corner to enhance the exchange of air between the pen and the surrounding 23^{o} C temperature-controlled room environment.

The background, or standard 300 lux lighting was provided by a single, ceiling-mounted twin-tube fluorescent luminaire located directly above the pen. The luminaire was fitted with Philips TL5 HE 28W/840 fluorescent tubes (Philips Lighting Holdings, B.V., http://www.lighting.philips.com) which have a nominal colour temperature of 4100K (Philips-Lighting 2015). The height of the pen above the floor (i.e. distance from the ceiling-mounted luminaire) was set so that an average illuminance of 310 ± 7 lux was measured with an LX-105 lux meter (Lutron, http://www.lutron.com.tw) at estimated guinea pig eye height across the floor of the pen. The spectral irradiance distribution of the fluorescent illumination measured at 300 lux illumination (Figure 3.2) shows the spectral profile typical of a tri-phosphor fluorescent source (van Bommel and Rouhana 2011).

As the 10,000 lux high intensity illumination periods were provided by the combination of the background fluorescent room lighting with the addition of the Solux™ halogen rig lighting, the spectral irradiance distribution of the high intensity illumination illustrated in Figure 3.3 shows both the typically broad and continuous profile of a halogen incandescent source with the overlaid peaks of a fluorescent source (van Bommel and Rouhana 2011).

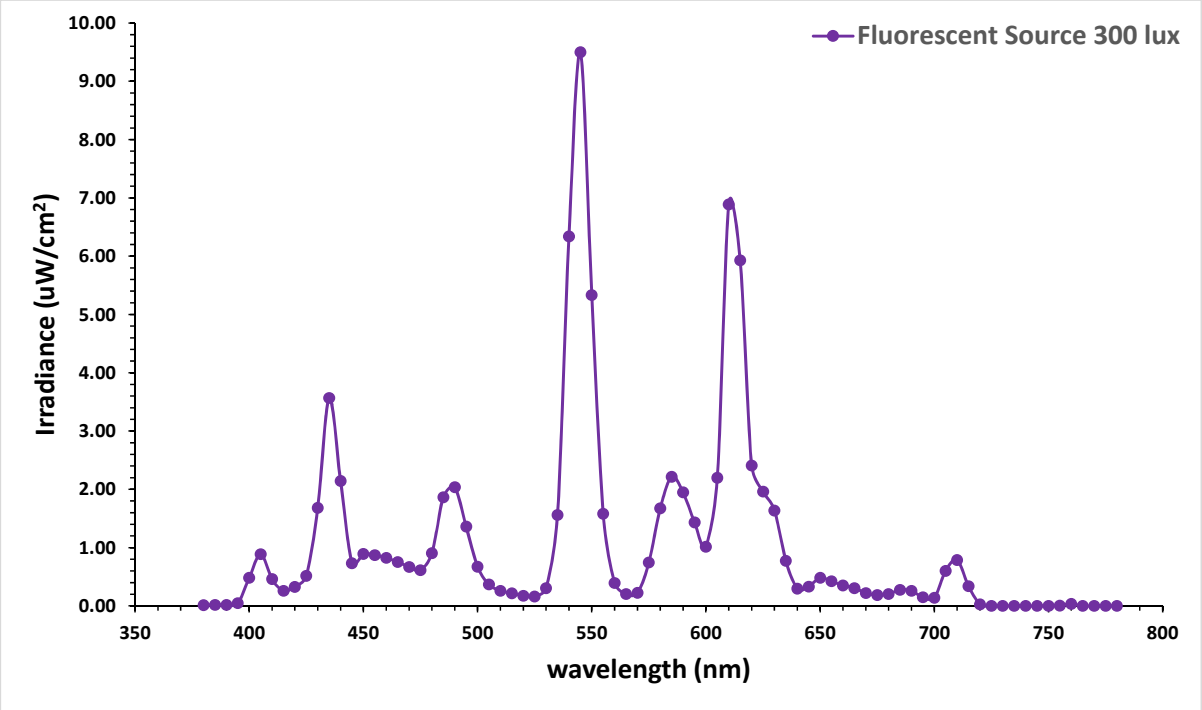

Figure 3.2. Spectral irradiance distribution (μW/cm^2) of the Philips TL5 HE 28W/840 fluorescent tubes measured at 300 lux illuminance. The fluorescent illumination shows the spectral profile typical of a tri-phosphor source. The measured colour temperature of the fluorescent light was 4000 K. Spectral irradiance of the fluorescent tubes tended to zero at the UV (380 nm) and IR (780 nm) ends of the human visible spectrum.

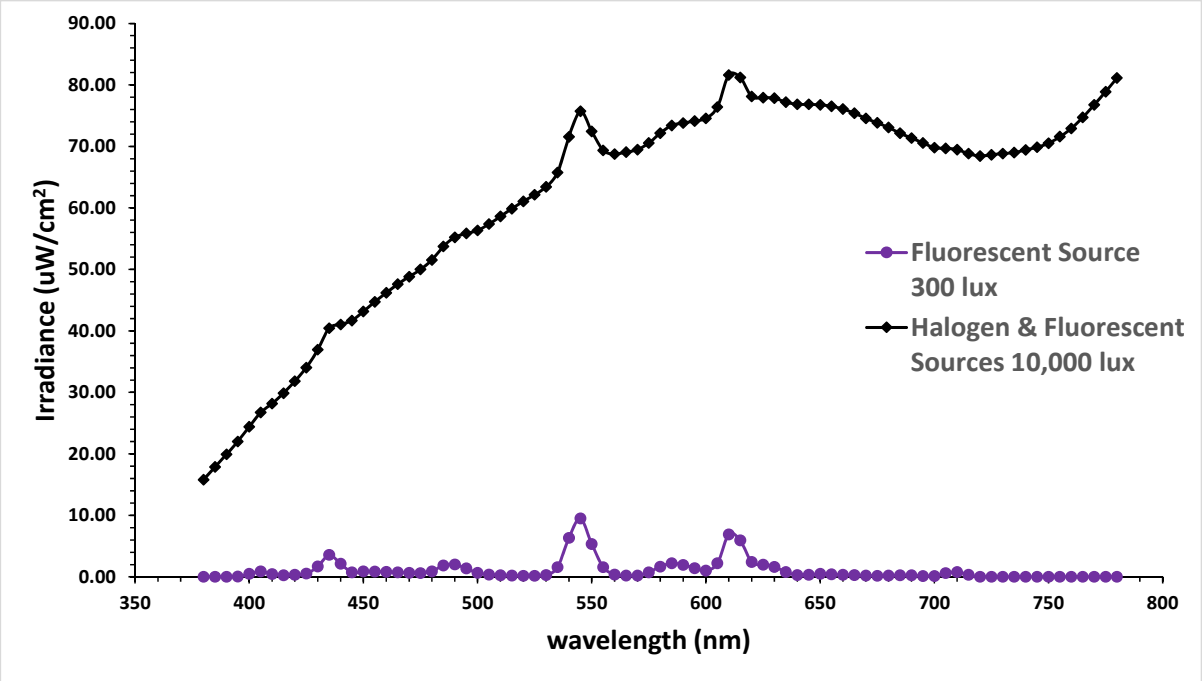

Figure 3.3. Spectral irradiance distribution (μW/cm^2) of the combination of the Solux™ 4700K halogen lamps and background fluorescent lighting measured at 10,000 lux illuminance. The combined irradiance has been plotted as the fluorescent room lighting was on continuously during the daylight period, with the halogen lamps switched on additionally during the high intensity illumination periods.

3.2.2 Animal Cohorts and Lighting Protocols

Outcome measures including refraction, biometry and retinal tissue collection for dopamine assay, were completed on a total of 20 guinea pigs in 10 cohorts of two animals following two weeks of monocular form deprivation (Section 2.1.2) under one of two lighting conditions: (i) constant 300 lux fluorescent illumination during the light phase of a 12:12 hour light:dark cycle (control group; n = 10); and (ii) periodic high-intensity lighting, where 15 minutes of high intensity illumination (e.g. 10,000 lux) was alternated with 15 minutes of 300 lux illumination during the light phase of a 12:12 hour light:dark cycle (treatment group; n = 10). The experiments were replicated with the 10 experimental cohorts taking place between the months of February and July, which correspond to the Southern Hemisphere Summer to Winter seasons.

The fluorescent room lighting was under the central control of the animal unit, with the daylight period running for 12 hours from 06.00 – 18.00 daily. Therefore, the 300 lux control group received 12 hours of constant 300 lux illumination during the light period. The high intensity Solux™ halogen lighting was controlled independently of the room lighting by the custom control system. The system was set to switch the halogen lighting on periodically for 15 minutes duration, beginning 15 minutes after the start of the daylight period (i.e. 06.15), and then switch off 15 minutes later. This cycle continued for the rest of the 12 hour period until 18.00 when the halogen lights remained off through the night phase from 18.00 to 06.00 the next morning (Figure 3.4). Therefore, the 10,000 lux treatment group received 6 hours of cumulative high intensity illumination (50% duty cycle) over the 12 hour light period.

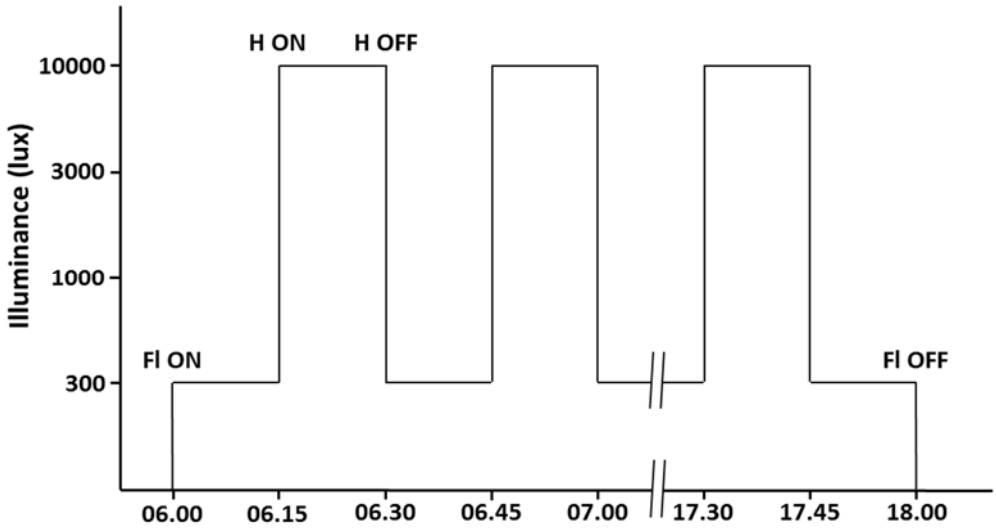

Figure 3.4. Illustration of the periodic high intensity lighting paradigm. The light phase started at 06.00 when the room fluorescent lighting switched on (Fl ON), giving 300 lux illumination in the pen. At 06.15 (15 minutes later) the halogen lights switched on (H ON) providing 10,000 lux illumination in the pen. After 15 minutes, the halogen light switched off (H OFF) returning the pen to the 300 lux condition. This pattern then repeated until both the room lights and halogen lights switched off at 18.00 until 06.00 the next day.

The periodic nature of the high intensity illumination paradigm was intended to simulate the movement of a person between relatively low intensity indoor lighting conditions, and high intensity outdoor lighting conditions during the day (Backhouse 2011). It also attempted to replicate the changing spectral composition between artificial indoor light and outdoor light (Backhouse, Collins et al. 2013).

Both the standard 300 lux intensity and periodic high intensity light cohorts of animals were maintained under their respective lighting conditions for 14 days after which outcome measurements were made immediately following removal from their pen. The lighting system was monitored in real-time using a remotely viewable webcam.

3.2.3 Retinal Dopamine Assay

Tissue Sample Collection

Tissue collection for mass spectrometry analysis of retinal dopamine concentration was commenced immediately following euthanasia of the guinea pig being measured (section 2.3.1). Tissue collection was timed to take place between approximately 12.00 and 1.00pm for each cohort of animals (median 12.50, range 12.10 - 13.35) as retinal dopamine levels exhibit a circadian rhythm in a range of vertebrate retinas including rats (Cahill and Besharse 1995) pigeons (Adachi, Nogi et al. 1998) and chicks (Megaw, Boelen et al. 2006). Tissue collection was completed within approximately 5 minutes of euthanasia.

Example Timeline: 2 GPs measured simultaneously

Task		GP1	GP2
Lighting Cycle start time		0600	0600
Cycloplegia	(1st gtt)	1010	1025
	(2nd gtt)	1017	1031
Refraction		1035	1100
Keratometry		1055	1110
Anaesthesia		1107	1117
Ultrasound		1130	1145
Euthanasia		1155	1200
Dissection completed		**1215**	**1225**
tissues (vitreous, retina, sclera) frozen in LN on dissection			
Stored in -80 freezer			

Figure 3.5. Example timeline of guinea pig experimental outcome measurements and tissue collection. Pairs of animals (GP1 and GP2) were processed in an overlapping sequence to minimise time differences between measurement points and tissue collection times. Measurements commenced approximately 4 hours after the daily lighting cycle onset.

As illustrated in Figure 3.5 the guinea pigs were maintained under their experimental lighting condition for approximately 4 hours from 6 am until they were removed to the outcome measurement room at about 10 am. The outcome measurement room was illuminated with standard daylight fluorescent luminaires resulting in an illumination of approximately 300 lux at desktop level. An interval of between 2 to 3 hours existed between when the animals were removed from their experimental lighting condition and when the dissection was completed and retinal tissue samples frozen in liquid nitrogen. As the light adaptation state of the guinea pig retina has been shown to affect the dopamine content of the retina (Nichols, Jacobowitz et al. 1967) then it is possible that the retinal dopamine content may have declined in the high intensity light treated animals during the measurement period.

Retinal tissue samples were collected from both the deprived and non-deprived eyes. The right eye was dissected first, as this resulted in alternating collection of deprived and non-deprived eye samples between consecutive animals. Each entire globe was removed in turn by dissecting through the conjunctival fornix and sectioning the extraocular muscles and optic nerve. The globe was then sectioned in half at the equator with a new razor blade. This separated the anterior components of the eye including iris and lens, from the posterior half, with the majority of the vitreous in place. The vitreous was gently removed from the posterior eye cup using forceps while viewed under a binocular microscope. This generally resulted in the partial detachment of the retina from the underlying pigmented choroid. The retina was then carefully dissected away from any remaining attachment points to the eye cup. The retina was then placed in a 1.5 mL Eppendorf tube and snap frozen in liquid nitrogen. No additional fluids such as buffers were used during the dissection or storage procedures. The sample tubes were then stored in a -80^{o} C freezer until processing for mass spectrometry.

Mass Spectrometry

Mass spectrometry sample preparation and analysis for retinal dopamine was undertaken by the Mass Spectrometry Centre (Martin Middleditch, Manager), Auckland Science Analytical Services (ASAS), Faculty of Science, The University of Auckland. Retinal dopamine concentration was determined by HPLC – MS/MS (Product Ion Scan) analysis using a QSTAR XL hybrid Quadrupole Time-of-Flight mass spectrometer (Applied Biosystems, Foster City CA, USA). The following procedure is a description of the process as undertaken by the ASAS.

Retinal tissue samples were removed from storage at -80^{o} Celsius and processed at 4^{o} Celsius in conjunction with the addition of a perchloric acid-containing solubilisation buffer to minimise degradation (Boix, Woien et al. 1997). A 150 µl aliquot of Solubilisation buffer (0.6% perchloric acid, 3.3 mM cysteine, 0.3 mM EDTA) was added to each retinal tissue sample. Dihydroxybenzoic acid (DHBA, Sigma) was then added at 0.5 µg/ml to all samples as an internal standard. Samples and standards were sonicated on ice for 30 seconds, then centrifuged at 16000 g for 5 minutes at 4^{o} Celsius. Following centrifugation, the supernatant was sampled and 200µl of 2 mg/ml 2-aminoethyl diphenylboronate (Sigma) in 2M ammonium bicarbonate (ABC) was added to 50 µl of supernatant. Dopamine standards (Sigma, St Louis, MO) were also prepared at 0, 2, 4, 8, 16, 32, and 64 ng/ml concentrations in 150 µl of Solubilisation buffer.

Solid phase extraction (filtering of tissue remnants) was then performed on 10 mg Oasis HLB cartridges (Waters, Milford, MA) using the following protocol: condition with 0.5 ml Methanol, then 0.5 ml of 0.2M ABC, load sample, wash with 0.5 ml of 0.2 M ABC in 5% acetonitrile, then 1 ml of 5% acetonitrile in water, elute with 0.3 ml of 1% formic acid in 5% acetonitrile in water. Samples were then concentrated in a vacuum centrifuge to 40 µl, centrifuged at 16000 g for 5 minutes and then 6 µl of extract was diluted 10-fold in 1% formic acid in acetonitrile.

Duplicate 10 µl injections were made for each sample and standard, and the analytes separated on a 0.3 x 150 SeaQuant ZIC HILIC column (Merck, Darmstadt, Germany) using the following gradient at 6 µl/min: 0-2 mins 70% B (30% A), 14 mins 50% B, 15 mins 5% B, 17 min 5% B, 18 min 70% B, held until 26 minutes; where Buffer A was 5 mM ammonium formate in 0.1% formic acid and Buffer B was acetonitrile. The liquid chromatography (LC) effluent was directed into the Ionspray source of QSTAR XL hybrid Quadrupole Time-of-Flight mass spectrometer (Applied Biosystems, Foster City CA, USA) and HPLC – MS/ MS (Product Ion Scans) performed for dopamine (m/z 154, and DHBA m/z 123). The mass spectrometer and high performance liquid chromatography (HPLC) system were under the control of the Analyst QS 1.1 software package (Applied Biosystems). Peak areas were obtained by integrating the intensities for the specific fragment ions at m/z 91.0 for dopamine and 51.0 for DHBA. Normalised peak intensities were used to create calibration curves for the standards and to then calculate dopamine concentrations for the retina samples (see Appendix A1).

3.3 Results: Guinea Pig

3.3.1 Refractive Outcome

Paired-Eye Comparison – Relative Mean Sphere

Relative mean-sphere refraction was calculated as the difference in refraction between the deprived eye and the non-deprived eye for each animal in both the standard 300 lux (S300) and periodic high intensity 10,000 lux (H10KP) illumination group. Paired eye comparisons are a means of controlling for individual variation in growth rates between animals which might influence biometric parameters. The non-occluded eye effectively acts as an internal control eye for comparison of refractive outcome with the occluded eye (Wallman and Adams 1987). The average relative mean-sphere refractions for the two illumination groups are illustrated in Figure 3.6.

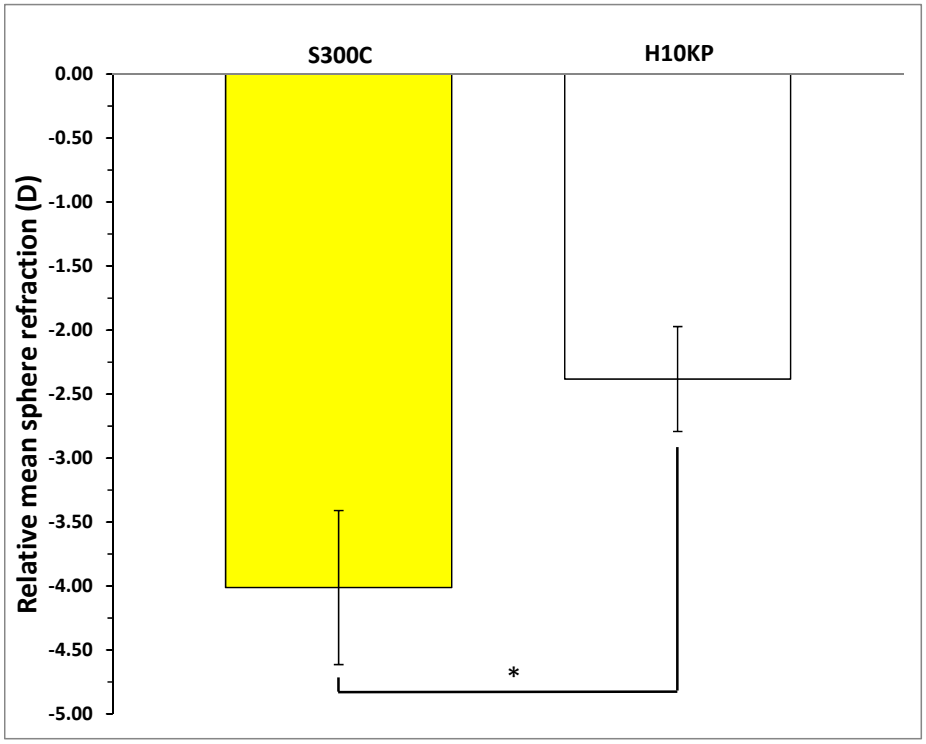

Figure 3.6. A significant difference in relative mean sphere refraction (deprived eye - non-deprived eye) was found for guinea pigs raised with unilateral form-deprivation between the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). (*t-test: $t_{(18)} = -2.234$, $p = 0.038$). Error bars are ± 1 SEM.

A significant difference in the primary outcome measurement, relative mean sphere refraction, was found between the two illumination groups (t-test: $t_{(18)} = -2.234$, $p = 0.038$). A lower degree of relative myopia was found in the group of guinea pigs that had been exposed to the periodic high intensity 10,000 lux illumination (H10KP) condition when compared to the group that was raised under the standard 300 lux illumination (S300C) condition. While the calculated p-value of 0.038 was significant

based on an α = 0.05, post-hoc power analysis using G*Power (University of Dusseldorf, Germany) (Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) found that the statistical power of the t-test analysis was only 56%. Further G*Power calculations showed that in order to achieve a statistical power of 80%, a larger sample size of 18 per group would have been required, rather than the 10 per group used in this study. The underlying refractive components that contribute to the primary outcome measurement, relative mean sphere refraction, are considered in the following sections.

Individual Eye Comparison – Absolute Mean Sphere

Average absolute mean sphere refraction was also calculated for both the deprived eyes and the non-deprived eyes in each of the two illumination groups. This calculation allowed consideration of whether there was any significant variation in the refractive development of either group of eyes due to illumination conditions, such as the relatively higher illumination levels experienced by the non-deprived eye compared to the form-deprived eye when the reduced transmittance of the translucent diffusers is taken into account (see Section 2.1.2). Figure 3.7 illustrates the average absolute mean-sphere refractions for the deprived and contralateral non-deprived eyes by each illumination condition.

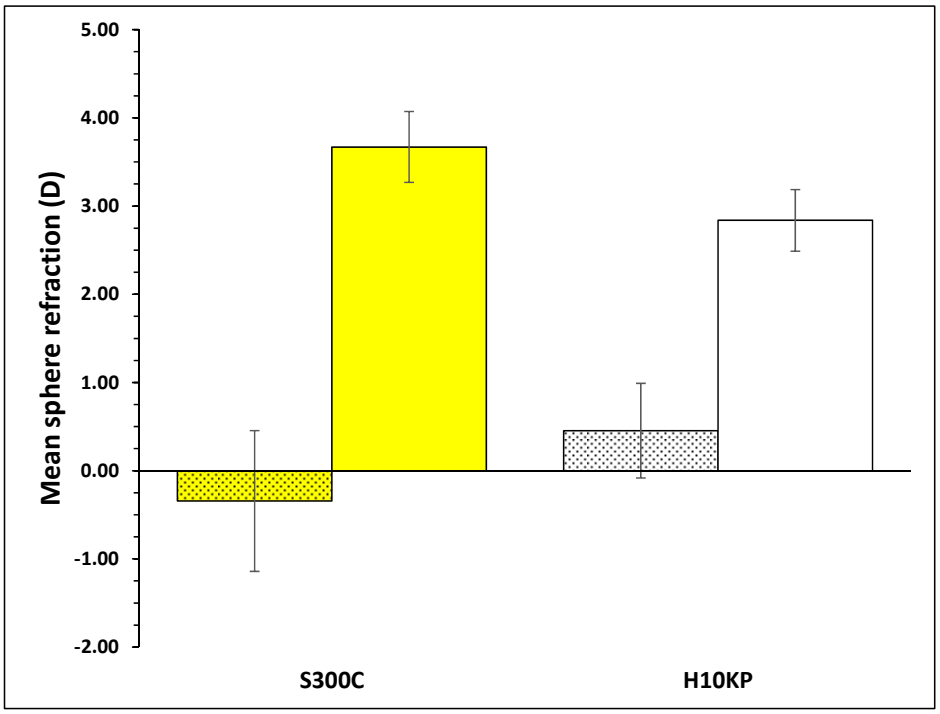

Figure 3.7. Absolute mean sphere refractions for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant differences in refractive error by illumination condition were found for either the form-deprived eyes (*t*-test; $t_{(18)} = -0.829$, $p = 0.418$), or for the non-deprived contralateral eyes (*t*-test; $t_{(18)} = 1.565$, $p = 0.135$). Error bars are ± 1 SEM.

Irrespective of lighting condition, overall the deprived eyes were found to be significantly more myopic/less hyperopic than the non-deprived eyes (Paired *t*-test; t = -7.973, $p < 0.001$). Therefore, form-deprivation did successfully induce relative myopia in the guinea pigs under both illumination conditions.

On average, form-deprived eyes in the S300C group developed a small degree of myopia, while those in the H10KP group developed a small hyperopic refractive error. Although these refractive outcomes were not significantly different (*t*-test; $t_{(18)} = -0.829$, $p = 0.418$), the apparent trend does indicate that the statistically significant difference in relative refraction (Figure 3.6) is due at least in part to a reduction in the degree of induced myopia in the deprived eye under the high intensity illumination condition. Conversely, the non-deprived eyes in both illumination groups developed a moderately hyperopic refraction, which again were not significantly different (*t*-test; $t_{(18)} = 1.565$, $p = 0.135$). The refractive outcomes for both the form-deprived and non-form deprived eyes, and relative mean sphere refraction are summarised in Table 3.1 below.

Illumination Condition	Form-deprived Eyes Mean sphere (D)	Non-deprived Eyes Mean sphere (D)	Difference in Refraction Relative mean sphere (D)
S300C	-0.34 ± 0.80	+3.67 ± 0.40	-4.01 ± 0.61
H10KP	+0.45 ± 0.54	+2.84 ±0.35	-2.38 ± 0.41
t-test	$p = 0.418$	$p = 0.135$	$p =$ **0.038**

Table 3.1 Refractive outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant (S300C, n = 10), High Intensity 10,000 lux periodic (H10KP, n = 10). *t*-test *p*-values for comparison of means by illumination condition are given in the final row of the table (significant values in **bold**).

The greater statistical power of the relative refraction measure is demonstrated in these results where a significant difference was found between the illumination groups for relative refraction, but not for the form-deprived or non-deprived eye groups. For example, G*Power (University of Dusseldorf, Germany) calculations show that for the form-deprived eye t-test, the statistical power achieved with a sample size of 10 animals was only 15%. In order to achieve a statistical power of 80%, a sample size of 85 would have been required. Whereas, for the non-deprived eye t-test, a sample size of 34 would have yielded 80% power. As noted above, relative or paired eye comparisons control for individual variation in growth rates between animals which increases the power of the comparison where the non-occluded eye acts as an internal control eye for outcome comparisons with the occluded eye (Wallman and Adams 1987).

The following sections investigate the variations in the ocular biometric components that produce the overall refractive outcomes of the eyes.

3.3.2 Corneal Curvature

Paired-Eye Comparison – Relative Corneal Radius

Relative corneal radius of curvature was calculated as the difference in central radius between the deprived eye and the non-deprived eye for each animal in the two illumination groups. The average relative corneal radius for each illumination group is illustrated in Figure 3.8.

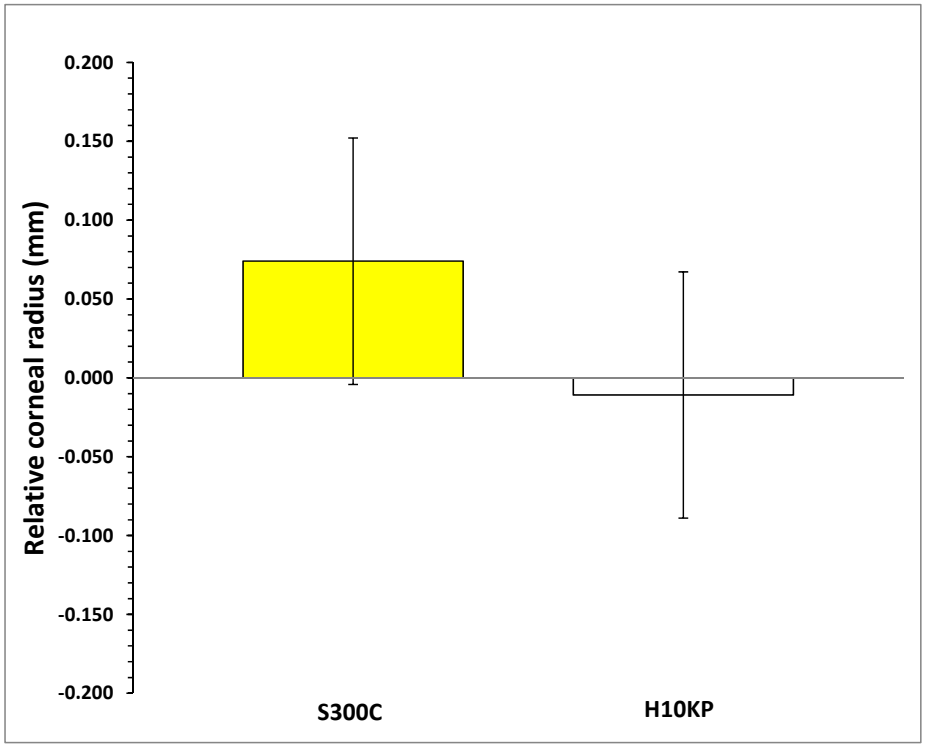

Figure 3.8. Relative corneal radius of curvature (deprived eye - non-deprived eye) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative corneal radius by illumination condition was found (*t*-test; $t_{(18)} = -0.857$, $p = 0.403$). Error bars are ± 1 SEM.

While no significant difference in relative corneal radius was found between guinea pigs raised in standard lighting conditions (S300C) when compared to those raised under periodic high intensity lighting (H10KP), there is an apparent trend for the relative corneal radius of the S300C group to be more positive than for the H10KP group, however this difference is small and unlikely to explain the significant difference in relative refraction found by illumination condition (Figure 3.6, Table 3.1). Post-hoc power analysis using G*Power (University of Dusseldorf, Germany) found that the statistical power of the t-test analysis was only 13%. Further G*Power calculations showed that in order to achieve a

statistical significance, a larger sample size of 62 per group would have been required, although the actual clinical or practical significance of this small difference would still be debatable.

The relationship between relative corneal radius and relative mean sphere refraction was investigated further by linear regression analysis of the pooled data (Figure 3.9). The linear regression analysis showed that corneal radius of curvature was not significantly correlated with relative mean sphere refraction (R^2 = 0.025, $F_{1,18}$ = 0.455, p = 0.508). Therefore the relatively small difference in corneal radius between groups does not appear to have had a significant influence on overall refractive outcome, however this relationship is investigated further using a Multivariate Linear General Model (MLGM) in Section 3.3.8.

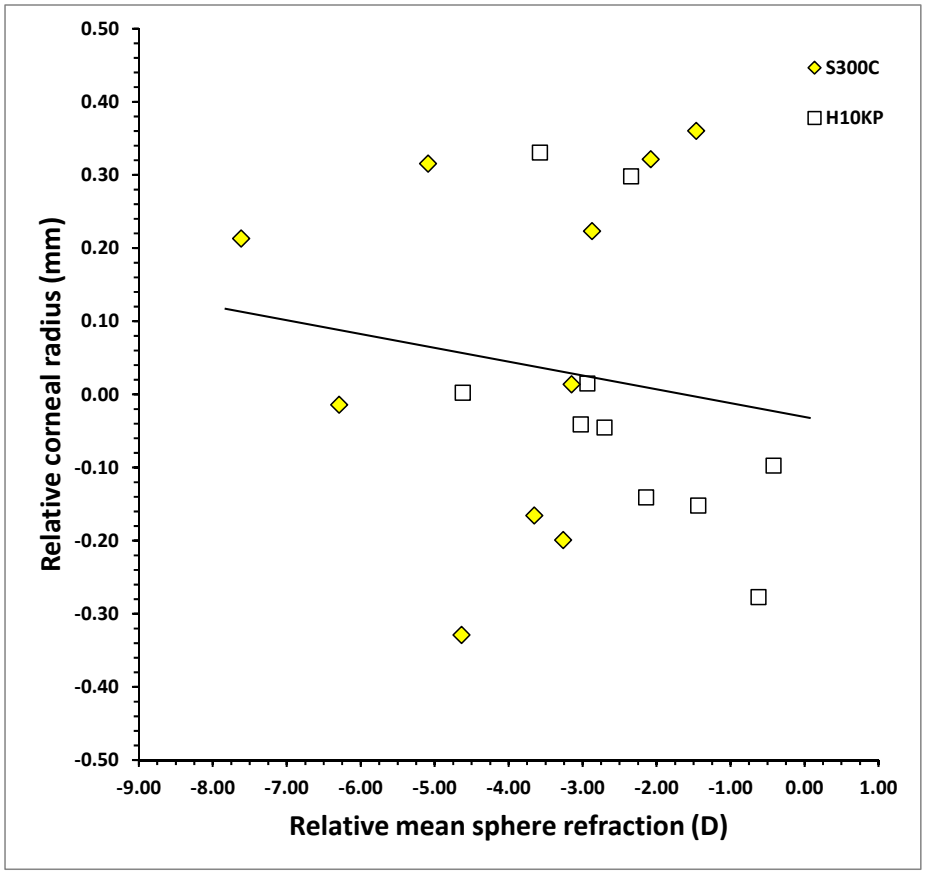

Figure 3.9. Correlation between relative mean sphere refraction and relative corneal radius (deprived eye - non-deprived eye) for all guinea pigs (n = 20): Linear regression revealed no significant correlation between the two measures (R^2 = 0.025, p = 0.508). The linear regression line is for the pooled data of all guinea pigs.

Individual Eye Comparison – Absolute Corneal Radius

As noted previously, one effect of the translucent diffuser used to produce form-deprivation is to reduce illumination of the occluded eye which may result in a differential effect of the lighting condition on corneal radius for each eye (Cohen, Belkin et al. 2008). The comparison of the absolute corneal radii of both deprived and non-deprived eyes, and by lighting conditions, allows for an investigation of this

potential effect. The average absolute corneal radius for the deprived and contralateral non-deprived eyes by illumination condition is illustrated in Figure 3.10. The relative and absolute values of corneal radius of curvature, and associated statistics, are summarised in Table 3.2.

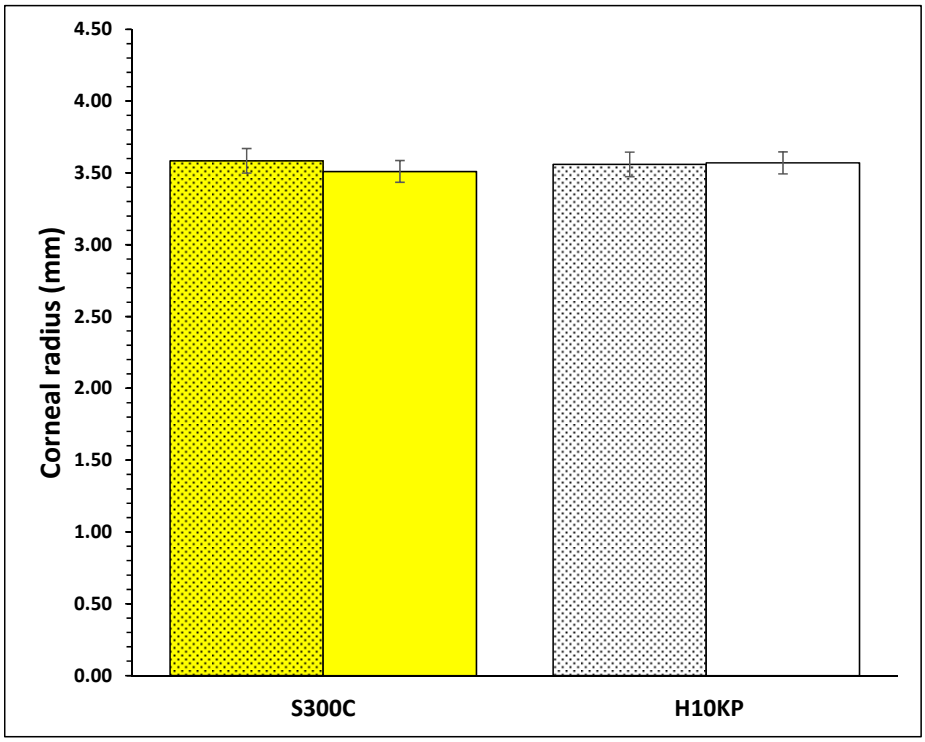

Figure 3.10. Absolute corneal radii of curvature for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative corneal radius by illumination condition was found for either the deprived eyes (*t*-test; $t_{(18)} = 0.207$, $p = 0.838$) or the non-deprived eyes (*t*-test; $t_{(18)} = -0.549$, $p = 0.590$). Error bars are ± 1 SEM.

Illumination Condition	Form-deprived Eyes Corneal radii (mm)	Non-deprived Eyes Corneal radii (mm)	Difference between Eyes Relative radii (mm)
S300C	+3.58 ± 0.08	+3.51 ± 0.08	-0.07 ± 0.08
H10KP	+3.56 ± 0.09	+3.57 ± 0.08	-0.01 ± 0.06
t-test	$p = 0.838$	$p = 0.590$	$p = 0.403$

Table 3.2. Corneal radius of curvature outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant (S300C, n = 10), High Intensity 10,000 lux periodic (H10KP, n = 10). *t*-test *p*-values for comparison of means by illumination condition are given in the final row of the table.

No significant difference in absolute corneal radius by illumination condition was found for either the deprived eyes (*t*-test; $t_{(18)} = 0.207$, $p = 0.838$) or the non-deprived eyes (*t*-test; $t_{(18)} = -0.549$, $p = 0.590$). Furthermore, the average corneal radii of eyes did not differ significantly by whether they were form-deprived or not (Paired *t*-test; $t_{(19)} = 0.653$, $p = 0.522$) irrespective of lighting condition. Therefore, neither form-deprivation nor lighting condition had a significant effect on corneal radius of curvature in this experiment.

3.3.3 Anterior Chamber Depth

Paired-Eye Comparison – Relative Anterior Chamber Depth

Relative anterior chamber depth (ACD) was calculated as the difference in ACD between the deprived eye and the non-deprived eye for each animal in the two illumination groups. The average relative ACD for each illumination group is illustrated in Figure 3.11.

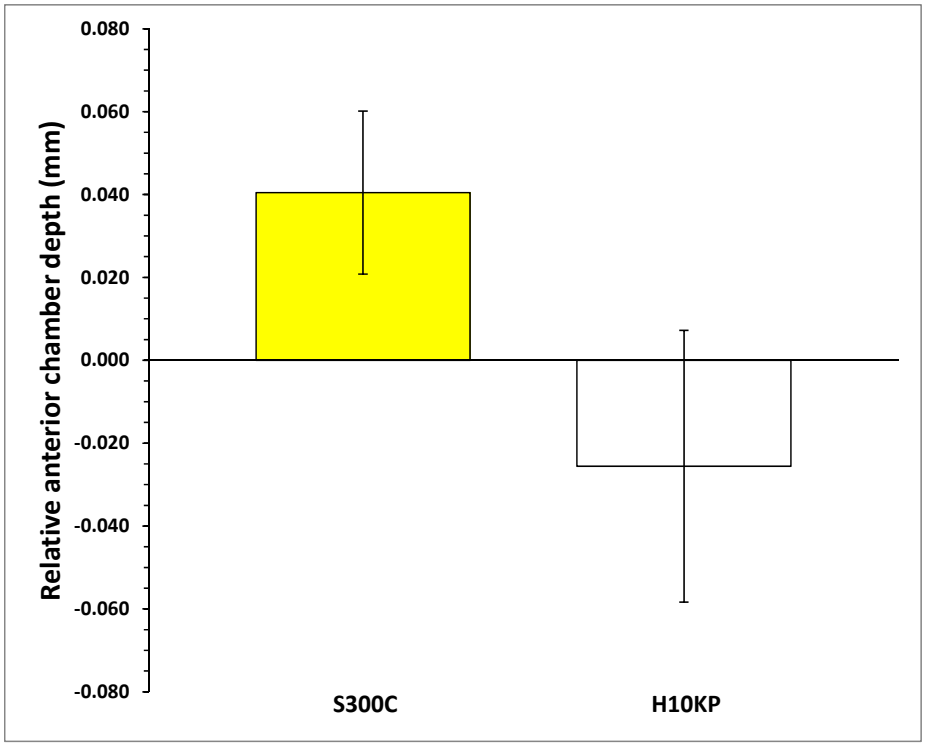

Figure 3.11. Relative anterior chamber depth (deprived eye - non-deprived eye) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative ACD was found by illumination condition (*t*-test; $t_{(18)} = 1.726$, $p = 0.102$). Error bars are ± 1 SEM.

No significant effect of illumination condition on the relative ACD was found between the two illumination groups (*t*-test; $t_{(18)} = 1.726$, $p = 0.102$). There is an apparent trend in the data for the relative ACD for the S300C group to be positive (indicating that the ACD of the deprived eye was greater than that of the non-deprived eye), while the H10KP group was negative (i.e. the ACD of the deprived eye was less than that of the non-deprived eye). Post-hoc power analysis (G*Power, University of Dusseldorf) found that the statistical power of the t-test analysis for relative ACD was low at 37%, and that a total sample size of 17 animals per group would be required for the variation in relative ACD by illumination group to reach statistical significance, although again the actual practical significance of this small difference would still be debatable.

The relationship between relative corneal ACD and relative mean sphere refraction was investigated further by linear regression analysis of the pooled data (Figure 3.12). The linear regression analysis showed that relative ACD was not significantly correlated with relative mean sphere refraction (R^2 = 0.005, $F_{1,18}$ = 0.087, p = 0.771). Therefore, the relatively small difference in relative ACD between groups does not appear to have had a significant influence on overall refractive outcome, however this relationship is investigated further using a Multivariate Linear General Model (MLGM) in Section 3.3.8.

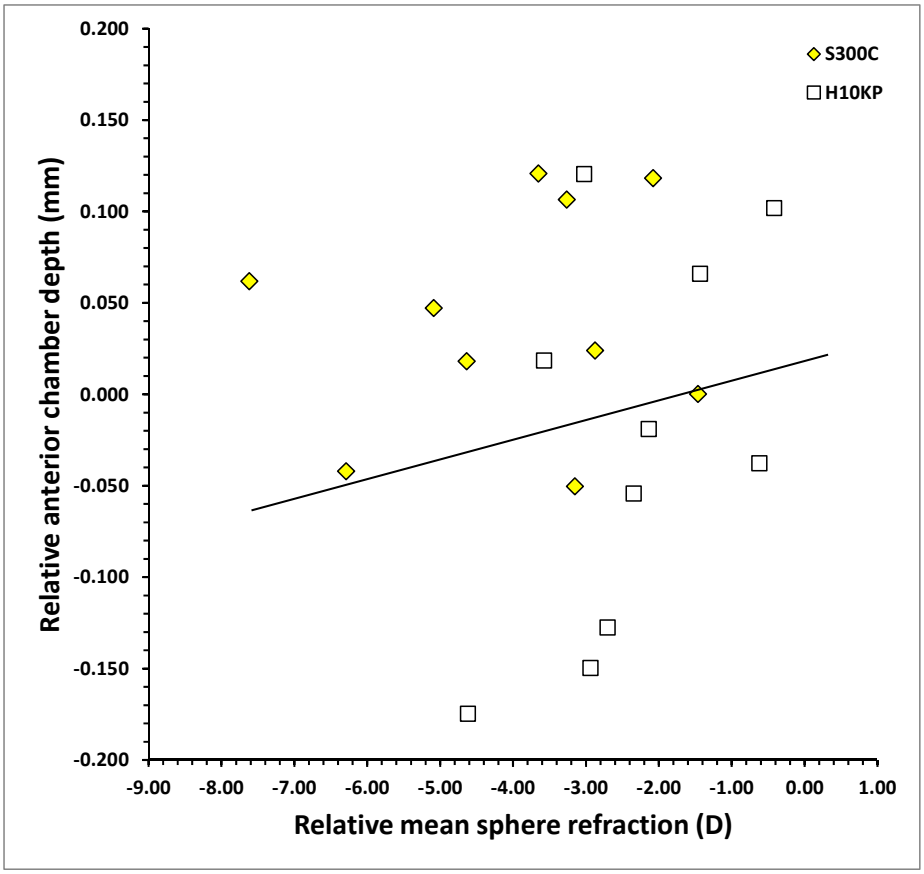

Figure 3.12. Correlation between relative mean sphere refraction and relative anterior chamber depth (deprived eye - non-deprived eye) for all guinea pigs (n = 20). Linear regression revealed no significant correlation between the factors (R^2 = 0.005, p = 0.771). The linear regression line is for the pooled data of all guinea pigs.

Individual Eye Comparison – Absolute Anterior Chamber Depth

The comparison of the absolute ACD of both deprived and non-deprived eyes, and by lighting conditions, allows for an investigation of the potential effect of retinal illumination differences between the groups of eyes. The average absolute ACDs for the deprived and contralateral non-deprived eyes by illumination condition are illustrated in Figure 3.13 and summarised in Table 3.3.

The deprived eyes exposed to the S300C lighting condition had a significantly greater average ACD (+1.22 ± 0.02 mm) than deprived eyes of the H10KP group (1.13 ± 0.02 mm)(*t*-test; $t_{(18)}$ = 2.804,

p = 0.012). Conversely, no significant difference in absolute ACD was found for the non-deprived eyes (t-test; $t_{(18)}$ = 0.902, p = 0.379). Therefore, the periodic high intensity lighting condition may have had more effect on overall refractive outcome in the deprived eyes than in the non-deprived eyes. The potential contribution of this significant difference in deprived eye ACD by illumination condition to refractive outcome is investigated further using a Multivariate Linear General Model (MLGM) in Section 3.3.8.

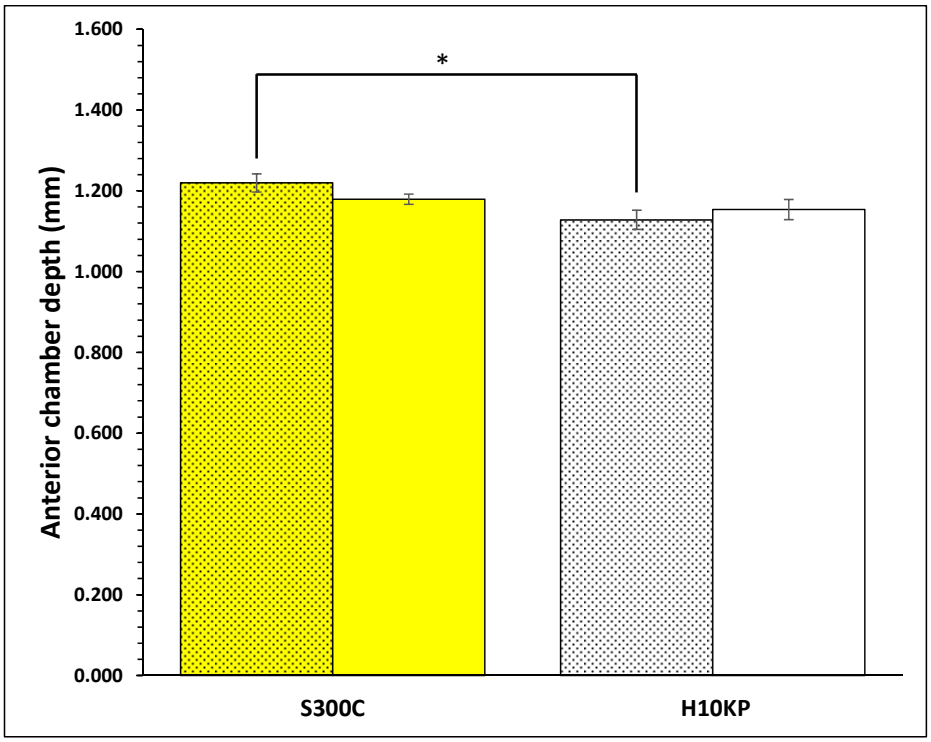

Figure 3.13. Absolute anterior chamber depth for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). For the deprived eyes, a significant difference in ACD was found by illumination condition (t-test; $t_{(18)}$ = 2.804, p = 0.012), however no significant difference was found for the non-deprived eyes (t-test; $t_{(18)}$ = 0.902, p = 0.379). Error bars are ± 1 SEM.

Illumination Condition	Form-deprived Eyes ACD (mm)	Non-deprived Eyes ACD (mm)	Difference between Eyes Relative ACD (mm)
S300C	+1.220 ± 0.022	+1.179 ± 0.013	+0.040 ± 0.020
H10KP	+1.128 ± 0.024	+1.154 ± 0.025	-0.026 ± 0.033
t-test	***p* = 0.012**	*p* = 0.379	*p* = 0.102

Table 3.3 Anterior chamber depth outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). t-test p-values for comparison of means by illumination condition are given in the final row of the table (significant values in **bold**).

On average, the ACD of deprived and non-deprived eyes were similar in depth (+1.17 ± 0.02 mm versus +1.16 ± 0.01 mm) irrespective of lighting condition and not significantly different statistically (pooled data, Paired t-test; t = 0.370, *p* = 0.716). Therefore, form-deprivation, in isolation, did not have a significant effect on ACD in this experiment.

3.3.4 Lens Thickness

Paired-Eye Comparison – Relative Lens Thickness

Relative lens thickness (LT) was calculated as the difference in LT between the deprived eye and the non-deprived eye for each animal in the two illumination groups. The average relative LT for each illumination group is illustrated in Figure 3.14.

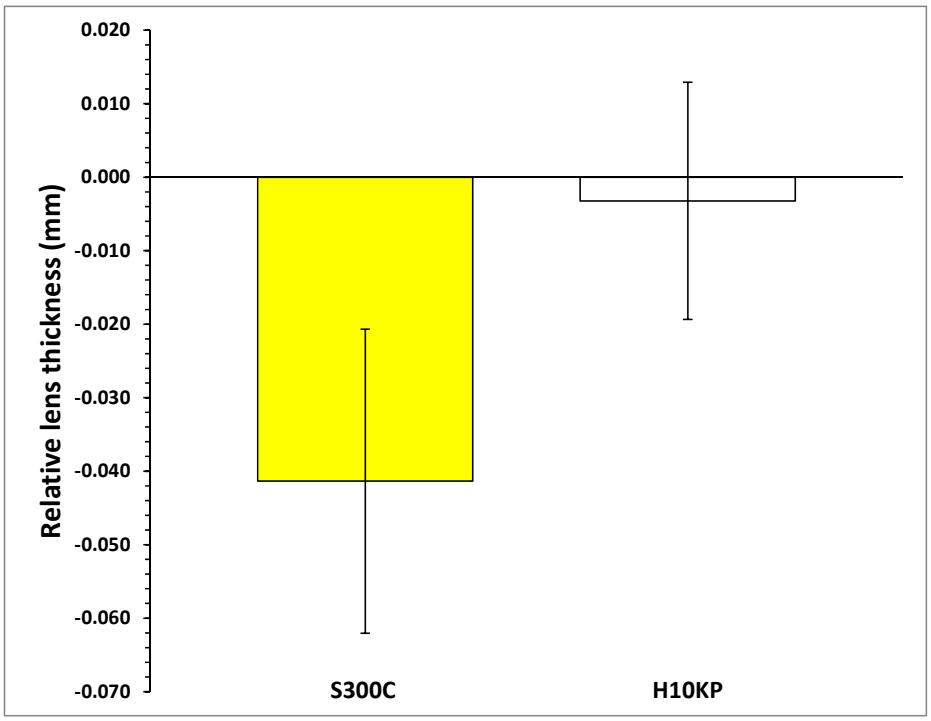

Figure 3.14. Relative lens thickness (deprived eye - non-deprived eye) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative LT was found by illumination condition (*t*-test; $t_{(18)}$ = -1.453, *p* = 0.163). Error bars are ± 1 SEM.

No significant effect of illumination condition on the relative LT was found between the two illumination groups (*t*-test; $t_{(18)}$ = -1.453, *p* = 0.163). However, there is an apparent trend in the data for the relative LT for the S300C group to be more negative (indicating that the LT of the non-deprived eye was greater than that of the deprived eye), than for the H10KP group. Post-hoc power analysis (G*Power, University of Dusseldorf) found that the statistical power of the t-test analysis for relative LT was low at 28%, and that a total sample size of 23 animals per group would be required for the variation in relative

LT by illumination group to reach statistical significance, although whether this small difference (0.038 mm) would represent a practically significant effect on refraction is debateable. For example, linear regression of relative LT against relative mean sphere refraction for all groups revealed no significant correlation between these variables (R^2 = 0.002, $F_{1,18}$ = 0.032, p = 0.859) (Figure 3.15). Therefore, variation in relative LT does not appear to contribute in any significant manner to the overall refractive status of the eyes, although this relationship is investigated further using a Multivariate Linear General Model (MLGM) in Section 3.3.8.

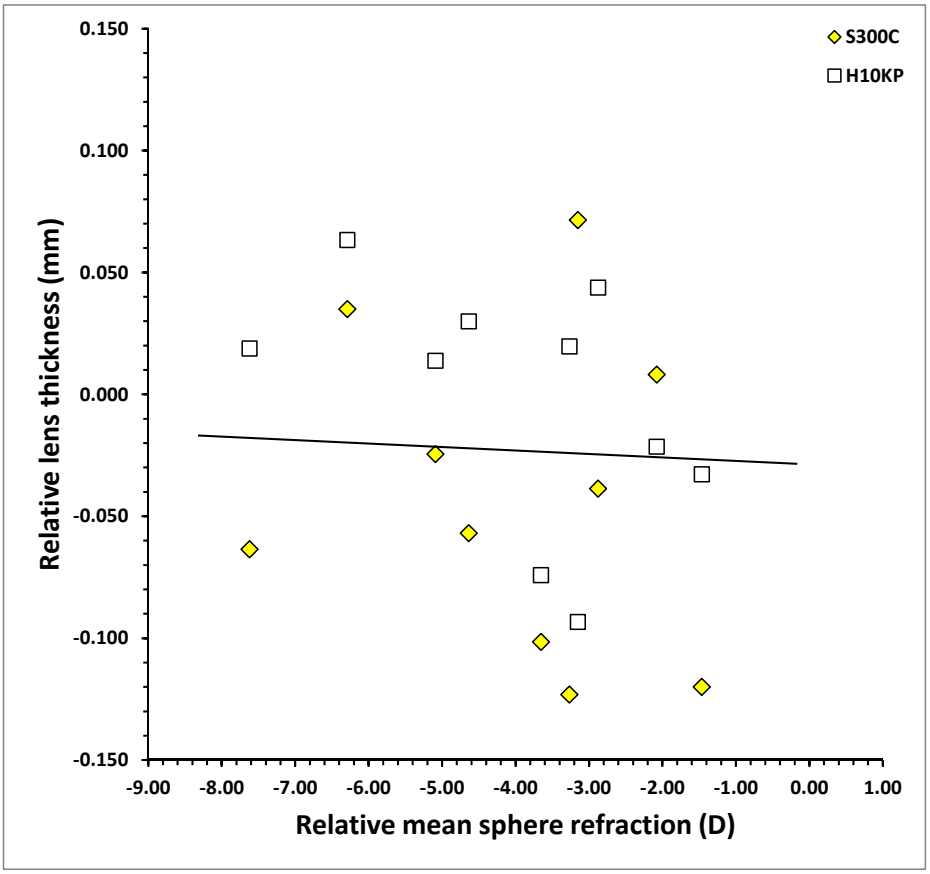

Figure 3.15. Correlation between relative mean sphere refraction and relative lens thickness (deprived eye - non-deprived eye) for all guinea pigs (n = 20). Linear regression revealed no significant correlation between these variables (R^2 = 0.002, p = 0.859). The linear regression line is for the pooled data of all guinea pigs.

The following section explores whether the individual eye LTs (deprived, non-deprived) differ by illumination condition.

Individual Eye Comparison – Absolute Lens Thickness

A comparison of the absolute LT of both deprived and non-deprived eyes was conducted to investigate whether there was any significant effect of the illumination differences between the form-deprived and non-derived eyes due to the use of translucent diffusers to induce FDM. The average absolute ACDs for the deprived and contralateral non-deprived eyes by illumination condition are illustrated in Figure 3.16 and summarised in Table 3.4.

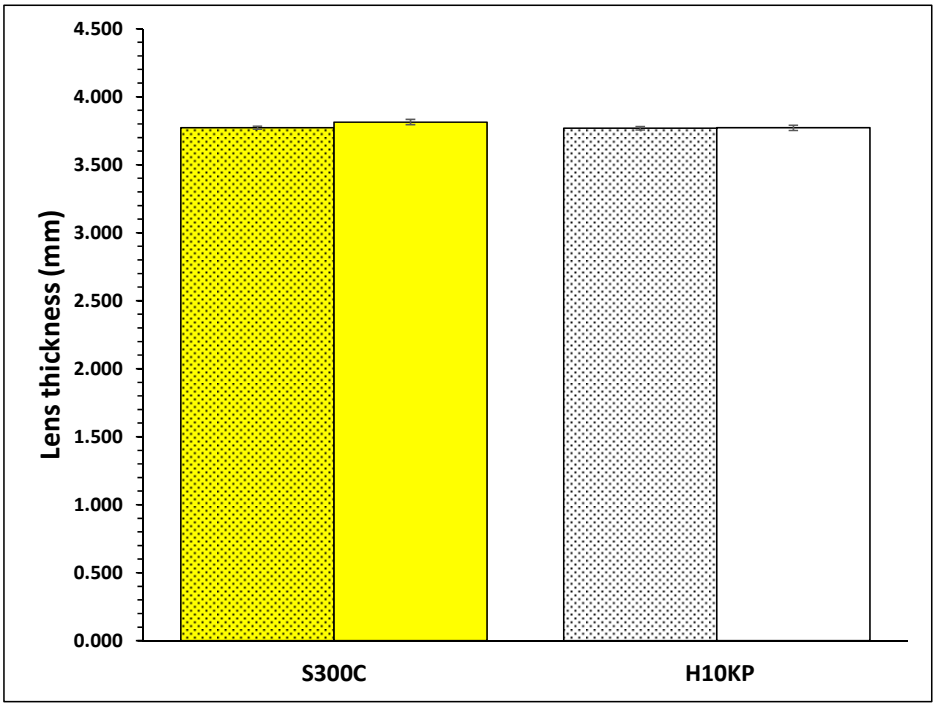

Figure 3.16. Absolute lens thickness for the deprived (shaded bars) and non-deprived eyes (non-shaded bars for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in absolute LT by illumination condition was found for either the deprived eyes (*t*-test; $t_{(18)} = 0.253$, $p = 0.803$) or the non-deprived eyes (*t*-test; $t_{(18)} = 1.577$, $p = 0.132$). Error bars are ± 1 SEM.

Illumination Condition	Form-deprived Eyes LT (mm)	Non-deprived Eyes LT (mm)	Difference between Eyes Relative LT (mm)
S300C	+3.772 ± 0.011	+3.814 ± 0.019	-0.041 ± 0.021
H10KP	+3.768 ± 0.014	+3.771 ± 0.014	-0.003 ± 0.016
t-test	$p = 0.132$	$p = 0.132$	$p = 0.163$

Table 3.4. Lens thickness outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). *t*-test *p*-values for comparison of means by illumination condition are given in the final row of the table.

No significant effect of illumination condition on the absolute LT was found for either the form-deprived eyes (*t*-test; $t_{(18)} = 0.253$, $p = 0.803$) or for the non-deprived eyes (*t*-test; $t_{(18)} = 1.577$, $p = 0.132$). Furthermore, the absolute LT of eyes did not differ significantly by whether they were form-deprived or not (Paired *t*-test; $t_{(19)} = -1.614$, $p = 0.177$) irrespective of illumination condition. Therefore, neither form-deprivation status nor lighting condition appears to have had a significant effect on lens thickness in this experiment, although these relationships are investigated further in Section 3.3.8.

3.3.5 Vitreous Chamber Depth

Paired-Eye Comparison – Relative Vitreous Chamber Depth

Relative vitreous chamber depth (VCD) was calculated as the difference in VCD between the deprived eye and the non-deprived eye for each animal in the two illumination groups. The average relative VCD for each illumination group is illustrated in Figure 3.17.

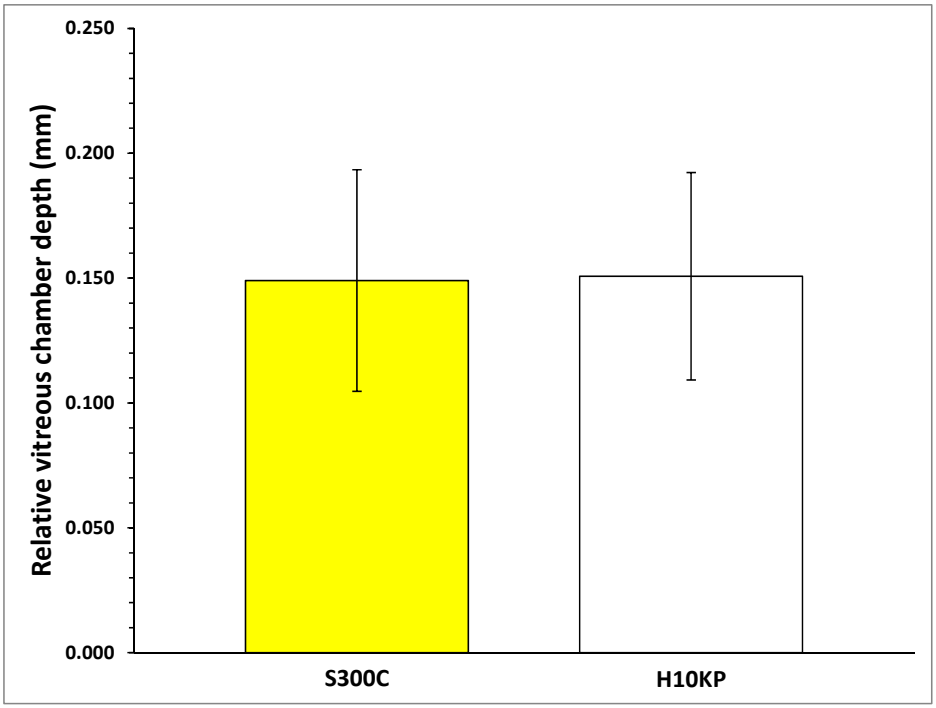

Figure 3.17. Relative vitreous chamber depth (deprived eye - non-deprived eye) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative VCD was found by illumination condition (*t*-test; $t_{(18)} = -0.028$, $p = 0.978$). Error bars are ± 1 SEM.

No significant effect of illumination condition on relative VCD was found between the lighting conditions following statistical analysis (*t*-test; $t_{(18)} = -0.028$, $p = 0.978$). Post-hoc power analysis (G*Power, University of Dusseldorf) showed that the statistical power of the t-test analysis for relative VCD was very low at 5%, due to a low effect size of 0.013. Although it was possible to calculate a sample size which would yield a statistically significant result, the required sample size was not realistically achievable, and demonstrates that the relative VCD values for the two lighting conditions were both statistically and practically no different. In order to determine if the weak statistical power of the statistical analysis was due to the presence of outliers in the data, Grubbs' test for outliers (Grubbs 1950) was carried out using GraphPad™ QuickCalcs Outlier online calculator (http://www.graphpad.com/quickcalcs, USA), however no significant outliers were identified.

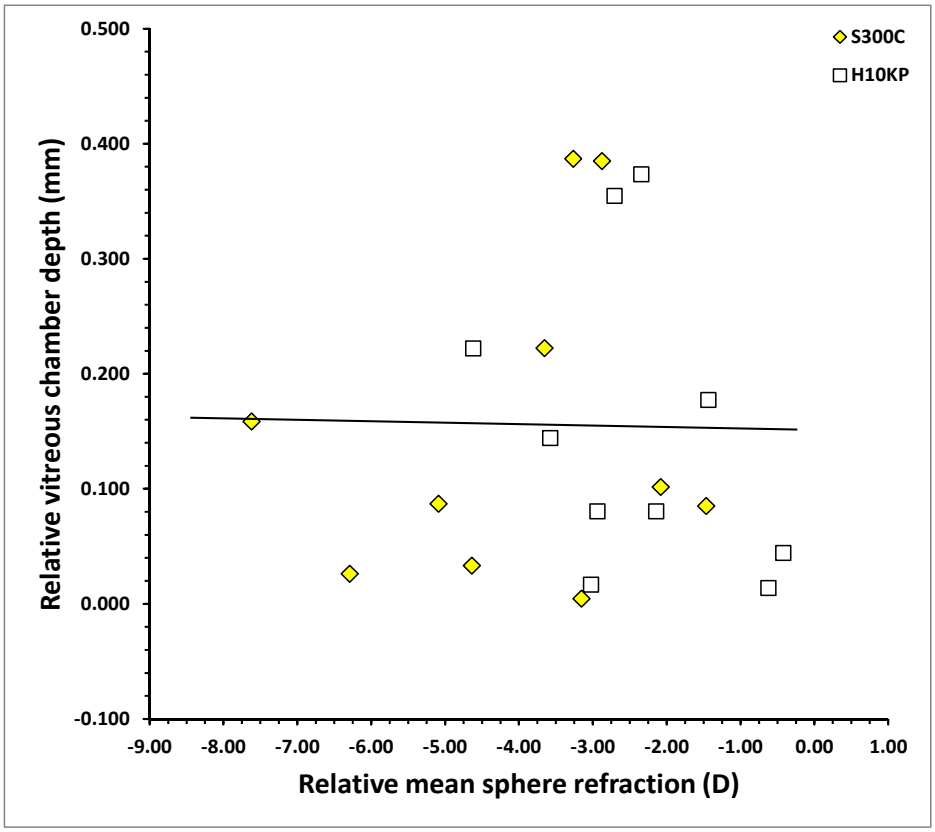

Figure 3.18. Correlation between relative mean sphere refraction and relative vitreous chamber depth (deprived eye - non-deprived eye) for all guinea pigs (n = 20). Linear regression revealed no significant correlation between these variables (R^2 < 0.001, p = 0.975). The linear regression line is for the pooled data of all guinea pigs.

Linear regression of relative VCD against relative mean sphere refraction for pooled data also revealed no significant correlation between these variables (R^2 < 0.001, $F_{1,18}$ = 0.001, p = 0.975) (Figure 3.18). Therefore, variation in relative VCD did not appear to contribute in any significant manner to the overall variation in the relative refractive status of the eyes, which is an unexpected finding as relative refractive error has been shown to be strongly correlated with relative VCD previously in this strain of guinea pigs raised under similar conditions (Backhouse and Phillips 2010). The following section explores whether the absolute VCD values vary by deprivation condition, and whether these values are correlated with absolute refractive error.

Individual Eye Comparison – Absolute Vitreous Chamber Depth

The comparison of the absolute VCD of both deprived and non-deprived eyes, and by lighting conditions, permits an investigation of the potential effect of retinal illumination differences between the groups of eyes due to the use of translucent diffusers to produce form-deprivation. The average absolute VCDs for the deprived and contralateral non-deprived eyes by illumination condition are illustrated in Figure 3.19 and summarised in Table 3.5 below. Also illustrated below are the regression relationships between absolute VCD and absolute refraction for both deprived (Figure 3.20) and non-deprived (Figure 3.21) eyes.

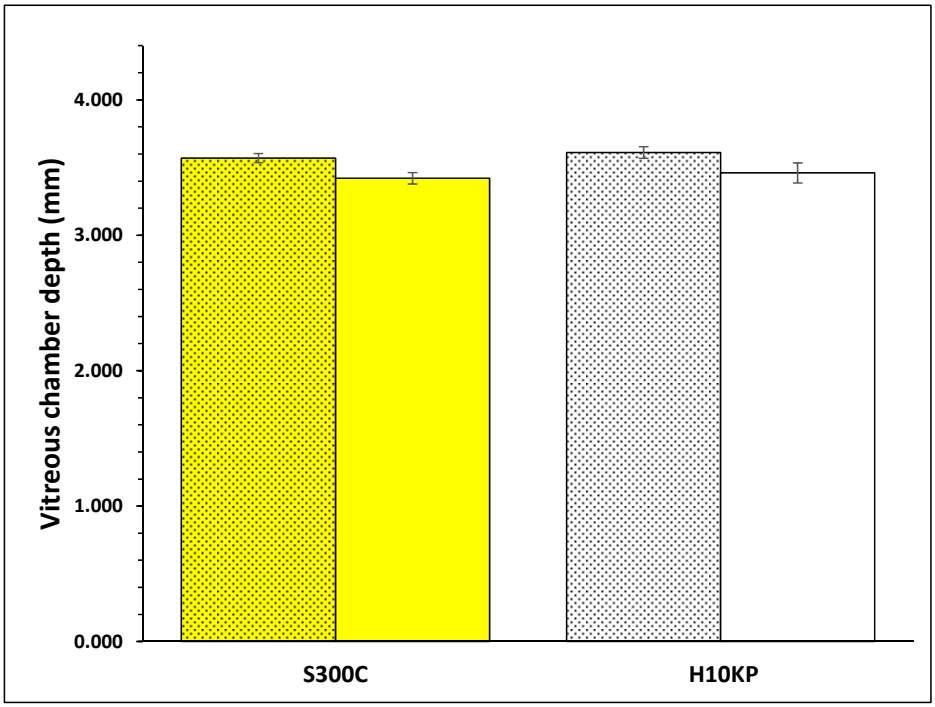

Figure 3.19. Absolute vitreous chamber depth for the deprived (shaded bars) and non-deprived eyes (non-shaded bars for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in absolute VCD was found by illumination condition for either the deprived eyes (*t*-test; $t_{(18)} = -0.764$, $p = 0.455$) or for the non-deprived eyes (*t*-test; $t_{(18)} = -0.468$, $p = 0.645$). Error bars are ± 1 SEM.

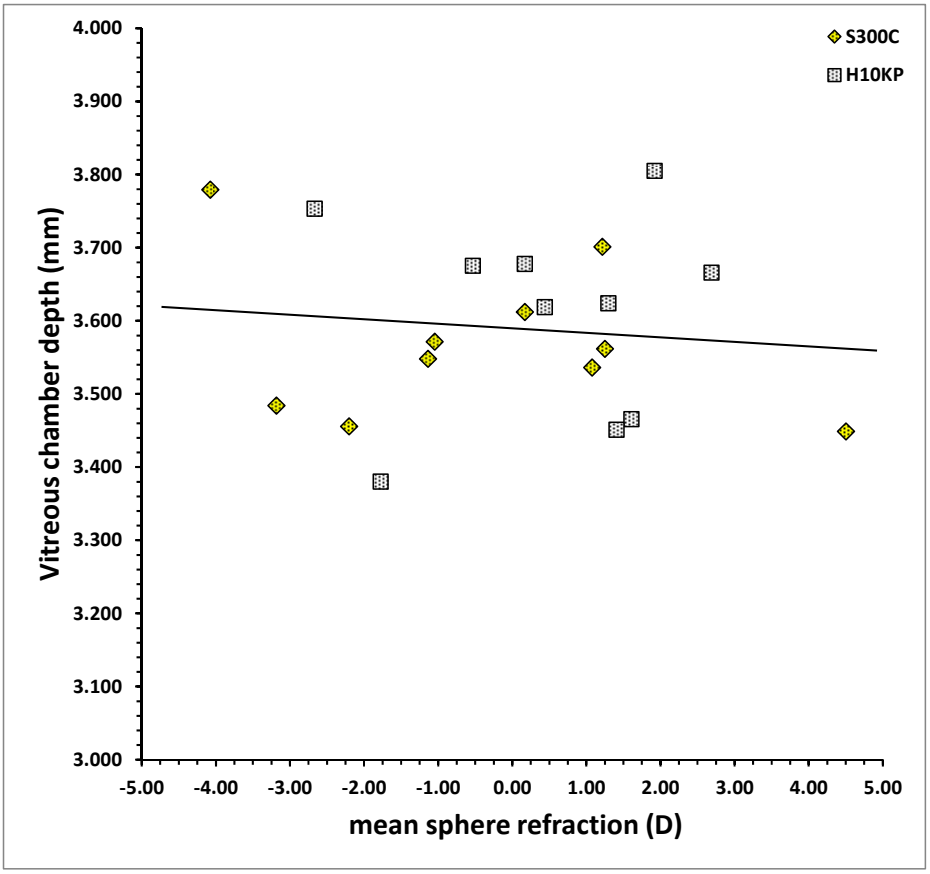

Figure 3.20. Correlation between deprived-eye absolute mean sphere refraction and absolute vitreous chamber depth for all guinea pigs (n = 20). Linear regression revealed no significant correlation between these variables ($R^2 = 0.009$, $p = 0.685$). The linear regression line is for the pooled data of all guinea pigs.

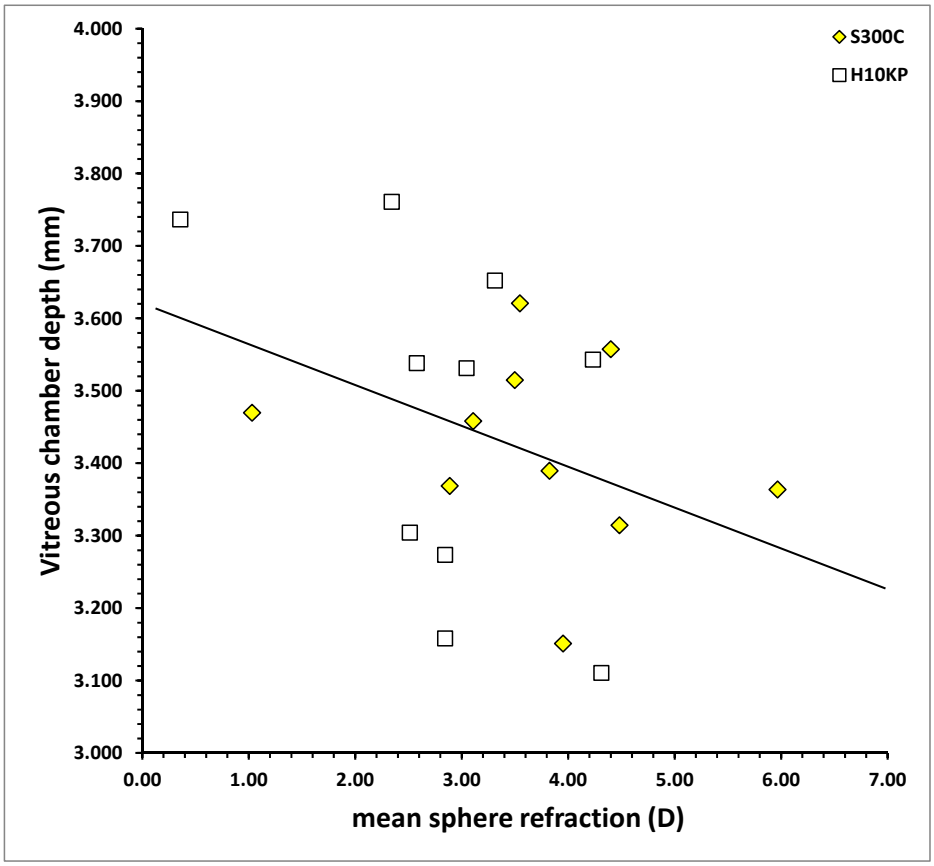

Figure 3.21. Correlation between non-deprived eye absolute mean sphere refraction and absolute vitreous chamber depth for all guinea pigs (n = 20). Linear regression revealed a weak (R^2 = 0.136) but non-significant correlation between these variables (p = 0.109). The linear regression line is for the pooled data of all guinea pigs.

Illumination Condition	Form-deprived Eyes VCD (mm)	Non-deprived Eyes VCD (mm)	Difference between Eyes Relative VCD (mm)
S300C	+3.570 ± 0.033	+3.421 ± 0.042	+0.149 ± 0.044
H10KP	+3.612 ± 0.043	+3.461 ± 0.074	+0.151 ± 0.041
t-test	p = 0.455	p = 0.645	p = 0.978

Table 3.5 Vitreous chamber depth outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). *t*-test *p*-values for comparison of means by illumination condition are given in the final row of the table (significant values in **bold**).

No significant effect of illumination condition on the absolute VCD was found for either the form-deprived eyes (*t*-test; $t_{(18)}$ = -0.764, p = 0.455) or for the non-deprived eyes (*t*-test; $t_{(18)}$ = -0.468, p = 0.645)(Figure 3.19, Table 3.5). However, when considering the effect of form-deprivation alone on VCD, deprived eyes exhibited a significantly longer VCD (+3.591 ± 0.027 mm) when compared to non-deprived eyes (+3.441 ± 0.042), irrespective of illumination condition (Paired t-test; t = 5.066, p < 0.001). As form-deprivation resulted in greater VCD values as expected (Backhouse and Phillips 2010), further regression analysis was carried out to investigate the relationship between absolute

values of VCD and refractive outcome for deprived (Figure 3.20) and non-deprived eyes (Figure 3.21) separately.

The regression plots for deprived (Figure 3.20) and non-deprived eyes (Figure 3.21) suggest a differing pattern of relationship between the two treatments, although the regression outcomes are non-significant. For the deprived eyes (Figure 3.20), VCD does not display any clear relationship to refractive outcome, as evidenced by the near horizontal regression line. Conversely, the regression plot for non-deprived eyes shows an apparent negative correlation between VCD and refraction, where a shorter VCD is associated with a more hyperopic refraction. Unfortunately, the regression is statistically non-significant and so only an indication of a possible trend in the relationship can be taken from this analysis. However, it does suggest that the statistically significant difference in relative refractive outcome (Figure 3.6, Table 3.1) may be underpinned by the variation in the refraction and VCD of non-deprived eyes more so than of the deprived eyes. In their seminal study of FDM in the guinea pig, Howlett and McFadden (Howlett and McFadden 2006) noted that although overall differences in VCD between the deprived and non-deprived eyes were correlated with relative refractive error, between 4-6 D of “optical myopia” could occur in animals with no difference in VCD. Therefore, the difference in refraction between the eyes is most likely due to differences in corneal curvature (power), ACD, and/or LT, although it is possible that a difference in refractive index of the media could also exist. The relationship between refractive outcome and biometric variables, including VCD, is therefore complex and investigated further in Section 3.3.8 using Multivariate Linear General Model (MLGM) analysis.

3.3.6 Axial Length

Paired-Eye Comparison – Relative Axial Length

Relative axial length (AXL) was calculated as the difference in AXL between the deprived eye and the non-deprived eye for each animal in the two illumination groups. The average relative AXL for each illumination group is illustrated in Figure 3.22.

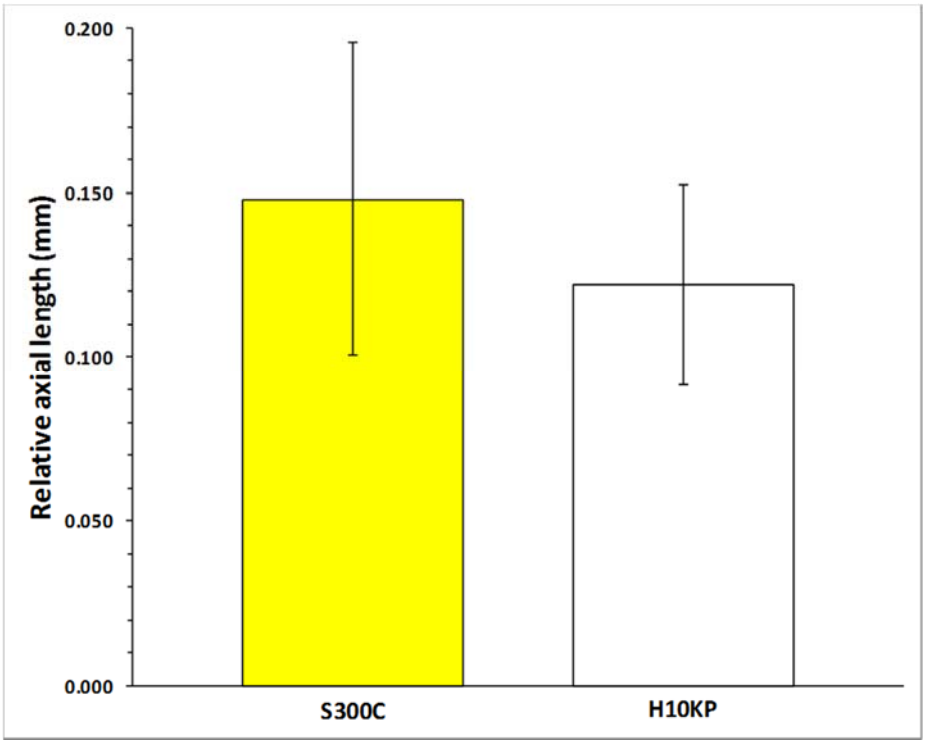

Figure 3.22. Relative axial length (deprived eye - non-deprived eye) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative AXL was found by illumination condition (*t*-test; $t_{(18)} = 0.463$, $p = 0.649$). Error bars are ± 1 SEM.

No significant effect of illumination condition on relative AXL was found between the lighting conditions following statistical analysis (*t*-test; $t_{(18)} = 0.463$, $p = 0.649$). Post-hoc power analysis (G*Power, University of Dusseldorf) showed that the statistical power of the t-test analysis for relative VCD was very low at 7%, due to a low effect size of 0.021, and that a total sample size of 209 animals per group would be required for the variation in relative AXL by illumination group to reach statistical significance, although whether this small difference (0.027 mm) would represent a practically significant effect is questionable. In order to determine if the weak statistical power of the statistical analysis was due to the presence of outliers in the data, Grubbs' test for outliers (Grubbs 1950) was carried out using GraphPad™ QuickCalcs Outlier online calculator (http://www.graphpad.com/quickcalcs, USA), however no significant outliers were identified.

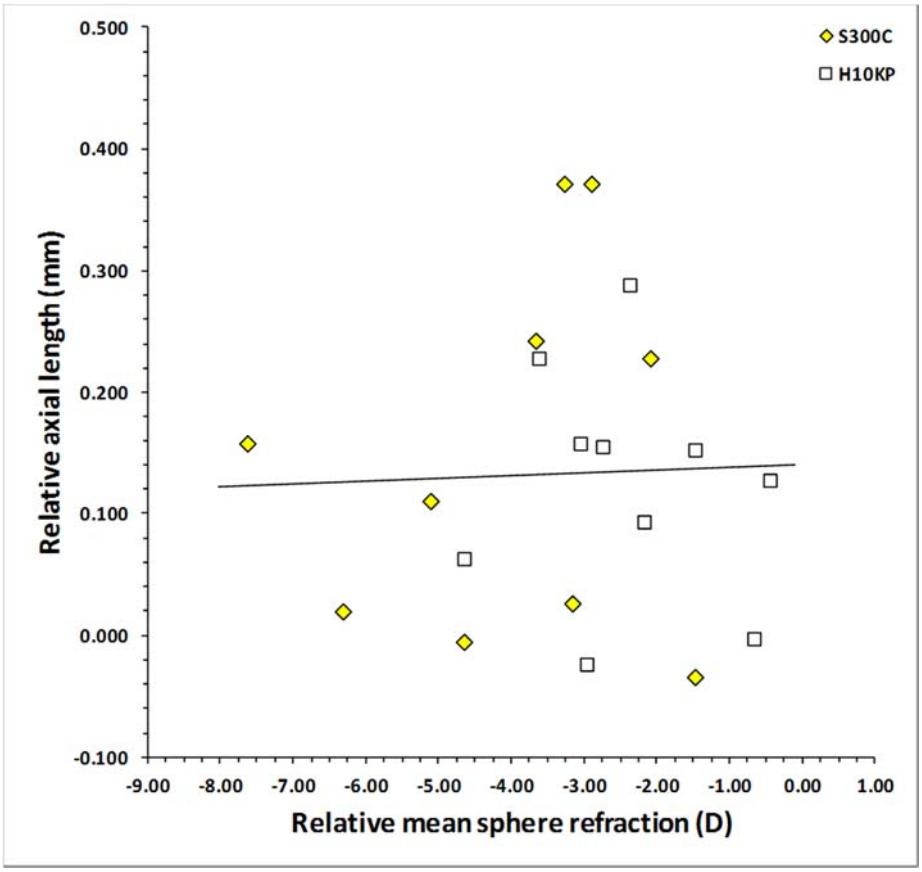

Figure 3.23. Correlation between relative mean sphere refraction and relative axial length (deprived eye - non-deprived eye) for all guinea pigs (n = 20). Linear regression revealed no significant correlation between these variables (R^2 < 0.001, p = 0.927). The linear regression line is for the pooled data of all guinea pigs.

Linear regression of relative AXL against relative mean sphere refraction for pooled data also revealed no significant correlation between these variables (R^2 < 0.001, $F_{1,18}$ = 0.009, p = 0.927) (Figure 3.23). The results for relative AXL are similar to those found for relative VCD (Figure 3.17) as this variable also was not found to contribute in a statistically significant manner to the overall variation in the relative refractive status of the eyes. However, there is an apparent trend for the average relative AXL of the guinea pigs raised under the standard 300 lux lighting condition (S300C) to be larger than for those raised under the high intensity (H10KP) lighting condition (Table 3.6). This apparent trend is consistent with the significantly greater relative refractive error exhibited by the S300C group in comparison to the H10KP group (Figure 3.6). The following section explores whether the absolute AXL values vary by deprivation condition, and whether these values are correlated with absolute refractive error.

Individual Eye Comparison – Absolute Axial Length

The comparison of the absolute AXL of both deprived and non-deprived eyes, and by lighting conditions, permits an analysis of the effect of retinal illumination differences between the groups of eyes due to the use of translucent diffusers to produce form-deprivation. The average absolute AXLs

for the deprived and contralateral non-deprived eyes by illumination condition are illustrated in Figure 3.24 and summarised in Table 3.6 below. The regression relationships between absolute AXL and absolute refraction for both deprived (Figure 3.25) and non-deprived (Figure 3.26) eyes are shown below.

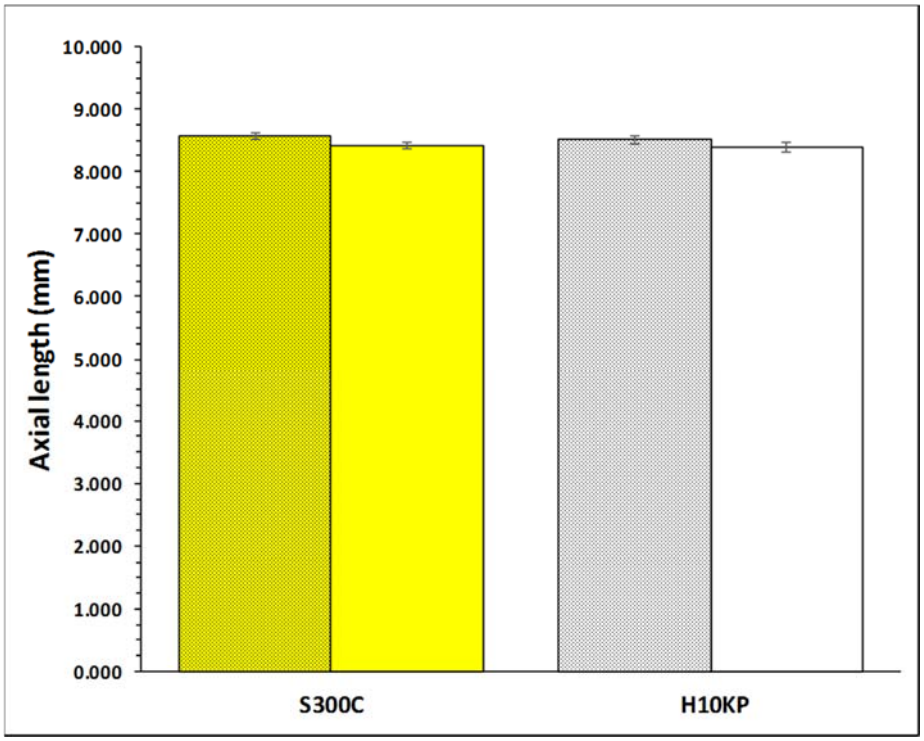

Figure 3.24. Absolute axial length for the deprived (shaded bars) and non-deprived eyes (non-shaded bars for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in absolute VCD was found by illumination condition for either the deprived eyes (*t*-test; $t_{(18)} = 0.621$, $p = 0.542$) or for the non-deprived eyes (*t*-test; $t_{(18)} = 0.341$, $p = 0.737$). Error bars are ± 1 SEM.

No significant effect of illumination condition on the absolute AXL was established for either the form-deprived eyes (*t*-test; $t_{(18)} = 0.621$, $p = 0.542$) or for the non-deprived eyes (*t*-test; $t_{(18)} = 0.341$, $p = 0.737$)(Figure 3.24, Table 3.6). However, when considering only the effect of form-deprivation on AXL, deprived eyes exhibited a significantly longer AXL (+8.535 ± 0.043 mm) when compared to non-deprived eyes (+8.399 ± 0.040), irrespective of illumination condition (Paired t-test; t = 4.881, $p < 0.001$). As form-deprivation resulted in significantly longer AXL values as expected (Backhouse and Phillips 2010), further regression analysis was carried out to investigate the relationship between absolute values of AXL and refractive outcome for deprived (Figure 3.25) and non-deprived eyes (Figure 3.26) separately.

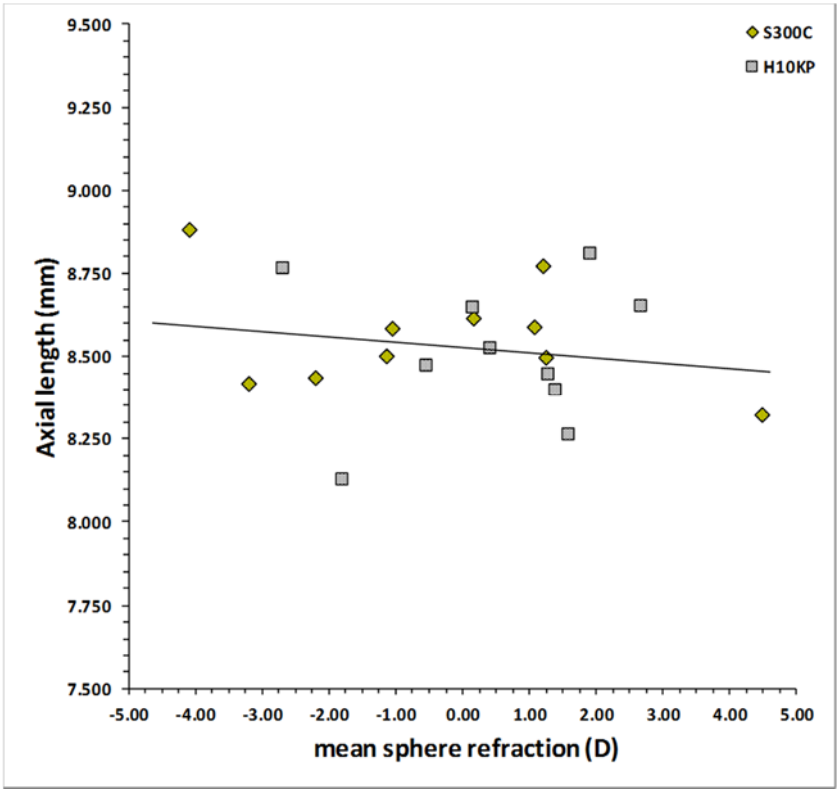

Figure 3.25. Correlation between deprived-eye absolute mean sphere refraction and absolute axial length for all guinea pigs (n = 20). Linear regression revealed no significant correlation between these variables (R^2 = 0.026, p = 0.500). The linear regression line is for the pooled data of all guinea pigs.

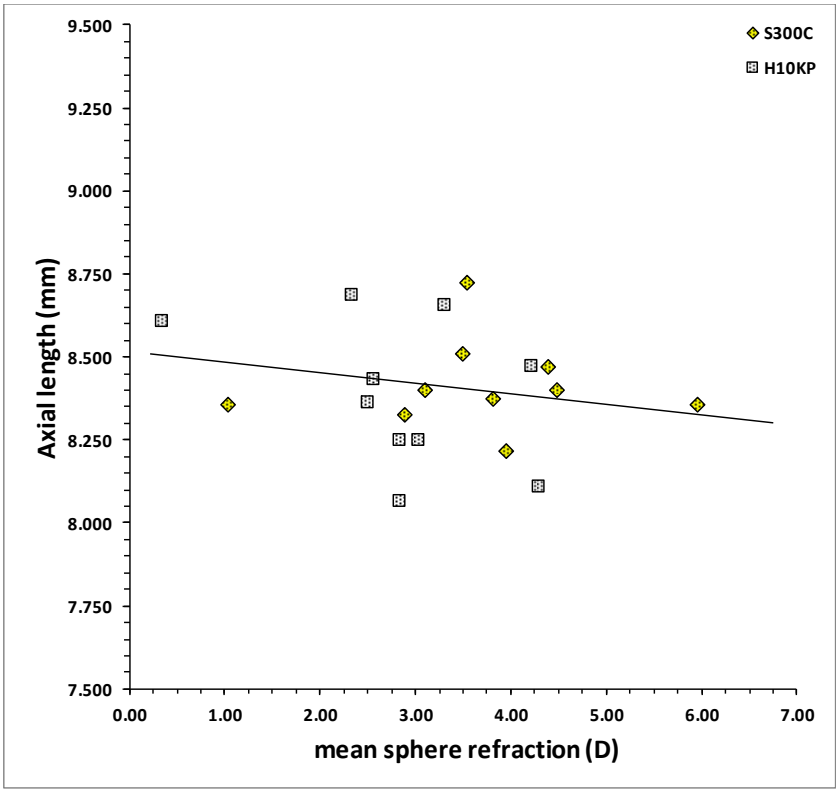

Figure 3.26. Correlation between non-deprived eye absolute mean sphere refraction and absolute axial length for all guinea pigs (n = 20). Linear regression revealed a weak (R^2 = 0.034) but non-significant correlation between these variables (p = 0.437). The linear regression line is for the pooled data of all guinea pigs.

Illumination Condition	Form-deprived Eyes AXL (mm)	Non-deprived Eyes AXL (mm)	Difference between Eyes Relative AXL (mm)
S300C	+8.562 ± 0.053	+8.414 ± 0.042	+0.148 ± 0.048
H10KP	+8.508 ± 0.068	+8.386 ± 0.042	+0.122 ± 0.030
t-test	$p = 0.542$	$p = 0.737$	$p = 0.649$

Table 3.6. Axial length outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). *t*-test *p*-values for comparison of means by illumination condition are given in the final row of the table.

The regression plots for deprived (Figure 3.25) and non-deprived eyes (Figure 3.26) suggest a similar apparent trend in the relationship between refraction and axial length for both treatments, where a shorter axial length is associated with a more hyperopic refractive outcome, although the regression outcomes are non-significant. While the same comparison for VCD (Figures 3.20 & 3.21) revealed an apparent asymmetry in the relationship between VCD and refraction by deprivation status, this asymmetry is not apparent for AXL. Therefore, complementary variations in ACD and LT must exist between the deprived and non-deprived eyes, which effectively mask the apparent differences in VCD. As noted in the discussion of VCD, this suggests that the difference in refraction between the eyes by lighting condition (Figure 3.6, Table 3.1) is most likely due to differences in the anterior segment dimensions including corneal radius (figure 3.8), ACD (Figure 3.11) and LT (Figure 3.14). The relationship between refractive outcome and biometric variables is complex and investigated further in Section 3.3.8 using Multivariate Linear General Model (MLGM) analysis.

3.3.7 Retinal Dopamine Concentration

Paired-Eye Comparison – Relative Dopamine Concentration

Relative retinal dopamine concentration (DAC) was calculated as the difference in dopamine concentration measured for the deprived eye and the non-deprived eye for each animal in the two illumination groups. The average relative DAC for each illumination group is illustrated in Figure 3.27.

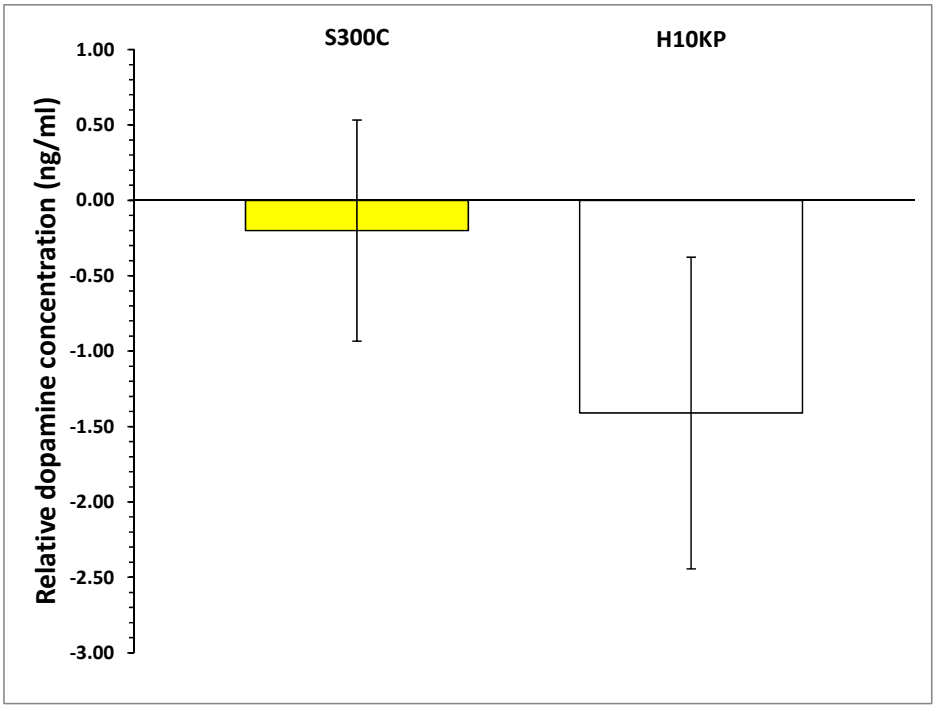

Figure 3.27. Relative retinal dopamine concentration (deprived eye - non-deprived eye) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in relative DAC was found by illumination condition (*t*-test; $t_{(18)} = 0.954$, $p = 0.353$). Error bars are ± 1 SEM.

No significant effect of illumination condition on relative DAC was found between the lighting conditions following statistical analysis (*t*-test; $t_{(18)} = 0.954$, $p = 0.353$). Post-hoc power analysis using G*Power (University of Dusseldorf, Germany) found that the statistical power of the t-test analysis was only 23%. In order to determine if the weak statistical power of the statistical analysis was due to the presence of outliers in the data, Grubbs' test for outliers (Grubbs 1950) was carried out using GraphPad™ QuickCalcs Outlier online calculator (http://www.graphpad.com/quickcalcs, USA), however no significant outliers were identified. Further G*Power calculations showed that in order to achieve statistical significance at a power of 80%, a larger sample size of 88 per group would be required, which suggests that despite the lack of identified outliers, the underlying data has a large degree of variability associated with it.

While no significant difference in relative DAC was found, Figure 3.27 illustrates that there is an apparent trend for the relative DAC of the H10KP group to be greater in magnitude than for the S300C group. This suggests that a greater difference in retinal DAC existed between the deprived and non-deprived eyes of the guinea pigs exposed to the high intensity lighting condition (H10KP) when compared with those exposed to the standard lighting condition (S300C). This difference is investigated further in the individual eye comparison section below.

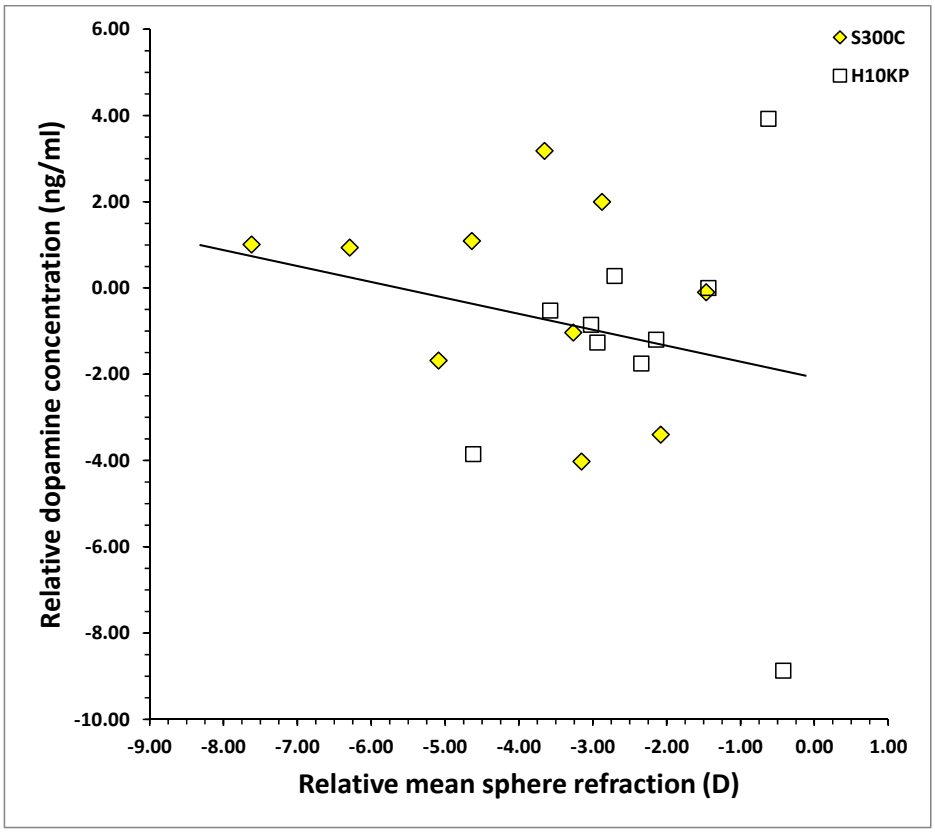

Figure 3.28. Correlation between relative mean sphere refraction and relative retinal dopamine concentration (deprived eye - non-deprived eye) for all guinea pigs (n = 20). Linear regression revealed no significant correlation between these variables (R^2 = 0.055, p = 0.319). The linear regression line is for the pooled data of all guinea pigs.

Linear regression of relative DAC against relative mean sphere refraction for pooled data revealed no significant correlation between these variables (R^2 = 0.055, $F_{1,18}$ = 1.051, p = 0.319) (Figure 3.28). However the relationship between retinal DAC and refraction is investigated further using a Multivariate Linear General Model (MLGM) in Section 3.3.8. The following section explores whether the individual eye DACs (deprived, non-deprived) differ by illumination condition as proposed above.

Individual Eye Comparison – Absolute Retinal Dopamine Concentration

Average absolute DAC was calculated for both the deprived eyes and the non-deprived eyes to investigate whether there was any significant effect of the illumination differences between the eyes due to the use of translucent occluders to produce form-deprivation. The translucent diffusers had a

transmission factor of approximately 78%, therefore under the high intensity 10,000 lux illumination condition (H10KP) the deprived eye would have received the lower illumination level of approximately 7800 lux, when compared with the non-deprived eye. Figure 3.29 shows the variation in average absolute DAC for the deprived and non-deprived eyes by illumination group.

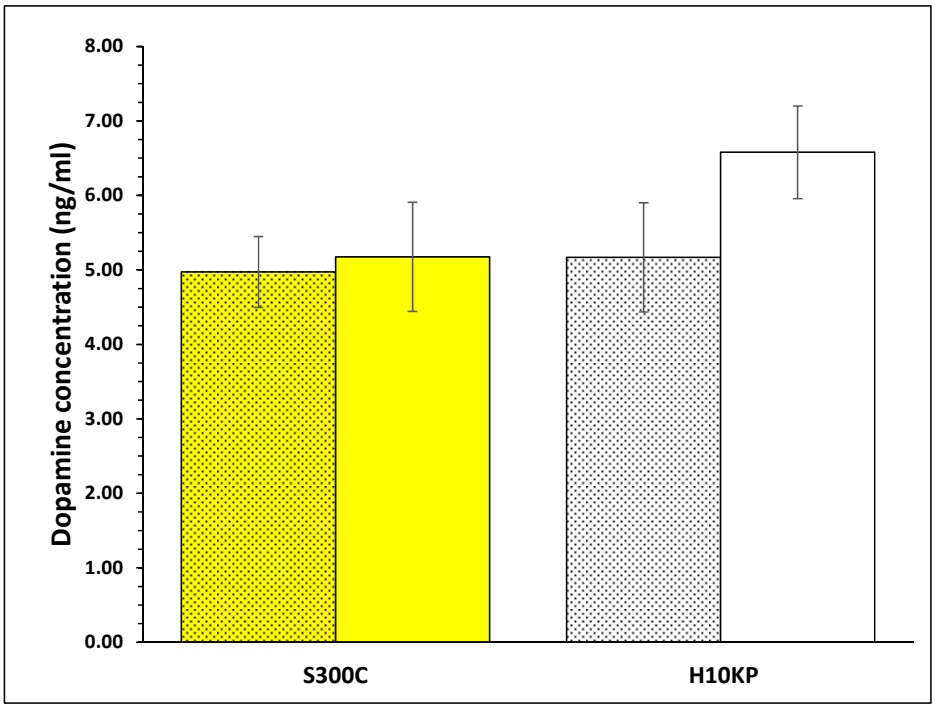

Figure 3.29. Absolute retinal dopamine concentration for the deprived (shaded bars) and non-deprived eyes (non-shaded bars for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). No significant difference in absolute DAC was found by illumination condition for either the deprived eyes (*t*-test; $t_{(18)} = -0.224$, $p = 0.825$) or for the non-deprived eyes (*t*-test; $t_{(18)} = -1.461$, $p = 0.161$). Error bars are ± 1 SEM.

No significant effect of illumination on absolute retinal dopamine concentration was found for either the deprived eyes (*t*-test; $t_{(18)} = -0.224$, $p = 0.825$) or for non-deprived eyes (*t*-test; $t_{(18)} = -1.461$, $p = 0.161$). However, observation of Figure 3.29 reveals an apparent trend where under the high intensity condition (H10KP) the non-deprived eyes exhibited a higher retinal DAC (+6.58 ± 0.62 ng/ml) than under the standard lighting condition (S300C; +5.17 ± 0.73 ng/ml). This apparent trend is consistent with the comments above where such a difference was proposed as being responsible for the greater relative DAC value found for the high intensity light condition. However, as this difference was not statistically significant, further G*Power (University of Dusseldorf, Germany) calculations showed that in order to achieve statistical significance at a power of 80%, a sample size of 30 per group would have been required. Nevertheless, the results suggest that exposure to the high intensity lighting condition (H10KP) potentially increases retinal DAC in the non-deprived eye when compared with the deprived eye.

The absolute DAC measures for both the form-deprived and non-deprived eyes, and relative DAC measures are summarised in Table 3.7 below.

Illumination Condition	Form-deprived Eyes DAC (ng/ml)	Non-deprived Eyes DAC (ng/ml)	Difference between Eyes Relative DAC (ng/ml)
S300C	+4.97 ± 0.48	+5.18 ± 0.73	-0.20 ± 0.73
H10KP	+5.17 ± 0.73	+6.58 ± 0.62	-1.41 ± 1.03
t-test	p = 0.825	p = 0.161	p = 0.353

Table 3.7. Retinal dopamine concentration outcome measures (mean ± 1 SEM) for the two illumination conditions: Standard 300 lux constant light (S300C, n = 10) versus High Intensity 10,000 lux periodic light (H10KP, n = 10). *t*-test *p*-values for comparison of means by illumination condition are given in the final row of the table.

Further consideration of the effect of form-deprivation showed that the absolute DAC levels between deprived eyes and non-deprived eyes were not significantly different for either the standard (S300C; Paired t-test; t = -0.274, p = 0.790) or high intensity (H10KP; Paired t-test; t = -1.364, p = 0.206) illumination condition. The relationship between DAC, refraction and illumination condition is investigated further using a Multivariate Linear General Model (MLGM) in the following section.

3.3.8 MGLM Analysis of Refractive and Retinal Dopamine Outcomes

Multivariate General Linear Model (MGLM) analysis of the data was undertaken in order to further investigate the relationships between the biometric components, illumination condition, and the retinal dopamine concentration and refractive outcomes of the experimental groups. The illumination group (S300C, H10KP) was set as a fixed factor, while radius of curvature, ACD, LT and VCD were set as covariates. Mean sphere refraction and DAC were set as the dependent variables. AXL was not included in the model as it is the sum of ACD, LT and VCD and therefore it is not an independent measure. The MGLM analysis yielded a measure of the effect of the covariates and the fixed factor on the model as well as the statistical significance (F test and associated p values), effect size (partial η^2) and observed power for the model. The test statistics for Pillai's Trace analysis were reported as it is considered the most robust multivariate test and its value increases as the size of the effect increases (IBM Knowledge Center 2016). Partial η^2 is interpreted as a measure of the effect size (i.e. an estimate of the practical significance) of each component of the model (Richardson 2011). The MGLM analysis also calculated the Univariate (ANOVA) analysis for each dependent variable separately. The MGLM analysis was carried out on both the relative (paired-eye) and absolute (individual eye) data.

MGLM - Paired-Eye Analysis

The results of the MGLM analysis of the relative mean sphere refraction and relative DAC, by illumination condition, and with associated relative biometric covariates, are summarised in Tables 3.8 3.9 and 3.10 below.

Multivariate Test: Δ Rx & Δ DAC	**F statistic Pillai's Trace**	**Significance (*p*)**	**partial η^2**	**Observed Power**
Intercept	14.075	**0.001**	0.684	0.991
Δ Corneal radius	0.328	0.726	0.048	0.092
Δ ACD	0.389	0.685	0.057	0.100
Δ LT	0.397	0.680	0.058	0.101
Δ VCD	0.100	0.906	0.015	0.062
Illumination	2.807	0.097	0.302	0.456

Table 3.8. Summary of MGLM analysis for relative mean sphere refraction and relative dopamine concentration as related dependent variables. Pillai's Trace tests for strength of contribution of each effect to the overall model. Only the Intercept of the model was statistically significant, however the Illumination factor has a partial η^2 of 0.302 which indicates a large effect size. Statistically significant p values (p <0.05) in **bold**.

MGLM analysis is applicable where two or more conceptually related dependent variables are modelled for the effects of certain contributing factors or covariates. Relative refraction and relative DAC can be considered the two main outcome measures of this study, where the experimental hypothesis is that both variables are affected by lighting condition (i.e. high intensity illumination suppressed relative myopia development and increases relative DAC).

Only the intercept component of the model demonstrated statistical significance (Table 3.8). This suggests that the dependent variable model has a fixed value, which does not change significantly with respect to the other factors of relative corneal radius, ACD, LT and VCD. The illumination condition is also non-significant, however it does display a large effect size (partial η^2 = 0.302) by Cohen's criteria (Richardson 2011). Therefore, while not statistically significant (most likely due to sample size limitations), illumination may have a practically significant effect on relative refraction and DAC.

The MLGM analysis also included calculation of the univariate model (ANOVA) for each dependent variable separately. The univariate analysis of relative refraction (Table 3.9) found that the illumination factor was statistically significant (p = 0.031) and had a large effect size (partial η^2 = 0.292) when only the one dependent variable was included in the model. This result was comparable to the t-test analysis of the effect of illumination condition on relative refraction in Section 3.3.1 (Figure 3.6, Table 3.1). However, none of the biometric values were statistically significant for the model, although relative corneal radius did display a medium-large effect strength (partial η^2 = 0.099).

Univariate Test: Δ Rx	**F statistic ANOVA**	**Significance (p)**	**partial η^2**	**Observed Power**
Corrected Model	1.281	0.326	0.314	0.324
Intercept	26.026	**< 0.001**	0.650	0.997
Δ Corneal radius	0.001	0.982	0.099	0.557
Δ ACD	0.683	0.422	0.047	0.120
Δ LT	0.495	0.493	0.034	0.101
Δ VCD	0.146	0.708	0.010	0.065
Illumination	5.784	**0.031**	0.292	0.610

Table 3.9. Summary of UGLM (ANOVA) analysis for relative mean sphere refraction as the dependent variable (R^2 = 0.314). Illumination factor was shown to have a significant effect on relative refraction (p = 0.031) with a large effect size (partial η^2 = 0.610). Statistically significant p values (p <0.05) in **bold**.

The univariate analysis of relative DAC (Table 3.9) found no statistically significant effects, for either the biometric components or illumination condition. While the illumination effect was not statistically significant, this factor had a medium effect size (partial η^2 = 0.060) and so may be practically related to relative DAC. This result is comparable to the t-test analysis of the effect of illumination condition on relative DAC in Section 3.3.7 (Figure 3.27, Table 3.7).

Univariate Test: Δ DAC	F statistic ANOVA	Significance (*p*)	partial η^2	Observed Power
Corrected Model	0.528	0.752	0.159	0.148
Intercept	1.210	0.290	0.080	0.176
Δ Corneal radius	0.690	0.420	0.047	0.121
Δ ACD	0.291	0.598	0.020	0.080
Δ LT	0.212	0.652	0.015	0.071
Δ VCD	0.108	0.747	0.008	0.061
Illumination	0.891	0.361	0.060	0.142

Table 3.10. Summary of UGLM (ANOVA) analysis for relative dopamine concentration as the dependent variable (R^2 = 0.159). No factors were found to be statistically significant. However, illumination was shown to have a medium effect size (partial η^2 = 0.060) on the model.

As the translucent diffusers used to produce form-deprivation also reduced transmission of incident illumination (Section 2.1.2), the MGLM analysis was also applied to the deprived and non-deprived eyes separately to assess any differential effects of illumination between the eyes.

MGLM – Individual Eye Analysis: Deprived Eyes

The results of the MGLM analysis of the mean sphere refraction, retinal dopamine concentration and associated biometric components by illumination condition, for the deprived and eyes, are summarised in Tables 3.11, 3.12 and 3.13 below.

Deprived Eyes Rx & DAC	F statistic Pillai's Trace	Significance (*p*)	partial η^2	Observed Power
Intercept	0.208	0.815	0.031	0.076
Corneal radius	2.858	0.094	0.305	0.463
ACD	0.116	0.891	0.018	0.064
LT	0.067	0.935	0.010	0.058
VCD	0.095	0.910	0.014	0.062
Illumination	0.261	0.774	0.039	0.083

Table 3.11. Summary of MGLM analysis of deprived-eye values for mean sphere refraction and dopamine concentration as dependent variables. No factors were found to be statistically significant. However corneal radius was shown to have a large effect size (partial η^2 = 0.305), while illumination was shown to have a small-medium effect size (partial η^2 = 0.039) on the model.

The MGLM analysis for the deprived eyes revealed no statistically significant factors for the model. However, corneal radius showed the largest effect size (partial η^2 = 0.305) of all the factors. Therefore, while the statistical power is low, most likely due to sample size limitations, corneal radius may have a practically significant effect on absolute refraction and DAC for the deprived eyes. This complements the finding above that relative corneal radius also displayed a medium-large effect size on relative refraction (Table 3.9).

The univariate analysis of absolute refraction for deprived eyes (Table 3.12) found no significant relationships between the dependent variable and the biometric covariates or illumination factor, however illumination displayed a small-medium effect size (partial η^2 = 0.028). This result is comparable to the t-test analysis of the effect of corneal radius on deprived-eye refraction in Section 3.3.2 (Figure 3.10, Table 3.2). The lack of significant effects in the model of the biometric components on refraction in the deprived eye suggests that the reduction in relative myopia (Figure 3.6, Table 3.1) is more likely to be due to the effects of the lighting conditions on the non-deprived eye, than the deprived eye.

Deprived Eyes Rx	F statistic ANOVA	Significance (*p*)	partial η^2	Observed Power
Corrected Model	0.218	0.949	0.072	0.086
Intercept	0.200	0.662	0.014	0.070
Corneal radius	0.069	0.796	0.005	0.057
ACD	0.021	0.886	0.002	0.052
LT	0.137	0.717	0.010	0.064
VCD	0.089	0.769	0.006	0.059
Illumination	0.397	0.539	0.028	0.090

Table 3.12. Summary of UGLM (ANOVA) analysis of deprived eye values for absolute mean sphere refraction as the dependent variable (R^2 = 0.072). No factors were found to be statistically significant. However, illumination was shown to have a small-medium effect size (partial η^2 = 0.028) on the model.

The univariate analysis of absolute DAC (Table 3.13) found that corneal radius was statistically significant (p = 0.037) and had a large effect size (partial η^2 = 0.276). However, neither illumination nor any of the other biometric values were statistically significant for the model. The other effects also displayed small partial η^2 values. The lack of effect of illumination in the deprived eye model may be related to the reduced retinal illumination due to the reduced light transmission of the diffusers.

Deprived Eyes DAC	F statistic ANOVA	Significance (*p*)	partial η^2	Observed Power
Corrected Model	1.100	0.404	0.282	0.280
Intercept	0.358	0.559	0.025	0.086
Corneal radius	5.335	**0.037**	0.276	0.575
ACD	0.179	0.679	0.013	0.068
LT	0.034	0.857	0.002	0.053
VCD	0.062	0.806	0.004	0.056
Illumination	0.051	0.824	0.004	0.055

Table 3.13. Summary of UGLM (ANOVA) analysis of deprived eye values for retinal dopamine concentration as the dependent variable (R^2 = 0.282). Corneal radius was shown to have a significant effect on absolute DAC (p = 0.037) with a large effect size (partial η^2 = 0.276). Statistically significant p values (p <0.05) in **bold**.

MGLM – Individual Eye Analysis: Non-deprived Eyes

The results of the MGLM analysis of the mean sphere refraction, retinal dopamine concentration and associated biometric components by illumination condition, for the non-deprived eyes, are summarised in Tables 3.14, 3.15 and 3.16 below.

Non-deprived Eyes Rx & DAC	F statistic Pillai's Trace	Significance (*p*)	partial η^2	Observed Power
Intercept	0.292	0.752	0.043	0.087
Corneal radius	1.341	0.295	0.171	0.238
ACD	1.265	0.315	0.163	0.227
LT	0.537	0.597	0.076	0.120
VCD	1.103	0.361	0.145	0.202
Illumination	0.494	0.621	0.071	0.114

Table 3.14. Summary of MGLM analysis of non-deprived eye values for mean sphere refraction and dopamine concentration as dependent variables. No factors were found to be statistically significant. However corneal radius (partial η^2 = 0.171) and VCD (partial η^2 = 0.145) were shown to have a large effect size, while illumination was shown to have a small-medium effect size (partial η^2 = 0.071) on the model.

The MGLM analysis for the non-deprived eyes revealed no statistically significant factors for the model. However, corneal radius (partial η^2 = 0.171) and VCD (partial η^2 = 0.145) showed the largest effect sizes. Therefore, while the statistical power is low, most likely due to sample size limitations, corneal radius and VCD may also have practically significant effects on absolute refraction and DAC for the non-deprived eyes.

Non-deprived Eyes Rx	F statistic ANOVA	Significance (*p*)	partial η^2	Observed Power
Corrected Model	1.117	0.395	0.285	0.284
Intercept	0.163	0.693	0.011	0.066
Corneal radius	0.250	0.625	0.018	0.075
ACD	0.380	0.548	0.026	0.089
LT	0.452	0.512	0.031	0.096
VCD	1.179	0.296	0.078	0.173
Illumination	0.951	0.346	0.064	0.149

Table 3.15. Summary of UGLM (ANOVA) analysis of non-deprived eye values for absolute mean sphere refraction as the dependent variable (R^2 = 0.285). No factors were found to be statistically significant. However, both VCD (partial η^2 = 0.078) and illumination (partial η^2 = 0.064) were shown to have a medium effect size on the model.

The univariate analysis of absolute refraction for non-deprived eyes (Table 3.16) found no significant relationships between the dependent variable and the biometric covariates or illumination factor. However, both VCD (partial η^2 = 0.078) and illumination (partial η^2 = 0.064) were shown to have a medium effect size on the model, as found for the MGLM analysis (Table 3.14).

Non-Deprived Eyes DAC	F statistic ANOVA	Significance (*p*)	partial η^2	Observed Power
Corrected Model	2.307	0.100	0.452	0.562
Intercept	0.551	0.470	0.038	0.107
Corneal radius	2.287	0.153	0.140	0.291
ACD	2.609	0.129	0.157	0.325
LT	0.891	0.361	0.060	0.142
VCD	1.604	0.226	0.103	0.219
Illumination	0.251	0.624	0.018	0.075

Table 3.16. Summary of UGLM (ANOVA) analysis of non-deprived eye values for retinal dopamine concentration as the dependent variable (R^2 = 0.452). No factors were found to be statistically significant. However, both corneal radius (partial η^2 = 0.140) and ACD (partial η^2 = 0.157) were shown to have a large effect size on the model.

The univariate analysis of absolute DAC (Table 3.16) for non-deprived eyes found no significant relationships between the dependent variable and the biometric covariates or illumination factor. However, both corneal radius (partial η^2 = 0.140) and ACD (partial η^2 = 0.157) were shown to have a large effect size on the model.

In summary, MGLM analysis revealed that when considering relative refraction and relative retinal DAC as related dependent variables, no statistically significant effects were found for illumination condition or for any of the biometric covariates. However, illumination condition was found to have a large effect size as measured by partial η^2, which suggests that it may have a practical influence on the dependent variables, but the effect is masked by insufficient sample sizes. Furthermore, univariate analysis of relative refraction as a single dependent variable, did reveal a statistically significant effect of illumination condition, which confirms the initial t-test analysis of this relationship in Section 3.3.1.

Analysis of the absolute values of the refraction and DAC of the deprived eyes only, also revealed no statistically significant effects for either illumination condition or for the biometric covariates. Illumination condition showed a small-medium effect size, while corneal radius exhibited a large effect size. This large effect size was confirmed by the univariate analysis of DAC for the form-deprived eyes which revealed that corneal radius had a significant effect on this dependent variable. The finding of a relationship between corneal radius changes and retinal DAC levels is not intuitive, as one is a feature of the anterior segment of the eye while the other is a feature of the posterior segment.

MGLM analysis of the absolute values of the refraction and DAC for the non-deprived eyes also revealed no statistically significant effects for either illumination condition or for the biometric covariates, although both corneal radius and VCD exhibited large effect sizes. While the effect of VCD was non-significant, the large effect size does support the proposal made in Section 3.3.5 that the difference in relative refractive outcomes (Section 3.3.1) may be the consequence of an effect of the high intensity lighting condition on the non-deprived eye more so than on the deprived eye, as the translucent diffusers transmitted only approximately 78% of the incident illumination.

3.3.9 Summary

The following statistically significant effects of illumination condition were identified in this experiment:

1. A significantly lower degree of relative myopia was found in the group of guinea pigs that had been exposed to the periodic high intensity 10,000 lux illumination (H10KP) condition when compared to the group that was raised under the standard 300 lux illumination (S300C) condition (*t*-test: $t_{(18)} = -2.234$, $p = 0.038$). This finding was replicated in the UGLM analysis of relative refraction (Table 3.9).

2 Under the H10KP group lighting condition, deprived eyes exhibited a significantly shallower average ACD (1.13 ± 0.02 mm) when compared to the eyes of the S300C group (+1.22 ± 0.02 mm) (*t*-test; $t_{(18)} = 2.804$, $p = 0.012$). Conversely, no significant difference in absolute ACD was found for the non-deprived eyes (*t*-test; $t_{(18)} = 0.902$, $p = 0.379$).

UGLM Analysis also identified the following statistically significant effect:

1. Univariate analysis of absolute DAC of deprived eyes (Table 3.13) found that corneal radius was a statistically significant ($p = 0.037$) component of, and had a large effect size (partial $\eta^2 = 0.276$) on, the linear model of factors that predicted DAC outcome.

3.4 Discussion: Guinea Pig

3.4.1 Suppression of Form Deprivation Myopia: Overview

This is the first study to demonstrate that in monocularly form-deprived pigmented guinea pigs, daily exposure to periodic high intensity white light (10,000 lux) suppresses development of form-deprivation myopia when compared to guinea pigs raised under continuous daily exposure to 300 lux standard room illumination (Figure 3.6, Table 3.1). Guinea pigs raised under 300 lux illumination developed -4.01 D of relative myopia on average, while those raised under periodic 10,000 lux illumination only developed -2.38 D of relative myopia, representing a statistically significant reduction of 1.63 D (~40%) in the degree of myopia induced. The difference in the magnitude of relative myopia developed under the two illumination conditions was due to an apparent trend under the high intensity lighting for a reduction in the degree of myopia induced in the deprived eye, as well as a decrease in the degree of hyperopia developed in the contralateral non-deprived eye (Figure 3.7, Table 3.1).

The ability of high intensity ambient illumination to at least partially suppress the development of FDM has been demonstrated previously in the chick (Ashby, Ohlendorf et al. 2009; Backhouse, Collins et al. 2013), rhesus monkey (Smith, Hung et al. 2012) and tree shrew (Siegwart, Ward et al. 2012). Furthermore, Karouta and Ashby (Karouta and Ashby 2015) have shown recently that the suppression of FDM in chicks by high intensity light is dose-dependent as they demonstrated a significant correlation between the logarithm of illumination intensity and magnitude of FDM suppression. Although this current study is the first to demonstrate the finding of the suppressive effect of high intensity light on FDM in the guinea pig, a similar effect has been reported previously for lens-induced myopia (LIM) (Li, Lan et al. 2014). Of particular relevance is that Li *et al* (Li, Lan et al. 2014) also utilised a combination of fluorescent lamps and Solux™ (Tailored Lighting Inc., USA) halogen lamps to provide the high and low intensity illumination conditions which were similar conditions to those used in this study. Conversely, they induced myopia binocularly with -4 D lenses, rather than by form-deprivation. Therefore, this current study completes the circle regarding the examination of whether both FDM and LIM can be suppressed by high intensity illumination in the guinea pig model of myopia.

The guinea pig is a well-established mammalian model for the study of both experimentally induced and spontaneously developing myopia. The development of both form-deprivation and lens-induced myopia in the pigmented guinea pig was described by Howlett and McFadden initially in 2002 and then further expanded upon in subsequent publications (Howlett and McFadden 2002; McFadden, Howlett

et al. 2004; Howlett and McFadden 2006; Howlett and McFadden 2007; Howlett and McFadden 2009). Like the chick, the guinea pig is a precocial species (Wagner and Manning 1976), and the development of experimentally-induced myopia is primarily associated with an increase in vitreous chamber depth (VCD), although changes in the anterior segment including steepening of the cornea and increasing anterior chamber depth have also been reported (Howlett and McFadden 2006; Howlett and McFadden 2009). In particular, corneal power was found to be well-correlated with vitreous chamber depth in deprived eyes, which led Howlett and McFadden to postulate that the growth of the lens may be controlled by a visually-guided feedback mechanism. This would suggest that the anterior segment changes may be under central (cortical) control while posterior segment changes are generally accepted to be primarily guided by a local control mechanism (Schmid and Wildsoet 1996; Wallman and Winawer 2004; Howlett and McFadden 2006).

Evidence that corneal curvature and anterior chamber growth in general may be under central (cortical) control comes from experiments which suppressed or eliminated ganglion cell activity in chicks using tetrodotoxin (McBrien, Moghaddam et al. 1995), colchicine (Fischer, Morgan et al. 1999) and optic nerve section (Troilo and Wallman 1991) and resulted in changes in the anterior segment, particularly changes in corneal curvature. Furthermore, intact ciliary innervation is necessary for normal corneal development (Li and Howland 2000), and the induction of form-deprivation myopia in the chick (Schmid, Papastergiou et al. 1999). While ciliary ganglionectomy inhibits the development of FDM, it does not inhibit either lens-induced hyperopia (LIH) or myopia (LIM) in chicks (Schmid and Wildsoet 1996), suggesting that these two responses have different underlying mechanisms. While ciliary ganglionectomy can inhibit the development of FDM, sectioning input to the pterygopalatine ganglia does not inhibit FDM development. However when combined as a double parasympathectomy, anterior chamber growth is also inhibited (Nickla and Schroedl 2012), This provides further evidence that anterior segment changes in experimental myopia are under central (cortical) control, while posterior segment changes such as an increase in VCD to a myopiagenic stimulus are subject to greater local control.

While monocular form-deprivation resulted in a significant increase in the VCD of deprived eyes when compared to non-deprived eyes as expected for the strain of guinea pigs used in this study (Backhouse and Phillips 2010), no significant difference in either relative VCD, or overall relative AXL, was found between the two illumination conditions. Therefore, the difference in relative myopia found in this study was not clearly related to an equivalent difference in relative VCD or AXL and so the

relationships between the various biometric components of refraction were investigated further with General Linear Model (GLM) analysis (Section 3.4.4).

Spontaneous axial myopia has been reported to develop in selected strains of guinea pigs, both in pigmented (Jiang, Schaeffel et al. 2009), but more predominantly in albino guinea pigs where some 70% were reported to be myopic at 2 weeks of age (Jiang, Long et al. 2011). As the colony from which experimental animals were drawn for this study displayed a moderate incidence of congenital central cataract in new-born guinea pig pups, which could potentially affect both refractive state and visual function, one week-old animals were screened to ensure the presence of clear media before they were accepted into the experiment. The guinea pigs were also screened for the ability to have refractive error successfully measured using an autorefractor (Section 2.4.1), which appeared to be related to the qualitative clarity of the ocular media. A hyperopic refractive error (+2.25 ± 0.21 D) was found on average for all eyes at screening, which was similar to the average refraction reported previously for pigmented guinea pigs (+2.13 D) at 2 weeks of age (Jiang, Long et al. 2011). Therefore, the development of myopic refractive errors following form-deprivation (Table 3.1) in these pre-screened guinea pigs can be assumed to be an effect of the deprivation treatment, rather than due to spontaneous development of myopia.

This study found no statistically significant difference in retinal dopamine concentration (DAC) by illumination condition either in terms of relative values (difference between deprived and non-deprived eye concentrations) or for absolute values (Table 3.7), despite the statistically significant difference found in relative refraction. This finding therefore does not support the experimental hypothesis that the high intensity illumination condition should have not only suppressed myopia development, but also increased retinal dopamine concentration, when compared to the guinea pigs raised under 300 lux. However, the trends visible within the data (Figure 3.27 and 3.28) suggest that the high intensity lighting condition did lead to an increase in retinal DAC in at least the non-deprived eye, but that the statistical power of the sample size was insufficient to reach statistical significance.

As the significant difference in relative myopia found in this study by illumination condition was not clearly related to an equivalent difference in retinal DAC, the relationships between the various components of refraction and retinal DAC were investigated further with General Linear Model (GLM) analysis in Section 3.4.4.

3.4.2 Refractive and Biometric Outcomes

Refractive Outcome and Illumination Condition

As noted above, this study demonstrated that daily exposure to periodic high intensity white light (10,000 lux) suppressed development of form-deprivation myopia in guinea pigs when compared to those raised under standard (300 lux) room illumination. A statistically significant difference was found in the degree of relative myopia induced by form-deprivation under the 300 lux condition (-4.01 ± 0.61 D) when compared to 10,000 lux condition (-2.38 ± 0.41 D; $p = 0.038$), representing a 40% reduction in induced myopia. The use of a relative, or paired-eye measurement as the primary outcome measurement has the advantage of accounting for individual variation between animals in terms of eye size due to factors such as differential growth rates (Wallman and Adams 1987). As such it tends to increase statistical power of small sample sizes by decreasing the variability between individual outcome measures allowing for the use of smaller sample sizes (Anderson and Vingrys 2001). This effect is seen when considering the absolute refractive outcomes by eye for the two illumination conditions. While the deprived eyes developed a low degree of myopia under the standard illumination condition and a low degree of hyperopia under the high intensity illumination condition, the difference was not statistically significant. Statistical power analysis determined that a much larger sample size of 88 would have been required to find a significant difference in the absolute refractive outcomes of the deprived eyes alone. A second effect that may have contributed to the significant difference of relative refraction between the lighting conditions was the trend under high intensity lighting for a decrease in the degree of hyperopia developed in the contralateral non-deprived eye (Figure 3.7), however this difference between deprived eye outcomes by illumination condition was not statistically significant.

The suppression of FDM in guinea pigs by high intensity illumination in this study has many parallels to the recent publication of Li *et al* (Li, Lan et al. 2014) who reported partial suppression of LIM in guinea pigs by 10,000 lux illumination. While Li *et al* induced myopia binocularly with -4 D lenses, rather than by monocular form-deprivation, both studies utilised a combination of fluorescent and Solux™ halogen sources to provide ambient illumination during the induction phases of the experiments. In this study, 4100K triphosphor fluorescent lamps (Section 3.2.1) were used to provide the standard or background 300 lux illumination condition of 12 hours daylight per day, while 4700K Solux™ halogen lamps were used to provide the high intensity (10,000 lux) periodic illumination condition. The use of a combination of fluorescent lighting to provide the standard intensity lighting condition with an additional period of

high intensity halogen lighting superimposed for part of the daylight phase (e.g. 6 hours out of 12) is a common paradigm that has been used to demonstrate suppression of induced myopia by high ambient illumination in chicks (Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010; Backhouse, Collins et al. 2013; Lan, Feldkaemper et al. 2014) and rhesus monkeys (Smith, Hung et al. 2012). Conversely Li *et al* (Li, Lan et al. 2014) used fluorescent and Solux™ halogen lamps to provide both standard and high intensity lighting conditions as they were investigating whether the spectral composition of the sources had any effect on the degree of suppression. While high intensity (10,000 lux) ambient illumination was found to significantly reduce the degree of LIM when compared to guinea pigs raised under the low intensity (500 lux) condition by about 0.75 D (~35%), no significant differences were found in the refractive outcomes between the effect of fluorescent or halogen sources. Therefore, they concluded that the ambient lighting intensity, rather than spectral properties of the source (including the presence or absence of UV), was more influential on refractive outcome (Li, Lan et al. 2014). In contrast, in this study in a second set of experiments conducted on chicks (Chapter 4), the spectral energy distribution of LED sources did appear to have influenced the induction of FDM (Section 4.4.1). Nevertheless, the proportional effects of high intensity illumination on induction of experimental myopia in guinea pigs are similar for this study (FDM; reduction in myopia ~40%) and for the study of Li *et al* (LIM; reduction in myopia ~35%) (Li, Lan et al. 2014).

Refractive Outcome and Corneal Curvature

This study utilised a monocular-deprivation paired-eye design (Wallman and Adams 1987; Backhouse, Collins et al. 2013), where the primary outcome measures were based on intra-animal (or relative) differences in measurements, such as relative mean sphere refraction (Figure 3.6). However, a secondary analysis of the individual eye, or absolute, measures was also undertaken to assess differences in biometric variables between the deprived and non-deprived eyes. Although no significant effect of illumination condition on refraction was found for either the deprived or non-deprived eyes, there was a trend on average for a smaller hyperopic refraction to develop in the non-deprived eyes exposed to the high intensity lighting condition when compared to those from the standard lighting condition (Figure 3.7). This finding is in contrast to Li *et al* (LIM; reduction in myopia ~35%) (Li, Lan et al. 2014) where control animals raised under the high intensity lighting conditions exhibited an increasing hyperopic shift in refraction. The authors proposed that this hyperopic shift was due to the flattening of the cornea exceeding any refractive change due to axial elongation in the animals raised without lenses (Li, Lan et al. 2014). However, corneal flattening is a normal feature of ocular development in pigmented guinea pigs (Howlett and McFadden 2007) and no significant

difference in the degree of corneal flattening was noted between the different treatment groups (Li, Lan et al. 2014). Comparatively, in this study no significant difference in relative corneal radius was found by illumination condition (Table 3.2), and relative corneal radius was not significantly correlated with the relative refractive outcome (Figure 3.9). Therefore, while exposure to continuous light has been shown to lead to corneal flattening in chicks (Li, Troilo et al. 1995) (Cohen, Belkin et al. 2008), there is no clear evidence in this study that the periodic high intensity light condition has produced excessive corneal flattening in either non-deprived or deprived eyes. Conversely, in guinea pigs, an increase in corneal curvature following form-deprivation has been reported (Howlett and McFadden 2006). The increase in corneal curvature resulted in a concomitant increase in corneal power and contributed up to 68% of the magnitude of the form-deprivation induced relative myopia (Howlett and McFadden 2006). However, a statistically significant relationship between corneal curvature and refractive outcome was not established in this study.

Although the difference in relative myopia found in this study was not clearly related to an equivalent difference in relative corneal radius this relationship is investigated further using General Linear Model (GLM) analysis (Section 3.4.4).

Anterior Chamber Depth

Relative anterior chamber depth (ACD) was not found to differ significantly by lighting condition, however the trend was for relative ACD to be positive for the standard illumination condition, and negative for the high intensity condition. To understand the implications of this trend, the absolute ACD results need to be considered (Figure 3.13). The absolute ACDs of deprived eyes were statistically different by illumination condition, while the ACDs of non-deprived eyes were not. For example, the deprived eyes' average ACD under the standard light condition was deeper at +1.220 ± 0.022 mm when compared to the high intensity light condition at +1.128 ± 0.022 mm (p = 0.012). Although not statistically significant, the deprived eyes' ACDs were also deeper than those of the contralateral non-deprived eyes for the standard light condition, and shallower for the high intensity light condition.

In guinea pigs, ACD has been shown to vary significantly in FDM between deprived (deeper) and non-deprived (shallower) eyes (Howlett and McFadden 2006). Therefore, the results of this study can be interpreted as demonstrating that the high intensity lighting condition has indeed suppressed the development of deeper anterior chambers under form-deprivation conditions. The underlying mechanism by which form-deprivation and light can affect growth of the anterior segment is not well

understood, however it is clear from chick experiments that parasympathetic input from higher cortical centres to both the anterior and posterior segments can strongly influence the development of form-deprivation myopia (Schmid, Papastergiou et al. 1999; Nickla and Schroedl 2012).

The effect of the high intensity lighting condition in this study was to reduce the ACD of the deprived eyes, which suggests that this is one of the factors that leads to the lower degree of relative myopia found under this lighting condition. This relationship is investigated further using General Linear Model (GLM) analysis (Section 3.4.4).

Lens Thickness

No significant difference in relative lens thickness (LT) was found by lighting condition. However, an apparent trend was identified for relative LT to be of greater magnitude (more negative) for the standard illumination condition when compared to the relative LT found for the high intensity condition. Statistical power calculations found that the sample size per group would need to have been more than doubled (10 to 23) for this difference to become statistically significant at 80% power. However, when the absolute LT values are considered (Table 3.4) it is reasonable to propose, given the relatively small absolute differences in LT, that it is unlikely that there was any practical effect of lighting condition on the LT of either the deprived or non-deprived eyes. This result is consistent with the similar lack of significant difference in LT found for guinea pigs raised with LIM under either high or low intensity lighting conditions (Li, Lan et al. 2014). This relationship is also investigated further using General Linear Model (GLM) analysis (Section 3.4.4).

Furthermore, form-deprivation itself did not significantly influence lens thickness under either lighting condition. The report of the initial form-deprivation experiment on guinea pigs noted that a significant difference in LT between deprived and non-deprived eyes did not develop until day 16 post deprivation (Howlett and McFadden 2006). However, as the measurements in this study took place on day 14, it is possible that any difference between deprived and non-deprived eyes may have become significant with time, particularly in the cohort raised under standard illumination conditions where the relative difference was larger (-0.041 ± 0.021 mm) than in the high intensity condition (-0.003 ± 0.016 mm).

Vitreous Chamber Depth and Axial Length

Form-deprivation produced a significant increase in both the absolute vitreous chamber depth (VCD: +3.591 ± 0.027 mm vs +3.441 ± 0.042 mm; p <0.001) and axial length (AXL: +8.535 ± 0.043 mm vs +8.399 ± 0.040 mm; p <0.001) of the deprived eyes of guinea pigs raised under either illumination condition when compared to the non-deprived eyes as expected (Howlett and McFadden 2006). Backhouse and Phillips (Backhouse and Phillips 2010) also found a significant difference in VCD in the same breeding population of guinea pigs as used in this study after 14 days of monocular form-deprivation. However, Howlett and McFadden (Howlett and McFadden 2006) found that the greatest significant difference in VCD due to form-deprivation was measured at day 11, but that the difference reduced and became non-significant by day 16. Therefore, the authors proposed that there was a degree of yoking of growth between deprived and non-deprived eyes in the guinea pig. This was further supported by the comparison with the eyes of the age-matched normal visual experience control group, which displayed significantly shorter VCDs at day 16 (difference = 91 μm, p<0.001) (Howlett and McFadden 2006). While a degree of yoking may exist in the guinea pig at 16 days, both this study and that of Backhouse and Phillips (Backhouse and Phillips 2010) found a similar degree of difference in VCD (+0.149 ± 0.044 mm vs. 0.169 ± 0.049 mm respectively, p = 0.99) following 14 days of monocular form deprivation under similar levels of fluorescent room illumination (300 vs. 350 lux respectively).

However, in this study, no significant differences in relative VCD (Figure 3.17, Table 3.5) or relative AXL (Figure 3.22, Table 3.6) were found by illumination condition. In other words, while form-deprivation resulted in an increase of VCD in deprived eyes, the high intensity illumination condition had no significant effect (reduction or otherwise) on this increase in VCD relative to the standard intensity lighting condition. This finding is perhaps unexpected as a significant effect of lighting condition was found for relative refraction (Figure 3.6, Table 3.1). Previous studies have shown that refraction is highly correlated with VCD in guinea pigs during normal development (Howlett and McFadden 2007), as well as under both form-deprivation (Howlett and McFadden 2006; Backhouse and Phillips 2010) and lens-induced myopia (Howlett and McFadden 2009). However, after 16 days of form-deprivation, Howlett and McFadden found that VCD ceased being significantly different between the eyes, while anterior segment depth (equivalent to ACD) remained significantly different, and LT became significantly different (Howlett and McFadden 2006).

Despite the loss of a significant difference in VCD between the eyes, relative axial length was still significantly different between form-deprived and normal control animals. Therefore, it appears that the anterior segment components also contributed in a considerable manner to the overall refractive state of the eyes, which remained relatively myopic (Howlett and McFadden 2006). This relationship also appears to exist in this study where the significant difference in relative refraction was not matched by an equivalent significant difference in relative VCD. However, while an apparent difference in relative AXL was found in this study, which would be consistent with the difference in relative refraction, it was not statistically significant. Therefore, it can only be proposed that the effect of illumination condition on relative refraction was mediated by changes in the anterior segment components (corneal radius, ACD, and LT), as well as any (non-significant) change in VCD, resulting in an apparent difference in AXL by illumination condition. Interestingly, considering the interactions between refractive error and VCD for deprived (Figure 3.20) and non-deprived (Figure 3.21) eyes separately, linear regression analysis shows that the inverse relationship between these factors is stronger (R^2 = 0.136) for non-deprived eyes than for deprived eyes (R^2 = 0.009). Therefore, there may be a differential effect of the illumination condition between the eyes. These potentially complex relationships are investigated further using General Linear Model (GLM) analysis in Section 3.4.4.

3.4.3 Retinal Dopamine and Lighting Condition

Relative retinal dopamine concentration (DAC) was not found to differ significantly by lighting condition (Table 3.7), however an apparent trend was revealed for relative DAC to be of greater magnitude (more negative) under the high intensity condition than under the standard illumination condition (Figure 3.27). This trend was also visible in the regression analysis of relative DAC by relative refraction (Figure 3.28) where a weak negative correlation (R^2 = 0.055) was found between these variables. As the relative DAC was calculated as (deprived eye DAC - non-deprived eye DAC), then this apparently increased (negative) magnitude must have been due to either a decrease in retinal DAC in the deprived eye, or an increase in retinal DAC in the non-deprived eye.

The absolute retina DAC results (Figure 3.29) confirm that the relative difference is due to an apparently (though not statistically) greater absolute retinal DAC in the non-deprived eye under the high intensity lighting condition. While this difference is not statistically significant, it follows the expected trend for high intensity illumination to produce an increase in retinal dopamine synthesis as previously demonstrated in the chick (Cohen, Peleg et al. 2012). Furthermore, although the measured

values for retinal DAC were on average lower in the deprived eyes than in the non-deprived eyes as expected (Stone, Lin et al. 1989; Mao, Liu et al. 2010; Stone, Cohen et al. 2016), these differences were also not statistically significant. A secondary effect which could result in a reduced retinal DAC in the deprived eyes may arise from the differential illumination received by the deprived and non-deprived eyes due to the transmission characteristics of the monocular FD diffusers (Section 2.1.2). As the diffusers had a transmission of approximately 78%, then the deprived eyes would have been exposed to a lower level of illumination (~7800 lux) compared to the non-deprived eyes (10,000 lux). This difference in illumination may in itself be sufficient to explain the apparent trend in retinal DAC differences between the deprived and non-deprived eyes found in this experiment. In chicks, the suppression of FDM by light declines rapidly below 10,000 lux of ambient illumination (Karouta and Ashby 2015), which suggests that the underlying mechanism of suppression (hypothesised in this experiment to be related to retinal DAC) also declines in parallel. Therefore, the apparent difference in retinal DAC between the deprived eyes and non-deprived eyes may be due to a simple difference in illumination level reaching the eyes.

Although higher illumination levels (15,000 to 20,000 lux) may have produced a greater effect which could have reached statistical significance with the current sample size, statistical power calculations indicate that a sample size of 30 per group (3 x current size) would have been sufficient for the absolute retinal DAC values to reach significance for the current experimental paradigm, which is greater than the numbers required to demonstrate significant differences in retinal DAC in guinea pigs (Mao, Liu et al. 2010), but smaller than the sample sizes used to demonstrate the effect of form-deprivation on vitreous DOPAC in the chick (Stone, Lin et al. 1989).

Conversely an alternative argument can be made that the failure to find statistically significant differences in relative DAC and relative VCD are consistent, and suggest that the illumination condition did not have any measurable effect on the posterior segment. Therefore, the significant difference in relative refraction between lighting conditions would be dependent on anterior segment effects, such as the significantly shallower average ACD found for deprived eyes under the high intensity lighting condition. However, such a conclusion would be less consistent with the literature regarding the effects of ambient illumination intensity on retinal dopamine levels, and so it is likely that the dopamine analysis was unable to measure the effect of light either due to the periodic nature of the high intensity illumination, an insufficient sample size, or due to retinal DAC being a relatively weak measure of dopaminergic activity. For example, studies have suggested that measurement of vitreal DOPAC is a more appropriate record of retinal dopamine synthesis as the vitreous acts as an inert reservoir of the

dopamine metabolite, providing an effectively historical record of activity (Megaw, Morgan et al. 1997; Ohngemach, Hagel et al. 1997; Feldkaemper and Schaeffel 2013).

Nevertheless, dopamine, a key retinal neurotransmitter, is now widely held to have a primary role in the signalling pathway which regulates eye growth in a number of species (Feldkaemper and Schaeffel 2013). For example, retinal dopamine levels have been shown to be reduced when myopia is induced experimentally in the chick (FDM) (Stone, Lin et al. 1989; Stone, Cohen et al. 2016), guinea pig (FDM and LIM) (Mao, Liu et al. 2010; Dong, Zhi et al. 2011), tree shrew (FDM) (McBrien, Cottriall et al. 2001) and mouse (FDM) (Park, Tan et al. 2013). Conversely the administration of either dopamine precursors or promotors in guinea pigs (Mao, Liu et al. 2010; Mao, Liu et al. 2016), or dopamine agonists in chicks (Stone, Lin et al. 1989; Ashby, McCarthy et al. 2007), guinea pigs (Dong, Zhi et al. 2011), rhesus monkeys (Iuvone, Tigges et al. 1991) and rabbits (Lin, Chen et al. 2008) can suppress the development of experimental myopia. Furthermore, dopaminergic agonists have also been shown to suppress the progression of naturally-occurring myopia in albino guinea pigs (Jiang, Long et al. 2014).

The demonstration that high intensity light could suppress experimentally-induced myopia in a range of animal models, and that retinal dopamine synthesis (measured as vitreal DOPAC concentrations) was related to log ambient illuminance (up to 10,000 lux) in chicks (Cohen, Mackey et al. 2012), resulted in the hypothesis that the inhibitory effect of high intensity light on myopia is mediated by retinal dopamine, released by dopaminergic amacrine cells (Witkovsky 2004), and under control of intrinsically photosensitive retinal ganglion cells (ipRGCs) (Norton and Siegwart 2013).

The interest in the role of dopamine and light exposure in the control of refractive development initially originated from human epidemiological studies where an increased duration of outdoor activity was found to reduce the risk of a child developing myopia (Jones, Sinnott et al. 2007; Rose, Morgan et al. 2008; Rose, Morgan et al. 2008; Dirani, Tong et al. 2009). In particular, Rose *et al* (Rose, Morgan et al. 2008b), in a comparison of prevalence and risk factors for myopia in 6-7 year old children of Chinese ethnicity resident in Sydney and Singapore, found that the 14 hours a week that Sydney children spent on outdoor activity significantly reduced their risk of developing myopia in comparison with the 3 hours reported by the Singaporean children. The protective effect of outdoor activity against myopia development was also effective even when high levels of near work were recorded (Rose, Morgan et al. 2008; Dirani, Tong et al. 2009).

The periodic high intensity lighting paradigm used in this study (Section 3.2.2) was intended to simulate the intermittent exposure patterns experienced by school children when tracked with light-sensitive data-loggers (Backhouse 2011), as animal experiments had demonstrated that the suppression of FDM and rate of retinal dopamine synthesis was dependent on temporal aspects (flicker) of the lighting condition (Rohrer, Iuvone et al. 1995; Schwahn and Schaeffel 1997; Kee, Marzani et al. 2001). However, it is also possible that the periodic nature of the high intensity illumination (15 min 10,000 lux:15 minutes 300 lux repeating for 12 hours) may have reduced or masked the difference in retinal DAC between the standard and high intensity lighting conditions. For example, the light adaptation state of the retina has been shown to be directly related to retinal catecholamine content of the retina and choroid in the guinea pig, and similar mammalian models of the rat and rabbit (Nichols, Jacobowitz et al. 1967), therefore the periodic nature of the high intensity illumination may have allowed retinal dopamine levels to fluctuate around an average value, rather than increase with time as might be expected with a continuous illumination paradigm.

Flickering, or stroboscopic illumination has been shown to influence eye growth in visually-competent guinea pigs (Di, Liu et al. 2013; Di, Lu et al. 2013; Zhi, Pan et al. 2013; Di, Lu et al. 2014). Exposure to 6 Hz flicker reduced the degree of myopia induced when guinea pigs were raised in a grey (low spatial frequency) background environment under 50 lux of illumination (Zhi, Pan et al. 2013). Conversely raising guinea pigs under lighting conditions with flicker rates of either 0.5 Hz or 5 Hz at an average illuminance of ~300 lux, resulted in the development of up to 5.5D of axial myopia with concomitant changes in flash ERGs and morphological changes to the photoreceptor outer disc membranes (Di, Liu et al. 2013; Di, Lu et al. 2013; Di, Lu et al. 2014). The authors (Di, Lu et al. 2014) postulated that the flickering retinal image triggered the blur detection circuitry of the retina which in turn resulted in excessive axial growth causing myopia, while the photoreceptor outer segment changes represented excessive retinal activity triggered by the flicker.

While flicker has been shown to both suppress and induce myopia in visually-competent guinea pigs, flicker has been shown to suppress FDM in chicks (Gottlieb and Wallman 1987; Rohrer, Iuvone et al. 1995; Schwahn and Schaeffel 1997). Rohrer *et al* (Rohrer, Iuvone et al. 1995) in particular demonstrated that 10 Hz stroboscopic illumination could overcome the suppression of retinal dopamine synthesis by form-deprivation in chicks. This effect was shown to be most likely related to upregulation of tyrosine hydroxylase by the gene regulator *c-fos* in dopaminergic amacrine cells, resulting in greater dopamine synthesis. However, Schwahn and Schaeffel (Schwahn and Schaeffel 1997) were unable to

clearly establish a similar relationship between suppression of FDM in chicks by 12 Hz flicker (with a short duty 4% cycle) and an increase in retinal dopamine synthesis or vitreal DOPAC levels.

Therefore, the potential effect of the periodic high intensity lighting paradigm as used in this study is complex as the stimulus contains both a high intensity illumination component (10,000 lux) as well as a low frequency "flicker" component. However the fundamental frequency of the periodic 15 minute high:standard light cycle in this study is several orders of magnitude lower at 0.00056 Hz (1 cycle per 1800 s) than used in the studies described above. More recently, Lan *et al* (Lan, Feldkaemper et al. 2014) investigated the effect of a similar lighting protocol of low frequency periodic, high intensity light on FDM in the chick. The study utilised a 50% duty cycle paradigm alternating between 500 lux and 15,000 lux in 60, 30, 15, 7 and 1 minute illumination periods over a 10 hour daylight period. Therefore, the 15:15 minute high:standard light cycle of Lan *et al* was similar in design to the high intensity light paradigm used in this study, but 1 hour shorter in total high illumination exposure time at 5 rather than 6 hours per day. Their study also found that 5 hours of continuous exposure to high intensity illumination (15,000 lux) required to significantly suppress FDM, while 10 hours exposure produced no significant additional suppression effect. When the 5 hours of high intensity illumination was provided in periodic cycles of 1:1, 7:7, 15:15, 30:30 or 60:60 minute high:standard light cycles, it was found that both the 1:1 and 7:7 minute cycles produced additional suppression of FDM over the continuous presentation (Lan, Feldkaemper et al. 2014). Of particular relevance to this study however, was the finding that the 15:15 minute light cycle did not produce any additional benefit beyond that exhibited by the continuous 5 hour lighting condition on suppression of FDM. Therefore, based on the response found in the chick there is no strong evidence that the 15:15 minute periodic lighting cycle used in this study would have influenced either FDM development or retinal DAC in the guinea pig, beyond the effect expected for a continuous high intensity lighting paradigm of the same total duration (6 hours) per daylight phase.

However, one possible explanation for why a low frequency periodic high intensity lighting paradigm might influence refractive development is that the square wave lighting cycle utilised in this study and by Lan *et al* (Lan, Feldkaemper et al. 2014) has higher temporal frequency components associated with the switching boundaries when compared with a sinusoidal illumination cycle (Cornsweet 1970). These higher temporal frequency components may be sufficient to stimulate a putative growth suppression mechanism, resulting in a reduction of axial growth and increase in retinal dopamine synthesis. Lan *et al* (Lan, Feldkaemper et al. 2014) hypothesised that this may be due to stimulation of the retinal ON-pathway by flickering light (or in this case the high frequency components of the low

frequency square wave) resulting in more dopamine production than that stimulated by continuous high intensity illumination.

Therefore, in this experiment, three competing factors may have been in play; suppression of retinal dopamine production by FDM, a complex relationship between retinal dopamine synthesis and the periodic nature of the high intensity illumination, and flicker potentially either inducing myopia in the non-deprived eyes, or suppressing myopia in the deprived eyes. Interestingly, although not statistically significant, the refractive error of the non-deprived eyes was on average less hyperopic under the high intensity lighting condition, while more hyperopic under the standard intensity lighting condition (Figure 3.6). Moreover, the deprived eyes exhibited a low degree of hyperopia on average under the high intensity lighting condition, while under standard illumination the deprived eyes were slightly myopic. Although these changes are only apparent trends within the data they do fit the hypothesis that there may have been multiple effects at play here, with high intensity illumination suppressing FDM in the deprived eyes, while the "flicker" associated with the square wave switching in the periodic high intensity lighting trials, inducing a small myopic shift (less hyperopia) in the non-deprived eye. The relationship between low frequency periodic illumination and intensity of illumination is potentially complex and requires further investigation, including replicating the experiment to increase sample size to determine if these trends are both statistically and practically significant.

3.4.4 MGLM Analysis

The use of a Multivariate General Linear Model (MGLM) procedure allows for the regression analysis and analysis of variance of more than one dependent (or outcome) variable by one or more factor variables and independent variables (or covariates) (IBM Knowledge Center 2016). For the MGLM analysis of the experimental results, the biometric components were set as covariates, illumination condition as a factor variable, and the retinal dopamine concentration (DAC) and refractive status as dependent variables. The rationale for this approach was that based on the existing literature, both the refractive status and retinal dopamine concentration were likely to be influenced by the lighting condition, but that the relationship between these variables was unknown, or at least inconsistent depending on the study.

As in the chick, the refractive status of the guinea pig is best predicted by its overall axial length, where axial length is the sum of both the anterior segment (including corneal thickness, anterior chamber

depth [ACD] and lens thickness [LT]) and the posterior segment (primarily vitreous chamber depth [VCD] and retinal thickness) depth (Howlett and McFadden 2007). Note that in this study, corneal thickness was not measured separately and is included in the reported ACD value. In guinea pigs, emmetropisation was found to occur within the first 35 days post-birth, during which the growth of both the anterior segment and posterior segment was approximately linear (Howlett and McFadden 2007). Therefore, the duration of this study on the induction of myopia by form-deprivation fell within the reported emmetropisation period of the guinea pig, and so the biometric changes within the eye that led to the production of relatively myopic refractions can be assumed to represent a failure of the emmetropisation mechanism (Wallman and Winawer 2004).

The pattern of response in the form-deprived guinea pig eye (Howlett and McFadden 2006) is perhaps somewhat more complex than in the chick (Wallman and Adams 1987). Initially, the response to form-deprivation was shown to be primarily an increase in relative VCD for the first 11 days, after which the VCD of the contralateral non-deprived eye also increased in length, reducing the relative VCD by some 25% after a further 5 days (Howlett and McFadden 2006). The anterior segment also increased in depth significantly in response to form-deprivation by 6 days, however this difference was maintained at 16 days post-deprivation. Conversely the lens thickness difference between eyes only became significant after 16 days of deprivation, which is beyond the duration of form-deprivation in this study. Howlett and McFadden concluded that a degree of yoking existed in the axial growth of the vitreous chamber between the deprived and non-deprived eyes which only became manifest after 11 days of form-deprivation. Conversely, the anterior segment did not display a yoking effect, and so they proposed that the anterior and posterior segment growth may be under the control of separate emmetropisation mechanisms (Howlett and McFadden 2006). Further evidence for the existence of yoking between the posterior segments of deprived and non-deprived eyes in guinea pigs comes from the study on ocular biomechanical compliance by Backhouse and Phillips (Backhouse and Phillips 2010). They reported that both eyes of a monocularly form-deprived animal showed a statistically significant increase in VCD in response to an experimentally-manipulated acute rise in IOP, whereas control eyes from visually-normal animals did not show this expansion. The authors concluded that the difference in response between the non-deprived eyes and the control eyes supported the concept of an interocular yoking signal in guinea pigs (Backhouse and Phillips 2010).

The aim of the MGLM analysis of the refractive and retinal dopamine concentration outcomes by lighting condition, and with the biometric components, was to determine whether the lighting conditions had a significant effect of the development of FDM, whether any difference in FDM was related to a

difference in retinal DAC, and what weighting, or effect size, could be attributed to each biometric component. MGLM analysis in this case is effectively the weighted linear regression of multiple variables and factors against the two primary outcome measures, refractive status and retinal dopamine concentration.

MGLM – Paired-Eye Analysis

The MGLM analysis of the paired eye data for relative mean sphere refraction and relative DAC is summarised in Table 3.8. No statistically significant relationship was revealed between either the biometric covariates, or the illumination factor and the dependent variables. This suggests that the relative refraction and retinal DAC outcomes are not strongly related. While the illumination factor was not statistically significant (p = 0.097), in GLM analysis it is also important to consider the effect size, represented by partial η^2, which can be interpreted as an estimate of the practical significance of the variable (Richardson 2011). Illumination factor had by far the greatest associated effect size of η^2 = 0.302 and observed power (0.456) of any of the variables contributing to the model, therefore the lack of statistical significance may be due more to an insufficient sample size, rather than a lack of an underlying relationship. This would be consistent with previous findings in guinea pigs that dopaminergic agonists or promoters can suppress FDM (Mao, Liu et al. 2010; Dong, Zhi et al. 2011; Mao, Liu et al. 2016) and that high intensity illumination can suppress LIM (Li, Lan et al. 2014).

The MGLM analysis procedure in SPSS (version 22; IBM, USA) also calculates the univariate analysis output for each dependent variable separately. Univariate analysis of relative refraction (Table 3.9) confirmed that the illumination factor had a statistically significant effect (p = 0.031, partial η^2 = 0.292) on relative refraction. Therefore, again confirming that the periodic high intensity illumination paradigm used in this study did suppress the induction of form-deprivation myopia in the guinea pig. As noted earlier, this study in association with the demonstration of at least partial suppression of LIM (Li, Lan et al. 2014), completes the examination of whether high intensity illumination can suppress both FDM and LIM in the guinea pig model of myopia. However, no statistically significant relationships were revealed between the biometric covariates, including VCD, and relative refraction, although relative corneal radius did display a medium-large effect strength (partial η^2 = 0.099). While not statistically significant, this result does display the pattern of response associated with “optical myopia” as defined by Howlett and McFadden (Howlett and McFadden 2006) where up to 4-6 D of FDM may be due to primarily an anterior segment response, particularly differences in corneal power.

Univariate analysis of relative DAC (Table 3.10) revealed no statistically significant relationships between either the biometric covariates, or the illumination factor, and the dependent variable. Again, while the illumination factor was not statistically significant (p = 0.361), it had a medium effect size of η^2 = 0.060. As discussed in Section 3.4.3, retinal DAC was reduced on average in the deprived eyes relative to the non-deprived eyes as expected (Stone, Lin et al. 1989; Mao, Liu et al. 2010) and therefore it is reasonable to propose that the lack of statistical significance is due to insufficient sample sizes rather than an actual lack of effect.

MGLM – Individual Eye Analysis: Deprived Eyes

The MGLM analysis of the deprived eye data for absolute mean sphere refraction and absolute DAC is summarised in Table 3.11. No statistically significant relationships were revealed between either the biometric covariates, or the illumination factor, and the dependent variables. This suggests that the absolute refraction and absolute retinal DAC are not strongly related in the deprived eye. While the corneal radius covariate was not statistically significant (p = 0.094), it was calculated to have the relatively large effect size of η^2 = 0.305 and observed power of 0.463. As noted above for the relative or paired eye analysis, this result does display the pattern of response associated with "optical myopia" (Howlett and McFadden 2006) where FDM may be due to primarily an anterior segment response, particularly changes in corneal power.

Univariate analysis of absolute refraction for the deprived eyes (Table 3.12) revealed no statistically significant relationship between either the biometric covariates, or the illumination factor, and the dependent variable. Again, while the illumination factor was not statistically significant (p = 0.539), it had a small-medium effect size of η^2 = 0.028, suggesting there may be some underlying relationship between the refractive state and illumination condition, which is masked by the insufficient sample size.

Univariate analysis of absolute DAC for the deprived eyes (Table 3.13) found that the corneal radius covariate had a statistically significant effect (p = 0.037, partial η^2 = 0.276) on the model. This is a somewhat difficult relationship to explain intuitively as corneal radius is an anterior eye property while DAC was measured from retinal samples. However, it most likely indicates that form-deprivation has a correlated, but not causative, relationship between these two factors. That is, form-deprivation both influences corneal radius as demonstrated by Howlett and McFadden (Howlett and McFadden 2006) and simultaneously affects retinal DAC, as found in the chick model (Stone, Lin et al. 1989).

MGLM – Individual Eye Analysis: Non-deprived Eyes

The MGLM analysis of the non-deprived eye data for absolute mean sphere refraction and absolute DAC is summarised in Table 3.14. No statistically significant relationships were revealed between either the biometric covariates, or the illumination factor, and the dependent variables. This suggests that the absolute refraction and absolute retinal DAC are also not strongly related in the non-deprived eyes. The two covariates which had the largest effect sizes were corneal radius (partial η^2 = 0.171) and VCD (partial η^2 = 0.145). Therefore, despite lack of statistical significance, in the non-deprived eyes there is an indication that both an anterior eye factor (corneal radius) and posterior eye factor (VCD) contribute to the overall refractive outcome. The relationship between VCD and DAC is also easier to explain in terms of the retinal dopaminergic system being associated with control of vitreous chamber growth both in guinea pigs and chicks (Feldkaemper and Schaeffel 2013).

Univariate analysis of absolute refraction for the non-deprived eye (Table 3.16) revealed no statistically significant relationship between either the biometric covariates, or the illumination factor, and the dependent variable. However, the two covariates which displayed the largest effect sizes were VCD (partial η^2 = 0.078) and illumination factor (partial η^2 = 0.064). These are medium effect sizes and so are consistent with the hypothesis that the statistically significant difference in relative refraction by illumination condition (Figure 3.6, Table 3.1) is due to the effect of the high intensity illumination on the non-deprived eye rather than the deprived eye in this study (Section 3.4.2).

Univariate analysis of absolute DAC for the non-deprived eyes (Table 3.16) revealed no statistically significant relationship between either the biometric covariates, or the illumination factor, and the dependent variable. However, large effect sizes were demonstrated by the two covariates of corneal radius (partial η^2 = 0.140) and ACD (partial η^2 = 0.157). This finding parallels the large effect size found for corneal radius in the deprived eyes. As both corneal radius and ACD are anterior segment components, it is again likely that this represents correlation, rather than causation, where form-deprivation both influences anterior segment growth (Howlett and McFadden 2006) and simultaneously affects retinal DAC, as in the chick (Stone, Lin et al. 1989).

In summary, MGLM analysis did not reveal a statistically significant relationship between illumination condition and the dependent variables of relative refraction and retinal DAC when considered together. Therefore, this study has not produced evidence that the statistically significant difference in relative refraction by illumination condition, as demonstrated by both t-test (Section 3.4.1) and univariate analysis above, is related to changes in retinal DAC. However, when considering effect sizes there is a

suggestion that the lighting condition affects the anterior segment (corneal radius and ACD) differentially from the posterior segment (VCD) depending on whether the eye is form-deprived or not.

3.4.5 Limitations

Sample Size and Statistical Power

The sample size of 10 guinea pigs per illumination condition was sufficient to reveal a statistically significant difference in relative mean sphere refraction, however no significant difference was found in the other primary outcome measurement, relative retinal dopamine concentration (DAC), despite a numerical difference in DAC of 1.21 ng/ml being found between the illumination conditions (in comparison to the overall average value of 5.48.ng/ml). *Post-hoc* calculations of power and sample size required to reach statistical significance for the relative DAC outcome were carried out using G*Power (University of Dusseldorf)(Faul, Erdfelder et al. 2007). These calculations revealed that the statistical power of the t-test analysis was low at 23%, while a total sample size of 88 animals per group would have been required to reach statistical significance at $p < 0.05$. This large sample size requirement most likely reflects the large variability of DAC values obtained from the HPLC analysis, which according to the manager of the Mass Spectrometry Centre, Auckland Science Analytical Services (ASAS) who carried out the extraction and analysis, was technically challenging (M. Middleditch, *personal communication*).

While a final sample size of 10 animals per lighting condition was achieved, this was lower than the design sample size of 12 per group, due to difficulty in obtaining cataract-free guinea pigs from the University's breeding colony. The results reported in this chapter are based on data collected from guinea pigs supplied once new externally-sourced breeding sires were added to the colony and sufficient numbers of non-cataractous animals could be identified on screening both by visual inspection of the optical media by ophthalmoscopy, and by the ability to measure the refraction of the animals successfully by autorefraction (Section 2.1.1).

During the design phase of this experiment, a sample size of 12 per group was estimated as statistically appropriate based on both previous studies on form deprivation in guinea pigs (Howlett and McFadden 2006; Backhouse and Phillips 2010) and on the conclusions of Anderson and Vingrys (Anderson and Vingrys 2001) regarding small sample sizes. Anderson and Vingrys propose that in an interventional study where the outcome of the intervention should be stereotypical and consistent (i.e. the induction of FDM), small sample sizes such as 12 per group as calculated for this study are

appropriate (Anderson and Vingrys 2001). However, while the final sample size of 10 per group was sufficient to obtain a statistically significant result for relative refraction, the *post hoc* analysis suggests that doubling the proposed sample size to 24 per group would have potentially resulted in differences in both relative ACD and LT being found to be statistically significant by lighting condition.

Periodic High Intensity Lighting Paradigm and Retinal Dopamine Concentration

While a numerical difference in relative retinal DAC was found by lighting condition this difference was not statistically significant, which may be related to the degree of variability in results obtained from the HPLC analysis. This variability could have been due to the technical challenge associated with the extraction process for HPLC as noted above, or due to variation in the actual retinal dopamine concentrations due to the use of a periodic high intensity illumination lighting paradigm. While samples were collected at approximately the same time of day (Section 3.2.3) to avoid the diurnal/circadian variation in retinal dopamine levels found in both mammalian and avian retinas (Feldkaemper and Schaeffel 2013), the animals were maintained under the high intensity lighting cycle until they were required for outcome measures. Therefore, the guinea pigs may have been exposed to up to 15 minutes of standard intensity illumination prior to the commencement of outcome measurements, and then at least a further hour of room illumination before euthanasia and collection of retinal samples. Therefore, the question arises as to whether this time delay between exposure to the last period of high intensity illumination and freezing of samples in liquid nitrogen was sufficient to result in a decrease in retinal dopamine levels. Furthermore, the lighting paradigm itself alternated between the high intensity 10,000 lux condition and standard intensity 300 lux condition every 15 minutes during the daylight phase, which in itself may have resulted in an averaging of retinal dopamine concentration over time.

The time course of the acute effect of light on retinal DAC and vitreal DOPAC levels was investigated in chicks by Megaw *et al* (Megaw, Morgan et al. 1997). Following lights-off at the beginning of the night phase, retinal DAC decreased by approximately 25% within the first 3 hours of darkness, and remained low for the rest of the night phase. Following lights-on, retinal DAC increased significantly by about 40% relative to night phase levels. Vitreal DOPAC levels followed a similar pattern, falling by some 55% of the initial level after 3 hours of darkness. Vitreal DOPAC levels also continued to fall over the entire night phase, unlike retinal DAC. Following lights-on, vitreal DOPAC rose by 400% (from 3 pmol to 12 pmol) within 3 hours, and kept rising for the entire daylight period. Therefore, in chicks, both the levels of retinal DAC and vitreal DOPAC changed rapidly within 3 hours following either the

start of the dark phase or the light phase of a diurnal cycle. Assuming a similar pattern exists in guinea pigs, some assumptions can be made regarding the effect of both the 15-minute periodic change in light intensity of the high intensity lighting paradigm, and the delay between removing the animals from the experimental lighting pen to the final collection of the retinal sample.

The 15 minute periods of standard intensity illumination during the periodic high intensity lighting condition are unlikely to have caused a large decrease in retinal DAC level overall. This is based on the assumption from the chick experiment described above where retinal DAC took 3 hours to decrease 25%, that 15 minutes of standard intensity illumination would produce at least a 2% drop in retinal DAC on a *pro-rata* basis. If the effect was cumulative however, the worst-case scenario would be that the periodic illumination would result in a steady-state retinal DAC which averaged out at about half the peak value that might have resulted from the high intensity illumination alone. Furthermore, assuming that the time period from removal of the guinea pig from the experimental pen, to the collection of the retinal sample was 90 minutes on average, then retinal DAC may have fallen by around 13% from the peak value based on the chick time-course (Megaw, Morgan et al. 1997). Therefore, the combination of the periodic high intensity lighting paradigm, and duration of data collection, is unlikely to have resulted in retinal DAC levels declining fully from the level influenced by the high intensity illumination, to the levels found in the guinea pigs raised under the standard illumination condition. This is supported by numerical difference in relative retinal DAC levels between the two lighting conditions found in this study (Figure 3.26, Table 3.7), however it may be sufficient to explain the relatively low power of the statistical analysis (23%) which resulted in *post-hoc* calculations requiring the much larger sample size of 88 per group to achieve statistically significant difference. In any future experiments efforts should be made to ensure that the animals are removed from the experimental pen at the end of a full 15 minute high intensity illumination period, and that the time between the start of outcome measures to tissue collection is minimised as far as possible.

A further factor that may have affected the ability of this experiment to detect significant differences in retinal DAC was the use of ketamine hydrochloride (44 mg/kg) in the anaesthetic protocol. A relatively low dose of ketamine hydrochloride (30 mg/kg) has been shown to increase DA turnover in the forebrain region of mice with 10 minutes of injection (Irifune, Shimizu et al. 1991) and an increase in CNS neuronal activity as measured by c-fos expression in rats (Imre, Fokkema et al. 2006). While an increase in DA turnover in the forebrain region of mice, as measured by a statistically significant increase in DOPAC, was found at 20 minutes post-injection, DAC itself was not changed (Irifune, Shimizu et al. 1991). Nevertheless, in future experiments an alternative form of anaesthesia such as

the use of isoflurane should be considered (Ashby, Zeng et al. 2014) to avoid any possible ketamine-induced changes in retinal DAC or DOPAC levels.

Finally, it would be better to measure vitreal DOPAC, rather than retinal DAC levels in this type of experiment. DOPAC is a metabolite of dopamine, and its measurement in the vitreous has been shown to be less variable than retinal DAC, but potentially more responsive to lighting conditions (Megaw, Morgan et al. 1997). Furthermore, vitreal DOPAC or the DOPAC/dopamine ratio is a better indicators of retinal dopamine release as the vitreous acts as a simple reservoir for retinal dopamine, with no further synthesis or degradation taking place in the vitreous (Ohngemach, Hagel et al. 1997; Feldkaemper and Schaeffel 2013).

3.4.6 Conclusions

Periodic high intensity illumination is able to suppress the development of form-deprivation myopia in pigmented guinea pigs. This complements the previous demonstration of the suppression of lens-induced myopia by Li *et al* (Li, Lan et al. 2014). While no significant difference in relative retinal dopamine concentration by lighting condition was demonstrated in this study, a numerical difference was found which suggests the high intensity illumination paradigm did raise retinal DAC, particularly in the non-deprived eyes, however the sample size was insufficient due to the degree of variability in the measurements.

The myopia induced by form deprivation was associated with a significant increase in vitreous chamber depth in the deprived eyes, however no difference in depth was found between the illumination conditions. Therefore, the significant difference in relative refraction by illumination condition must have been the result of the sum of effects of illumination on the other biometric variables that contribute to refraction. In particular, the absolute anterior chamber depth of the deprived eyes varied significantly by illumination condition, with the eyes exposed to the high intensity lighting condition exhibiting smaller ACD. Multivariate General Linear Model analysis was undertaken with the aim of determining the relative contributions of each of the biometric components to the overall refractive outcomes. However, while some moderate to large effect sizes were identified, only the illumination condition was revealed again as having a statistically significant effect on relative refractive outcome. A consideration of *post-hoc* statistical power calculations suggests that the sample size of 24 per illumination condition group would have been more suitable for this experiment, which may have resulted in a number of these effects becoming statistically significant.

4 The Chick: Periodic Quasi-monochromatic High Intensity Illumination and Form Deprivation Myopia

4.1 Introduction

In this chapter, the Cobb strain of domestic chick (*Gallus gallus domesticus*) is utilised in a series of experiments to investigate whether periodic, high intensity, white and quasi-monochromatic (coloured) light can suppress FDM development in an avian model of myopia (Wallman, Turkel et al. 1978; Wallman and Adams 1987). The chick is a widely used model of experimentally-induced myopia (Schaeffel and Feldkaemper 2015) with a major role in the investigation of many aspects of myopia from the demonstration of the local control of ocular growth (Troilo, Gottlieb et al. 1987), to the inhibition of both FDM and LIM by high ambient illumination (Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010).

In order to investigate the potential mechanisms by which white and quasi-monochromatic ambient lighting might influence the development of FDM in the chick, measurement and analysis of the spectral irradiance distributions of the both the LED-based light sources used in this experiment, and the fluorescent-based white light source used previously (Backhouse, Collins et al. 2013), was undertaken. This analysis leads to an extensive discussion in this chapter on the spectral energy distribution of fluorescent versus LED sources of white light, and how lighting stimuli should be measured and defined in animal experiments.

4.2 Methods: Chick Experiment Specific

4.2.1 Lighting System – RGBW LED

The chick experiments utilised an LED-based lighting system consisting of an array of QTX SL-Q8 stage lights (AVSL Group Ltd, UK, http://www.avsl-qtx.com) mounted above the pen to provide both the high intensity 10,000 lux white (and equivalent irradiance quasi-monochromatic) lighting and standard 300 lux lighting.

The QTX SL-Q8 stage lights consisted of eight 12 W quad-colour RGBW LED lamps, with a 35^{o} beam angle, and a total power consumption of 100 W (Figure 4.1). These units can be controlled by an industry-standard DMX (Digital MultipleX) serial interface system, with 6 separate 8-bit DMX channels, providing 256 (0 – 255) control levels for each channel. Four channels were dedicated to independently controlling the dimming of the RGBW LEDs, with a fifth channel providing a master dimming control. Each unit could be assigned a starting DMX address (e.g. A001, A007, A013…) and so multiple units (fixtures) could be managed by one DMX controller by "daisy-chaining" successive units together with DMX cables.

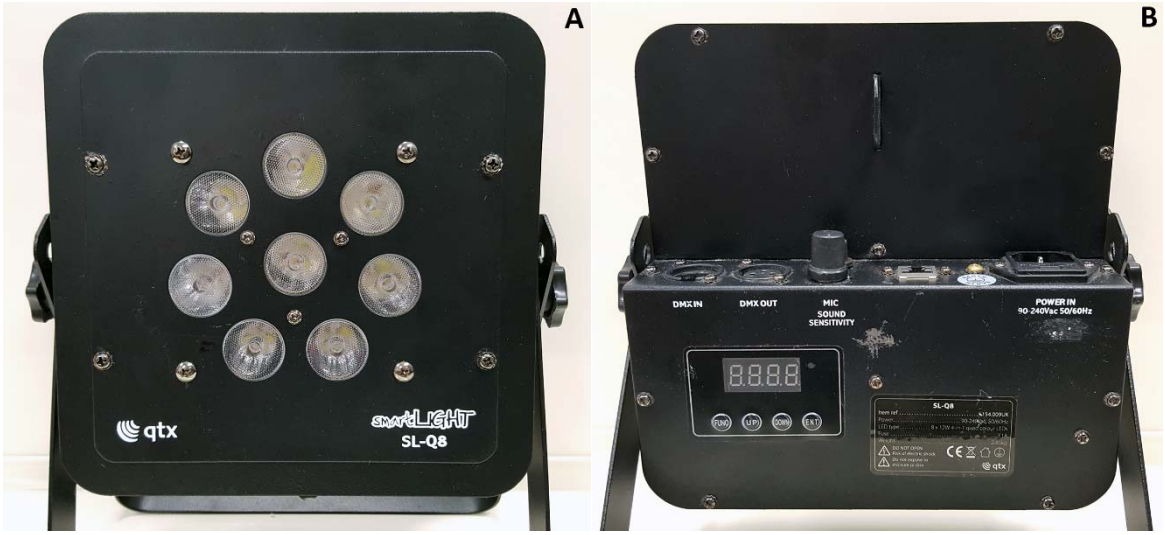

Figure 4.1. (A) Front view of the QTX SL-Q8 quad-colour RGBW-LED stage lights used to illuminate the chick experimental pens. Each of the 8 lamp units on the front panel contained a 12 W RGBW LED. (B) Rear view of the stage light showing the DMX control display and DMX input and output sockets. Each unit was assigned a starting DMX address (e.g. A001, A007, A013…) and so multiple units could be managed by one DMX controller by "daisy-chaining" successive units together with DMX cables.

Lightfactory version 2 Pro software (DreamSolutions Ltd., NZ, http://www.lightfactory.net) was used with a DMXKing ultraDMXmicro USB interface (DMXKing.com, NZ, http://dmxking.com) to control the QTX SL-Q8 stage lights (Figure 4.2). The Lightfactory software supported the scripting of lighting cue playlists which were used to control the lighting conditions for the various illumination protocols used in

the chick experiments. The lighting rig used for the guinea pig experiments consisted of a 2 x 3 array of the QTX SL-Q8 stage lights mounted on aluminium rails and attached to a PVC pipe frame. The PVC pipe frame was self-supporting, and so the animal pen was located under the rig independently of the frame. This allowed the pen to be raised or lowered relative to the stage lights as a secondary method of achieving the required illuminance (or irradiance) at chick eye level within the pen (Figure 4.3).

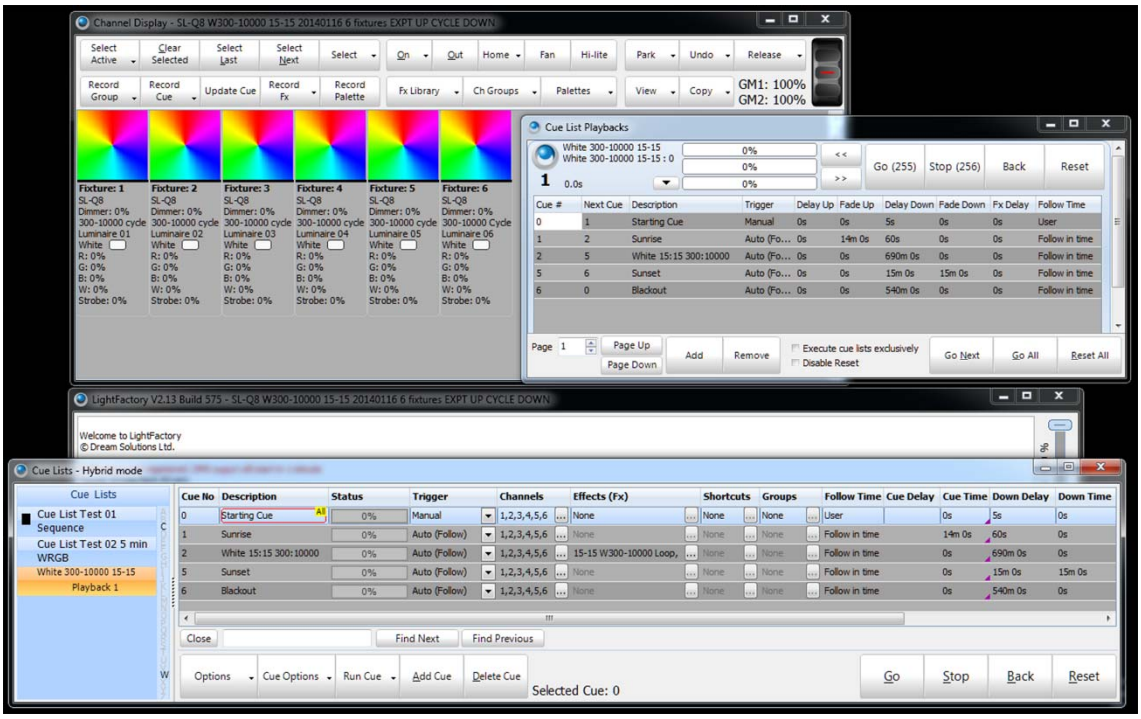

Figure 4.2. Screen capture of the Lightfactory software interface showing the Channel (Fixture) Display Window, Clue List and Cue Playlist. Lighting playlists were set to start at 08.00 hours daily, and run until 20.00. The lighting cue list included a 15 minute sunrise and sunset period at each end of the daylight phase. The Playlist was triggered each morning using Windows Task Scheduler.

Figure 4.3. View of the lighting rig from above showing the 3 x 2 array of QTX SL-Q8 LED stage lights mounted on rails attached to the PVC pipe frame. Each stage light (fixture) was connected to the software controller/hardware interface by DMX serial cables in a “daisy-chain” sequence.

Illumination Levels and Irradiance Matching

The presence of quad-colour RGBW LEDs in the stage lights allowed the lighting rig to be used to provide not only the standard 300 lux white light condition, but also the high intensity white, blue green and red illumination conditions for the chick form-deprivation experiments. A secondary consideration which led to the use of the LED system to provide the 300 lux white light, rather than the ceiling mounted fluorescent light as used in the guinea pig experiments (Section 3.2.1), was that the size and location of the stage lights above the pen (Figure 4.3) produced uneven illumination of the pen by the ceiling mounted fluorescent light due to shadowing.

The lighting rig was initially set up with a 60 cm distance between the stage lights and the floor of the pen. This distance could be used to provide both the 300 lux white illumination (DMX setting W8), and 10,000 lux white illumination (DMX setting 255) when measured at estimated chick eye level using a standard illumination (lux) meter (LX-105, Lutron, http://www.lutron.com.tw). The average illuminance levels measured across the floor of the pen under the DMX W8 and DMX W255 settings were 293 ± 14 lux and 10,698 ± 89 lux respectively.

As a standard photometric lux meter such as the Lutron LX-105 used in these experiments to measure illuminance is calibrated for the human photopic relative luminous efficiency function, it would be inappropriate to attempt to use it to match the illuminances of the high intensity blue, green and red lighting conditions with that of the high intensity white lighting conditions when considering the effective stimulus to the chick. No initial assumption was made regarding the photo-transduction mechanism of light intensity detection in the chick with regard to the previously demonstrated suppression of form-deprivation myopia (Ashby, Ohlendorf et al. 2009). Therefore, the decision was made to match the high intensity blue green and red lighting conditions for irradiance rather than illuminance. Irradiance is a measure of incident radiant energy flux per unit area, with the SI unit of watt per square metre (W/m^2) (Wyszecki and Stiles 1982). However, for consistency with the calculation methods of Lucas *et al* (Lucas, Peirson et al. 2014) used later in the discussion, irradiance has been reported in $\mu W/cm^2$ in the following text and figures.

A Spectrascan PR655 spectrophotometer and SRS-3 reflectance standard (PhotoResearch, USA, http://www.photoresearch.com), was used to measure the irradiance of the various lighting conditions at estimated chick eye level (5 cm) above the floor of the pen. The high intensity white lighting condition was initially set to 10,000 lux when measured with a Lutron LX-105 lux meter. The irradiance of this lighting condition was then measured with the PR655 spectrophotometer, and this value was

used as the irradiance target for the blue, green and red lighting conditions. The irradiance level was primarily controlled by the DMX setting of lighting control system, and secondarily by adjusting the pen to lighting rig distance, by raising the pen as required. The coloured lighting conditions then were set to have approximately the same irradiance as the high intensity white light condition (which was set to 10,000 lux illuminance) within the limits of the equipment setup. The white, blue, green and red high intensity light conditions were encoded as W10K, B10K, G10K, and R10K respectively. The suffix "P" was appended to indicate that the lighting condition alternated with white 300 lux illumination every 15 minutes during the form-deprivation experiments. The irradiance values for each lighting condition are summarised in Table 4.1.

Lighting Condition	DMX Setting	Distance (cm)	Illuminance (lux)	Irradiance ($\mu W/cm^2$)	Ratio to W10K
W300C	8	60	303.2 ± 3.5	**91.3 ± 1.1**	n/a
W10KP	255	60	10,016.4 ± 34.5	**3149.9 ± 11.7**	1.00
B10KP	210	60	1800.8 ± 10.5	**3193.9 ± 10.5**	1.01
G10KP	255	60	12244.9 ± 45.0	**2786.6 ± 10.1**	0.89
R10KP	255	50	5389.9 ± 30.0	**2994.9 ± 15.4**	0.95

Table 4.1 Summary of irradiance values measured with a Spectrascan PR655 spectrophotometer (Photoresearch, USA) for each lighting condition. The high intensity blue (B10KP), green (G10KP) and red (R10KP) irradiances were set to have approximately the same irradiance as the high intensity white condition (W10KP), when it measured 10,000 lux illuminance. The standard intensity white (W300C) values are given for comparison. Illuminance and irradiance values are the mean ± 1 SEM for 3 measurements.

Using the nominal white high intensity illuminance of 10,000 lux, the irradiance target was set at ~3150 $\mu W/cm^2$ for the blue, green and red lighting conditions. The column labelled "Ratio to W10K" in Table 4.1 gives the ratio of the measured irradiance for each coloured condition to the reference W10KP measured irradiance. For example, the blue high intensity condition (B10KP) produced a ratio of 1.01 (or 1% difference in irradiance) for a DMX setting of 210 at a lamp to pen distance of 60 cm. The R10KP condition produced a ratio 0.95 (5% difference) with a maximum DMX setting of 255 and a reduced distance of 50cm. The largest difference between the target and achieved irradiance was for the G10KP condition where the ratio was 0.89 or 11% difference. In retrospect, it would have been preferable to reduce this difference by decreasing the lamp to pen distance, however the 11% difference is still relatively small when considering that the suppression of form-deprivation myopia in chicks is usually demonstrated for several orders of magnitude difference in intensity (i.e. 100s versus 10,000s of lux) (Ashby, Ohlendorf et al. 2009; Backhouse, Collins et al. 2013; Karouta and Ashby 2015).

The spectral irradiance profiles of the five lighting conditions measured with a Spectrascan PR655 spectrophotometer (PhotoResearch) at 5 nm wavelength steps are given in Figure 4.4 for the standard and high intensity white light conditions, and in Figure 4.5 for the high intensity white, blue, green and red light conditions. Under both the standard and high intensity conditions, the white LED source demonstrated the typical bimodal spectral profile produced by a blue LED (peak at ~450 nm) with associated phosphor coating giving a broader peak at ~550 nm (van Bommel and Rouhana 2011). The total irradiance of the condition is proportional to the area under the curve for that condition. The positions of the two spectral peaks were invariant with total irradiance.

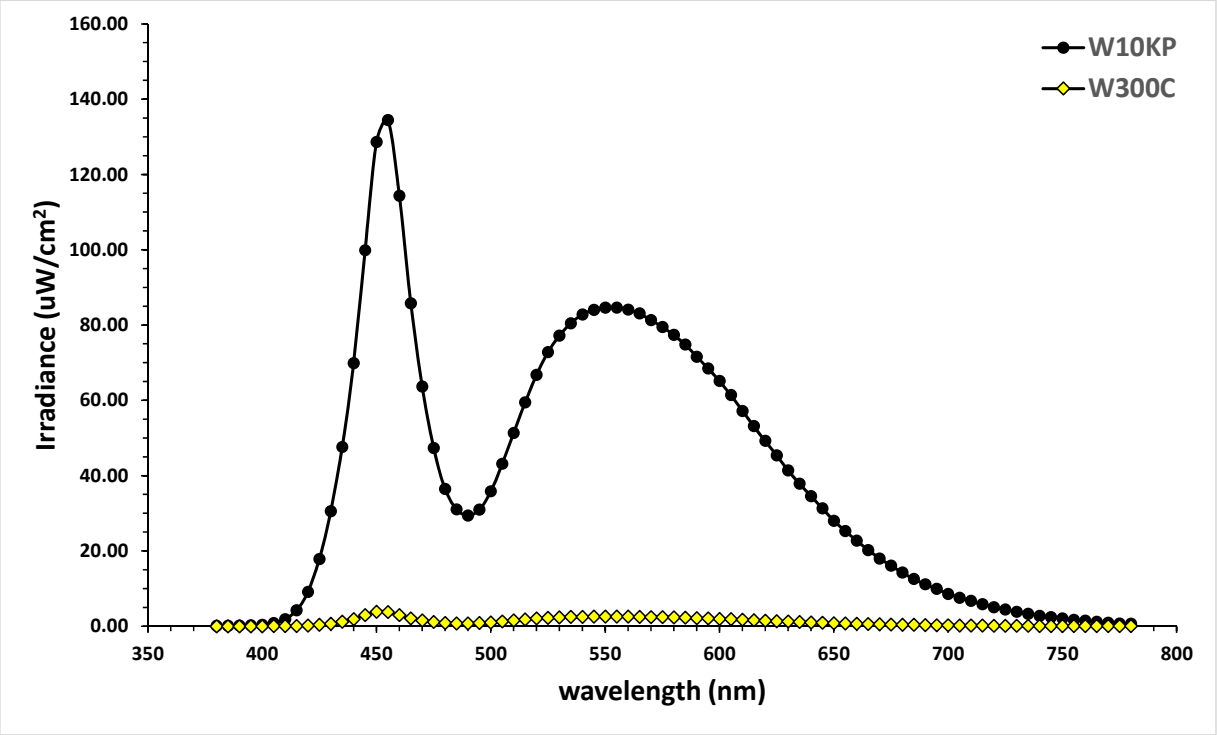

Figure 4.4. Spectral irradiance distributions (μW/cm^2) of the QTX-SL8 LED stage lights for the white 300 lux (W300C) and white 10,000 lux (W10KP) lighting conditions. The white LED demonstrates the typical bimodal spectral profile produced by a blue LED (peak at ~450 nm) with associated phosphor coating giving a broader peak at ~550 nm.

The quasi-monochromatic nature of the blue, green and red spectral irradiance distributions is illustrated in Figure 4.5. The spectral distributions the blue (B10KP), green (G10KP) and red (R10KP) high intensity lighting conditions display spectral peaks at 455, 515 and 635 nm respectively, with 1/2 height bandwidths of < 50 nm. In comparison, the spectral distribution of white (W10KP) condition is broader and biphasic due to the underlying construction from a phosphor-coated blue LED. However, the total irradiances of the four conditions, which is proportional to the area under each curve, is approximately equal (Table 4.1).

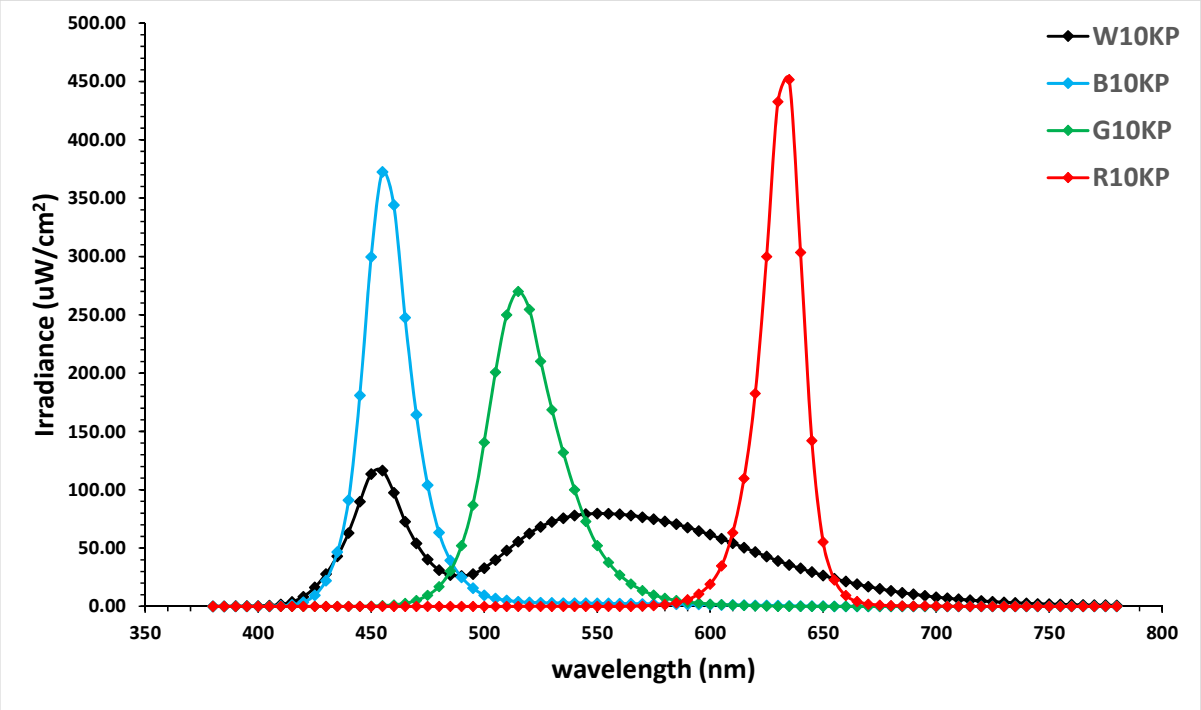

Figure 4.5. Spectral irradiance distributions (μW/cm^2) of the QTX-SL8 LED stage lights under high intensity conditions. The white LED (W10KP) demonstrates the typical bimodal spectral profile of a phosphor coated LED. The blue (B10KP), green (G10KP) and red (R10KP) spectral irradiance distributions display the quasi-monochromatic nature of the sources with spectral peaks at 455, 515 and 635 nm respectively.

4.2.2 Animal Cohorts and Lighting Protocols

Refractive and ocular biometry outcome measurements were completed on a total of 56 Cobb chicks, in 10 cohorts, on the 4th day of monocular form-deprivation using translucent occluders as described in Section 2.1.2. During form-deprivation the chicks were exposed to one of five LED-based lighting conditions during the light phase of a 12:12 hour light:dark daily cycle:

1) W300C constant white LED 300 lux illumination during the 12 hour light phase.
2) W10KP periodic high intensity white LED 10,000 lux illumination alternated with standard intensity white LED 300 lux illumination in a 15:15 minute cycle.
3) B10KP periodic high intensity blue LED irradiance-matched lighting alternated with standard intensity white LED 300 lux illumination in a 15:15 minute cycle.
4) G10KP periodic high intensity green LED irradiance-matched lighting alternated with standard intensity white LED 300 lux illumination in a 15:15 minute cycle.
5) R10KP periodic high intensity red LED irradiance-matched lighting alternated with standard intensity white LED 300 lux illumination in a 15:15 minute cycle.

Note that the irradiance of the blue, green and red conditions was matched to the irradiance of the white 10,000 lux condition (Table 4.1).

The number of chicks with refractive outcome measures for each lighting condition was (replication dates in brackets): W300C, n = 10 (Dec, Jan); W10KP, n = 11 (Jan, Aug); B10KP, n = 12 (Mar, Apr); G10KP, n = 10 (Mar, Apr); and R10KP, n = 13 (Aug, Dec). The variation in numbers between lighting condition cohorts was due to exclusion of small numbers animals from the study due to health issues (failure to thrive condition – see Section 2.1.1).

The LED stage lights and lighting conditions were controlled by lighting playlists run in Lightfactory v2 Pro software (DreamSolutions Ltd.) (Section 4.2.1). The room fluorescent lighting was disabled centrally, so that all experimental lighting was supplied by the LED stage lights. The appropriate playlist was triggered each morning using Windows Task Scheduler. Lighting playlists were set to start at 08.00 daily and run until 20.00. The lighting playlists included a 15 minute sunrise (0 – 300 lux white illumination) and sunset period (300 – 0 lux white illumination) at each end of the daylight phase to replicate the standard lighting conditions of the animal unit under which the chicks were initially acclimatised. Following the 15 minute sunrise period, the playlist was cued to periodically run the standard intensity white lighting on for 15 minutes duration, beginning 15 minutes after the start of the daylight period (i.e. 08.15), and then switch to the appropriate high intensity coloured 15 minutes later. This cycle continued for the rest of the 12 hour period until 19.45 when the sunset phase commenced. All lights remained off through the dark phase from 20.00 to 08.00 the next morning (Figure 4.6). Therefore, the periodic high intensity lighting groups received 5.75 hours of cumulative high intensity illumination (50% duty cycle) over the 12 hour light period.

The periodic nature of the high intensity illumination paradigm was designed to simulate the movement of a person between relatively low intensity indoor lighting conditions, and high intensity outdoor lighting conditions during the day (Backhouse 2011). The use of quasi-monochromatic illumination, in addition to broadband white light, was to investigate whether the suppression of form-deprivation myopia by high intensity light was wavelength-sensitive. White light, rather than the respective quasi-monochromatic light, was used for the standard 300 lux illumination phase to ameliorate any possible effect of longitudinal chromatic defocus on the axial growth (and hence refractive status) of the non-deprived eye (Wildsoet, Howland et al. 1993; Seidemann and Schaeffel 2002; Liu, Qian et al. 2011; Qian, Dai et al. 2013).

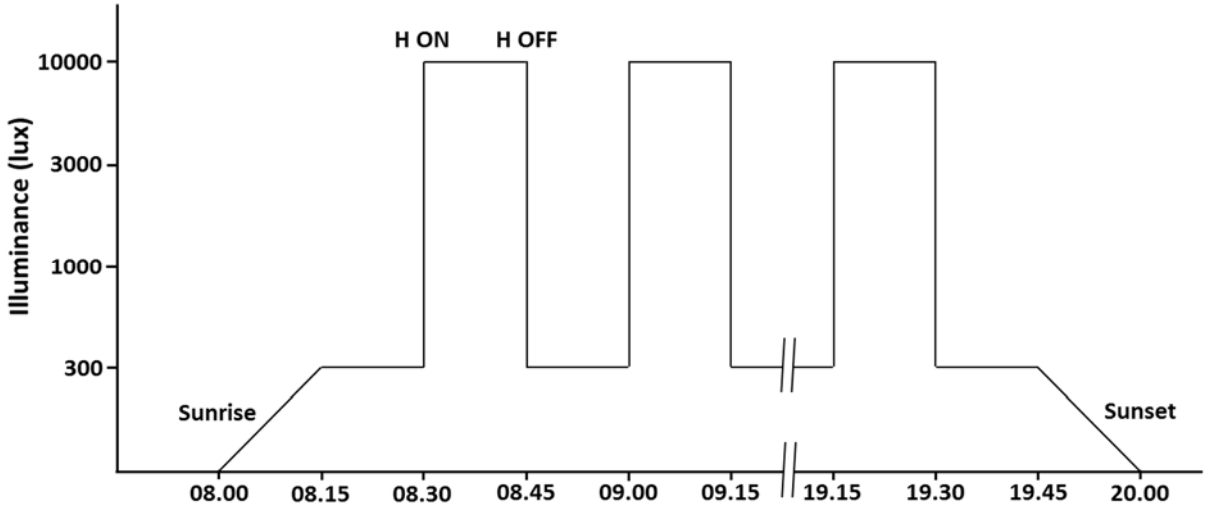

Figure 4.6. Illustration of the periodic high intensity lighting paradigm. The light phase started at 08.00 with the sunrise phase, peaking at 300 lux illumination in the pen. At 08.15 (15 minutes later) the high intensity lighting condition switched on (H ON). After 15 minutes, the high intensity lighting switched off (H OFF) returning the pen to the 300 lux condition. This pattern then repeated until the sunset phase commenced at 19.45. At 20.00 the LED stage lights switched off until 08.00 the next day.

Both the standard 300 lux intensity and periodic high intensity light cohorts of chicks were maintained under their respective lighting conditions for 3 days (Section 2.1.2) after which outcome measurements were made on the 4th day (8 days post-hatch) immediately following removal from their pen. The lighting system was monitored in real-time using a remotely viewable webcam.

4.3 Results: Chick

4.3.1 Refractive Outcome

Paired-Eye Comparison – Relative Mean Sphere

Relative mean-sphere refraction was calculated as the difference in refraction between the deprived eye and the non-deprived eye for each animal in each illumination group. Paired eye comparisons are a means of controlling for individual variation in growth rates between animals which might influence biometric parameters. The non-occluded eye effectively acts as an internal control eye for comparison of refractive outcome with the occluded eye (Wallman and Adams 1987). The average relative mean-sphere refraction for each illumination group is illustrated in Figure 4.7.

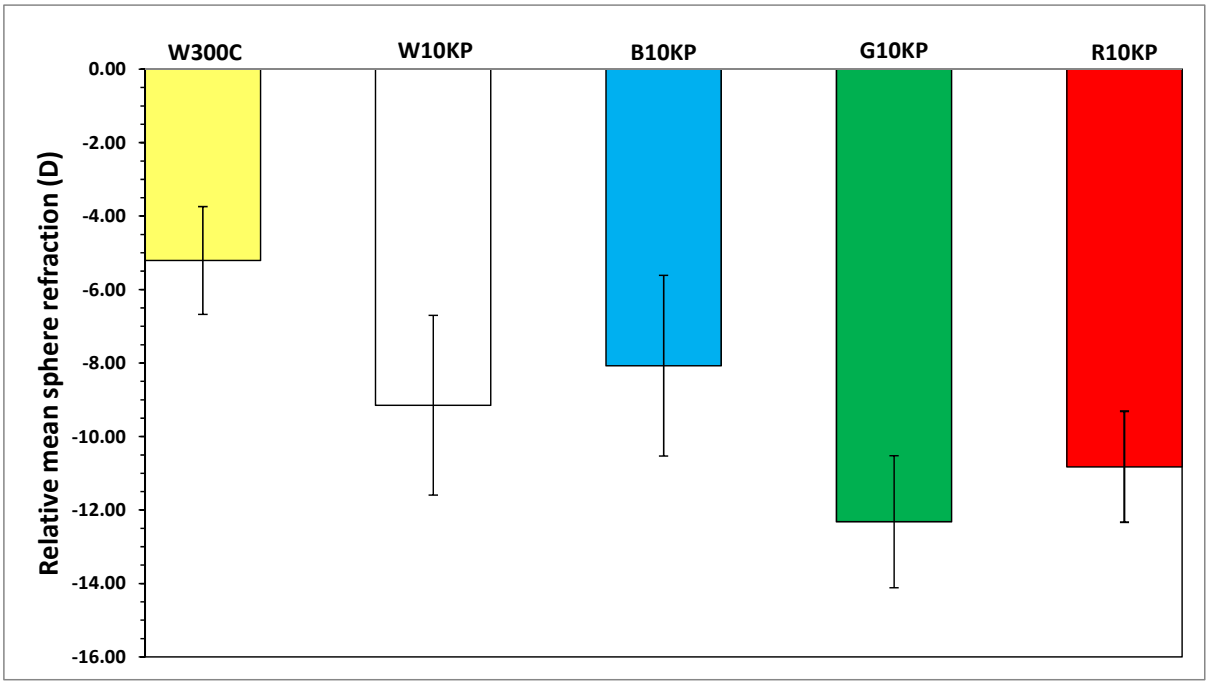

Figure 4.7. Relative mean sphere refractions (deprived eye - non-deprived eye) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 10), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). No significant differences in relative mean sphere refraction by illumination condition were found (ANOVA; $F_{(4, 51)} = 1.701$, $p = 0.164$). Error bars are ± 1 SEM.

All illumination groups developed a relative myopic refraction on average, although no significant difference in relative mean-sphere refraction was found between the five illumination groups (ANOVA; $F_{(4, 51)} = 1.701$, $p = 0.164$). However, inspection of Figure 4.7 demonstrates the trend that on average, the least myopia was induced in group W300C which was exposed to constant, but relatively lower white light illumination levels while under form deprivation. Conversely the group of chicks exposed to the G10KP illumination condition developed the highest degree of relative myopia on

average. Post-hoc power analysis (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) shows that the statistical power of the ANOVA analysis for relative Mean Sphere was low at 51%, and that a total sample size of 100 animals (20 per group) would be required for the variation in refraction by illumination group to reach statistical significance. The underlying refractive components that contribute to these trends are considered in the following sections.

Individual Eye Comparison – Absolute Mean Sphere

The average absolute mean sphere refraction was also calculated for both the deprived eyes and the non-deprived eyes in each illumination group. This allowed consideration of whether there was any significant variation in the refractive development of each group of eyes due to illumination conditions, including the relatively higher illumination levels experienced by the non-deprived eye compared to the form-deprived eye when the reduced transmittance of the translucent diffusers is taken into account (Section 2.1.2). The average absolute mean-sphere refraction for the deprived and contralateral non-deprived eyes by illumination group is illustrated in Figure 4.8.

On average, form-deprived eyes developed a myopic refractive error, and non-deprived eyes developed a hyperopic refractive error, irrespective of illumination condition. The deprived eyes were significantly more myopic than the non-deprived eyes (Paired t-test; $t = -9.975$, $p < 0.0001$). No significant differences in refractive error by illumination condition were found for either the form-deprived eyes (ANOVA; $F_{(4, 51)} = 1.095$, $p = 0.369$), or for the non-deprived contralateral eyes (ANOVA; $F_{(4, 51)} = 1.509$, $p = 0.214$). Therefore, the illumination conditions did not have a significant effect on refractive error development for either the non-deprived eyes, which received the full illumination under each lighting condition, or for the form-deprived eyes. The refractive outcomes for both the form-deprived and non-form deprived eyes, and relative mean sphere refraction are summarised in Table 4.2 below.

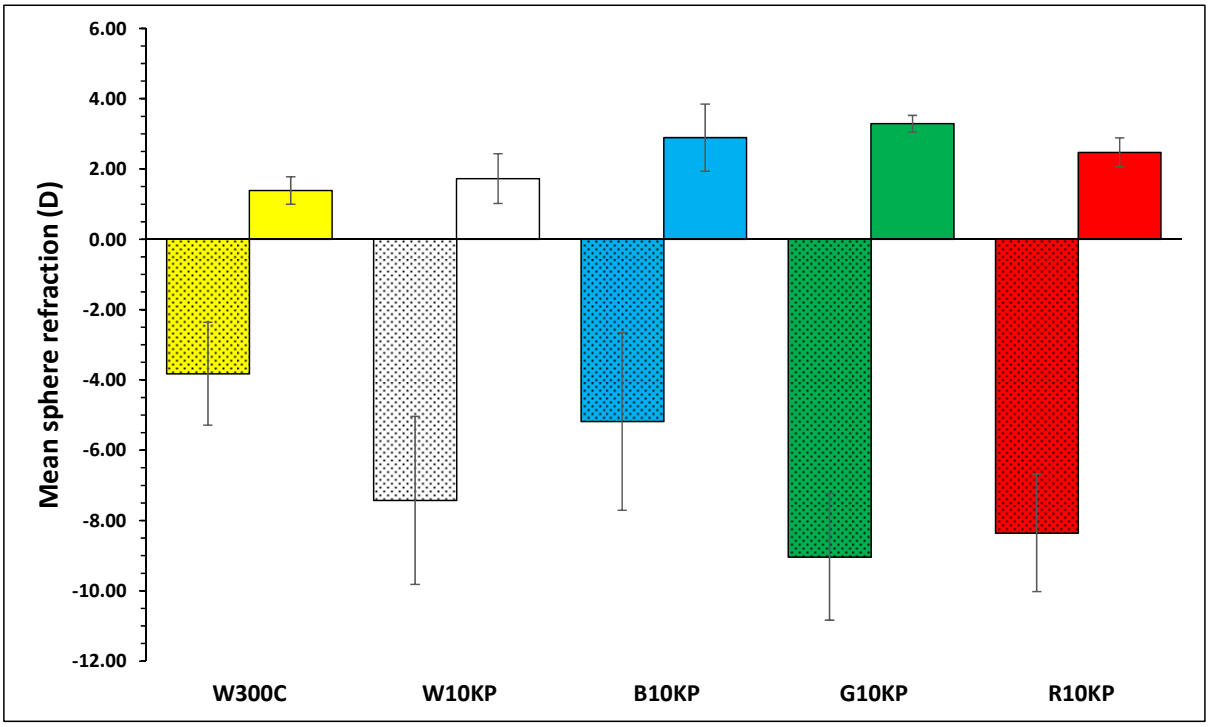

Figure 4.8. Absolute mean sphere refractions for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 10), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). No significant differences in refractive error by illumination condition were found for either the form-deprived eyes (ANOVA; $F_{(4, 51)} = 1.095$, $p = 0.369$), or for the non-deprived contralateral eyes (ANOVA; $F_{(4, 51)} = 1.509$, $p = 0.214$). Error bars are ± 1 SEM.

Illumination Condition	Form-deprived Eyes Mean sphere (D)	Non-deprived Eyes Mean sphere (D)	Difference in Refraction Relative mean sphere (D)
W300C	-3.82 ± 1.47	+1.39 ± 0.39	-5.21 ± 1.47
W10KP	-7.42 ± 2.39	+1.72 ±0.71	-9.15 ± 2.44
B10KP	-5.18 ± 2.53	+2.89 ±0.95	-8.07 ± 2.46
G10KP	-9.03 ± 1.80	+3.29 ± 0.24	-12.32 ± 1.80
R10KP	-8.35 ± 1.67	+2.47 ± 0.41	-10.82 ± 1.51
ANOVA	$p = 0.368$	$p = 0.214$	$p = 0.164$

Table 4.2 Refractive outcome measures (mean ± 1 SEM) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 10), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). ANOVA *p*-values for comparison of means by illumination condition are given in the final row of the table.

While this study has found no significant difference in the paired-eye relative mean sphere refractive outcome by illumination condition, a general trend can be seen in Figure 4.7 where on average the W300C lighting condition resulted in the lowest degree of induced myopia. A previous study from the same laboratory by Backhouse *et al (Backhouse, Collins et al. 2013)*, reported a greater degree of

induced myopia in the same strain of Cobb chicks raised under constant 300 lux illumination on a 12L:12D light cycle, where the source used was a ceiling-mounted luminaire containing two Philips Linear T5 28 W fluorescent tubes (Philips, http://www.philips.com). In contrast, this study utilised LED-based QTX SL-Q8 (QTX, http://avslgroup.com/en/about/qtx) stage lights (Section 4.2.1) to provide an average illumination of 300 lux at the chick eye level. The difference in relative mean sphere refraction induced under these two conditions (this study and Backhouse *et al*) is illustrated in Figure 4.9.

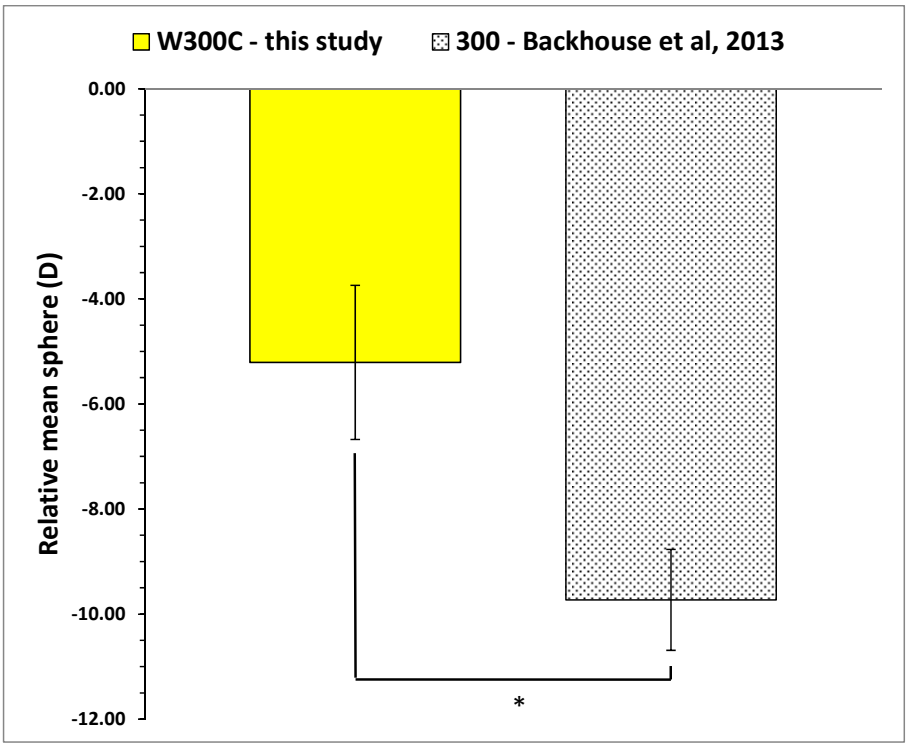

Figure 4.9. A comparison of the relative mean sphere refractions obtained for Cobb chicks raised with unilateral form-deprivation under either LED-based white illumination of 300 lux (W300C - this study, -5.21 ± 1.47 D, n = 10), or raised under fluorescent lamp-based white illumination of 300 lux (300, (Backhouse, Collins et al. 2013), -9.73 ± 0.96 D, n = 11). **t* test: $p = 0.0168$. Error bars are ± 1 SEM.

Significantly less myopia was induced in this study using LED-based luminaires at an average illumination of 300 lux (-5.21 ± 1.47 D, n = 10), when compared with the level of relative mean sphere refractive error reported by Backhouse *et al* (Backhouse, Collins et al. 2013) for chicks raised under fluorescent lamp-based white illumination of 300 lux (-9.73 ± 0.96 D, n = 11) ($t_{19} = 2.6216$, $p = 0.0168$). As both cohorts of Cobb chicks were sourced from the same supplier and raised under the same conditions (room, temperature, feeding regime), then the difference in refractive outcome between the studies may be explained by differences in spectral distributions of the fluorescent and LED illumination sources (Section 4.4.1). However, while the Cobb chicks were obtained from the same supplier, two years had elapsed between experiments which may be an additional source of variability in the response to form-deprivation.

4.3.2 Corneal Curvature

Paired-Eye Comparison – Relative Corneal Radius

Relative corneal radius of curvature was calculated as the difference in central radius between the deprived eye and the non-deprived eye for each animal in each illumination group. The average relative corneal radius for each illumination group is illustrated in Figure 4.10.

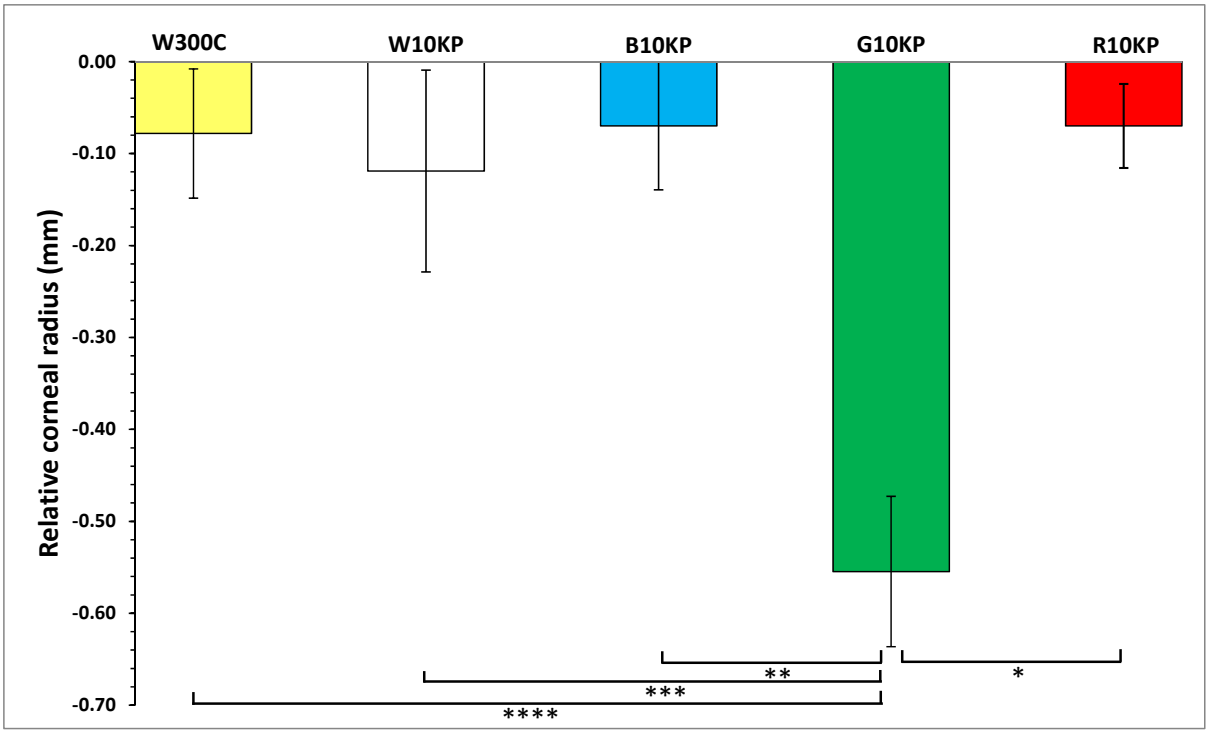

Figure 4.10. Relative corneal radius of curvature (deprived eye - non-deprived eye) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 8), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 11). For the G10KP condition, the difference in corneal radius between the deprived eye and the non-deprived eye was significantly more negative than all other conditions (ANOVA; $F_{(4, 47)} = 6.883$, $p = 0.0002$; *Post-hoc* Tukey: *$p = 0.001$, **$p = 0.001$, ***$p = 0.003$, ****$p = 0.003$). Error bars are ± 1 SEM.

ANOVA analysis revealed a significant effect of illumination condition on average relative corneal radius (ANOVA; $F_{(4, 47)} = 6.883$, $p < 0.0001$), and post-hoc testing confirmed that chicks raised under the G10KP condition had a significantly more negative relative corneal radius of curvature when compared with the other four illumination conditions. While the G10KP group demonstrated a significant difference in relative corneal radius, which might be expected to influence the overall refractive state of the eye, this group did not exhibit a statistically significant difference in either relative or absolute refraction (Table 4.2) when compared with the other illumination groups.

The relationship between relative corneal curvature and relative mean sphere refraction was investigated further by linear regression analysis of the pooled data (Figure 4.11).

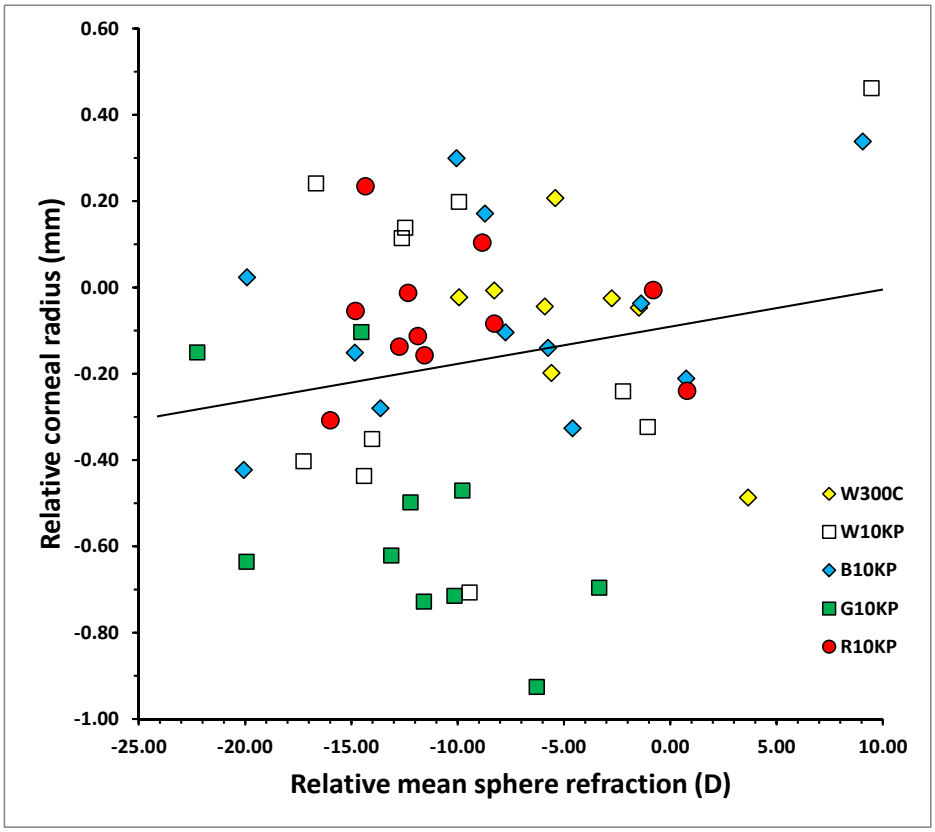

Figure 4.11. Correlation between relative mean sphere refraction and relative corneal radius (deprived eye - non-deprived eye) for all chicks (n = 55): Linear regression revealed a weak, non-significant, correlation (R^2 = 0.036, p = 0.177). The linear regression line is for the pooled data of all chicks.

The linear regression analysis showed that corneal radius of curvature was not significantly correlated with relative mean sphere refraction (R^2 = 0.036, $F_{1,51}$ = 1.878, p = 0.177). Therefore, the relatively small differences in corneal curvature between groups do not appear to have had a significant influence on overall refractive error in this experiment, however this relationship is investigated further using a Univariate Linear General Model (ULGM) in Section 4.3.6.

Individual Eye Comparison – Absolute Corneal Radius

As the negative values obtained for relative corneal radius indicate that the corneas of the non-deprived eyes were on average flatter (longer radii) than the contralateral non-deprived eyes (under all illumination conditions), the question arises as to whether this was due to flattening of the non-deprived corneas, or steeping of the deprived eye corneas. As noted above, the effect of the translucent occluder is to reduce illumination of the occluded eye which may result in a differential effect of the illumination condition on corneal radius for each eye (Cohen, Belkin et al. 2008). The average absolute corneal radius for the deprived and contralateral non-deprived eyes by illumination group is illustrated in Figure 4.12.

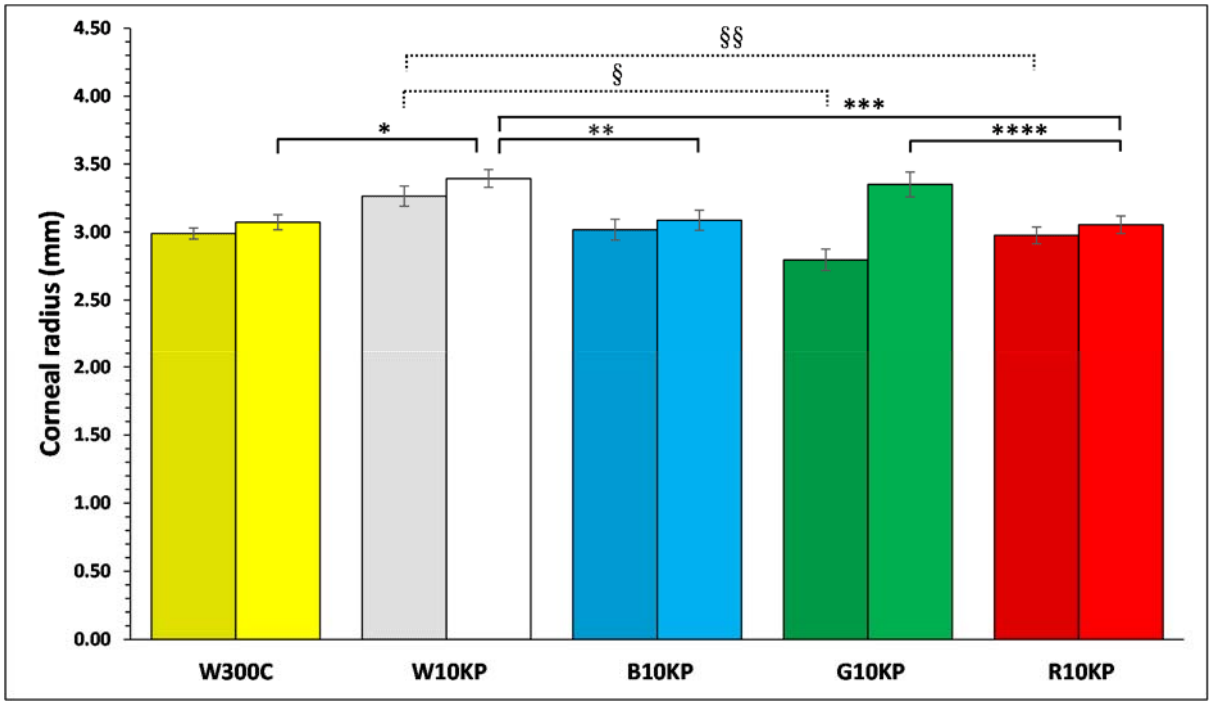

Figure 4.12. Absolute corneal radii of curvature for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 8), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 11). Both the deprived eyes and the non-deprived eyes exhibited significant differences in average corneal radius by illumination condition. Deprived eyes: ANOVA; $F_{(4, 47)} = 5.674$, $p < 0.001$; *Post-hoc* Tukey: *§ < 0.001, **§ = 0.036. Non-deprived eyes: ANOVA; $F_{(4, 51)} = 5.386$, $p = 0.001$; *Post-hoc* Tukey: *$p = 0.025$, **$p = 0.025$, ***$p = 0.008$, ****$p = 0.037$. Error bars are ± 1 SEM.

On average, non-deprived eyes exhibited a significantly greater corneal radius of curvature (+3.19 ± 0.04 mm) than the deprived eyes (+3.01 ± 0.04 mm), irrespective of illumination condition (pooled data, Paired t-test; t = -4.156, $p < 0.001$). This relationship is generally consistent with the data trends reported by Backhouse *et al* (Backhouse, Collins et al. 2013) however the differences between deprived and non-deprived eyes were not statistically significant in their study. The relationships between corneal curvature and illumination condition by deprivation state are complex with the corneal radii of curvature for the W10KP condition showing the greatest number of significant relationships with the other illumination conditions for both the deprived and non-deprived eyes (Figure 4.12). For deprived eyes, those exposed to the W10KP condition had a significantly greater average corneal radius (+3.26 ± 0.07 mm) than: (i) the G10KP group (2.80 ± 0.08 mm)(*post-hoc* Tukey; $p < 0.001$); and (ii) the R10KP group (+2.98 ± 0.06 mm)(*post-hoc* Tukey; $p = 0.036$). For non-deprived eyes, the eyes exposed to the W10KP condition also had a significantly greater average corneal radius (+3.39 ± 0.07 mm) than: (i) the W300C group (+3.07 ± 0.06 mm)(*post-hoc* Tukey; $p = 0.025$); (ii) the B10KP group (+3.09 ± 0.07 mm)(*post-hoc* Tukey; $p = 0.025$); and (iii) the R10KP group (+3.05 ± 0.06 mm)(*post-hoc* Tukey; $p = 0.008$). Furthermore the average corneal radius non-deprived eyes of the G10KP group (+3.35 ± 0.09 mm) were significantly greater than for the R10KP group

(+3.05 ± 0.07 mm)(*post-hoc* Tukey; $p = 0.025$). The corneal radii of curvature for both the form-deprived and non-form deprived eyes, and relative corneal radii are summarised in Table 4.3 below.

Illumination Condition	Form-deprived Eyes Corneal radii (mm)	Non-deprived Eyes Corneal radii (mm)	Difference between Eyes Relative radii (mm)
W300C	+2.99 ± 0.04	+3.07 ± 0.06	-0.08 ± 0.07
W10KP	+3.26 ± 0.07	+3.39 ± 0.07	-0.12 ± 0.11
B10KP	+3.02 ± 0.08	+3.09 ± 0.07	-0.07 ± 0.07
G10KP	+2.80 ± 0.08	+3.35 ± 0.09	-0.55 ± 0.08
R10KP	+2.98 ± 0.06	+3.05 ± 0.06	-0.07 ± 0.05
ANOVA	***p* < 0.001**	***p* = 0.001**	***p* = 0.0002**

Table 4.3 Corneal radius of curvature outcome measures (mean ± 1 SEM) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 8), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 11). ANOVA *p*-values for comparison of means by illumination condition are given in the final row of the table.

The G10KP group displayed the largest difference in corneal radii between the deprived and non-deprived eyes, which was sufficient to produce a significant difference in relative corneal radius of curvature when compared with the other illumination conditions (Figure 4.4). However, this difference was not reflected in a significant difference in relative mean sphere refraction for the G10KP group (Figure 4.7, Table 4.2), suggesting that the other components of refraction (anterior chamber, lens and vitreous chamber depth) compensate for, or mask the corneal effect on refraction.

4.3.3 Anterior Chamber Depth

Paired-Eye Comparison – Relative Anterior Chamber Depth

Relative anterior chamber depth (ACD) was calculated as the difference in ACD between the deprived eye and the non-deprived eye for each animal in each illumination group. The average relative ACD for each illumination group is illustrated in Figure 4.13.

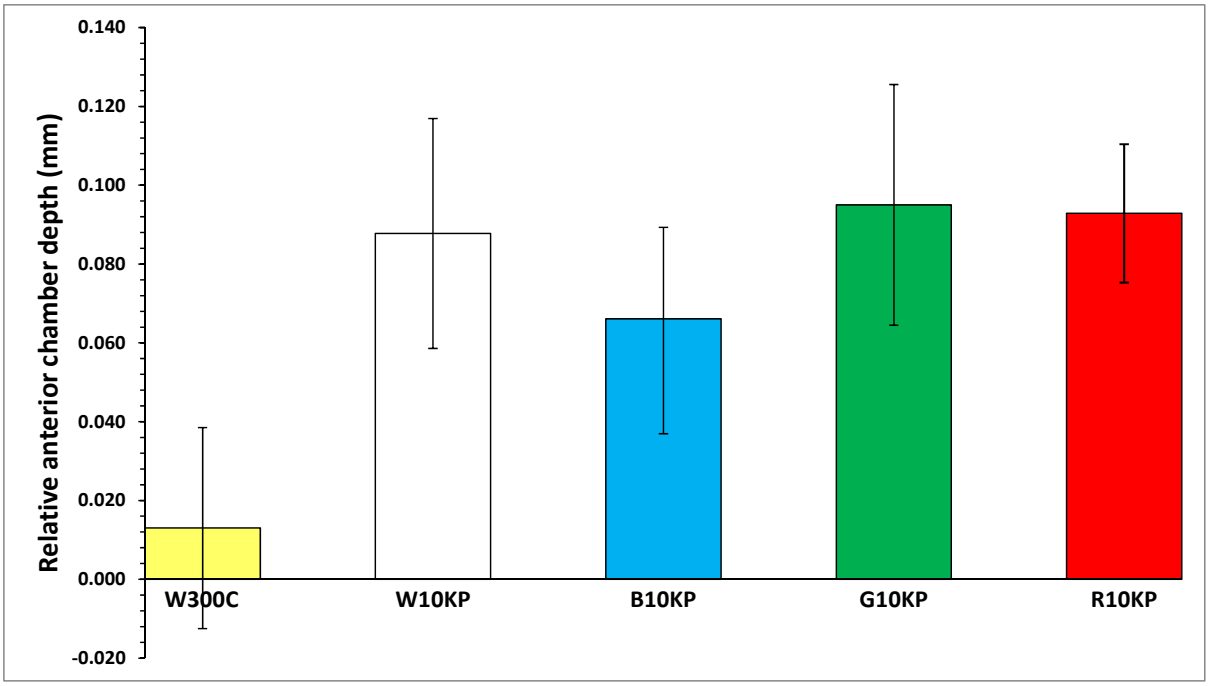

Figure 4.13. Relative anterior chamber depth (deprived eye - non-deprived eye) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 11). No significant difference in ACD was found by illumination condition (ANOVA; $F_{(4, 50)} = 1.674$, $p = 0.177$). Error bars are ± 1 SEM.

No significant effect of illumination condition on the relative ACD was found between the five groups (ANOVA; $F_{(4, 50)} = 1.674$, $p = 0.177$). However, Figure 4.13 demonstrates the trend that on average, the shallowest relative ACD was found in group W300C which was exposed to constant, but relatively lower white light illumination levels while under form deprivation. Post-hoc power analysis (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) shows that the statistical power of the ANOVA analysis for relative ACD was low at 44%, and that a total sample size of 105 animals (21 per group) would be required for the variation in relative ACD by illumination group to reach statistical significance.

Although no significant effect of illumination was found, linear regression of relative ACD against relative mean sphere refraction for all groups, revealed that increased relative ACD was significantly,

but weakly, correlated with increased relative myopic refraction (R^2 = 0.170, $F_{1,53}$ = 10.876, p = 0.002) (Figure 4.14).

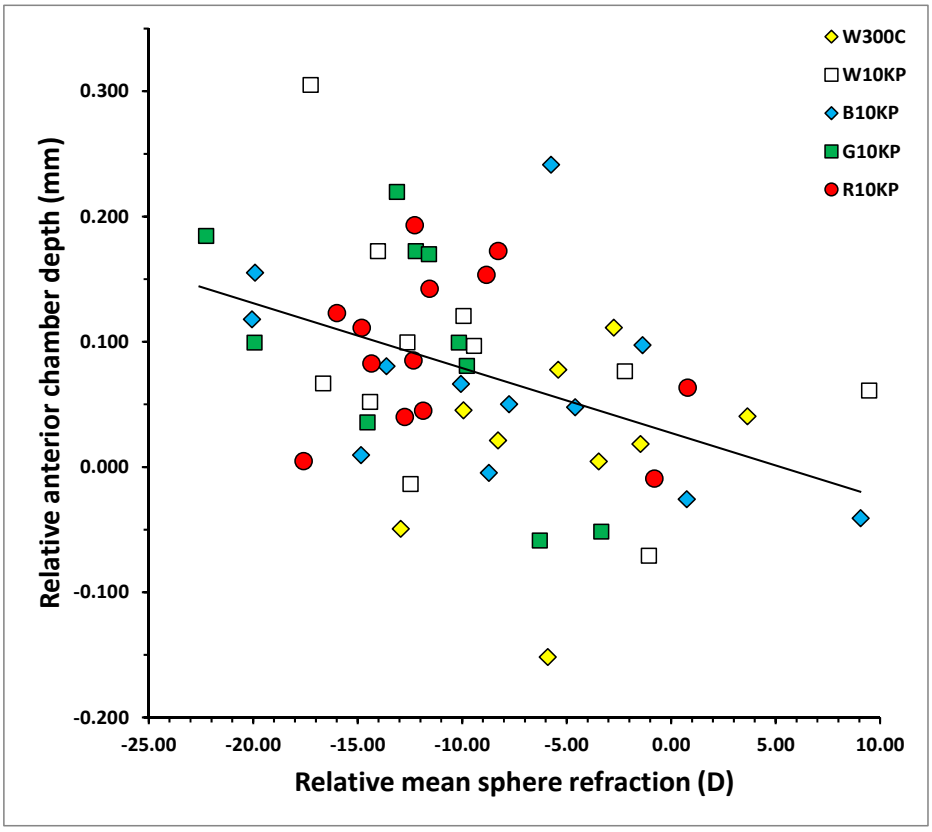

Figure 4.14. Correlation between relative mean sphere refraction and relative anterior chamber depth (deprived eye - non-deprived eye) for all chicks (n = 55): Linear regression revealed a significant, but weak, correlation (R^2 = 0.170, p = 0.002). The linear regression line is for the pooled data of all chicks.

Therefore, the difference in ACD between the deprived and non-deprived eyes contributed to the overall relative refractive error outcome, but was not influenced by illumination condition. The strength of this relationship is investigated further by ULGM analysis in Section 4.3.6. The following section explores whether the individual eye ACDs (deprived, non-deprived) differ by illumination condition.

Individual Eye Comparison – Absolute Anterior Chamber Depth

As noted previously, the calculation of average absolute ACD for both the deprived eyes and the non-deprived eyes for each illumination group allows for an investigation of whether there was any significant effect of the illumination differences between the groups of eyes due to the use of translucent occluders to produce form-deprivation. Figure 4.15 demonstrates the variation in average absolute ACD for the deprived and non-deprived eyes by illumination group.

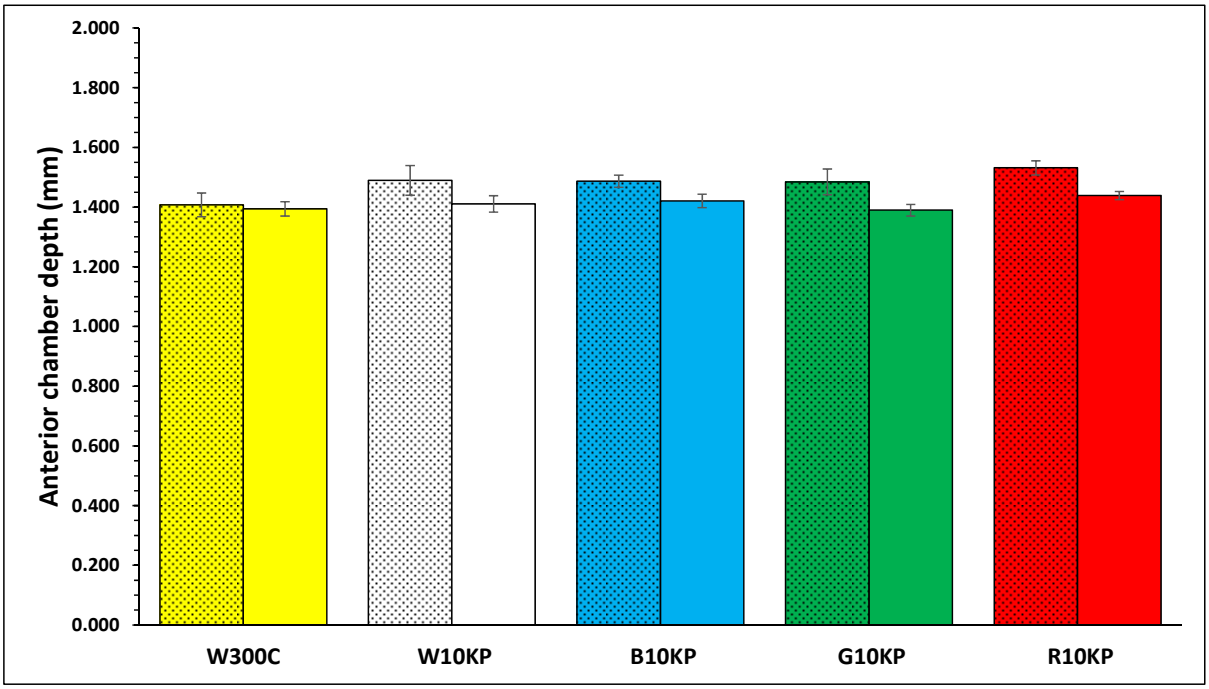

Figure 4.15. Absolute anterior chamber depth for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). No significant differences in ACD were found by illumination condition for either the deprived (ANOVA; $F_{(4, 50)} = 1.482$, $p = 0.222$) or non-deprived eyes (ANOVA; $F_{(4, 50)} = 0.879$, $p = 0.483$). Error bars are ± 1 SEM.

The absolute ACD outcomes for both the form-deprived and non-form deprived eyes, and relative ACD values are summarised in Table 4.4 below.

Illumination Condition	Form-deprived Eyes ACD (mm)	Non-deprived Eyes ACD (mm)	Difference between Eyes Relative ACD (mm)
W300C	+1.407 ± 0.040	+1.394 ± 0.024	+0.013 ± 0.026
W10KP	+1.490 ± 0.050	+1.411 ± 0.027	+0.088 ± 0.029
B10KP	+1.487 ± 0.020	+1.421 ± 0.023	+0.066 ± 0.023
G10KP	+1.485 ± 0.041	+1.390 ± 0.020	+0.095 ± 0.031
R10KP	+1.531 ± 0.024	+1.439 ± 0.014	+0.093 ± 0.018
ANOVA	$p = 0.222$	$p = 0.482$	$p = 0.177$

Table 4.4 Anterior chamber depth outcome measures (mean ± 1 SEM) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). ANOVA *p*-values for comparison of means by illumination condition are given in the final row of the table.

On average, form-deprived eyes exhibited a small but significantly greater ACD (+1.485 ± 0.016 mm) than non-form-deprived eyes (+1.413 ± 0.009) irrespective of illumination condition (Paired t-test; t = 6.226, $p < 0.0001$). This result is consistent with that found previously in the same laboratory by

Backhouse *et al* (Backhouse, Collins et al. 2013) where the ACD was also found to be significantly longer in deprived eyes of the same strain of chicks under all illumination conditions. This difference in ACD is therefore likely to be in response to the form-deprivation, rather than differences in illumination, as no significant difference in ACD by illumination condition was found for either the form-deprived eyes (ANOVA; $F_{(4, 50)} = 1.479$, $p = 0.222$), or for the non-deprived contralateral eyes (ANOVA; $F_{(4, 50)} = 0.881$, $p = 0.482$)(Figure 4.15, Table 4.4).

4.3.4 Lens Thickness

Paired-Eye Comparison – Relative Lens Thickness

Relative lens thickness (LT) was calculated as the difference in LT between the deprived eye and the non-deprived eye for each animal in each illumination group. The average relative LT for each illumination group is illustrated in Figure 4.16. Non-parametric statistical analysis was applied to the LT data as it failed normality testing using the Kolmogorov-Smirnov with Lilliefors correction test.

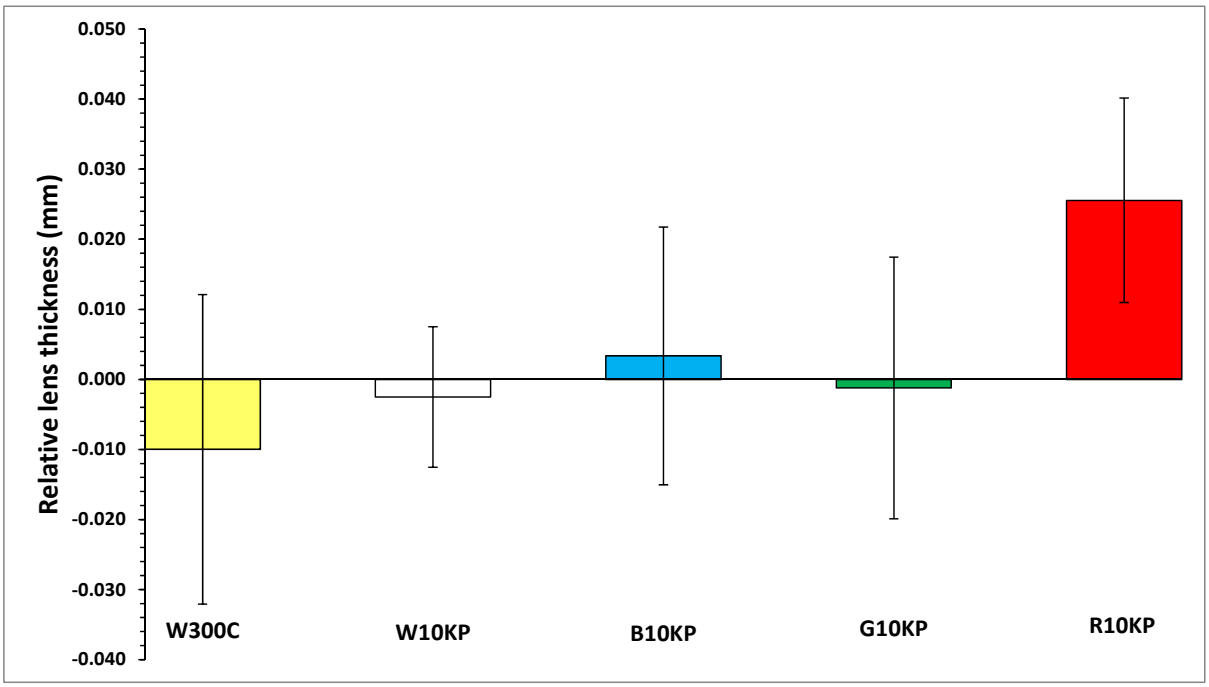

Figure 4.16. Relative anterior chamber depth (deprived eye - non-deprived eye) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 11). No significant difference in ACD was found by illumination condition (Kruskal-Wallis ANOVA; $\chi^2_{(4)} = 2.764$, $p = 0.598$). Error bars are ± 1 SEM.

No significant effect of illumination condition on the relative LT was found between the five groups (Kruskal-Wallis ANOVA; $\chi^2_{(4)} = 2.764$, $p = 0.598$). Post-hoc power analysis (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) shows that the statistical power of

an ANOVA analysis for relative LT was low at 21%, and that a total sample size of 230 animals (46 per group) would be required for the variation in relative LT by illumination group to reach statistical significance. This weakness in power is illustrated by both the non-normal nature of the data, and the relatively large SEM values associated with the average data for each group. Furthermore, linear regression of relative LT against relative mean sphere refraction for all groups revealed no significant correlation between these variables (R^2 = 0.042, $F_{1,53}$ = 2.303, p = 0.135) (Figure 4.17). Therefore, variation in LT does not appear to contribute in any significant manner to the overall refractive status of the eyes.

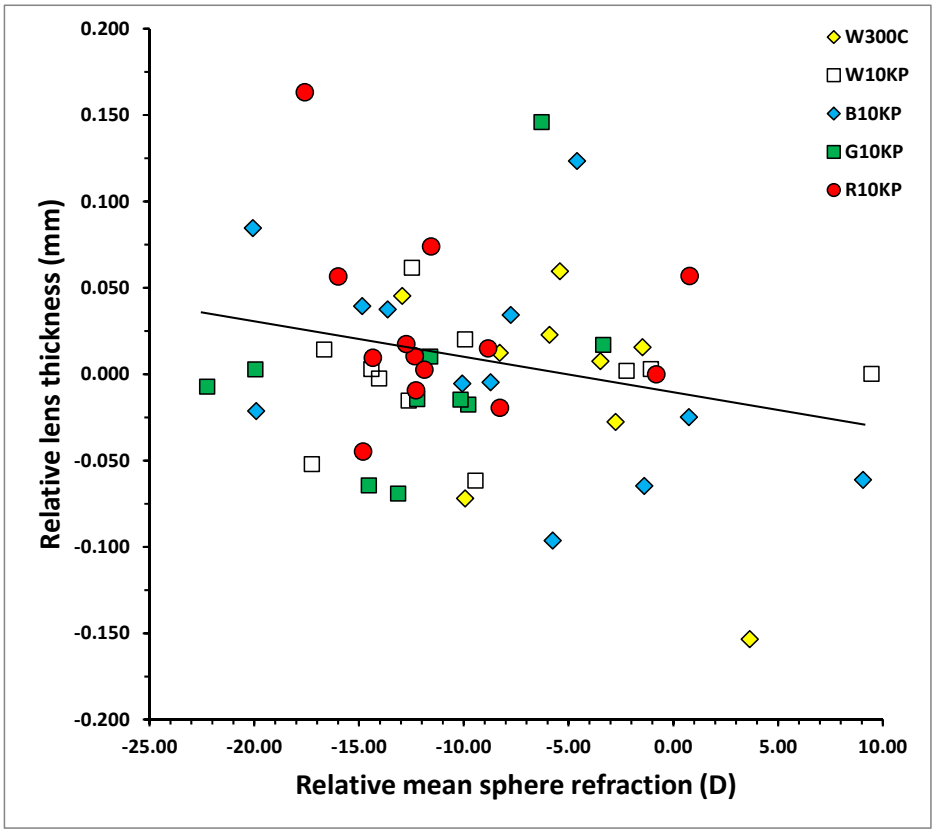

Figure 4.17. Correlation between relative mean sphere refraction and relative lens thickness (deprived eye - non-deprived eye) for all chicks (n = 55): Linear regression revealed no significant correlation between these variables (R^2 = 0.042, p = 0.135). The linear regression line is for the pooled data of all chicks.

Individual Eye Comparison – Absolute Lens Thickness

A non-parametric Wilcoxon Signed Ranks Test was carried out in order to investigate whether there was any significant effect of the illumination differences between the form-deprived and non-derived eyes due to the use of translucent occluders. The outcome of this test (Z = -0.574, p = 0.566) demonstrated that no significant difference exists between the LT of the deprived and non-deprived eyes. The minimal differences in LT between form-deprived and non-form deprived eyes, and between illumination groups is shown in Figure 4.18.

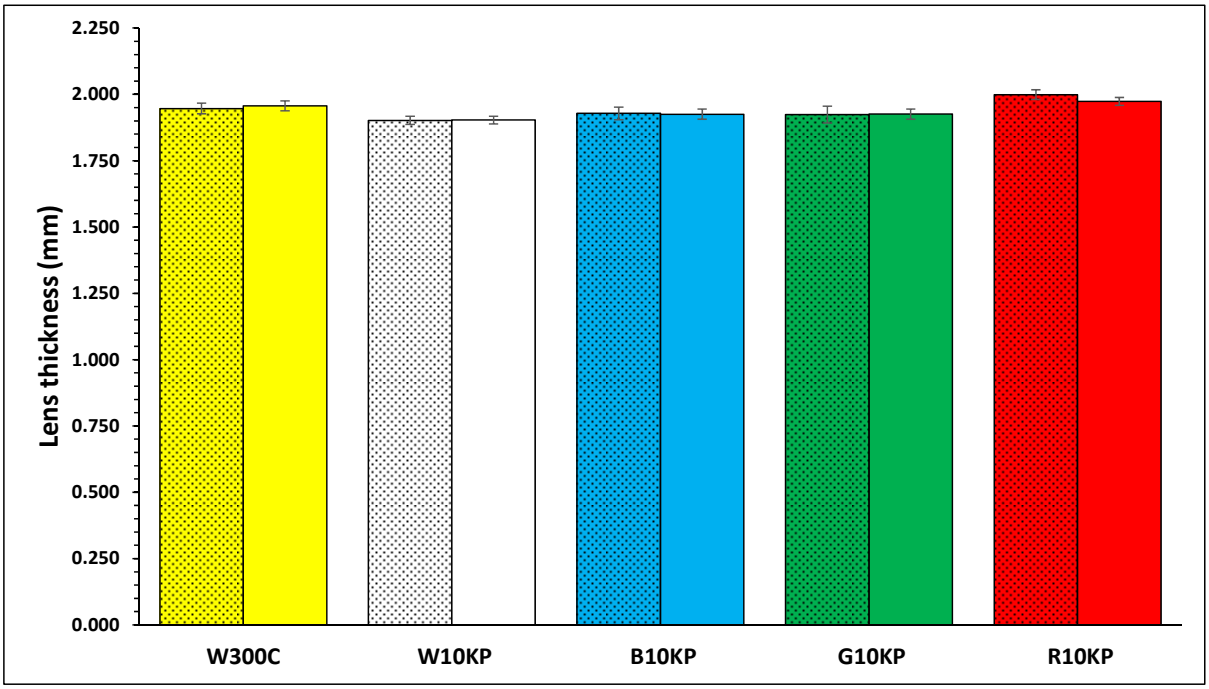

Figure 4.18. Absolute lens thickness for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). No significant difference in LT between deprived and non-deprived eyes (Wilcoxon Signed Ranks Test; Z = -0.574, p = 0.566. Error bars are ± 1 SEM.

The absolute LT outcomes for both the form-deprived and non-form deprived eyes, and relative LT values are summarised in Table 4.5 below.

Illumination Condition	Form-deprived Eyes LT (mm)	Non-deprived Eyes LT (mm)	Difference between Eyes Relative LT (mm)
W300C	+1.947 ± 0.020	+1.957 ± 0.019	-0.010 ± 0.022
W10KP	+1.902 ± 0.016	+1.903 ± 0.014	-0.003 ± 0.010
B10KP	+1.929 ± 0.024	+1.925 ± 0.019	+0.003 ± 0.018
G10KP	+1.924 ± 0.032	+1.925 ± 0.019	-0.001 ± 0.019
R10KP	+1.999 ± 0.019	+1.974 ± 0.015	+0.026 ± 0.015
	p = 0.566*		p = 0.598**

Table 4.5 Lens thickness outcome measures (mean ± 1 SEM) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). *Wilcoxon Signed Ranks Test p-value for comparison of LT between pooled data for form-deprived and non-deprived eyes. **Kruskal-Wallis ANOVA p-value for comparison of average LT by illumination condition.

As (i) no significant effect of illumination group on relative LT; and (ii) no significant effect of form-deprivation on absolute LT, was found, this experiment supports the conclusions of Backhouse *et al* (Backhouse, Collins et al. 2013) who suggested that LT was not influenced by form-deprivation (and

therefore the difference in retinal illumination produced by the use of a translucent occluder), but rather was a product of a non-visually-guided mechanism.

4.3.5 Vitreous Chamber Depth

Paired-Eye Comparison – Relative Vitreous Chamber Depth

Relative vitreous chamber depth (VCD) was calculated as the difference in VCD between the deprived eye and the non-deprived eye for each animal in each illumination group. The average relative VCD for each illumination group is illustrated in Figure 4.19.

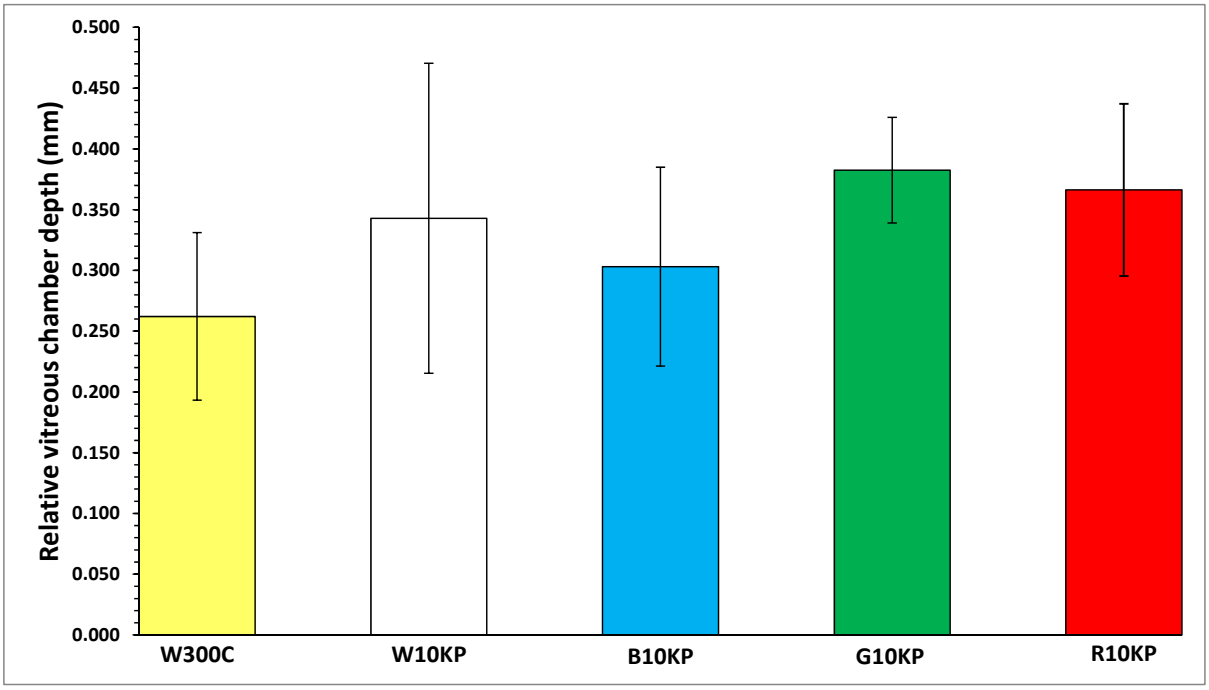

Figure 4.19. Relative vitreous chamber depth (deprived eye - non-deprived eye) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). No significant difference in VCD was found by illumination condition (ANOVA; $F_{(4, 50)} = 0.301$, $p = 0.876$). Error bars are ± 1 SEM.

No significant effect of illumination condition on the relative VCD was found between the five groups (ANOVA; $F_{(4, 50)} = 0.301$, $p = 0.876$). Post-hoc power analysis (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) showed that the statistical power of the ANOVA analysis for relative VCD was very low at 12%, and that a total sample size of 475 animals (95 per group) would be required for the variation in relative VCD by illumination group to reach statistical significance. In order to determine if the weak statistical power of the ANOVA analysis was due to the presence of outliers in the data (as suggested by the relatively large SEM values associated with the data) (Figure 4.19), Grubbs' test for outliers (Grubbs 1950) was carried out using

GraphPad™ QuickCalcs Outlier online calculator (http://www.graphpad.com/quickcalcs, USA). This analysis identified two outliers in the data; one point in the W10KP group (-0.746 mm, Z = 2.575, $p < 0.05$), and one in the R10KP group (-0.300 mm, Z = 2.606, $p < 0.05$). Exclusion of these two data points from the ANOVA analysis did not change the non-significance of the effect of illumination on relative VCD (ANOVA; $F_{(4, 48)} = 1.418$, $p = 0.242$), however the statistical power increased to 44%, while the required total sample size decreased to 110 animals (22 per group). This required sample size is very similar to that calculated for relative Mean Sphere (Section 4.3.1), which supports the assertion that the values identified above were true outliers, and that a sample size of approximately 20 animals per group would have been more appropriate for this experiment than the 12 animals per group initially predicted using the method of Anderson and Vingrys (Anderson and Vingrys 2001).

Although no significant effect of illumination on relative VCD was found, linear regression of this variable against relative mean sphere refraction (pooled data), revealed that increased relative VCD was strongly correlated with increased relative myopic refraction ($R^2 = 0.594$, $F_{1,53} = 77.475$, $p < 0.0001$) (Figure 4.20).

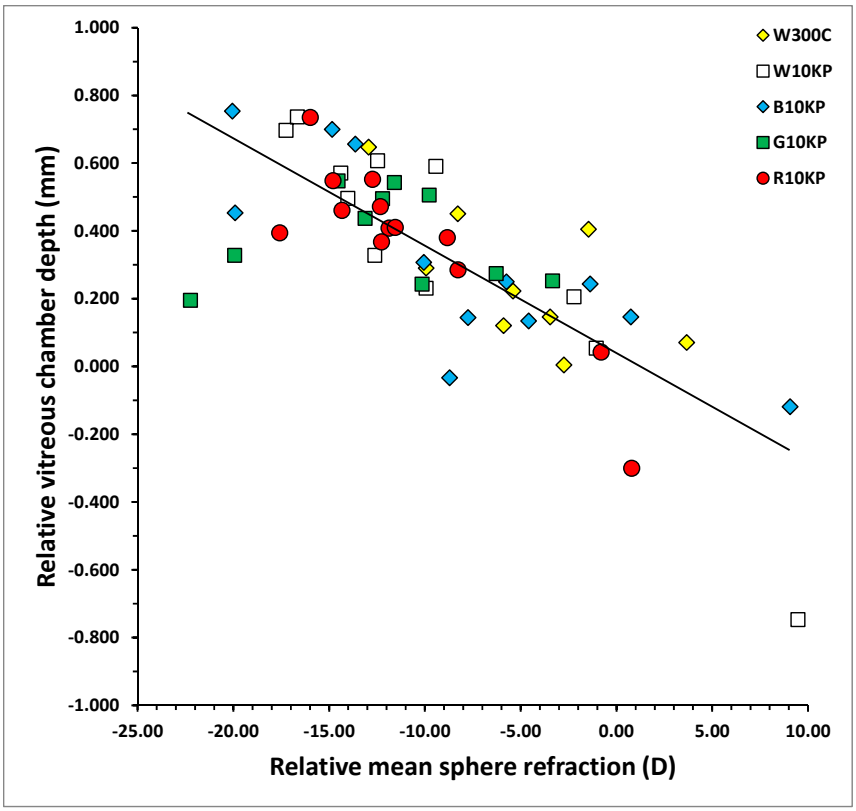

Figure 4.20. Correlation between relative mean sphere refraction and relative vitreous chamber depth (deprived eye - non-deprived eye) for all chicks (n = 55): Linear regression revealed a strong correlation ($R^2 = 0.594$, $p < 0.0001$). The linear regression line is for the pooled data of all chicks.

The strength of the relationship between relative VCD and relative means sphere refraction is investigated further using a Univariate Linear General Model (ULGM) in Section 4.3.6.

Individual Eye Comparison – Absolute Vitreous Chamber Depth

Average absolute VCD was calculated for both the deprived eyes and the non-deprived eyes to investigate whether there was any significant effect of the illumination differences between the eyes due to the use of translucent occluders to produce form-deprivation. Figure 4.21 shows the variation in average absolute VCD for the deprived and non-deprived eyes by illumination group.

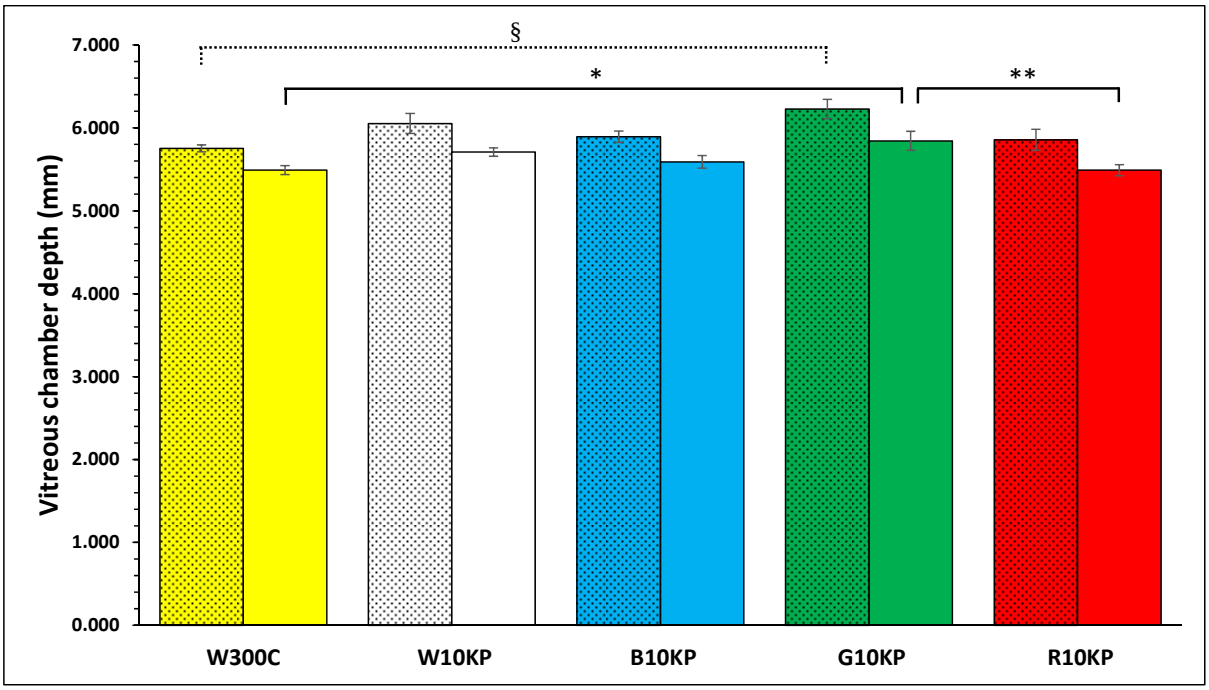

Figure 4.21. Absolute vitreous chamber depth for the deprived (shaded bars) and non-deprived eyes (non-shaded bars) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). Both the deprived eyes and the non-deprived eyes exhibited significant differences in average vitreous chamber depth by illumination condition. Deprived eyes: ANOVA; $F_{(4, 50)} = 2.852$, $p = 0.033$; *Post-hoc* Tukey: *§ = 0.036. Non-deprived eyes: ANOVA; $F_{(4, 50)} = 3.863$, $p = 0.008$; *Post-hoc* Tukey: *$p = 0.028$, **$p = 0.013$. Error bars are ± 1 SEM.

On average, form-deprived eyes exhibited a significantly longer VCD (+5.954 ± 0.050 mm) than non-form-deprived eyes (+5.621 ± 0.037) irrespective of illumination condition (Paired t-test; t = 9.019, $p < 0.0001$). This result is again consistent with that reported previously by Backhouse *et al* (Backhouse, Collins et al. 2013) where VCD was also found to be significantly longer in deprived eyes of the same strain of chicks under all illumination conditions.

A subset of illumination conditions (W300C, G10KP, R10KP) produced significant differences in absolute VCD for both deprived and non-deprived eyes (Figure 4.21). When compared with the W300C group, the G10KP group demonstrated significantly longer VCDs for both deprived (*post-hoc* Tukey; $p = 0.036$) and non-deprived (*post-hoc* Tukey; $p = 0.028$) eyes. The G10KP non-deprived eyes' VCDs were also found to be longer on average than those of the R10KP non-deprived eyes (*post-hoc*

Tukey; p = 0.013). However, these differences in VCD were not reflected in equivalent differences in absolute mean sphere refractive error (Figure 4.8, Table 4.2) for the same illumination groups. As overall axial length (and hence VCD) is a primary contributor to refractive status in chicks (Wallman, Turkel et al. 1978; Wallman and Adams 1987; Schaeffel and Howland 1988), this lack of direct relationship by illumination groups is unexpected and likely reflects the relatively low statistical power of the analyses due to the samples sizes used. However, the two parameters are strongly correlated when pooled data is considered (Figure 4.20).

The absolute VCD measures for both the form-deprived and non-deprived eyes, and relative VCD measures are summarised in Table 4.6 below.

Illumination Condition	Form-deprived Eyes VCD (mm)	Non-deprived Eyes VCD (mm)	Difference between Eyes Relative VCD (mm)
W300C	+5.753 ± 0.042	+5.491 ± 0.053	+0.262 ± 0.069
W10KP	+6.053 ± 0.120	+5.710 ± 0.051	+0.343 ± 0.128
B10KP	+5.894 ± 0.069	+5.591 ± 0.076	+0.303 ± 0.082
G10KP	+6.227 ± 0.116	+5.844 ± 0.115	+0.382 ± 0.043
R10KP	+5.856 ± 0.127	+5.490 ± 0.067	+0.366 ± 0.071
ANOVA	***p* = 0.033**	***p* = 0.008**	p = 0.876

Table 4.6 Vitreous chamber depth outcome measures (mean ± 1 SEM) for each of the 5 illumination conditions: White 300 lux constant (W300C, n = 9), White 10,000 lux periodic (W10KP, n = 11), Blue 10,000 lux equivalent periodic (B10KP, n = 12), Green 10,000 lux equivalent periodic (G10KP, n = 10) and Red 10,000 lux equivalent periodic (R10KP, n = 13). ANOVA p-values for comparison of means by illumination condition are given in the final row of the table.

As noted in Section 4.3.1, this study produced an atypical refraction result for the illumination condition W300C when compared to results from a similar study by Backhouse *et al* (Backhouse, Collins et al. 2013). This divergence may be due to differences in illumination sources, namely the use of fluorescent lighting by Backhouse *et al* versus LED-based lighting in this study. A comparison of relative mean sphere refractions obtained for the nominally-equivalent 300 lux white light conditions in the two studies revealed a significant difference in refractive outcome (Figure 4.9). A similar analysis for VCD results from the two studies (this study and Backhouse *et al*) is illustrated in Figure 4.22.

While there is an apparent trend for the relative VCD to be smaller in this study for the W300C group (+0.262 ± 0.069 mm) than for the 300 lux illumination group from Backhouse *et al* (+0.364 ± 0.047 mm), statistical analysis reveals that these average VCD values are not significantly different (t_{18} = 1.2544, p = 0.2257). However, the trend in average VCD is consistent with the

difference in relative mean sphere refraction established in Section 4.3.1, where a shorter VCD would be expected to be associated with a less myopic refraction.

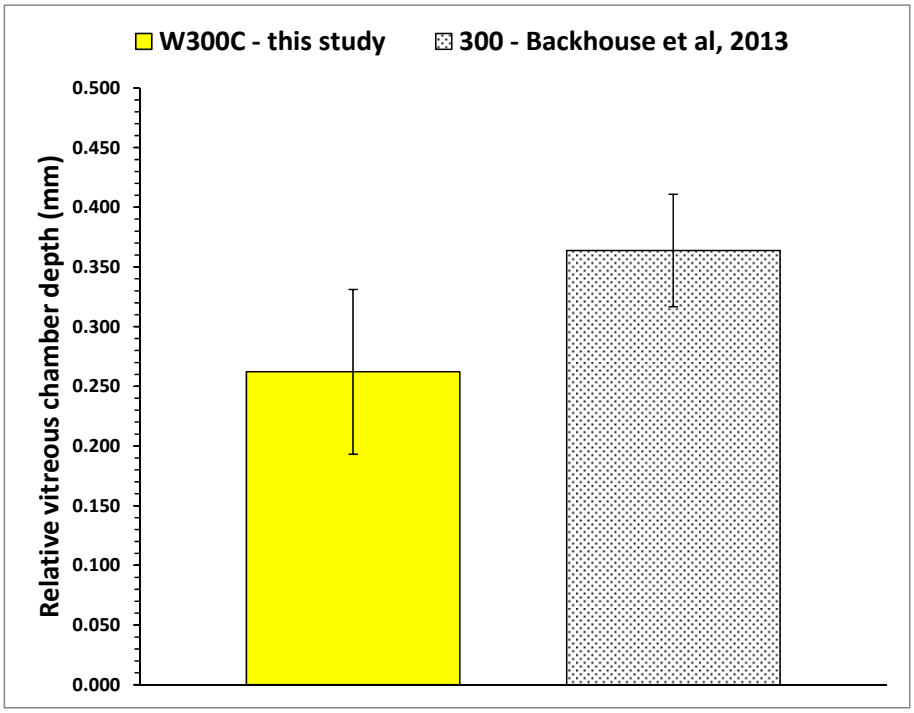

Figure 4.22. A comparison of the relative vitreous chamber depths obtained for Cobb chicks raised with unilateral form-deprivation under either LED-based white illumination of 300 lux (W300C - this study, +0.262 ± 0.069 mm, n = 9), or raised under fluorescent lamp-based white illumination of 300 lux (300, (Backhouse, Collins et al. 2013), +0.364 ± 0.047 mm, n = 11). No significant difference in relative VCD was found between the results. Error bars are ± 1 SEM.

4.3.6 UGLM Analysis of Components of Refraction

Univariate General Linear Model (UGLM) analysis of the data was undertaken in order to estimate the contribution of each biometric component (radius of curvature, anterior chamber depth, lens thickness and vitreous chamber depth) and illumination condition to overall refractive outcome. The illumination group (i.e. W300C, W10KP, B10KP, G10KP and R10KP) was set as a fixed factor, while radius of curvature, ACD, LT and VCD were set as covariates. Mean sphere refraction was set as the dependent variable. The output of the UGLM analysis yielded a measure of statistical significance (F test and associated *p* value) for the model and for each factor, as well as partial η^2 and observed power. Partial η^2 is interpreted as a measure of the effect size (i.e. an estimate of the practical significance) of each component of the model (Richardson 2011). The UGLM analysis was carried out on both the relative (paired-eye) and absolute (individual eye) data.

UGLM - Paired-Eye Analysis

The results of the UGLM analysis of the relative mean sphere refraction and associated relative biometric components by illumination condition are summarised in Table 4.7 below.

Relative Rx	F statistic	Significance (*p*)	partial η^2	Observed Power
Corrected Model	14.256	**< 0.0001**	0.731	1.000
Δ Corneal radius	4.637	**0.037**	0.099	0.557
Δ ACD	8.295	**0.006**	0.165	0.803
Δ LT	3.779	0.059	0.083	0.476
Δ VCD	63.478	**< 0.0001**	0.602	1.000
Illumination	1.916	0.126	0.154	0.530

Table 4.7 Summary of UGLM analysis for relative mean sphere refraction as the dependent variable. The overall model was highly statistically significant ($p < 0.0001$). Relative corneal radius, ACD and VCD were also statistically significant, but with varying sizes of effect. Of the statistically significant components, relative VCD showed the greatest effect size (partial $\eta^2 = 0.602$) on relative mean sphere refraction. Statistically significant p values ($p < 0.05$) in **bold**.

ULGM analysis produced a highly statistically significant model ($p < 0.0001$) with a large effect size (partial $\eta^2 = 0.731$) for relative mean sphere as the dependent variable. The analysis revealed that (in descending order of effect size) VCD, ACD and corneal radius produced statistically significant contributions to the relative mean sphere refraction outcome. While it is common to suggest that the partial η^2 value of 0.602 for VCD represents some 60% of the contribution to the refractive outcome, this is a somewhat erroneous interpretation as the sum of the partial η^2 values can be greater than 1. However, this value does represent a large effect size (Richardson 2011). This analysis is consistent with the strong correlation between relative VCD and relative mean sphere refraction shown in Figure 4.20.

Illumination condition, however, did not show a statistically significant effect on relative mean sphere refraction ($p = 0.126$) in the model. This is consistent with the ANOVA analysis of the effect of illumination group on relative mean sphere refraction illustrated in Figure 4.7. Therefore, it is evident that the various illumination conditions did not have a significant effect on refractive development when considered as a paired-eye (difference in refraction between the eyes: deprived - non-deprived) model. As the translucent occluders used to produce form-deprivation also reduced transmission of incident illumination (Section 2.1.2), the UGLM analysis was also applied to the deprived and non-deprived eyes separately.

UGLM – Individual Eye Analysis

The results of the UGLM analysis of the mean sphere refraction and associated relative biometric components by illumination condition for both deprived and non-deprived eyes are summarised in Tables 4.8 and 4.9 respectively.

Deprived-eyes	F statistic	Significance (*p*)	partial η^2	Observed Power
Corrected Model	9.257	**< 0.0001**	0.638	1.000
Corneal radius	6.875	**0.012**	0.141	0.726
ACD	0.242	0.625	0.006	0.077
LT	7.001	**0.011**	0.143	0.734
VCD	50.842	**< 0.0001**	0.548	1.000
Illumination	1.194	0.327	0.102	0.341

Table 4.8 Summary of UGLM analysis for deprived-eye mean sphere refraction as the dependent variable. The overall model was highly statistically significant ($p < 0.0001$) with a large effect size (partial $\eta^2 = 0.638$). Absolute corneal radius, LT and VCD were also statistically significant, but with varying sizes of effect. Of the statistically significant components, VCD showed the greatest effect size (partial $\eta^2 = 0.548$) on deprived-eye mean sphere refraction. Statistically significant p values (p <0.05) in **bold**.

Non-deprived eyes	F statistic	Significance (*p*)	partial η^2	Observed Power
Corrected Model	5.440	**< 0.0001**	0.486	0.998
Corneal radius	1.101	0.299	0.023	0.177
ACD	18.549	**< 0.0001**	0.287	0.988
LT	0.461	0.500	0.010	0.102
VCD	18.313	**< 0.0001**	0.285	0.987
Illumination	4.851	**0.002**	0.297	0.936

Table 4.9 Summary of UGLM analysis for non-deprived eye mean sphere refraction as the dependent variable. The overall model was highly statistically significant ($p < 0.0001$), although the effect size was less than for the deprived eye model (partial $\eta^2 = 0.486$). Absolute ACD, VCD and Illumination group were also statistically significant. These components had similar effect sizes (partial η^2 range: 0.285 – 0.297) on non-deprived eye mean sphere refraction. Statistically significant p values (p <0.05) in **bold**.

ULGM analysis with deprived-eye mean sphere refraction as the dependent variable, produced a highly statistically significant model ($p < 0.0001$) with a large effect size (partial $\eta^2 = 0.638$) (Table 4.8). The analysis revealed that (in descending order of effect size) VCD, LT and corneal radius produced statistically significant contributions to the deprived-eye mean sphere refraction outcome. While VCD has a large effect size (partial $\eta^2 = 0.548$), the other two significant components of the model; LT and

corneal radius have relatively smaller (but approximately the same) effect size of partial η^2 = 0.143 and partial η^2 = 0.141 respectively. Illumination condition, however, did not show a statistically significant effect on relative mean sphere refraction (p = 0.327) in the model. This is consistent with the ANOVA analysis of the effect of illumination group on deprived-eye absolute mean sphere refraction illustrated in Figure 4.8 and Table 4.2. Therefore, for the deprived eyes, the overall myopic refractive outcome can be explained primarily by variation in VCD.

ULGM analysis with non-deprived eye mean sphere refraction as the dependent variable, also produced a highly statistically significant model (p < 0.0001) but with a relatively smaller, but still strong, effect size (partial η^2 = 0.486) (Table 4.9). The analysis revealed that (in descending order of effect size) Illumination condition, ACD, and VCD produced statistically significant contributions to the deprived-eye mean sphere refraction outcome. Corneal radius and LT were not statistically significant for this model, unlike for the deprived eye model (Table 4.8). The effect sizes of Illumination condition (partial η^2 = 0.297), ACD (partial η^2 = 0.287) and VCD (partial η^2 = 0.285) were similar in magnitude, but still considered to be large according to Cohen's criteria (Richardson 2011). The significant contribution of Illumination condition to the model for non-deprived eye refraction is in contrast with the ANOVA analysis of the effect of illumination group illustrated in Figure 4.8 and Table 4.2. However, this demonstrates the difference in the two forms of analysis where the ANOVA assessed the contribution of illumination condition to refractive outcome in isolation, whereas the ULGM analysis assessed the relative strength of each components' contribution to the refractive outcome. Therefore, for the non-deprived eyes, the overall hyperopic refractive outcome can be explained approximately equally by variation in Illumination condition, ACD and VCD.

In conclusion, the UGLM analysis reveals that in this experiment, under form-deprivation the chick eye's refractive outcome is determined by (in descending effect order): VCD, LT and corneal radius. Whereas for the non-deprived eye (exposed fully to the illumination conditions without attenuation by an occluder) the refractive outcome is determined approximately equally by: Illumination condition, ACD and VCD.

4.4 Discussion: Chick

4.4.1 Refractive Outcome and Illumination Conditions

This study shows that periodic daily exposure of chicks to high intensity white light (10,000 lux), or equivalent intensity blue, green or red LED light, did not produce a significant reduction in the development of form-deprivation myopia when compared to chicks raised under continuous daily exposure to 300 lux white LED illumination (Figure 4.7, Table 4.2). However, this result requires further interpretation as the chicks raised under the 300 lux white LED light condition did not develop as high levels of FDM as have previously been found by the author for the same strain of chicks raised under the same environmental conditions, except for the provision of the 300 lux illumination by ceiling-mounted fluorescent luminaires (Figure 4.9) (Backhouse, Collins et al. 2013). Therefore these results pose several questions: (i) why did continuous exposure to white light at 300 lux result in lower levels of FDM than previously found; (ii) why did periodic exposure to white light at 10,000 lux result in higher levels of FDM than might be expected from similar studies (Ashby, Ohlendorf et al. 2009; Lan, Feldkaemper et al. 2014; Karouta and Ashby 2015); and (iii) was there any demonstrable effect of high intensity coloured light (differing spectral energy distributions) on FDM refractive or biometric outcomes?

As noted above, one potentially significant modification between this study on the effect of high intensity light on FDM in chicks, and the previous study of Backhouse *et al* (Backhouse, Collins et al. 2013) was the use of different lighting sources for both the standard and high intensity lighting conditions in the two studies. For example, Backhouse *et al* used an array of 50 W halogen lamps to provide both the 2000 and 10,000 lux lighting conditions, while the 300 lux standard room illumination was provided by a ceiling mounted fluorescent luminaire fitted with two 28 W fluorescent tubes (Backhouse, Collins et al. 2013). In the current study, both the 300 lux and 10,000 lux white light illumination conditions were provided by an array of LED-based commercial stage lights (see Section 4.2.1). Therefore, non-experimental features of the LED illumination, such as flicker rate and spectral energy distribution, may have influenced the outcomes at both 300 and 10,000 lux. Also, the use of a periodic, low frequency, high illumination paradigm, where 15 minutes of 300 lux illumination was alternated with 15 minutes of high intensity illumination (e.g. 10,000 lux white light) for 12 hours per day (50% duty cycle, 0.00056 Hz, 6 hours cumulative high intensity illumination) differs from previous continuous high intensity diurnal regimes in chick experiments reported to reduce the magnitude of

FDM myopia (Ashby, Ohlendorf et al. 2009) and alter the normal emmetropisation process (Cohen, Belkin et al. 2011).

In comparison, Karouta & Ashby (Karouta and Ashby 2015) recently utilised a white LED-based illumination system in experiments in which they demonstrated a log-linear dose-response between the light intensity and the inhibition of FDM in chicks. Seven days of exposure to 10,000 lux illumination for 6 hours per day was found to produce -3.44 ± 0.07 D of relative myopia, in comparison to -8.02 ± 0.21 D of relative myopia in chicks exposed to 500 lux in a 12:12 hour day:night cycle (Karouta and Ashby 2015). Similar exposure to 40,000 lux illumination for 6 hours per day for 7 days was found to almost entirely suppress FDM resulting in the development of only -0.73 ± 0.20 D of relative myopia (Karouta and Ashby 2015). In contrast, this study resulted in the development of -9.15 ± 2.44 D of relative myopia after 4 days exposure to the W10KP condition (10,000 lux, 50% duty cycle, 6 hours total exposure to high intensity light) (Figure 4.23, Table 4.10). Therefore, at initial consideration, it appears that the periodic nature of the exposure to 10,000 lux in this experiment, rather than the use of continuous exposure for 6 hours as by Karouta & Ashby, resulted in minimal, if any suppression, of FDM. The use of a periodic, low frequency, high illumination paradigm in this study was intended to simulate the intermittent exposure to high (outdoor) light levels that adolescent school-age children are exposed to in their daily activities (Backhouse 2011).

One possible mechanism to explain this lack of suppression of FDM by high intensity illumination, is that the periodic exposure to an alternating sequence of 10,000 lux and 300 lux illumination every 15 minutes interfered with the underlying mechanism(s) suppressing myopia development under high illumination conditions, such as an increase in retinal dopamine levels (see also Chapter 3) (Weiss and Schaeffel 1993; Cohen, Peleg et al. 2012; Feldkaemper and Schaeffel 2013), choroidal thickening (Lan, Feldkaemper et al. 2013), or modification of the ocular circadian rhythms (Nickla 2013; Nickla and Totonelly 2015; Nickla and Totonelly 2016).

Lan *et al* also recently investigated the effect of periodic, low frequency, high intensity light on FDM in chicks (Lan, Feldkaemper et al. 2014). Their study utilised a 50% duty cycle paradigm alternating between 500 lux and 15,000 lux in 60, 30, 15, 7 and 1 minute illumination periods. As the total daylight period was 10 hours, all animals were exposed to 5 hours of high intensity light per day, but at different periodic frequencies. Therefore, the 15:15 minute light cycle of Lan *et al* was similar in design to the high intensity light paradigm used in this study, but 1 hour shorter in total high illumination exposure time (5 vs. 6 hours in this study). However, Lan *et al* used the same illumination sources as described

by Ashby *et al* (Ashby, Ohlendorf et al. 2009), namely ceiling-mounted fluorescent lighting to produce the 500 lux condition, and quartz-halogen lights to produce the 15,000 lux condition. It is worthwhile noting that is a similar experimental lighting setup (room fluorescent lighting plus task halogen lighting) as used by Backhouse *et al* in their chick FDM experiments on periodic exposure to bright lights (Backhouse, Collins et al. 2013).

Thus, exploratory comparisons can be made between the results of this study and the relevant studies of Karouta & Ashby (Karouta and Ashby 2015) (use of LED lighting, 10,000 lux high intensity light condition), and Lan *et al* (Lan, Feldkaemper et al. 2014) (use of periodic, low frequency, high intensity light conditions) to put the apparently anomalous results of this study for the effect of high intensity illumination on FDM in chicks into some context. A comparison of the results with these 3 studies and Backhouse *et al* (Backhouse, Collins et al. 2013) is illustrated in Figure 4.23 and Table 4.10 below.

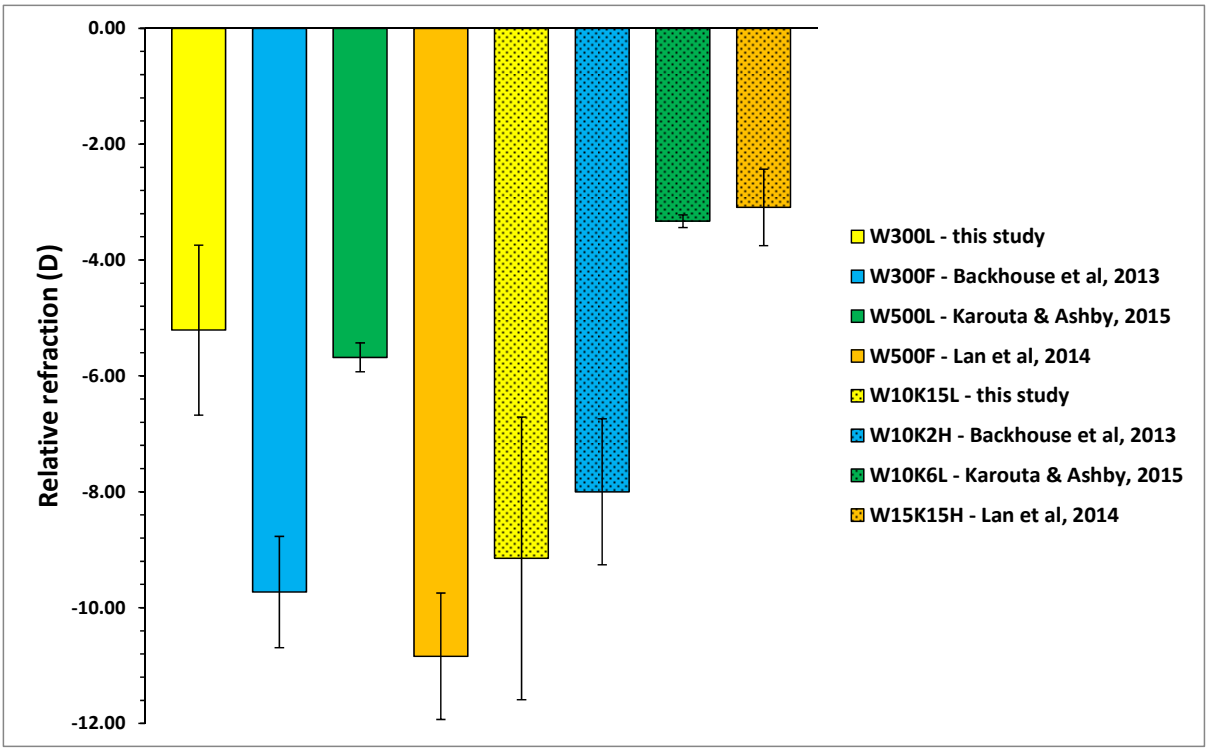

Figure 4.23. Relative refractions (deprived eye - non-deprived eye) for each of the 4 studies listed in Table 4.10: W300L – white 300 lux LED (this study); W300F – while 300 lux fluorescent (Backhouse, Collins et al. 2013); W500L – white 500 lux LED (Karouta and Ashby 2015); W500F – white 500 lux fluorescent (Lan, Feldkaemper et al. 2014); W10K15L – white 10,000 lux LED, 15 minute periodic (this study); W10K2H – White 10,000 lux halogen, 2 hour period (Backhouse, Collins et al. 2013); W10K6L – white 10,000 lux LED, 6 hour period (Karouta and Ashby 2015); W15K15H – white 15,000 lux halogen, 15 minute periodic (Lan, Feldkaemper et al. 2014). Error bars are ± 1 SEM.

Source	Strain of Chick	Months (Season) of Experiment	Lighting Sources	Lighting Paradigm (light phase)	Age at FD (days post-hatch)	Relative Refraction at (X) days of FD	Relative Refraction at 7 days of FD
This study	Cobb*	Jan, Dec (Summer#)	White 300 lux **LED**	Constant 12 hours	4	**-5.21 ± 1.47 D** (4)	NA
Backhouse *et al* (2013)	Cobb*	Dec, Jan, Feb (Summer#)	White 300 lux Fluorescent	Constant 12 hours	4	**-9.73 ± 0.96 D** (4)	NA
Karouta & Ashby (2015)	White Leghorn	Not reported	White 500 lux **LED**	Constant 12 hours	5	**-5.68 ± 0.25 D** (4)	**-8.02 ± 0.21 D**
Lan et al (2014)	White Leghorn	Not reported	White 500 lux Fluorescent	Constant 10 hours	8	**-10.84 ± 1.09 D** (5)	NA
This study	Cobb*	Jan, Aug (Summer/Winter#)	White 300: 10,000 lux **LED**	Periodic 15:15 min for 12 hours	4	**-9.15 ± 2.44 D** (4)	NA
Backhouse *et al* (2013)	Cobb*	Dec, Jan, Feb (Summer#)	White 300: 10,000 lux Fluorescent: Halogen	300 for 5 hours: 10,000 for 2 hours: 500 for 5 hours	4	**-8.00 ± 1.26 D** (4)	NA
Karouta & Ashby (2015)	White Leghorn	Not reported	White 500: 10,000 lux **LED**	500 for 4 hours: 10,000 for 6 hours: 500 for 2 hours	5	**-3.33 ± 0.11 D** (4)	**-3.44 ± 0.07 D**
Lan et al (2014)	White Leghorn	Not reported	White 500: 15,000 lux Fluorescent: Halogen	Periodic 15:15 min for 10 hours	8	**-3.09 ± 0.66 D** (5)	NA

Table 4.10 Comparison of relative refractive outcomes (mean ± 1 SEM) from this study and those of Backhouse *et al* (Backhouse, Collins et al. 2013), Karouta & Ashby (Karouta and Ashby 2015) and Lan *et al* (Lan, Feldkaemper et al. 2014). Yellow shading represents the standard illumination conditions for control groups (300 or 500 lux, LED or Fluorescent lighting). The unshaded cells represent selected high intensity lighting conditions from the various studies. Number in brackets represent days of form-deprivation at measurement. *Same supplier. #Southern hemisphere summer.

Standard, Normal or Background Illumination Conditions

The 300 and 500 lux conditions are often referred to a standard, normal or background illumination levels as they represent the light levels often found within offices or industrial locations such as laboratories (van Bommel and Rouhana 2011). Therefore, such illumination levels have become the *de facto* standard for light and myopia experiments such as the ones presented in Table 4.10 and Figure 4.23. While existing room fluorescent luminaires may be used to provide the standard illumination level for chick FDM experiments, such as by Ashby *et al* (Ashby, Ohlendorf et al. 2009) and Backhouse *et al (Backhouse, Collins et al. 2013)*, the increasing availability of high output LED systems, both commercial (as in this study) or custom-made (Karouta and Ashby 2015) has led to their use in such experiments. However, the spectral energy distribution of white LED lamps may be significantly different to both fluorescent and halogen light sources (van Bommel and Rouhana 2011) and so researchers have taken efforts to match the novel LED lighting systems' spectral output with that of previous systems. For example, Karouta & Ashby report that their custom-made LED system included both cool (400 - 650 nm, 450 nm peak) and warm (430 – 700 nm, 630 nm) LED modules, which produced light with a similar spectral composition to the halogen lamps used to generate high light levels used previously (Ashby, Ohlendorf et al. 2009; Ashby and Schaeffel 2010; Karouta and Ashby 2015).

However, on inspection of Table 4.10 and Figure 4.23 a potential relationship between the type (fluorescent vs. LED) of standard (300 or 500 lux) light source used to produce the illumination condition and the resultant degree of myopia induced by form-deprivation at 4-5 days post-diffuser mounting can be proposed. For example, in this study, 4 days of diffuser wear under a diurnal illumination of 300 lux by white LED lamps resulted in the development of -5.21 ± 1.47 D of relative myopia. However, the same Cobb strain of chicks, raised under similar conditions except for the use of the standard fluorescent luminaire to yield the 300 lux illumination by Backhouse *et al*, produced the significantly different figure of -9.73 ± 1.47 D of relative myopia (Figure 4.9). Therefore, raising form-deprived chicks under LED illumination yielded only 54% of the relative myopia expected given the otherwise similar conditions. As the same diffuser lenses and environmental conditions were used in this study and by Backhouse *et al* (Backhouse, Collins et al. 2013), one potential hypothesis is that some aspect of the LED illumination is sufficiently different from the fluorescent illumination to influence the development of FDM.

In support of the above conclusion is the observation that for the results of Karouta & Ashby (Table 4.10, Figure 4.23) under the 500 lux LED condition, only -5.68 ± 025 D of relative myopia was induced after 4 days. This figure is not significantly different from that found for the 300 lux LED condition in this study (t-test; t = 0.3448, p = 0.7339). Conversely, both studies reported in Table 4.10 that utilised standard room fluorescent illumination produced higher and near equal amounts of relative myopia: Backhouse *et al*, 300 lux, 4 days, -9.73 ± 0.96 D; in comparison to Lan *et al*, 500 lux, 5 days, -10.84 ± 1.09 D. The evidence suggests that the use of white LED-based illumination systems to provide the standard illumination levels of 300 or 500 lux slows the progression of FDM in chicks. For example, 4 days of form-deprivation under standard fluorescent lighting is usually sufficient to induce approximately 10D of relative myopia, while white LED-based illumination reduces the rate of progression by nearly 50%. Further evidence for this conclusion is given by the experiments of Karouta & Ashby where the induced refractive error had increased further to -8.02 ± 0.96 D of relative myopia by day 7 of form-deprivation under 500 lux of LED illumination (Karouta and Ashby 2015). Therefore, while a lower degree of relative myopia was found for this study in the W300C illumination condition, the cause may have been concluding the experiment on day 4 of diffuser wear, as had been typical previously in this laboratory (Backhouse, Collins et al. 2013).

While the hypothesis is advanced above that some aspect of LED-based illumination is responsible for the apparent pattern of a reduction in the degree of FDM developed in both Cobb (Backhouse, Collins et al. 2013) and Leghorn chicks (Lan, Feldkaemper et al. 2014; Karouta and Ashby 2015) when compared with the same strains raised under fluorescent lighting (Figure 4.23 and Table 4.10), a number of other sources of variation must be considered in this comparison.

Although the experiments reported in this study and that of Backhouse *et al* (Backhouse, Collins et al. 2013) were conducted on Cobb chicks provided by the same supplier, the individual experiments were separated by a period of two years. Therefore the two groups of chicks, although of the same strain, may have possessed a different intrinsic susceptibility to the development of FDM as has been demonstrated in the literature (Stone, Lin et al. 1995; Troilo, Li et al. 1995; Schmid and Wildsoet 1996; Guggenheim, Erichsen et al. 2002). As no age-matched normal (non-deprived) cohorts of chicks were included in either this study or that of Backhouse et al (Backhouse, Collins et al. 2013), due to the paired-eye experimental design employed, then no comparison of the normal refractive development of the Cobb chicks between the two time points can be made. Such a comparison would have been useful to provide evidence of whether the baseline refractive development of the Cobb chicks had remained constant over the intervening two-year period. If the baseline refractive development was

stable over this time period, it would have provided indirect evidence that the susceptibility to FDM was also likely to be similar. However, circumstantial evidence that the difference in refractive outcome between chicks raised under LED-based versus fluorescent lighting is not strain-dependent, but rather a property of the illumination is given by the fact that a similar refractive development pattern is seen in Table 4.10 for both the experiments utilising Cobb chicks and those utilising White Leghorn chicks (Lan, Feldkaemper et al. 2014; Karouta and Ashby 2015). For both strains, the chicks raised under standard levels of LED-based illumination developed less FDM than the same strain of chicks raised under standard levels of fluorescent-based illumination (Table 4.10). Therefore, circumstantial evidence exists to support the hypothesis that some aspect of LED-based illumination is responsible for the apparent pattern of a reduction in the degree of FDM developed.

Another potential source of variability in refractive outcome is the possibility of a seasonal effect of susceptibility to form-deprivation reported in the literature (Sivak, Barrie et al. 1989; Schmid and Wildsoet 1996). Seasonal timing of the experiments reported in Table 4.10 are available for this study and Backhouse *et al* (Backhouse, Collins et al. 2013). In both cases, the FD experiments were carried out during the months of December, January and February (although two years apart) which are within the Southern Hemisphere summer season. Therefore, it is unlikely that a purely seasonal effect is responsible for the difference in FDM induced in Cobb chicks under standard illumination levels for this study (LED-based) and that of Backhouse *et al* (fluorescent-based) (Backhouse, Collins et al. 2013).

In conclusion, while accepting that other causes of variation in the refractive outcomes outlined in Table 4.10 must be considered, the hypothesis that some aspect of the LED illumination is sufficiently different from the fluorescent illumination to influence the development of FDM is worthy of further investigation. As such, possible aspects of LED-based illumination that could result in this slowing of myopia induction are discussed later in this chapter

High Intensity Illumination Conditions

High intensity illumination conditions have been shown to reduce the level of myopia produced by form deprivation in chicks (15,000 lux for 6 hours)(Ashby, Ohlendorf et al. 2009), rhesus monkeys (~25,000 lux for 6 hours)(Smith, Hung et al. 2012) and in Tree Shrews (~16,000 lux for ~7.75 hours)(Siegwart, Ward et al. 2012). Halogen light sources have commonly been used in chick FDM experiments (Ashby, Ohlendorf et al. 2009; Backhouse, Collins et al. 2013; Lan, Feldkaemper et al. 2014) to provide the required levels of high intensity illumination, but these have the issue of all incandescent sources of also producing heat (van Bommel and Rouhana 2011). LED-based illumination systems

have the advantage of being able to produce both the standard and high intensities required, while producing minimal heat and so have been used in this study and by Karouta & Ashby (Karouta and Ashby 2015) with chicks, and by Liu et al (Liu, Qian et al. 2011) with guinea pigs.

In this study, high intensity white light (10,000 lux) was produced by commercial LED stage lights that could be dimmed to 300 lux in a 15:15 minute cycle using a computerised control system (Section 4.2.1). As a 12 hour daylight period was used, the chicks were exposed cumulatively to 6 hours of 10,000 lux and 6 hours of 300 lux illumination per day. Table 4.10 shows that a relatively high degree of relative myopia was induced in these animals after 4 days of form-deprivation (-9.15 ± 2.44 D) when compared to the results of Karouta & Ashby (-3.33 ± 0.11 D, 4 days) and Lan *et al* (-3.09 ± 0.66 D, 5 days). Conversely, in Backhouse *et al* the chicks exposed to 10,000 lux for only two hours a day (at midday) also developed -8.09 ± 1.26 D, a similar level of FDM as found in this study.

In contrast to the standard illumination conditions discussed above, there does not appear to be a clear relationship between the light source type (halogen vs. LED) and the degree of relative myopia induced (Table 4.10). Rather the differences in refractive outcome are potentially due to differences in the periodicity and duration of the high intensity light phases. For example, this study delivered 6 hours cumulatively of high intensity (10,000 lux) LED white light, presented in 15 minute periods, alternating with the same period of 300 lux illumination. Karouta and Ashby also provided 6 hours of high intensity (10,000 lux) LED white light, but presented as a continuous 6 hour dose, preceded by 4 hours, and followed by 2 hours, of 500 lux illumination. Although both experiments utilised LED-based sources, and presented high intensity white light for a total of 6 hours out of a 12 hour diurnal period, the chicks in Karouta and Ashby's study developed less than 40% of the relative myopia when compared to this study (Karouta and Ashby 2015). Therefore, it can be hypothesised that continuous exposure to high intensity LED light is more effective at suppression of FDM than low frequency, periodic exposure to high intensity light.

Conversely, Lan *et al* found that intermittent exposure of chicks to high intensity halogen light (15,000 lux) in either 1:1 or 7:7 minute cycles with the standard (500 lux) fluorescent illumination, was more effective at suppressing FDM than either 5 or 10 hours constant exposure to the high light level (Lan, Feldkaemper et al. 2014). The 1:1 minute cycle almost fully suppressed FDM with a relative refraction of -0.47 ± 0.38 D, while the 7:7 minute cycle produced -1.27 ± 0.56 D of relative myopia, at 5 days post-deprivation. In the 15:15 minute paradigm, which is most similar to that employed in this study, an intermediate degree of relative myopia was induced of -3.09 ± 0.66 D (Table 4.10). In comparison, the

15:15 minute paradigm in this study resulted in -9.15 ± 2.44 D of relative myopia, nearly 3 times the amount found by Lan *et al* (Lan, Feldkaemper et al. 2014). Therefore, for high intensity illumination conditions, the continuous exposure protocol of Karouta and Ashby, and the 15:15 minute intermittent exposure paradigm of Lan *et al*, produced similar degrees of relative myopia (approximately -3 D), both about 1/3rd of the myopia induced under 10,000:300 lux 15:15 minute exposure pattern used in this study (Table 4.10, Figure 4.23). Therefore, the question arises as to why the high intensity light protocol used in this study apparently failed to suppress FDM when compared to similar studies? Potential sources of variance in refractive outcome between the studies compared in Table 4.10 such as effect of strain or season have been discussed above, and so the discussion below will concentrate on other aspects of the lighting paradigms that might be responsible for the apparent failure of the high intensity light protocol to suppress FDM in this study.

While the 15:15 minute intermittent exposure protocol of Lan *et al* (Lan, Feldkaemper et al. 2014) is most similar to this study, several differences in the lighting paradigm exist; Lan *et al* utilised a combination of fluorescent and halogen sources, and both phases were of higher illumination than used in this study; 500 lux and 15,000 lux respectively. Previous studies show little difference in the degree of relative myopia induced between fluorescent illumination at 300 lux (Backhouse, Collins et al. 2013) and 500 lux (Ashby, Ohlendorf et al. 2009; Lan, Feldkaemper et al. 2014), while this study and Karouta & Ashby (Karouta and Ashby 2015) show a reduction in relative myopia induced at 4 days under white LED illumination (Table 4.10). Therefore, the standard light phases of these experiments may not have equivalent effects on induction of FDM in chicks due to differences in the sources (LED vs. fluorescent) used.

When considering the high intensity phase of the cycle, a comparison of the results of Karouta & Ashby (10,000 lux, LED source) with Lan *et al* (15,000 lux, halogen source) reveals little difference in the degree of suppression of FDM with relative myopic refractions of -3.33 ± 0.11 D and -3.09 ± 0.66 D respectively (Table 4.10, Figure 4.23). Therefore, it seems less likely that it was either the type of source (LED) or intensity of illumination (10,000 lux) that resulted in less suppression of FDM under high intensity light in this study when compared to other similar studies.

This study presented the periodic high intensity light cycle for the entire 12 hour diurnal period (6am – 6pm), apart from sunrise and sunset phases where the illumination ramped up to (or down from) 300 lux over a 15 minute period. One potential issue related to the chicks being exposed to periodic high intensity light from morning to evening is that Backhouse *et al* have shown a weak time of day effect of

two hours of exposure to high intensity light on FDM in chicks (Backhouse, Collins et al. 2013). For example, while two hours of 10,000 lux halogen illumination did not produce a significant reduction in FDM when compared to the 300 lux standard illumination control group, presentation of the two hour period in the evening (4pm – 6pm) produced significantly more relative myopia (-13.33 ± 1.16 D) than at midday (-8.00 ± 1.26 D) (Backhouse, Collins et al. 2013). Therefore, the continued presentation of the periodic high intensity illumination phase into the late afternoon/early evening in this experiment may have resulted in more relative myopia developing than if a "wash-out" period of standard lighting had been provided before the dark phase of the diurnal cycle.

An ancillary investigation was carried out to examine the effect of continuous high intensity light exposure using the white LED-based lighting system described in Section 4.2.1 on the time course of development of FDM in Cobb chicks. Chicks were raised under either 300 lux constant daily illumination for 12 hours per day, or under an additional period of 15,000 lux high intensity illumination for 6 hours in the middle of the day. The results of this ancillary experiment are detailed in Appendix A4. In particular, the time course of any potential inhibition of FDM development was assessed by taking outcome measurements at both 5 days and 10 days post-deprivation (9 and 14 days post-hatch respectively). In summary, there was an apparent trend for FDM to develop more slowly under the 15,000 lux condition (Figure A4.2), however this effect did not quite reach statistical significance ($p = 0.059$)(Table A4.1) most likely due to the relatively small sample size of 6 animals per measurement cohort. As expected, the degree of relative FDM developed by all cohorts was highly correlated with an increase in VCD ($p < 0.0001$)(Figure A4.4). Notably, a statistically significant effect of illumination condition on overall axial length (relative AXL) with time was found (Table A4.3), where a significant increase in relative AXL between 5 and 10 days of FD occurred for the chicks raised under 300 lux condition, but not for the chicks raised under the 15,000 lux condition.

Therefore, the 15,000 lux lighting condition, where the high intensity illumination was provided by white LED light sources for 6 out of 12 hours per day, was sufficient to suppress axial growth induced by FD, when compared to the 300 lux lighting condition. This finding validates both the Cobb-strain chick as a suitable FD model of myopia development, and the white LED-based lighting system as capable of suppressing the axial elongation associated with FD at sufficient intensity levels and duration of exposure. However, it does raise the possibility that the lack of significant difference in the degree of FDM induced under the periodic high intensity lighting conditions in the chick-model element of this study (i.e. Chapter 4) is that the effect is not apparent until more than 5 days of deprivation has occurred, whereas outcome measures in Chapter 4 were made at 4 days post-deprivation. This

outcome measurement point was originally chosen to be consistent with previous chick (Backhouse, Collins et al. 2013) FDM experiments reported from this laboratory, and one day less than the initial report in 2009 of the suppression of FDM by high intensity light by Ashby et al (Ashby, Ohlendorf et al. 2009). Therefore, future experiments into the effect of the high intensity illumination on FDM in chicks using the LED-based lighting system should be extended out to at least 7 to 10 days post-form deprivation, as utilised more recently by Karouta and Ashby (Karouta and Ashby 2015) in their investigation of the correlation between light levels and deprivation myopia in chicks.

Previous studies which have demonstrated suppression of FDM (Ashby, Ohlendorf et al. 2009; Karouta and Ashby 2015) and partial suppression or slowing of lens-induced myopia (LIM) (Ashby and Schaeffel 2010) using high illumination, have allowed for at least a two hour period of standard intensity light at the end of the diurnal light cycle. The apparent lack of suppression of FDM in this study suggests that a period of standard illumination following high intensity light exposure may be necessary for the suppression effect to predominate. For example, Backhouse *et al* suggest that high intensity light exposure in the evening may disrupt the emmetropisation process, resulting in increased development of FDM (Backhouse, Collins et al. 2013).

Form-deprivation has been shown to disrupt the circadian pattern of normal eye growth where the growth phase of the eye takes place during the day, with cessation or even reduction of growth at night. This disruption results in axial elongation and development of myopia, due to a loss of growth inhibition at night (Weiss and Schaeffel 1993; Nickla, Wildsoet et al. 2001; Nickla 2006; Nickla 2013). While high intensity light during the middle of the day may help restore the normal growth cycle, thereby suppressing FDM, bright light exposure in the evening may negate this effect. The possible mechanisms by which bright light exposure might suppress myopia development due to form deprivation include an increase in retinal dopamine (as reviewed by Feldkaemper and Schaeffel (Feldkaemper and Schaeffel 2013), also (Lan, Yang et al. 2016)), an entrainment or enhancement of circadian ocular growth rhythms (as reviewed by Nickla (Nickla 2013)), and through induction of choroidal thickening (Lan, Feldkaemper et al. 2013). These three mechanisms are not mutually exclusive, and may represent a triumvirate of linked responses that combine to reduce excessive axial elongation of the eye.

Retinal dopamine levels are reduced during the development of FDM in chicks (Stone, Lin et al. 1989; Weiss and Schaeffel 1993; Lan, Yang et al. 2016) and the rhesus monkey (Iuvone, Tigges et al. 1989); while the introduction of dopamine agonists and precursors into the eye inhibits the development of

FDM in chicks (Stone, Lin et al. 1989; Rohrer, Spira et al. 1993; Schmid and Wildsoet 2004; Nickla, Totonelly et al. 2010), guinea pigs (Mao, Liu et al. 2010) and rhesus monkeys (Iuvone, Tigges et al. 1991). Furthermore, the intraperitoneal injection of Citicoline, a drug known to stimulate dopamine production within the CNS, reduces the development of FDM in the guinea pig, while raising retinal dopamine levels (Mao, Liu et al. 2016). Therefore, an environmental intervention which raises retinal dopamine levels is also likely to suppress FDM.

High intensity light provides such a possible environmental intervention as (i) there is a positive log-linear relationship between illumination levels and retinal dopamine release in chicks (Megaw, Boelen et al. 2006; Cohen, Peleg et al. 2012) and (ii) a negative (suppressing) log-linear relationship with relative myopia due to form-deprivation in chicks (Karouta and Ashby 2015). Furthermore, light exposure during the night phase of a diurnal light cycle causes a rapid rise in ocular dopamine to daytime levels in the pigeon (Adachi, Nogi et al. 1998). Lan *et al* (Lan, Yang et al. 2016) have recently demonstrated the linkage between these two factors as high illumination levels (15,000 lux) both inhibit the development of FDM in chicks, while simultaneously reducing the decrease in vitreal DOPAC levels (-28.8% decrease with 15,000 lux vs -36.7% decrease with 500 lux) produced by form-deprivation. This gives further support to the hypothesis that the anti-myopiagenic effects of bright (or outdoor) light are mediated by a relative increase in retinal dopamine (Rose, Morgan et al. 2008; Norton and Siegwart 2013) (see also Section 3.4.3 for further discussion of the role of dopamine as an ocular growth mediator).

The choroid also is implicated in the control of eye growth and hence emmetropisation (Nickla and Wallman 2009) (Summers 2013). Rapid changes in the choroid can be induced in chicks using optical lenses to produce either (i) hyperopic defocus (image beyond the retina) which results in thinning of the choroid or (ii) myopic defocus (image in front of the retina) which results in choroidal thickening (Wallman, Wildsoet et al. 1995; Wildsoet and Wallman 1995). Similar defocus-induced choroidal thickness changes have been demonstrated in the tree shrew (Siegwart and Norton 1998), primates (Hung, Wallman et al. 2000; Troilo, Nickla et al. 2000), guinea pigs (Howlett and McFadden 2009) and humans (Read, Collins et al. 2010; Chiang, Phillips et al. 2015). Changes in choroidal thickness are thought to represent an initial rapid emmetropisation response to optical defocus (and form-deprivation) which attempts to move the retina back to the plane of the image (Zhu, Park et al. 2005), followed in turn by longer-term scleral growth modification (Guggenheim and McBrien 1996; Rada, Nickla et al. 2000).

A link between retinal dopamine, choroidal thickening and ocular growth regulation is given by the work of Nickla *et al* (Nickla, Totonelly et al. 2010) who demonstrated that dopaminergic agonists inhibited the development of negative lens-induced myopia in chicks, while also producing transient thickening of the choroid. Furthermore, Lan *et al* (Lan, Feldkaemper et al. 2013) have demonstrated that bright light (15,000 lux) produces choroidal thickening in chicks, completing the triumvirate of responses. However, the choroidal response in chicks to bright light was shown to have two components, a short term and a long term effect. The short term effect of 6 hours exposure to bright light was to produce an initial thinning of the choroid of approximately -5.2%, followed by a rebound effect where the choroid thickened on average by some +17.8% following 2 hours of 500 lux illumination and 2 hours of darkness. The long-term effect of 5 days exposure to daily bright light was choroidal thickening of +7.6 ± 26.0 % (8 out of 12 chicks showed thickening), while the control group exposed to 500 lux illumination showed thinning of -18.6 ± 26.0 % (Lan, Feldkaemper et al. 2013).

In chicks, initial choroidal thickness predicts ocular growth under normal visual conditions, where chicks with thinner choroids showed faster growth than those with thicker choroids. However, induction of experimental myopia by either form-deprivation or lenses appears to decouple the association of between choroidal thickness and growth rate (Nickla and Totonelly 2015), again suggesting a role for the choroid in modulation of ocular growth. Finally, recent research in chicks shows that two hours of exposure to bright light at night disrupts the normal diurnal cycle changes in axial length and choroidal thickness (so that they could no longer be fitted to a sinusoidal function with a period of 24 hours) and produced an acute transient period of ocular growth during the following 6 hours of darkness (Nickla and Totonelly 2016).

In summary, the fact that the chicks in this study were exposed to periodic high intensity light for the entire daylight phase into the early evening (6am – 6pm, 12 hours total, 6 hours cumulative high intensity light exposure) may have inhibited the usually anti-myopiagenic effect of high intensity light, by stopping the bright light from re-establishing the normal diurnal rhythms of dopamine and choroidal thickness changes within the eye. This speculation is supported by the demonstration of Backhouse *et al* of a time of day effect of high intensity light exposure in the evening on suppression of FDM in chicks (Backhouse, Collins et al. 2013).

4.4.2 LED Sources and Form Deprivation Myopia

As noted earlier in this discussion, this experiment found that daily exposure of chicks to periodic high intensity LED-based white light (10,000 lux) did not produce a significant reduction in the development of relative myopia due to form-deprivation, when compared to chicks raised under continuous daily exposure to standard room illumination levels (300 lux)(Figure 4.7, Table 4.2). Moreover, chicks raised under the 300 lux white LED light condition in this study did not develop as high levels of FDM as have previously been found by the author for the same strain of chicks raised under the same environmental conditions, apart from the provision of the 300 lux illumination by ceiling-mounted fluorescent luminaires (Figure 4.9) (Backhouse, Collins et al. 2013). Interestingly, the form-deprived chicks raised by Karouta & Ashby (Karouta and Ashby 2015) under 500 lux LED light, also developed only -5.68 ± 025 D of relative myopia after 4 days (Table 4.10, Figure 4.23). This figure is not significantly different from the -5.21 ± 1.47 D of relative myopia found for the 300 lux LED condition in this study (t-test; t = 0.3448, p = 0.7339). Moreover, by day 7, the chicks in Karouta & Ashby's study developed -8.02 ± 0.21 D of relative myopia. This suggests that the use of white LED-based lighting systems to provide the standard illumination levels of 300 or 500 lux slows the progression of FDM in chicks.

In summary, while 4 days of form-deprivation under standard fluorescent lighting is usually sufficient to induce approximately 10D of relative myopia, white LED-based illumination appears to have reduced the rate of progression by nearly 50%. Although a lower degree of relative myopia was found for this study under the 300 lux white LED-based illumination condition than expected, the cause may have been the result of concluding the experiment on day 4 of diffuser wear (as had been typical previously in this laboratory (Backhouse, Collins et al. 2013)) due to a possible slowing of induction of myopia by the use of LED-based illumination. The question arises as to what differences exist between these fluorescent and LED light sources that might account for the apparent difference in rate of myopia development. The aspects of the sources considered in more detail below are flicker, spectral energy distribution, and spectral sensitivity of chicks.

LED Sources - High Frequency Flicker

The 300 lux standard lighting condition used in this study was produced by dimming the array of commercial LED-based stage lights (QTX SL-Q8, http://avslgroup.com/en/about/qtx) via a computer-controlled DMX interface (using setting DMX 8)(Section 4.2.1). As the standard method for dimming LEDs is by pulse-width modulation (PWM) of an underlying square-wave voltage cycle (van Bommel

and Rouhana 2011), then the potentially confounding factor of flicker must be considered when analysing the outcome of the 300 lux lighting condition of this experiment.

Stroboscopic illumination has been shown to suppress both form-deprivation and lens-induced myopia in chicks (Gottlieb and Wallman 1987; Schwahn and Schaeffel 1997). Schwahn and Schaeffel investigated the effect of both the frequency (6 & 12 Hz) and duty cycle (4 – 75 %) of flickering light on form-deprivation and defocus-induced myopia. The extent of suppression of form-deprivation myopia was dependent on the length of the dark phase of the duty cycle, with statistically significant suppression occurring under 6 Hz flicker at both 25% and 50%; and at 12 Hz flicker at 4%, 12% and 50% duty cycles, but not at a 75% duty cycle. The 12 Hz flicker at 4% and 12% duty cycles produced a greater suppression effect than at the 50% duty cycle (Schwahn and Schaeffel 1997).

Therefore, as the degree of induced relative myopia produced in this experiment under 300 lux LED illumination was significantly less than produced by Backhouse *et al* (Backhouse, Collins et al. 2013) under fluorescent lighting for chicks under otherwise similar experimental conditions (Figure 4.9, Table 4.10) the flicker rate and duty cycle of the stage lights were investigated to see if there could have been unintended flicker-based suppression of FDM in this study.

The output profile of a QTX SL-Q8 stage light was measured at the School of Engineering & Advanced Technology, Massey University (Albany, Auckland) using a Sensing SL300 Spectroradiometer attached to a SPR-600M Photometric Sphere (Sensing Optronics Co., China, http://www.sensingm.com). The output waveform was visualised using a Keysight 1000B Series Oscilloscope (Keysight Technologies Inc, USA, http://www.keysight.com). Light output waveform frequency and duty cycle values were calculated from the raw data file of the oscilloscope, and confirmed by pixel analysis of the digital screen capture images (Figure 4.24) using ImageJ software (Version 1.50b, National Institute of Health, USA; http://rsb.info.nih.gov/ij/). Table 4.11 shows the peak to peak frequency and % duty cycle of the light output waveforms at a range of dimmer (DMX) settings with associated luminous flux measurements.

Figure 4.24 illustrates the output waveform recorded at a DMX 8 setting for the white LED components of the light fitting. The waveform has a triangular profile at this DMX setting and so the duty cycle was measured at the half-amplitude height. At higher DMX settings (e.g. 128) the waveform more closely resembled a square-wave. The amplitude of the waveforms was normalised on acquisition and so no direct measurement of waveform amplitude changes with DMX settings are available, however the total luminous flux was recorded simultaneously by the spectroradiometer. At DMX 8 (29.9 lumens) the

waveform had a frequency of 3905 Hz and an ON duty cycle of approximately 10%. Doubling the DMX setting to 16 doubled the frequency and doubled the ON duty cycle to 21%. The luminous flux only increased by a factor of 1.6 times. Further doubling of the DMX setting did not change the frequency or duty cycle of the output waveform until DMX 128 (ON duty cycle 32%). At DMX 255 the output was a constant DC signal (i.e. there was no PWM). As there was no change in frequency or duty cycle between DMX 16 and DMX64, the measured increase in luminous flux must be due to an (unmeasured) increase in amplitude of the waveform.

DMX Setting	Luminous Flux (lumens)	P-P frequency (Hz)	%ON	%OFF
8	29.9	**3905**	**10**	90
16	47.5	**7810**	**21**	79
32	81.6	**7810**	**21**	79
64	150.1	**7810**	**21**	79
128	292.8	**7810**	**32**	68
255	563.6	**DC**	**100**	0

Table 4.11 Summary of light output waveform peak to peak frequency and % ON and OFF duty cycle components for a range of DMX settings. DMX 8 was used for the 300 lux illumination condition. DMX 255 was used for the 10,000 lux condition. The frequency and ON duty cycle doubled between DMX 8 and 16. At DMX 255 the output was a constant DC signal. The luminous flux measurements were made simultaneously.

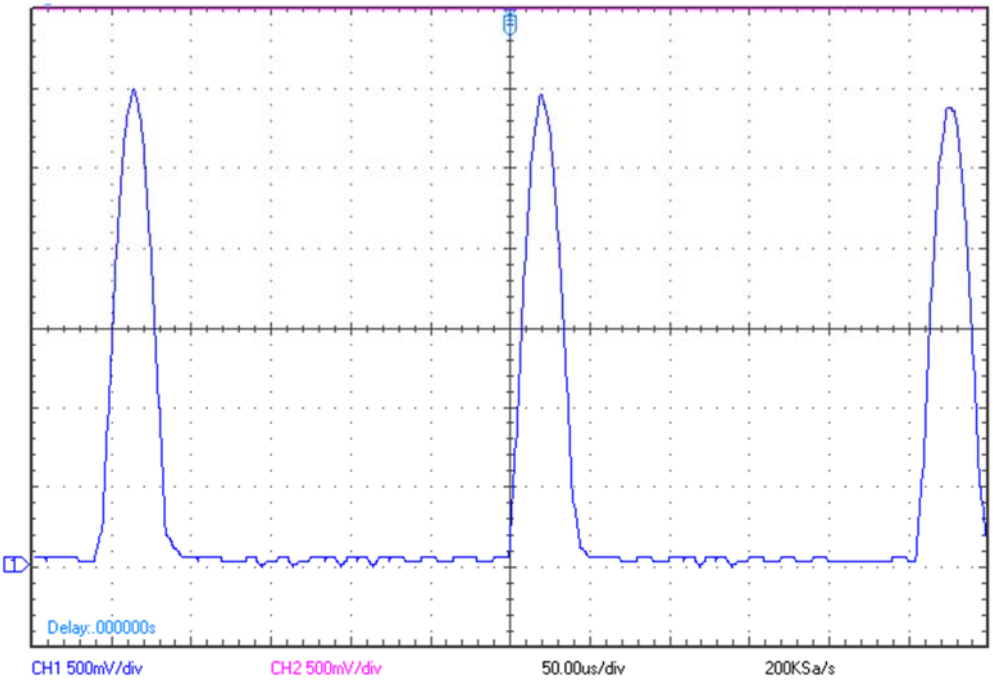

Figure 4.24. A screen capture of the triangular light output waveform of QTX-SL8 stage lamp recorded by the digital oscilloscope for white LED output setting DMX 8 (luminous flux 29.9 lumens). The peaks represent the ON phase of the duty cycle. The peak to peak frequency is 3905 Hz. At amplitude half-height the duty cycle is 10% ON: 90% OFF. The amplitude of the waveform was normalised during recording. Time base is 50µs/div.

The analysis of the QTX-SL8 white LED light output at the DMX 8 setting revealed an unexpectedly high modulation frequency for the output waveform. Typical commercial LED control gear employs a modulation frequency of 150 – 400 Hz (van Bommel and Rouhana 2011), while the 3905 Hz frequency measured for a DMX 8 setting is some 10 times higher. The high modulation frequency was confirmed independently using a Spectrascan PR655 spectrophotometer (PhotoResearch, USA, http://www.photoresearch.com), set to synchronise with the modulation rate of the source. Both methods confirmed a modulation frequency of approximately 3900 Hz at DMX 8. The high modulation rate may be used in these units as they are designed as stage lights and any stroboscopic effect produced by movement on stage would be unacceptable.

Considering the potential effect of flicker on the induction of form-deprivation myopia in this study, the high modulation frequency of 3905 Hz is several orders of magnitude higher than those found to suppress myopia development (6 – 12 Hz) in previous chick studies (Gottlieb and Wallman 1987; Schwahn and Schaeffel 1997). However, the 10% ON duty cycle of the light modulation is comparable to the 4% and 12% duty cycles (at 12 Hz) stroboscopic illumination which produced the greatest suppression of FDM previously (Schwahn and Schaeffel 1997). At 3905 Hz and a 10% ON duty cycle, the time period of the OFF phase is only in the order of 225 μs, and therefore the chick retina would require a very short temporal integration period in order to detect the length of this phase. The critical flicker frequency (CFF) of chicks has been estimated to be approximately 90 Hz by behavioural methods (Lisney, Rubene et al. 2011) and 105 Hz by electroretinogram (Lisney, Ekesten et al. 2012). The retinal CFF of 105 Hz is equivalent to a periodicity of 9.5 ms, suggesting that the chick retina has insufficient temporal resolution to detect the length of the OFF duty cycle phase at a modulation frequency of 3900 Hz. Therefore, it is unlikely that either the modulation frequency or duty cycle of the LED light at 300 lux (DMX 8) will have produced suppression of the development of FDM in this study, when compared to the results of Backhouse *et al* (Backhouse, Collins et al. 2013).

An alternative explanation for the difference in refractive outcome under 300 lux between the two studies could be that fluorescent lighting exacerbates the development of FDM in chicks. As modern fluorescent luminaires use electronic ballasts, the flicker frequency of fluorescent lamps such as the Philips TL5 used in the experiments of Backhouse *et al* (Backhouse, Collins et al. 2013) would be in the range of 25 – 125 kHz (van Bommel and Rouhana 2011) and well beyond the temporal resolution of the chick retina as discussed above. When the modulation frequency of the current fluorescent lamps installed in the laboratory used by both this study and Backhouse *et al* was assessed using a

Spectrascan PR655 spectrophotometer (PhotoResearch, USA, http://www.photoresearch.com), the device was unable to synchronise with the very high frequency of the lamps and gave a DC signal reading. Therefore, it appears unlikely that the flicker rate/modulation frequency of modern fluorescent lamps with electronic ballasts would have any effect on the induction of FDM in chicks as it would be orders of magnitude above the chick CFF. However, it is of interest to note that older (pre-1990s) fluorescent lamps with conventional electromagnetic ballasts flicker at double the mains frequency (e.g. 100 Hz in New Zealand and other countries with 50 Hz mains power frequency) (van Bommel and Rouhana 2011), and so chicks may be able to detect the flicker of older fluorescent luminaires.

LED Sources - Spectral Energy Distribution

When considering possible explanations for why differing degrees of form-deprivation myopia might be induced under white LED versus white fluorescent sources at similar illuminances, the spectral energy distributions of these sources needs to be considered. The spectra of white LED and fluorescent sources would be expected to be quite dissimilar as the light is generated by different mechanisms (van Bommel and Rouhana 2011).

Fluorescent lamps are low pressure mercury gas discharge lamps, which produce visible light through the excitation of a phosphor coating on the inside of the glass tube by the UV radiation generated by excitation of the mercury gas. The resulting visible white light can be tuned to various colour temperatures (i.e. cool-white versus warm-white) by altering the composition of the phosphor coating on the inner wall of the fluorescent tube. Standard fluorescent tubes with a moderate colour-rendering index of 80-90 typically have tri-phosphor coatings, with each phosphor having a distinct emission peak in the red, green and blue part of the visible spectrum (van Bommel and Rouhana 2011).

LEDs produce light by the passage of electrons (current) between two types of semiconductor material under the influence of a voltage difference. As the electrons move from the donor n-type to the recipient p-type semiconductor, they drop in energy state and therefore produce photons. The magnitude of the difference in energy state, and hence wavelength of emitted light, is dependent on the combination of semiconductor materials used in the LED construction. Combinations of aluminium, indium, gallium and phosphide are used to produce amber to red LEDs, while indium, gallium and nitride produce blue to green LEDs. White light emitting LEDs can be manufactured by combining red, blue and green LEDs in one unit to produce white light through additive colour mixing, or by adding an appropriate phosphor coating to a blue LED (as in this study, Figure 4.25) (van Bommel and Rouhana 2011).

Figure 4.25 illustrates the emission spectra of the commercial QTX-SL8 white LED source used in this study, and the standard room fluorescent luminaire fitted in the laboratory where both the experiments of this study and Backhouse *et al* (Backhouse, Collins et al. 2013) were conducted. The spectral irradiance measurements were taken at the same plane at which a standard lux meter (Lutron LX-105) gave a reading of 300 lux for each source. The luminaire contained two Philips TL5 HE 28W/840 tubes, which have a nominal colour temperature of 4100K and a nominal colour rendering index of 82 (Philips-Lighting 2015).

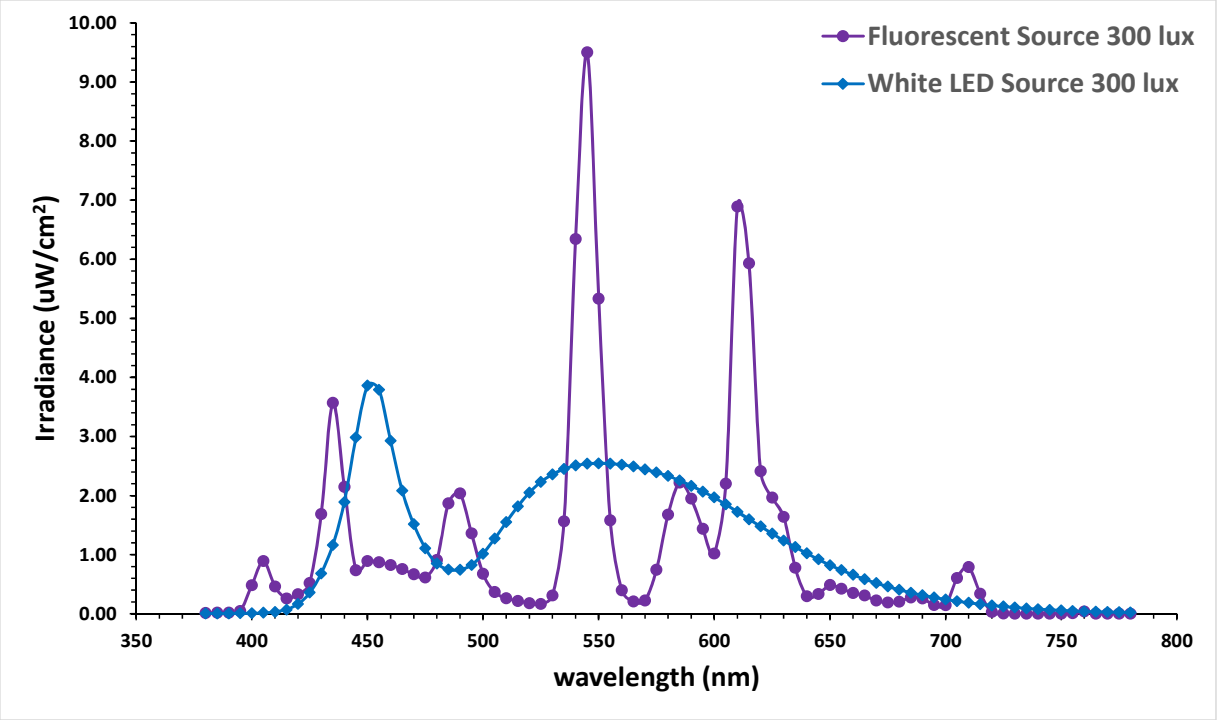

Figure 4.25. Spectral irradiance distributions (μW/cm^2) of the QTX-SL8 white LED source and Philips TL5 HE 28W/840 fluorescent source measured at 300 lux illuminance. The white LED demonstrates the typical bimodal spectral profile produced by a blue LED (peak at ~450 nm) with associated phosphor coating giving a broader peak at ~550 nm. The fluorescent source also shows the typical spectral profile of a tri-phosphor lamp. The measured colour temperatures of the LED and fluorescent sources were 5800 K and 4000 K respectively. Spectral irradiance tended to zero for both sources at the UV (380 nm) and IR (780 nm) ends of the human visible spectrum.

Figure 4.25 demonstrates a number of apparent differences between the spectral profiles of the LED and fluorescent sources. The white LED spectrum exhibits two distinct peaks, a narrow peak at approximately 450 nm, and a relatively broader and shallower peak at approximately 550 nm. The short wavelength peak represents the emission of the underlying blue LED, while the broader peak which spans around 490-720 nm represents the output of the phosphor coating of the LED. The colour temperature of the resulting white light was measured as 5800 K, which is defined as cool white (van Bommel and Rouhana 2011).

The fluorescent source exhibits the typical spectral profile of a tri-phosphor lamp with three main peaks at ~435 nm (blue), ~545 nm (green) and ~610 nm (red). Each peak is relatively narrow and the overall spectral profile is more discontinuous than the LED source, with smaller secondary peaks at ~490 nm and ~585 nm in particular. The colour temperature of the lamp was measured as 4000 K, which is defined as neutral white (van Bommel and Rouhana 2011).

Neither lamp produced spectral emissions in the near UV or near IR zones. This is typical of both standard white LED and fluorescent lamps which should emit no UV and minimal IR radiation. The combination of the glass and phosphor coating of the fluorescent tube blocks any UV transmission to the environment, while the output of an LED can be tuned to a quasi-monochromatic output (1/2 bandwidth of < 50 nm) to avoid production of UV (van Bommel and Rouhana 2011). Therefore, it is unlikely that any difference in development of FDM between experiments using LED and fluorescent sources would be due to an UV sensitive mechanism (French, Ashby et al. 2013).

Radiometry and Photometry: Human lux or Chick lux?

Measurement of the illuminance at a certain distance from a source requires the conversion from a radiometric measurement of energy, irradiance ($\mu W/cm^2$) to its equivalent photometric quantity, illuminance ($lumens/m^2$ or lux). The conversion is made by convolving the radiometric measurement with a spectral efficiency function V(λ) and multiplying by an appropriate constant (Wyszecki and Stiles 1982). The human V(λ) function is defined by the Commission Internationale de l'Éclairage (CIE, http://www.cie.co.at) for a standard observer as the CIE 1924 photopic relative luminous efficiency function. An equivalent scotopic function and constant are also defined (Wyszecki and Stiles 1982). The human photopic relative spectral efficiency function V(λ) can be thought of as representing our perceptual brightness response to the spectral energy of a source at a particular wavelength of electromagnetic radiation. The photopic V(λ) function is normalised to 1 at 555 nm, and the photopic constant k_m is defined as 683 lm/W (Wyszecki and Stiles 1982). As these experiments were conducted within the range of daylight light levels the photopic V(λ) has been used in calculations in this study (Figure 4.26).

For discrete measurement values (usually 1 or 5 nm steps between 380 and 760 nm), the following equation can be used to convert between equivalent radiometric (R: radiant flux, radiance, irradiance) and photometric (L: luminous flux, luminance, illuminance) quantities:

Equation 4.1 $$L = k_m \sum R_\lambda V_\lambda$$

While human photopic colour vision is considered trichromatic and is based on three retinal cone photoreceptors (long or red, medium or green, short or blue wavelength sensitive) (Wyszecki and Stiles 1982), the domestic chick has a substantially more complex retinal cone photoreceptor system (Hart 2001) and has tetrachromatic colour vision (Osorio, Vorobyev et al. 1999). The chick, like other diurnal birds, possesses five types of cone photoreceptors, one of which is a double-cone. The single cones are termed long (L), medium (M), short (S) and ultraviolet (UV) wavelength sensitive. Moreover, the L, M and S cones contain oil droplets which act as filters and narrow the spectral sensitivities of the cones (Bowmaker, Heath et al. 1997). The peak sensitivities of the cones are: UV 419 nm; S 455 nm; M 508 nm; L and Double 570 nm (Hart 2001). While the four single cones contribute to tetrachromatic colour vision, the double cone may have a significant role in luminance-based tasks (Osorio, Vorobyev et al. 1999). As both the colour and luminance perception of chicks is likely to be different to human perception (Kelber, Vorobyev et al. 2003) the use of the human relative spectral sensitivity function and hence photometric quantities, may be inappropriate when working with both avian and other mammalian species (Lewis and Morris 2000; Saunders, Jarvis et al. 2008). For example, behavioural measurement of the chick photopic spectral sensitivity function between 360 - 695 nm revealed a profile that shows greater relative sensitivity at both shorter and longer wavelengths than in humans (Figure 4.26) (Prescott and Wathes 1999).

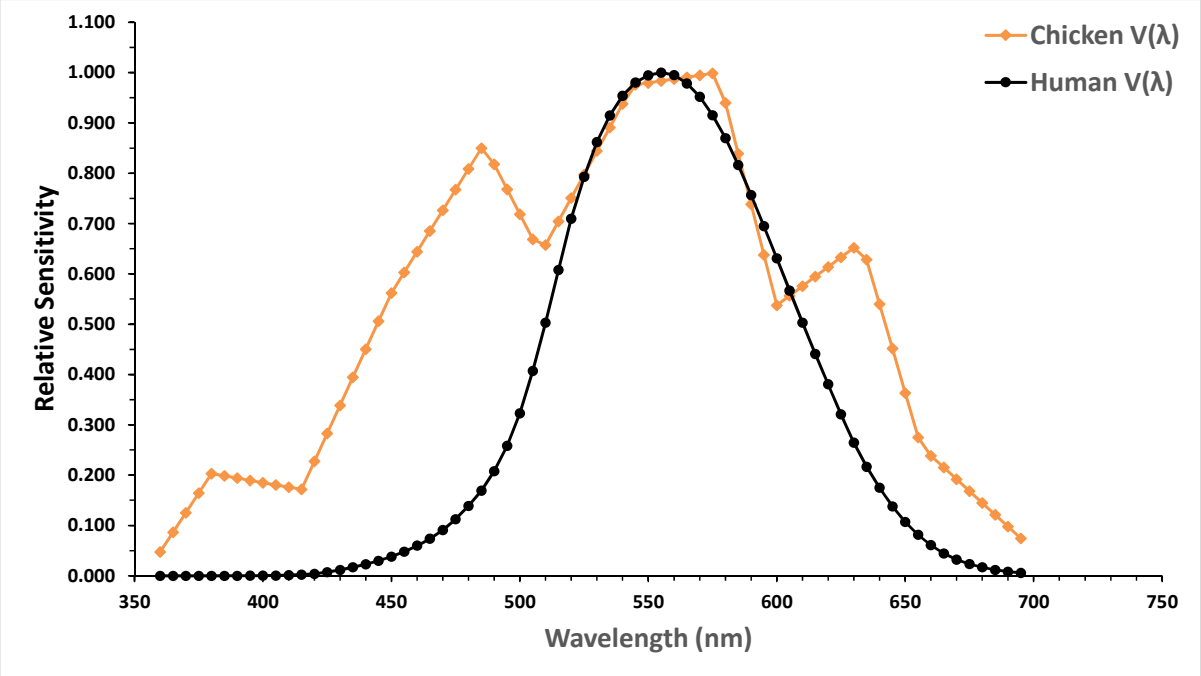

Figure 4.26. Relative Spectral Efficiency Functions V(λ) for the domestic chicken (interpolated from Prescott & Wathes,1999) and human observer (CIE 1924). The functions are normalised to 100% for humans at 555 nm and chickens at 575 nm. The chicken V(λ) function was measured psychophysically using an behaviour-based strategy (Prescott and Wathes 1999). The chicken V(λ) function shows greater relative sensitivity at the shorter wavelength (blue to UV) end of the spectrum, and to a lesser extent at the longer wavelength (red) end of the spectrum when compared to the human V(λ) function.

Although the profiles of the chick and human V(λ) functions overlap significantly in the range of peak wavelength sensitivity, the higher sensitivity of the chick function at the (human) blue end (360 - 520 nm) and red end (610 – 695) of the spectrum implies that a source which emits radiant energy in those regions will be perceived as a different (likely higher) intensity by a chick than by a human observer (Prescott and Wathes 1999; Lewis and Morris 2000). This issue has been investigated within the poultry industry with regard to growth, behaviour and reproductive success. For example, chickens exhibit poorer growth but enhanced photosexual responses under red light, while blue/green light results in higher growth rates (Lewis and Morris 2000).

One approach that has been suggested by several authors is to substitute the chicken V(λ) (Figure 4.26) into equation 4.1 in order to calculate an equivalent chicken photometric value (Lewis and Morris 2000; Saunders, Jarvis et al. 2008). Using this approach, a comparison can be made between a human photometric intensity and a chicken photometric intensity. While the commonly used unit for an illuminance measurement is lux (lumens/m^2) the equivalent chicken illuminance unit has been termed "gallilux" (Nuboer, Coemans et al. 1992) from the species name *Gallus gallus domesticus*, or perhaps more appropriately "clux" (Prescott and Wathes 1999). In this study, I have adopted the term *c-lux* (or chick lux) to differentiate it from another form of illuminance measurement *m-lux* (melanopic lux) which I will introduce in the following section.

Using the method outlined above, Lewis and Morris (Lewis and Morris 2000) calculated ratios of chick to human illuminance values for a range of light sources at a particular irradiance. For a cool white fluorescent tube, the ratio of illuminance calculated was 1.32, while for a blue fluorescent tube the ratio was much greater at 41.86, illustrating the effect of the greater sensitivity of the chick V(λ) function at short or "blue" wavelengths. While Lewis and Morris suggested that this ratio could be taken as a ratio of intensity perception, this interpretation should be treated with caution as the luminous value is not a direct measure of perceived brightness, however it is likely to represent at least an ordinal scale of brightness (Saunders, Jarvis et al. 2008). In these calculations, the human value for the photopic constant k_m of 683 lm/W, was used as it is the basis of the international definition of the lumen. That is, at 555 nm, 1W of radiant flux is equivalent to 683 lumens. However, a correction ratio of 1.22 for chick spectral sensitivity values has been suggested (Saunders, Jarvis et al. 2008) which would further increase the difference between human and chick luminous values.

In summary, for a broadband source which emits from the "blue" through to the "red" end of the spectrum, the chick luminous value will be higher than the equivalent human luminous value. The

higher luminosity of the source (in this case measured in c-lux) may be a better measure of the stimulus for suppression of FDM, and therefore at least partially explain the apparent slowing of development of FDM under white LED lights demonstrated in this study and potentially by Karouta and Ashby (Karouta and Ashby 2015). Table 4.12 summarises the difference in human lux versus c-lux values calculated (using equation 4.1) for the white LED source used in this experiment and the fluorescent source in the same laboratory as used by Backhouse *et al* (Backhouse, Collins et al. 2013). The calculations were based on the methods of Saunders *et al* (Saunders, Jarvis et al. 2008) and Lucas *et al* (Lucas, Peirson et al. 2014). Detailed calculations are presented in Appendix A2.

Radiometry and Photometry: Melanopic Lux

When investigating the effect of light on deprivation-induced myopia it is important to not only consider the role of cones as light intensity transducers under photopic conditions, but to also consider the input from a third class of photoreceptors located in the inner retina, the intrinsically photosensitive retinal ganglion cells (ipRGCs) (Berson, Dunn et al. 2002; Hattar, Liao et al. 2002; Valdez, Nieto et al. 2009; Bailes and Lucas 2010; Lok 2011; Phillips, Backhouse et al. 2012; Lucas, Peirson et al. 2014). ipRGCs are ganglion cells which contain the photopigment melanopsin, which is most sensitive to short wavelength blue light around 480 nm (Berson, Dunn et al. 2002; Hattar, Liao et al. 2002; Torii, Kojima et al. 2007; Valdez, Nieto et al. 2009). Initially it was shown that these photosensitive ganglion cells projected to the suprachiasmal nucleus, and could entrain circadian rhythms (Berson, Dunn et al. 2002; Berson 2003). However it is now clear that ipRGCs also project to visual centres including the lateral geniculate nucleus and superior colliculus, and have a role in the tonic and post-illumination phase of the pupillary light reflex (Bailes and Lucas 2010; Lucas, Peirson et al. 2014) and in visual grating discrimination in mice without functional rods and cones (Ecker, Dumitrescu et al. 2010). Of most interest to this study is the finding that in the mouse, ipRGCs may be encoding overall irradiance levels in the environment (Brown, Gias et al. 2010).

In chicks, light-mediated pupillary responses and circadian entrainment of feeding have been demonstrated in animals with a mutation (GUVY1*) which produces non-functional rods and cones in the outer retina. An investigation of the pupillary light reflex in the same (GUVY1*) chicks demonstrated that the spectral sensitivity of the reflex was best fitted by a single opsin/vitamin A-based photopigment with a peak sensitivity at 484 nm (Valdez, Nieto et al. 2009). In agreement, an isoform of melanopsin with the same peak absorption wavelength (484 nm) has been isolated from the chick

pineal gland (Torii, Kojima et al. 2007) and melanopsin-expressing ipRGCs have been identified in the inner retina and *in vitro* cultures of retinal ganglion cells of the chick (Guido, Valdez et al. 2010).

As discussed in Section 3.4.3, retinal dopamine synthesis exhibits a circadian rhythm in counterphase with melatonin, where dopamine levels are high during the day, while melatonin levels peak at night (Nickla 2013). As form-deprivation depresses levels of retinal dopamine in both chicks (Stone, Lin et al. 1989) and rhesus monkey (Iuvone, Tigges et al. 1989), the suppression of FDM by high light levels may be due to an increase in dopamine synthesis where the ipRGCs act as the photoreceptive driver (Norton and Siegwart 2013). This hypothesis is supported by the interconnectivity demonstrated between ipRGCs and amacrine cells in the inner plexiform layer of the retina, where both synaptic and gap junction connections may exist between the cells (Bailes and Lucas 2010). As a subset of amacrine cells produce dopamine (Iuvone, Galli et al. 1978), activation of these cells by light could increase retinal dopamine synthesis and suppress FDM. As direct signalling from ipRGCs to dopaminergic amacrine cells has been established in the mouse (Zhang, Wong et al. 2008) then a putative pathway exists for light exposure to increase dopamine production in the form-deprived eye, and therefore suppress the development of axial myopia.

Lucas *et al* (Lucas, Peirson et al. 2014) make the argument that the use of standard human-weighted photopic measurements such as illuminance (lux) are inappropriate for the investigation of non-visual responses to light such as light-entrainment of circadian rhythms, pupillary light response, stimulation of cortisol production, and the role of light as a neurophysiological stimulant (Lucas, Peirson et al. 2014). In particular, where melanopsin-based ipRGCs may have a primary light transduction role in a non-visual response, the use of a novel measure of photometric light intensity, the melanopic lux (or m-lux) has been proposed (Enezi, Revell et al. 2011; Lucas, Peirson et al. 2014). As the spectral sensitivity of melanopsin is generally conserved across species (Torii, Kojima et al. 2007; Bailes and Lucas 2013), a melanopic spectral efficiency function, based on an opsin:vitamin A-based photopigment with a maximum sensitivity (λ_{max}) at 480 nm, can be used in general to calculate melanopic lux (Enezi, Revell et al. 2011). The melanopic function needs to be corrected for the spectral absorption properties of the ocular media, and in this study an interpolation of transmission data for the visually similar galliform, the domestic turkey, has been used in calculations of m-lux in chicks (Hart, Partridge et al. 1999; Hart 2001). Lucas *et al* (Lucas, Peirson et al. 2014) provided equations and an “irradiance toolbox” for the calculation of both human and rodent receptor-based illuminance values for a given corneal spectral power distribution (Lucas 2014). Using equation 4.1 and the methods of Lucas *et al* (Lucas, Peirson et al. 2014) with appropriate modifications for the chick

V(λ) function and media transmission characteristics, I have calculated the human (h-lux) and chick (c-lux) illuminance values, as well as the human and chick melanopic illuminance (m-lux) values for the spectral power distributions of both the LED source used in this study and the fluorescent source as used by Backhouse *et al* (Backhouse, Collins et al. 2013). The spectral irradiance measurements were made at the appropriate distances (60 cm from the LED source and 1.6 m from the fluorescent luminaire) to produce a nominal 300 lux illuminance measured with a standard lux meter (Lutron LX-105) at chick eye level. These calculations are summarised in Table 4.12, and shown in detail in Appendix A2.

Source (300 lux nominal)	**Chick V(λ) (c-lux)**	**Human V(λ) (h-lux)**	**Chick N_Z(λ) (c-m-lux)**	**Human N_Z(λ) (h-m-lux)**
LED	**429.3**	**302.4**	**231.9**	**249.3**
Fluorescent	**401.4**	**301.2**	**167.4**	**178.6**
Ratio LED/Fl	1.069	1.004	1.385	1.396
Ratio as %	**6.9%**	**0.4%**	**38.5%**	**39.6%**

Table 4.12 Summary of modified illuminance calculations for the LED source used in this study (QTX-SL8) and the fluorescent luminaire (Phillips Master TL5 28/840 lamps) of Backhouse *et al* (2013). All calculations were based on the spectral irradiance distribution for a nominal 300 lux illuminance at the chick eye height within the pen. Chick c-lux calculations used the chick spectral sensitivity function of Prescott and Wathes (1999). Human h-lux calculations used the CIE 1924 V(λ) function. Chick and Human melanopic (m-lux) calculations used the melanopic response function N_Z(λ) of Lucas *et al* (2014) modified for chick and human media transmission characteristics respectively.

A comparison of the human illuminance values (h-lux column) calculated for the LED and fluorescent sources shows that both sources produce approximately 300 lux of illumination, effectively the same value as measured with a standard lux meter (Table 4.1). This is unsurprising as both sources were designed for use with the human visual system, and therefore despite the underlying difference in spectral irradiance distributions (Figure 4.25), the final photometric output, in this case measured as illuminance, is the same. These calculations therefore validate the method used to calculate illuminance from spectral irradiance measurements as detailed in Appendix A2.

While the calculated difference in illuminance for humans between the sources was negligible at 0.4%, the difference calculated for the chick was an order of magnitude greater at 6.9% (Table 4.12). While the fluorescent source produced a calculated chick illuminance of 401.4 c-lux, the LED source produced the higher value of 429.3 c-lux. This can be explained in part by the presence of the short wavelength (~450 nm) “blue” peak of the LED spectral irradiance distribution (Figure 4.25) which contributes more to the overall illuminance value when convolved with the chick V(λ) function (Figure

4.26) than the smaller short wavelength peaks of the fluorescent source. Although the ratio of 6.9% between the chick illuminance values for the LED and fluorescent sources should not be taken as a direct measurement of intensity perception by the chick (Saunders, Jarvis et al. 2008) it is reasonable to assume that the LED light would be brighter in an ordinal or ranking sense to the chick. The question then remains as to whether this relatively small difference in chick illuminance (6.9%) is sufficient to have any physiological (suppression) effect on myopia induction by form deprivation. The comparison of the degrees of FDM produced in this study and by Backhouse *et al* (Figures 4.9 & 4.23, Table 4.10) shows a reduced level of FDM of about 46% in this study after 4 days of deprivation. Therefore, if this effect is due to some aspect of the sources, such as spectral energy distribution, only about 15% of the difference in induced myopia can be explained by a simple difference in effective illuminance (i.e. 429.3 vs. 410.4 c-lux).

A comparison between the illuminance values calculated using human V(λ) and a chick V(λ) functions shows that the chick illuminance values are higher for both sources. While within species (chick or human) comparisons are valid, between species comparisons are less appropriate (Saunders, Jarvis et al. 2008). For example, the standard human photopic constant k_m was used to calculate both human and chick illuminance values as the exact value is not known for chicks. However, the chick photopic constant may actually be a factor of 1.22 greater than the human value (Saunders, Jarvis et al. 2008). So although it would be tempting to assume that chicks perceive the sources as being brighter than a human observer, this is a speculative interpretation.

A comparison of the melanopic illuminances calculated for the two sources reveals a much greater difference in values for both human and chick observers, than found for the overall V(λ) calculations (Table 4.12). As the underlying melanopic function for both humans and chicks is similar due to the conservation across species of the underlying photopigment (Enezi, Revell et al. 2011) the resulting melanopic lux values calculated for the two species are relatively similar for both sources. However, as the calculations only take into account the spectral sensitivity of melanopsin itself and the transmission characteristics of the human and chick eyes, the values obtained only represent the level of melanopsin photoactivation by the source, rather than any measure of whole retinal, or biological response (Enezi, Revell et al. 2011; Lucas, Peirson et al. 2014). Therefore, although the calculated values are similar, the physiological consequences, such as effect on retinal dopamine synthesis, may not be equivalent.

Nevertheless, relatively greater values for melanopic illuminance were calculated for the LED source when compared to the fluorescent source (Table 4.12) for both species. For a human observer, the white LED source calculations produced a melanopic illuminance value 39.6% higher than for the fluorescent source (249.3 vs. 178.6 m-lux respectively). A similar pattern was found for the chick where the LED value was 38.5% higher than for the fluorescent source (231.9 vs. 167.4 m-lux respectively). Therefore, it is reasonable to speculate that in this study under the 300 lux lighting condition, the white LED source could have produced greater photoactivation of the melanopsin photopigment in the inner retinal ipRGCs of the chick. This in turn would result in greater synthesis of dopamine by amacrine cells, triggering a signalling cascade which reduced overall axial growth and hence slowed myopia development due to form deprivation. Such a mechanism could also explain why the chicks in the recent study of Karouta and Ashby (Karouta and Ashby 2015), who also utilised an LED-based lighting system, developed a similar degree of myopia after 4 days of deprivation (Table 4.10). In contrast the chicks in the study of Backhouse *et al* (Backhouse, Collins et al. 2013) which were raised under 300 lux of fluorescent light, developed around 86% more myopia after 4 days of deprivation, in otherwise equivalent conditions to this study. While Karouta and Ashby (Karouta and Ashby 2015) used a combination of both cool and warm white LED lamps in their study, which reportedly had a spectral energy distribution biased more to the blue red (630 nm) and red (630 nm) ends of the spectrum respectively, it is likely that both types of lamps would have still exhibited an underlying blue peak due to the construction of white LEDs (van Bommel and Rouhana 2011).

Short-wavelength (Blue) Light and Circadian Rhythms

The potential health effects of the widespread use of LED lights and devices with LED-backlight screens, particularly in the evening, has become the subject of some discussion in both the academic literature and popular press (Stevens, Brainard et al. 2013; Knight 2015; Laber-Warren 2015). In general, night time lighting is considered a health risk due to its effects on circadian rhythms of the individual, where the light acts as a zeitgeber and delays or disrupts the normal 24 hour day/night rhythms of the sleep/wake cycle, melatonin production, body temperature, food consumption and even repair of DNA damage (Stevens, Brainard et al. 2013). Blue light, and the blue-biased light produced by white LEDs in particular, has been identified as most likely to disrupt circadian rhythms due to the presence of short-wavelength sensitive (~480 nm) melanopsin-based photoreceptors both in the eye of many species including humans (Brainard, Hanifin et al. 2001), and in more central brain structures such as the pineal gland in the chick (Torii, Kojima et al. 2007). A general room illumination level at night of approximately 200 lux has been shown to be sufficient to delay melatonin production (Gooley,

Chamberlain et al. 2011). More specifically, the light emitted from LED-backlit computer screens or handheld devices has also been shown to suppress the rise of melatonin levels in human participants in the evening (Cajochen, Frey et al. 2011). This was associated with increased sleepiness of the participants, as assessed by both subjective (self-reported scale) and objective (EEG recording) measurements (Cajochen, Frey et al. 2011). Conversely, exposure to 6.5 hours of blue light (460 nm) from midnight has been shown to improve auditory reaction times and attention, and decrease subjective sleepiness rating in participants (Lockley, Evans et al. 2006). In a recent experiment on chicks, Nickla and Totonelly (Nickla and Totonelly 2016) have shown that 2 hours of light exposure between 12:00 am and 2:00 am disrupted the normal circadian cycles of axial length and choroidal thickness, while also stimulating an increase in ocular growth rate in the following 6 hour period. The authors speculated that these circadian disruptions may have been responsible for the development of more myopic refractions in the chicks exposed to the nightly light regime (Nickla and Totonelly 2016).

In summary, exposure of both animals and humans to short-wavelength "blue" illumination in the evening or at night can cause disruption to normal circadian rhythms. However light exposure during the day can be beneficial and has been used to treat conditions such as seasonal affective disorder (Lam, Zis et al. 1996). Furthermore, Backhouse *et al* (Backhouse, Collins et al. 2013) demonstrated a weak time of day effect on suppression of FDM, where exposure to high intensity (10,000 lux) white halogen light for 2 hours in the middle of the day was more effective at suppressing the development of myopia in chicks than light in the evening. In this study, the use of white LED sources (with their underlying short-wavelength blue emission peak, Figure 4.25), to provide the standard 300 lux illumination throughout the day may have had a suppressive effect on the development of FDM through activation of an ipRGC-based growth suppression cascade including increased production of dopamine by amacrine cells as initially proposed by Norton and Siegwart (Norton and Siegwart 2013).

4.4.3 Quasi-monochromatic High Intensity Illumination and Form Deprivation Myopia

Chicks in this study were not only exposed to periodic high intensity white LED light illumination, but also to blue, green and red LED light following the same 15:15 minute paradigm, where 15 minutes of high intensity coloured light was alternated with 15 minutes of 300 lux white LED illumination for 12 hours per day. As the coloured light was produced by discrete blue, green and red LEDs, the spectral distribution of each coloured light exhibited a quasi-monochromatic, narrow-band profile, with a half-

height bandwidth of less than 50 nm (Figure 4.27) (van Bommel and Rouhana 2011). Rather than matching illuminance, the irradiance of the three coloured light conditions were matched to each other, and within the limits of the lighting system, to the irradiance of the white 10,000 lux light condition. Therefore, no assumption was made as to the underlying nature of the receptor or spectral sensitivity of the phototransduction process that might affect the refractive development of the chicks (Lucas, Peirson et al. 2014). For ease of comparison however, each coloured lighting condition was referred to by its equivalence to the periodic 10,000 lux white light (W10KP) as follows; blue – B10KP (peak λ 455 nm), green – G10KP (peak λ 515 nm) and red – R10KP (peak λ 635 nm)(Figure 4.27).

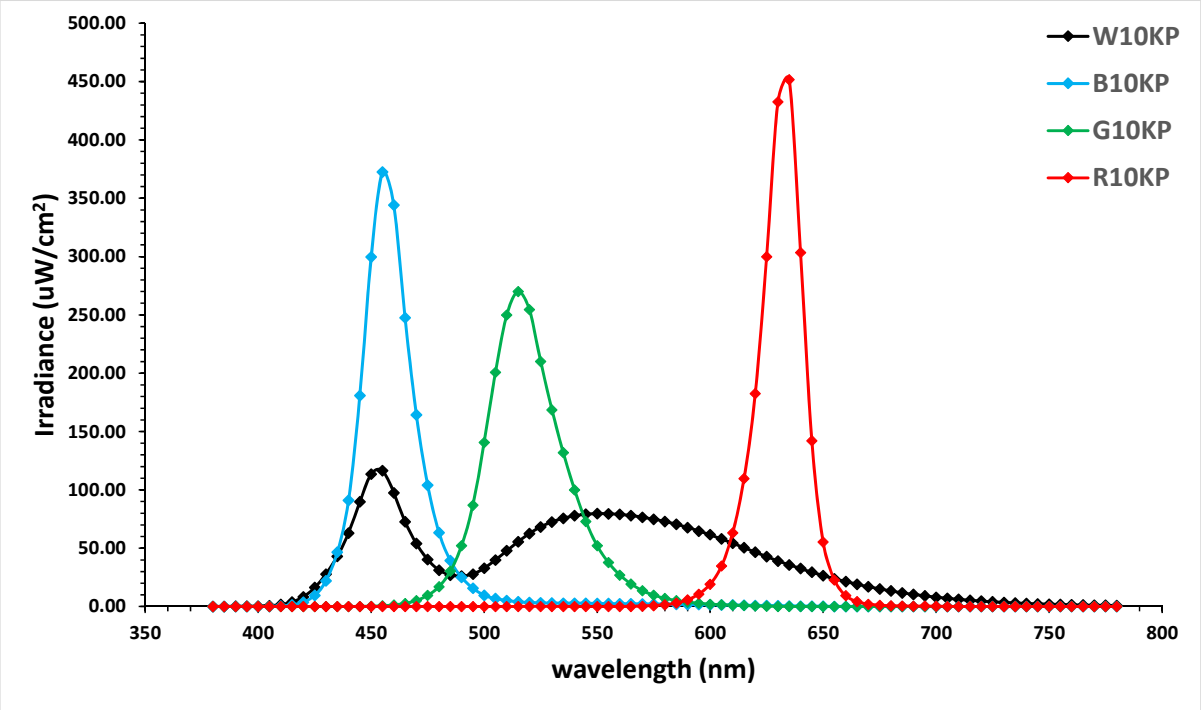

Figure 4.27. Spectral irradiance distributions (μW/cm^2) of the QTX-SL8 LED sources under high intensity conditions. The white LED (W10KP) demonstrates the typical bimodal spectral profile produced by a blue LED (peak at ~450 nm) with associated phosphor coating giving a broader peak at ~550 nm. The blue (B10KP), green (G10KP) and red (R10KP) spectral irradiance distributions display the quasi-monochromatic nature of the sources with spectral peaks at 455, 515 and 635 nm respectively. Spectral irradiance tended to zero for all sources at the UV (380 nm) and IR (780 nm) ends of the human visible spectrum.

As discussed in Section 4.4.1, no significant difference was found in the degree of deprivation myopia produced under any of the high intensity coloured lighting conditions when compared to either the high intensity white condition, or to the standard intensity 300 lux white lighting condition. This lack of a significant difference in refractive outcomes existed for both paired eye comparisons (relative mean sphere refraction, Figure 4.7) and for individual eye comparisons (absolute mean sphere refraction, Figure 4.8). However, when considering individual eye refractive outcomes by coloured light condition, deprived eyes developed on average a myopic refraction, while contralateral non-deprived eyes developed a hyperopic refraction as would be expected (Table 4.2).

As no differences in refractive outcomes were found for the blue, red and green high intensity lighting conditions, when compared to the high intensity white light condition, the arguments previously made regarding the apparent failure of high intensity white light to suppress FDM in this study are also likely to provide some explanation for the lack of effect of high intensity coloured light (see section 4.4.1). For example, Lan *et al* (Lan, Feldkaemper et al. 2014) found that intermittent exposure of chicks to high intensity halogen light (15,000 lux) in 15:15 minutes cycles with a standard (500 lux) fluorescent room illumination was sufficient to suppress FDM. The 15:15 minute cycle was less effective than both 1:1 or 7:7 minute cycles, but more effective at suppressing FDM than either 5 or 10 hours constant exposure to the high light level (Lan, Feldkaemper et al. 2014). Therefore, the 15:15 min periodic high intensity illumination paradigm of Lan *et al* was more effective at suppressing FDM than the equivalent paradigm in this study (Table 4.10, Figure 4.23). Possible explanations advanced earlier for this difference in outcome included: (i) the difference in illumination level (10,000 vs. 15,000 lux) could have demonstrated a threshold effect for FDM suppression by periodic illumination; (ii) the difference in illumination sources (LED vs. halogen) which might have differential suppression effects due to wavelength energy distribution (although the argument has been made above that the blue-biased spectral distribution of white LED light may have a greater suppressive effect); (iii) length of illumination period (6 hours vs. 5 hours at 50% duty cycle) and/or the lack of an period of standard light levels in the evening (a lack of a "twilight") resulting in a failure to entrain or restore normal retinal circadian rhythms which are disrupted by form-deprivation. It is likely that a combination of these factors led to the lack of FDM suppression by high intensity white light in this study, in particular the lack of a standard illumination period in the early evening. However, this does not necessarily explain why no difference in FDM suppression was found by high intensity illumination colour (peak wavelength).

The aim of this part of the study was to determine if the mechanism that results in suppression of FDM by high intensity light was wavelength-dependent. The use of quasi-monochromatic LED lighting system allowed for 3 distinct wavelength distributions of light to be used (peak λ: 455, 515 and 635 nm) to provide the high intensity illumination. As noted above, these 3 conditions were equated for irradiance rather than illuminance as no assumptions were made of the underlying spectral sensitivities of the phototransduction mechanism. However, the blue LED light with a peak emission at 455 nm would be expected to provide a greater stimulus to a melanopsin-based ipRGC mechanism, than either the green or red LED light. Melanopic illuminance (m-lux) values were calculated for each of the sources under high intensity conditions to investigate the potential differential stimulus effects on a putative melanopsin-based mechanism (Table 4.13, Appendix A3). Note that although each condition

was designed to have approximately the same irradiance as the 10,000 lux white LED condition, when considering melanopic lux, the m-lux value can actually be higher than the equivalent human photopic value as the putative melanopic pigment/receptor has effectively greater sensitivity at the relevant wavelengths when compared to the human V(λ) function.

Source	White LED W10KP	Blue LED B10KP	Green LED G10KP	Red LED R10KP
Chick $N_Z(\lambda)$ (m-lux)	7522.5	11903.0	10140.0	9.5

Table 4.13. Melanopic lux (m-lux) calculations for the four high intensity LED conditions. The blue LED (B10KP) condition provides the greatest potential stimulus for a melanopsin based receptor, followed by the green (G10KP) and white (W10KP) conditions. The red (R10KP) conditions provides a very minimal stimulus effect. Calculations used the melanopic response function $N_Z(\lambda)$ of Lucas *et al* (2014) modified for chick media transmission characteristics.

As can be seen from Table 4.13, the high intensity blue light condition should have provided the greatest stimulus for any melanopsin-based response at a value of 11,903.0 m-lux. The green light condition produced a melanopic illumination of 10,140.0 m-lux, around 85% of the blue light figure, while the white light condition resulted in 7522.5 m-lux, or about 63% of the blue light value. The red light condition produced a very small melanopic illuminance of only 9.5 m-lux, due to the limited degree of overlap between the spectral irradiance distribution of the red LED output and the spectral sensitivity of a putative chick melanopsin-based receptor (Appendix A3). Therefore, if the FDM suppression mechanism of high intensity illumination was based solely on signalling cascade initiated by a melanopsin-based photoreceptor such as the ipRGC, then the hypothetical pattern of FDM developed by the chicks in this study would have been predicted to be: most myopia - R10KP < W10KP < G10KP < B10KP – least myopia. However, as no significant difference in the degree of relative myopia induced by illumination condition was found in this study, this hypothesis is not currently supported. As discussed above, it is likely that other aspects of the high intensity light paradigm, such as the 15:15 minute periodic nature, or simply the magnitude of the illumination (10,000 lux for the high intensity white light) was insufficient to suppress the development of myopia due to form-deprivation.

Monochromatic light has been shown to influence refractive development of chicks (Wildsoet, Howland et al. 1993; Seidemann and Schaeffel 2002; Rucker and Wallman 2008; Foulds, Barathi et al. 2013), guinea pigs (Liu, Qian et al. 2011; Qian, Dai et al. 2013; Qian, Liu et al. 2013), rhesus monkeys (Liu, Hu et al. 2014), cichlid fish (Kroger and Wagner 1996) and squid (Turnbull, Backhouse et al. 2015). In an animal raised under monochromatic light but normal visual conditions (i.e. no form-deprivation or lens-induced error), the chromatic defocus of the eye may guide emmetropisation, influencing the final

refractive outcome, by altering the plane of focus within the eye due to the variation in refractive index (dispersion) of the ocular media with the wavelength of light. For example, measurements of the longitudinal chromatic aberration of the chick eye have yielded estimates of magnitude of between 1.24 D (Mandelman and Sivak 1983) to over 3 D (Schmid and Wildsoet 1997).

Under monochromatic light conditions, experiments have shown that chicks can emmetropise using luminance contrast cues alone (Rohrer, Schaeffel et al. 1992; Foulds, Barathi et al. 2013; Rucker 2013). Therefore, the non-deprived eyes of the chicks in this study should emmetropise, but the end point would be influenced by the chromatic defocus of the eye. For example, Foulds *et al* found that chicks raised under red light developed progressive myopia, while those raised under blue light developed progressive hyperopia. In both cases the induced refractive error was axial in nature (Foulds, Barathi et al. 2013). This effect has also been shown to occur in the Blue Acara cichlid fish, where the diameter of the eye increased in a wavelength dependent manner, from shorter in blue (485 nm) through to longer under red (623.5 nm) when raised under the monochromatic lighting condition for six months (Kroger and Wagner 1996). However, the influence of chromatic defocus on refractive outcome appears to be limited in the range of wavelength to which the chick eye will respond. Rohrer *et al* (Rohrer, Schaeffel et al. 1992) found that chicks raised under monochromatic near ultraviolet light (~380 nm) and far red light (> 655 nm) did not develop a different refractive outcome compared to those raised under white light. The monochromatic light sources used in this study (Figure 4.27) had spectral peaks at 455, 515 and 635 nm and so fell inside this range and so would be expected to influence refractive outcome based on previous reports (Foulds, Barathi et al. 2013). The LED sources used also produced little or no UV or IR emissions (van Bommel and Rouhana 2011) and so any effect would be due to what humans consider visible light. Finally, it is worth noting that any refractive error that develops due to chromatic defocus may only exist when measured with white light, as the emmetropisation mechanism would have increased axial length under red light, and reduced it under blue light, to maintain the image on the retina under the respective monochromatic conditions.

In contrast, the form-deprived eyes of the chicks in this study would have received chromatic input only in terms of wavelength of the monochromatic light as the diffusing effect of the occluder would degrade any spatial signal that might guide emmetropisation (Wallman and Winawer 2004; Rucker 2013). Therefore, the differential stimulation of cones and/or melanopsin-based ipRGC photoreceptors relative to the wavelength of the monochromatic light is likely to play a greater role in the control of refractive development in the form-deprived eye (as discussed above with regard to melanopic lux) than a luminance contrast or sign of defocus signal. Chicks have previously been shown to develop similar

degrees of FDM following lid suture with exposure to green monochromatic (550 nm) light or white light (-21.01 D vs. -24.33 D respectively) for 2 weeks (Wildsoet, Howland et al. 1993). While this study measured the refractive outcome on day 4 of form-deprivation, no significant difference was found in the degree of myopia induced under high intensity white light (W10KP: -9.15 ± 2.44 D) when compared to high intensity green light (G10KP: -12.32 ± 1.80 D)(Figure 4.7, Table 4.2). Overall, no difference in FDM was found in this study for any of the high intensity light conditions, despite high intensity white light having previously been demonstrated to inhibit FDM in chicks (Ashby, Ohlendorf et al. 2009; Karouta and Ashby 2015).

As discussed earlier with regard to the standard intensity (300 lux) white light condition, flicker may influence refractive development. High frequency (2 – 10 Hz) luminance and chromatic flicker have been shown to influence refractive development in chicks with changes in temporal luminance contrast (flicker) producing a hyperopic refractive shift in chicks, while changes in temporal chromatic contrast (flicker) produced a myopic refractive shift (Rucker and Wallman 2012; Rucker, Britton et al. 2015). However, the temporal frequencies present in this study are either many orders of magnitude lower (e.g. the 15:15 minute periodic change in intensity represents a temporal frequency of ~0.0006 Hz) or higher (e.g. the 7810 Hz flicker of the LED source) than those shown to influence emmetropisation (Rucker 2013) and so can most likely be discounted as having affected the refractive outcomes.

In summary, while it can be concluded that high intensity monochromatic light does not suppress FDM from the results of this study, it seems likely that some other aspect of the high intensity illumination paradigm has masked any effect of high intensity light, as even high intensity white light did not produce the predicted suppression effect. In particular, the periodic nature of the illumination with intervals of 15 minutes of high intensity light followed by 15 minutes of standard intensity light might have reduced the stimulus for suppression of FDM. Karouta and Ashby (Karouta and Ashby 2015) have shown that the suppression of FDM by high intensity light in chicks is dose-dependent. Although the high intensity white light condition was designed to produce the equivalent of 10,000 lux of illumination (while the monochromatic conditions were matched for irradiance), the effect of the 50% duty cycle of the periodic illumination paradigm would have reduced the average illuminance dose by half to 5,000 lux over the 12 hour period of daylight. As the dose-dependency of FDM suppression by light is best fitted by a logarithmic relationship (Karouta and Ashby 2015), then halving the average intensity from 10,000 lux to 5,000 lux, would increase the degree of myopia induced by some 35%.

Although analysis of overall refractive outcome, either as relative myopia (the difference between deprived and non-deprived eyes) or as absolute refractive error (the refractive outcomes of the deprived or non-deprived eyes considered separately), revealed no significant effects of illumination condition on outcome, it is still possible to investigate whether the underlying biometric components of refraction were influenced by these conditions. This analysis is presented in the next section.

4.4.4 Biometric Outcomes and UGLM Analysis

It has been well established that the refractive status of chicks is best predicted by the axial length of the globe (Schaeffel and Howland 1988), and that the myopic refractive error developed under form-deprivation conditions (Wallman, Turkel et al. 1978) is primarily due to an increase in vitreous chamber depth (VCD), resulting in an overall axial elongation of the eye (Wallman and Adams 1987). Other components of refraction; corneal radius of curvature, anterior chamber depth (ACD), and lens thickness (LT) also contribute in varying degrees to the overall refraction of the chick eye (Schaeffel and Howland 1988) and can be manipulated by environmental conditions. Chicks raised under constant light develop hyperopia due to corneal flattening which is severe enough to over-ride a concomitant increase in VCD (Li, Troilo et al. 1995). The degree of hyperopia developed by chicks raised under constant light conditions was found to vary with the intensity of the illumination, which in turn was directly correlated with the degree of corneal flattening (Cohen, Belkin et al. 2008). Chicks raised under constant light with concomitant form deprivation also resulted in hyperopic refractions, associated with shallow ACD (Stone, Lin et al. 1995). Interestingly, rearing chicks in constant darkness also leads to hyperopia due to corneal flattening irrespective of whether the eyes were form-deprived by the use of an occluder, or not (Gottlieb, Fugate-Wentzek et al. 1987).

In order to estimate the contribution of each biometric component (radius of curvature, anterior chamber depth, lens thickness and vitreous chamber depth) and illumination condition to the overall refractive outcome, Univariate General Linear Model (UGLM) analysis of the data was undertaken.

UGLM – Paired-Eye Analysis

The UGLM analysis of the paired eye data for relative mean sphere refraction (Figure 4.7) is summarised in Table 4.7. The model itself is robust with high statistical significance ($p< 0.0001$). The main component of overall relative refraction was relative VCD (~60% contribution), which is consistent with previously published literature on FDM in chicks (Wallman and Adams 1987; Wallman and Winawer 2004). Relative corneal radius and ACD also showed statistically significant contributions to

relative refraction, although the effects sizes were some 4 times smaller than VCD. LT was not found to be a significant variable for the relative refraction model, indicating it varies little in axial dimension with changes in refraction. The results for corneal radius, ACD and LT are consistent with previous findings (Schaeffel and Howland 1988).

The model also showed no significant effect for illumination condition on relative refraction. This is consistent with the ANOVA analysis of relative mean sphere refraction by illumination (Table 4.2) although unexpected with regard to previous studies both in this laboratory (Backhouse, Collins et al. 2013) and in the literature (Ashby, Ohlendorf et al. 2009; Karouta and Ashby 2015) in which light levels have been correlated with differences in refractive outcome with form deprivation. Possible explanations for this lack of effect have been discussed in the section on refractive outcomes above (Section 4.4.1). While ANOVA analysis of corneal curvature revealed that the green high intensity illumination condition (G10KP) exhibited a significantly different (more negative) relative corneal radius when compared to the other illumination conditions (Figure 4.10), this difference did not result in a significant difference in relative refraction (Figure 4.7) indicating that other biometric components compensated for this difference. Furthermore, relative corneal radius was not significantly correlated with relative refraction (Figure 4.11). Therefore, the UGLM analysis for relative refractive outcome is consistent with the ANOVA analysis for the paired-eye relative data, and both analyses demonstrate that lighting condition had no significant effect on refraction in this study. This is a rather unexpected result, particularly when comparing the standard (W300C) and high intensity white light (W10KP) conditions. Aspects of the LED-based illumination system such as spectral energy distribution of the white light, periodicity of the high intensity phase, time of day effects have been proposed as possible influences on the outcome of this experiment (Sections 4.4.1, 4.4.2 & 4.4.3) and require further investigation.

UGLM – Individual Eye Analysis

The UGLM analyses of the deprived and non-deprived eye refraction data (Figure 4.12) are summarised in Tables 4.8 and 4.9 respectively. The models are robust with high statistical significance ($p< 0.0001$) for both deprived and non-deprived eyes.

For the deprived eye model (Table 4.8), the most highly significant component of overall refraction was VCD ($p< 0.0001$, ~55% contribution). Corneal radius and LT showed statistically significant contributions to refraction ($p = 0.012$ and $p = 0.011$ respectively), although the effects sizes were small (~14% for both). ACD was not found to be a significant variable for the deprived eye model, indicating

it varied little in axial depth with changes in refraction. The model also showed no significant effect for illumination condition on deprived eye refraction. This is consistent with the ANOVA analysis of deprived eye mean sphere refraction by illumination (Figure 4.8, Table 4.2) although unexpected as noted above for the relative mean sphere outcome.

For the non-deprived eye model (Table 4.8), VCD was also a highly significant component ($p< 0.0001$), however the effect size was smaller (~29%) than for the deprived eyes, and approximately equal to the effect sizes for ACD (~29%) and illumination condition (~30%). Conversely, illumination condition was not significant for the deprived eye model ($p < 0.327$). Therefore, while illumination condition had no overall effect on refractive outcome for either the relative refraction or absolute refractions of deprived or non-deprived eyes by ANOVA analysis (Figures 4.7 and 4.8, Table 4.2), the UGLM analysis reveals that illumination condition did contribute to the refraction of the non-deprived eyes, however the ANOVA analysis suggests that the contributions of the biometric parameters (ACD and VCD) masked any overall change in refraction between the illumination conditions.

The biometric parameter that did show statistically significant differences (ANOVA) in outcome by illumination condition was corneal curvature for relative and absolute (deprived and non-deprived) comparisons (Table 4.3). Furthermore, a comparison of the pooled data for the corneal radii of deprived and non-deprived eyes showed that the non-deprived eyes had longer corneal radii (flatter corneal curvature) on average, which resulted in the relative corneal radii values being negative for all illumination conditions. In particular, the average relative corneal radius of the G10KP group was more negative when compared to the other illumination conditions (Figure 4.10) due to greater difference between the shorter (steeper) average corneal radii of the deprived eyes relative to the longer (flatter) average corneal radii of the non-deprived eyes (Figure 4.12). These results suggest that the high intensity lighting conditions employed in this study did produce corneal flattening in the non-deprived eye relative to the deprived eyes, and that there was a differential effect of illumination colour. In particular, the corneal radii of the non-deprived eyes of the white standard illumination W300C condition and the white high intensity W10KP condition were significantly different (Figure 4.12). This is consistent with previous reports of a dose-dependent corneal flattening effect of high intensity light in chicks (Cohen, Belkin et al. 2008). Furthermore, the corneal radii of deprived eyes of the W10KP, B10KP and R10KP lighting conditions were also found to be significantly different (Figure 4.12). Therefore, in this study the results of both ANOVA analysis (Table 4.3) and ULGM analysis (Table 4.9) indicate any corneal flattening effect is sensitive to both the colour (wavelength) and intensity of the lighting condition.

4.4.5 Limitations

Sample Size and Statistical Power

Relative mean sphere refraction, calculated as the difference between the mean sphere refractions of the deprived eye and the contralateral non-deprived eye, was the primary outcome measurement of this study. The use of a relative, or paired-eye measurement as the primary outcome measurement is supported by the assumption that it accounts for individual variation between animals in terms of eye size due to factors such as differential growth rates (Wallman and Adams 1987). During the design phase of this experiment, a sample size of 12 per group was estimated as statistically appropriate based on both previous studies on form deprivation in chicks (Wallman, Wildsoet et al. 1995; Ashby, Ohlendorf et al. 2009) (Backhouse, Collins et al. 2013) and on the conclusions of Anderson and Vingrys (Anderson and Vingrys 2001) regarding small sample sizes. While a sample size of 12 may seem small, it is important to understand that this is not an epidemiological or ecological study where samples are taken from a large population with the aim of statistically distinguishing sub-populations, but rather an interventional study where the outcome of the intervention should be stereotypical and consistent. Under these circumstances small sample sizes such as 12 per group as used in this study are appropriate (Anderson and Vingrys 2001).

However, as no significant difference in relative refraction by illumination condition was found (Figure 4.7, Table 4.2) *post-hoc* calculations of power and sample size required to reach statistical significance for the relative refraction data were carried out using G*Power (University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010). These calculations showed that the statistical power of the ANOVA analysis was low at 51%, while a total sample size of 100 animals (20 per group) would have been required to reach statistical significance at $p < 0.05$. Therefore, the question is why did the sample size used in this study not yield sufficient power as predicted?

Inspection of Tables 4.2 and 4.10 show that the standard error of the mean (SEM) values obtained in this study were consistently larger than those obtained in similar studies. In particular, the relative refraction SEM values of both this study and Backhouse *et al* (Backhouse, Collins et al. 2013) which used the same infra-red optometer, were at least four times as large as those of Karouta and Ashby (Karouta and Ashby 2015) who utilised a bespoke automated infrared photoretinoscopy system. Therefore, the failure to reach statistical significance for the relative refraction data may have been primarily due to an inherent variability in the refraction measurement system used this study, rather than a true underlying variability in the refractive outcome of form-deprivation in the chicks.

High Intensity Light Presentation

This study presented the high intensity light in a periodic manner where 15 minutes of 10,000 lux white light (or equivalent radiance coloured light) was alternated with 15 minutes of standard intensity 300 lux white light. Both the high intensity and standard intensity illumination was produced by the same LED-based lighting system. A periodic presentation paradigm was adopted to replicate the protocol used in the guinea pig experiments reported in Chapter 3, and to simulate the intermittent exposure to high (outdoor) light levels that adolescent school-age children are exposed to in their daily activities (Backhouse 2011).

However, a recent study has shown that a 15:15 minute high:standard intensity paradigm is not optimal at suppressing FDM in chicks, with greater suppression occurring for either 1:1 or 7:7 minute lighting cycles (Lan, Feldkaemper et al. 2014). Furthermore, Lan *et al* presented the high intensity light level at 15,000 lux, rather than the 10,000 lux (or equivalent irradiance); while the standard intensity light level was 500 lux, rather than 300 lux as used in this study. Therefore, although both studies utilised a 50% duty cycle, the average illuminance experienced by the chicks in this study was 2,575 lux (12 hour daylight period), versus 3,875 lux (10 hour daylight period) for Lan *et al* (Lan, Feldkaemper et al. 2014). This represents an increase over this study of about 50%, which may explain why a greater degree of FDM suppression was found by Lan *et al* for their 15:15 minute paradigm (Table 4.9). This conclusion is supported by the demonstration that the relative change in refraction of chicks under form-deprivation is inversely related to the logarithm of the light intensity under which they were raised (Karouta and Ashby 2015). Therefore, although the 15:15 minute periodic paradigm is not optimal, it may have been more effective at suppressing FDM in this study if it was combined with a greater level of illumination (e.g. 15,000 lux) for the high intensity periods.

Furthermore, the ancillary experiment investigating the time course of the development of FDM in Cobb chicks under continuous high intensity white LED-based illumination described in Appendix A4, indicates that any difference in outcome measurements (refractive or biometric) may not develop until more than 5 days of form-deprivation has occurred. As outcome measurements in this chapter were made at 4 days post-deprivation, insufficient time may have passed for the periodic high intensity lighting paradigm to have produced a significant effect. Future experiments should investigate the time course of refractive development under the lighting conditions, rather than at a single time point as in this study.

It would also be appropriate to repeat these experiments with either: (i) a 15:15 minute periodic lighting paradigm with 15,000 lux illumination under white light, and equivalent irradiance for the coloured lighting conditions; or (ii) a 6 hour continuous exposure high intensity lighting paradigm, preferably at 15,000 lux or equivalent, and with a 2 hour standard illumination evening phase as commonly utilised in similar studies (Ashby, Ohlendorf et al. 2009; Karouta and Ashby 2015) to avoid any circadian rhythm disruption (Stevens, Brainard et al. 2013).

4.4.6 Conclusions

The aim of this study was to investigate the effect of high intensity quasi-monochromatic coloured light on suppression of form deprivation myopia in the chick. While no significant difference was found in the degree of FDM induced under different coloured high intensity lights, the use of an LED-based illumination system produced an unexpected, but potentially important, finding of a significant difference between the effect of fluorescent-based and white LED-based standard 300 lux illumination levels on induction of myopia by form-deprivation in chicks. The direct comparison of the degree of FDM induced under 300 lux white LED light in this study, with the equivalent group raised under 300 lux fluorescent light by Backhouse *et al* (Backhouse, Collins et al. 2013), showed that significantly less myopia was induced under standard white LED illumination. This result is particularly noteworthy as not only were the chicks in each study of the same Cobb strain (and from the same supplier), but the studies were conducted in the same environment (including diffusers, rooms, food, and temperature), and the outcome measurements were made with the same equipment; while the only difference was the illumination sources (LED vs. fluorescent for the standard 300 lux condition).

The difference in refractive outcome between LED and fluorescent lighting has been discussed in terms of spectral energy distribution of the sources, both in terms of chick-defined illuminance (c-lux), and perhaps more importantly, melanopsin-defined illuminance (m-lux). As the white LEDs used in this study were constructed in the typical manner of a phosphor-coated blue LED, the resulting spectral energy distribution was more blue light-biased than the fluorescent sources used in the facility. Therefore, the melanopic illuminance values (m-lux) calculated for the nominal 300 lux LED white light condition were ~39% greater than that calculated for the nominal 300 lux fluorescent light condition (Table 4.13). If the suppression of FDM by light is mediated via a melanopic/ipRGC pathway leading to increased synthesis of dopamine by amacrine cells in the retina as hypothesised (Norton and Siegwart

2013), then the higher melanopic illuminance of the LED source can at least partially explain the apparent suppression of FDM under the standard white light condition in this study.

A recent study conducted in schools in China has shown that simply raising the ambient light levels in school classrooms from less than 100 lux, to greater than 500 lux at desk level, reduced the incidence of myopia, decreased the mean refractive error in non-myopic participants, and reduced axial growth in all school-aged children exposed to the higher illumination levels (Hua, Jin et al. 2015). The redesigned lighting systems in the study of Hua *et al* used fluorescent luminaires each containing two TL-28W 6500K fluorescent tubes. While this study compared the effect of 5800K LED sources with the TL-28W 4000K fluorescent lamps used by Backhouse *et al* (Backhouse, Collins et al. 2013), based on a comparison of the colour temperatures the use of white LED-based luminaires in a school environment to produce the required desk illuminance would be predicted to be at least, if not more, effective at reducing myopia incidence due to the underlying blue (~450 nm) component of LED light.

While there may be both antimyopiagenic and broader health benefits to utilising blue-biased LED lighting systems during the morning and middle of the day (Glickman, Byrne et al. 2006), the use of such lighting in the evening has been identified as being a health hazard due to the potential disruption of circadian rhythms such as the sleep/wake cycle and even DNA repair (Cajochen, Frey et al. 2011; Stevens, Brainard et al. 2013). Therefore, the use of colour-changing LED lamps such as the Philips Hue™ (Philips Lighting, http://www2.meethue.com) may provide a solution by allowing for the diurnal variation of the white light from “cooler” blue-biased lighting from the morning to the middle of the day, followed by a shift to a “warm” red-biased lighting in the evening. Such a regime could support the maintenance of both potentially antimyopiagenic retinal circadian rhythms as well as more general whole-body circadian rhythms.

5 Thesis Conclusions

The Guinea Pig, FDM and Ambient Light

This study has produced the novel finding that daily exposure to periodic high intensity white light (10,000 lux) suppresses development of form-deprivation myopia (FDM) in pigmented guinea pigs when compared to form-deprived animals raised under 300 lux ambient lighting. This finding complements the previous demonstration of the suppression of lens-induced myopia (LIM) in the guinea pig by high intensity ambient illumination (Li, Lan et al. 2014). It also contributes to the increasing weight of experimental literature which supports the proposal that the protective effect of outdoor activity on myopia development in children (Rose, Morgan et al. 2008a) is mediated by exposure to the high ambient light levels experienced outdoors.

While the induction of FDM was associated with a significant increase in vitreous chamber depth (VCD) in the deprived eyes as expected, no difference in relative VCD by illumination condition was found which corresponded to the difference in relative refraction. Therefore, the significant effect of high intensity ambient illumination on the degree of relative FDM induced must have been the result of the sum of effects of illumination on the other biometric variables that contribute to refraction. In particular, the absolute anterior chamber depth (ACD) of the deprived eyes varied significantly by illumination condition, with eyes exposed to high intensity lighting exhibiting a smaller ACD. Furthermore, consideration of effect sizes calculated by Univariate General Linear Model Analysis (UGLM) suggests that the ambient high intensity lighting condition affected the anterior segment (corneal radius and ACD) differentially from the posterior segment (VCD) depending on whether the eye was form-deprived or not. This finding is consistent with previous reports of anterior segment effects during the development of FDM in guinea pigs (Howlett and McFadden 2006) and indicates that the guinea pig model of myopia based on form-deprivation is more complex than the chick model.

No significant difference in relative retinal dopamine concentration (DAC) by lighting condition was established, however a numerical difference was apparent which suggests the high intensity illumination paradigm may have raised retinal DAC, particularly in the non-deprived eyes, but not to a statistically significant degree. Conversely, it may be that under the periodic high intensity illumination paradigm used in this study, anterior segment effects predominate over the more typical posterior segment VCD differences reported for the chick (Ashby, Ohlendorf et al. 2009). Therefore, the finding in this study of no significant difference in relative DAC is consistent with the parallel finding of no

significant difference in relative VCD. The effect of periodic high intensity illumination, and its interaction with a putative time of day effect on FDM suppression (Backhouse, Collins et al. 2013) deserves further investigation to determine the optimal temporal relationship between high intensity ambient lighting and myopia suppression in the guinea pig.

The Chick, FDM and Ambient Light

The aim of the chick experiment was to investigate the effect of periodic, low frequency, high intensity white and quasi-monochromatic coloured light on the suppression of FDM. This aim was motivated by the finding that such an ambient lighting paradigm did suppress FDM in the guinea pig (Chapter 3) and an interest in the role of the wavelength of light on the underlying suppressive mechanism which may be mediated by the short-wavelength sensitive intrinsically photosensitive retinal ganglion cells (ipRGCs) of the inner retina (Phillips, Backhouse et al. 2012). While no significant difference was found in the degree of FDM induced under different quasi-monochromatic high intensity lights, the use of an LED-based lighting system produced the unexpected, but potentially important finding that significantly less FDM (54%) was induced under 300 lux white LED light in this study, when compared to an equivalent control group previously raised under 300 lux fluorescent light in the same laboratory (Backhouse, Collins et al. 2013).

One explanation for the above effect could be that some aspect of LED lighting is sufficiently different from fluorescent lighting to influence the development of FDM at standard illumination levels. Therefore, this thesis has comprehensively examined the differences between the spectral energy distributions of the two type of sources, both in terms of chick-defined illuminance (c-lux), and perhaps more importantly, melanopsin-defined illuminance (m-lux). For a broadband source which emits from the “blue” through to the “red” end of the spectrum, the chick luminous value will typically be higher than the equivalent human luminous value. The higher chick luminosity of the source (in this case calculated as a ~7% increase as c-lux) may be a better measure of the stimulus for suppression of FDM, and therefore at least partially explain the apparent slowing of development of FDM under white LED lights demonstrated in this study.

Furthermore, the melanopic illuminance values (m-lux) calculated for the nominal 300 lux LED white light condition were ~39% greater than those calculated for the nominal 300 lux fluorescent light condition. Therefore, if the suppression of FDM by light is mediated via a melanopic/ipRGC pathway leading to increased synthesis of dopamine by amacrine cells in the retina, as hypothesised (Norton

and Siegwart 2013), then the higher melanopic illuminance of the LED source can more fully explain the apparent suppression of FDM under the standard white LED light condition in this study.

The relationship between the wavelength of ambient light and FDM suppression certainly requires further investigation to determine if the effect is mediated through a dopaminergic pathway. In order to isolate the effect of wavelength only, the illumination paradigm should be designed so that the high intensity ambient lighting phases are provided as a continuous 6 hour period in the middle of the day, to avoid both the confounding factors of periodic/intermittent lighting and time of day effects on FDM. The duration of exposure to the lighting condition following form deprivation should also be extended beyond the 4 days utilised in this study, with the time course of both refractive and biometric outcomes investigated over a period of up to 10 days post-deprivation.

In order to investigate the effect of such a lighting paradigm on a dopaminergic mechanism, it may be advisable to measure vitreal DOPAC, rather than retinal DAC levels. DOPAC is a metabolite of dopamine, and measurement of vitreous concentration has been shown to be less variable than retinal DAC, but potentially more responsive to lighting conditions (Megaw, Morgan et al. 1997). Furthermore, vitreal DOPAC or the DOPAC/dopamine ratio may be better indicators of retinal dopamine release as the vitreous acts as a simple reservoir for retinal dopamine, with no further synthesis or degradation taking place in the vitreous (Ohngemach, Hagel et al. 1997; Feldkaemper and Schaeffel 2013).

Summary

In conclusion, this thesis has demonstrated that high intensity ambient lighting can suppress FDM in the guinea pig, which provides further evidence that the protective effect of outdoor activity in children is mediated by outdoor light levels. Second, it has also demonstrated, perhaps unexpectedly, that white LED illumination at standard room illumination levels appears to reduce the development of FDM in the chick. This effect may be related to the fact that chicks have different luminous sensitivity to humans, and so the use of standard photometric measurements may not be appropriate when attempting to equate the luminous characteristics of different light sources such as LED and fluorescent lighting in animal experiments. Furthermore, if ipRGCs act as the light transducer for a dopaminergic axial growth suppression pathway as suggested, then the spectral sensitivity of melanopsin must also be considered when designing or measuring lighting systems for animal experiments on myopia suppression.

6 Appendices

6.1 Appendix A1: Dopamine Assay Methodology

A1.1. Calibration curve for Dopamine Assay by HPLC – MS/MS

A calibration curve for calculating the retinal tissue dopamine concentration was constructed by analysing known concentrations of dopamine (Sigma, St Louis, MO) at 0.5, 1.0, 2.0, 4.0, 8.0 and 16 ng/ml in 150 µl of Solubilisation buffer. Peak areas from HPLC – MS/MS analysis were obtained by integrating the intensities for the specific fragment ions at m/z 91.0 for dopamine (Section 3.2.3).

The resulting calibration equation for converting peak area counts (PAC) to dopamine concentration (DAC) in ng/ml was:

(PAC – 226.65)/664.17 = DAC

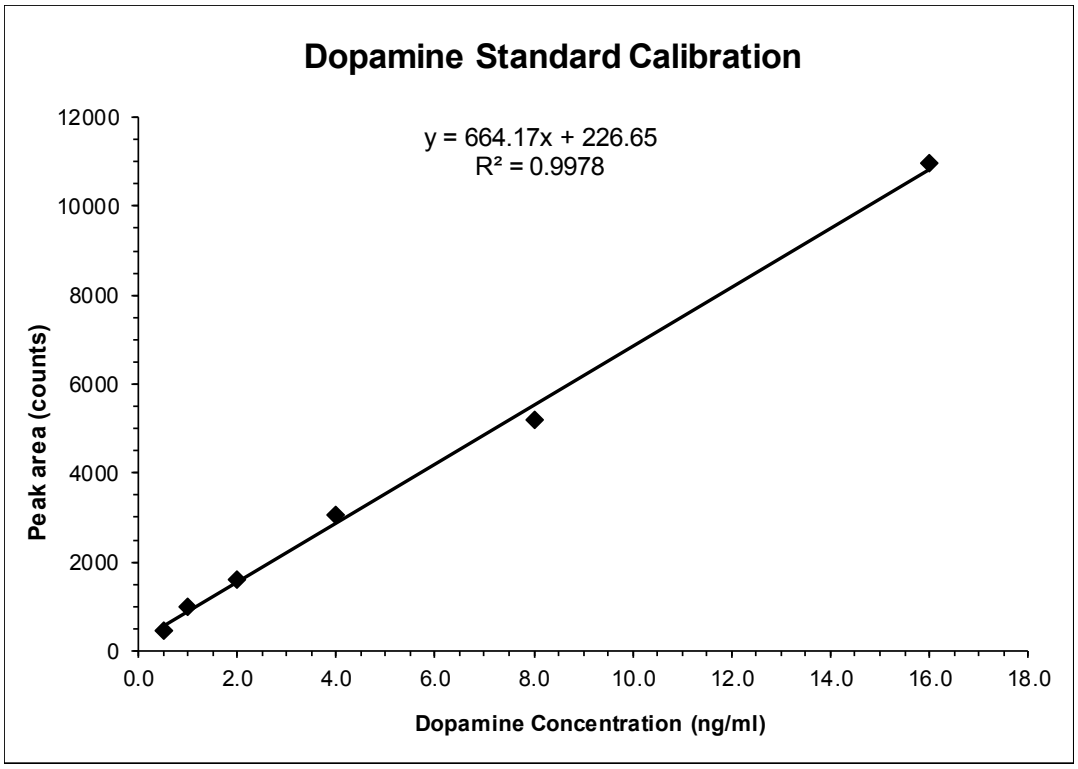

Figure A1.1. Dopamine standard calibration curve used to the determine calibration equation for calculating dopamine concentration in ng/ml from measured peak area counts for retinal tissue samples.

A1.2. Example of Retinal Tissue Assay Analysis by HPLC – MS/MS

A typical example of the HPLC – MS/MS scan output for a retinal tissue sample. The blue-filled peak at 11.6 minutes indicates the presence of dopamine in the sample. The area of the peak (575.09 counts) is proportional to the concentration of dopamine in the sample. Using the calibration equation from Appendix A.1.1 and accounting for a 10x dilution of the sample, the resulting concentration of dopamine present in the retinal tissue sample calculated was:

[(575.09 – 226.65)/664.17] x 10 = 5.25 ng/ml

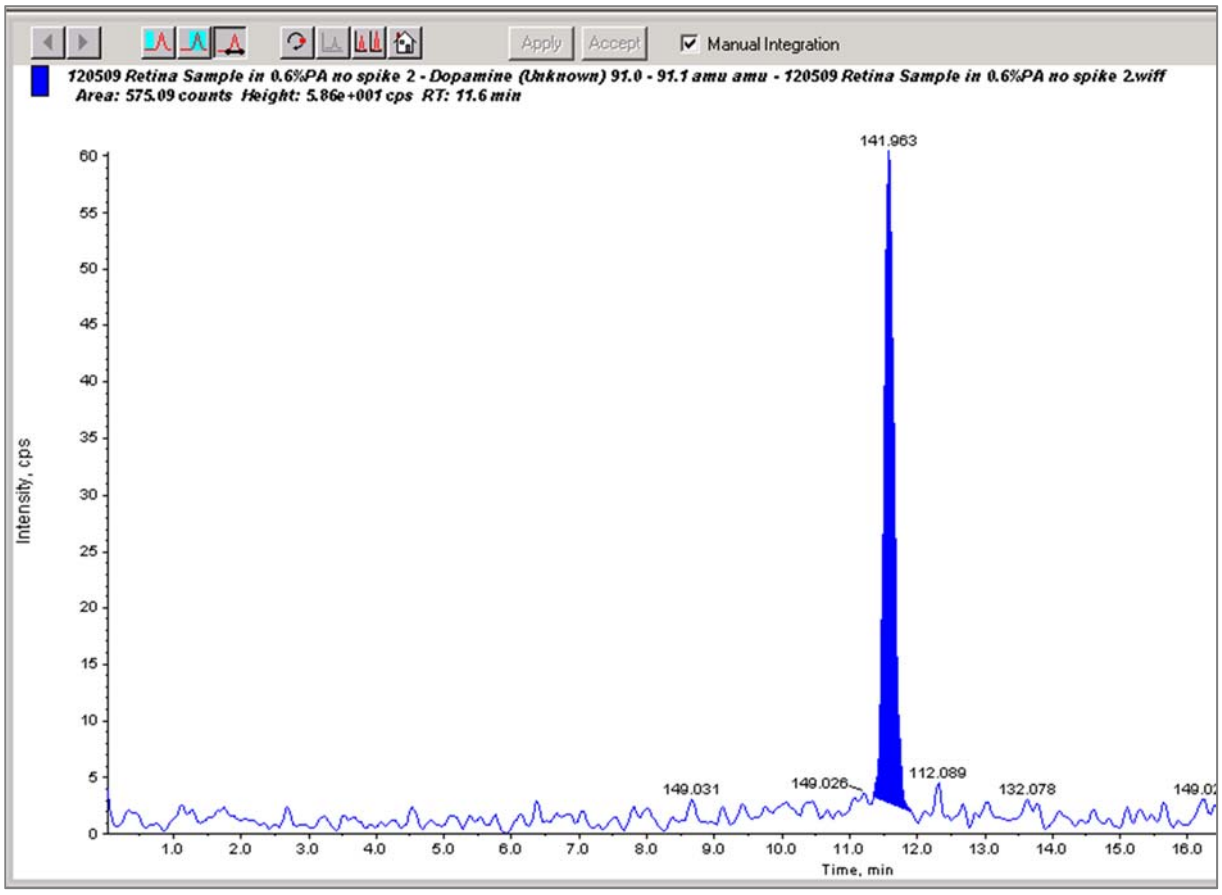

Figure A1.2. A typical example of the HPLC – MS/MS scan output for a retinal tissue sample. The blue-filled peak at 11.6 minutes indicates the presence of dopamine in the sample. The area of the peak (575.09 counts) is proportional to the concentration of dopamine in the sample.

A1.3. Examples of Signal to Noise Ratio achieved for HPLC – MS/MS Analysis

Figure A1.3 demonstrates the signal to noise ratio (SNR) achieved for the HPLC – MS/MS assay using the Extracted Ion Chromatogram for the dopamine transition m/z 154->91.0. For the lowest concentration calibration standard used of 2ng/ml dopamine, an appropriate SNR of approximately 10:1 was attained. In comparison, Figure A1.4 demonstrates the SNR found for the blank (0 ng/ml dopamine) standard where the peak at 9.96 min represents an SNR of approximately 1.5:1.

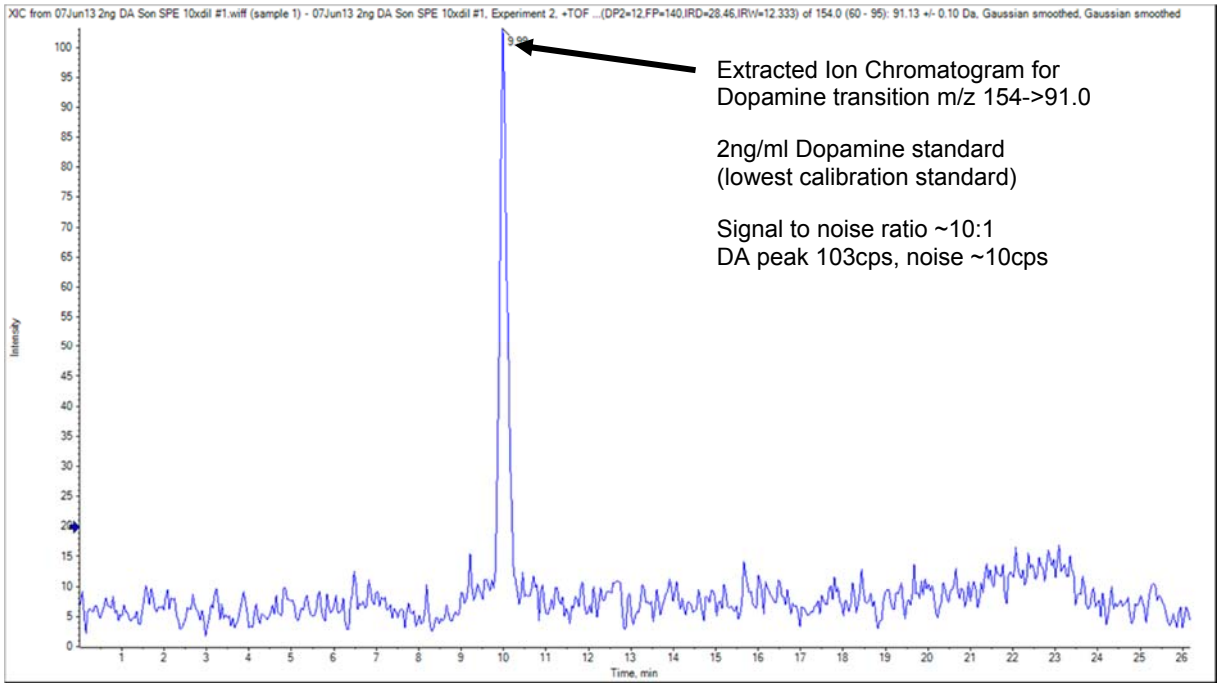

Figure A1.3. An example of the Extracted Ion Chromatogram for the dopamine transition m/z 154 – 91 for the 2 ng/ml dopamine standard. A signal to noise ratio of approximately 10:1 was achieved for the lowest concentration standard used in the assay procedure.

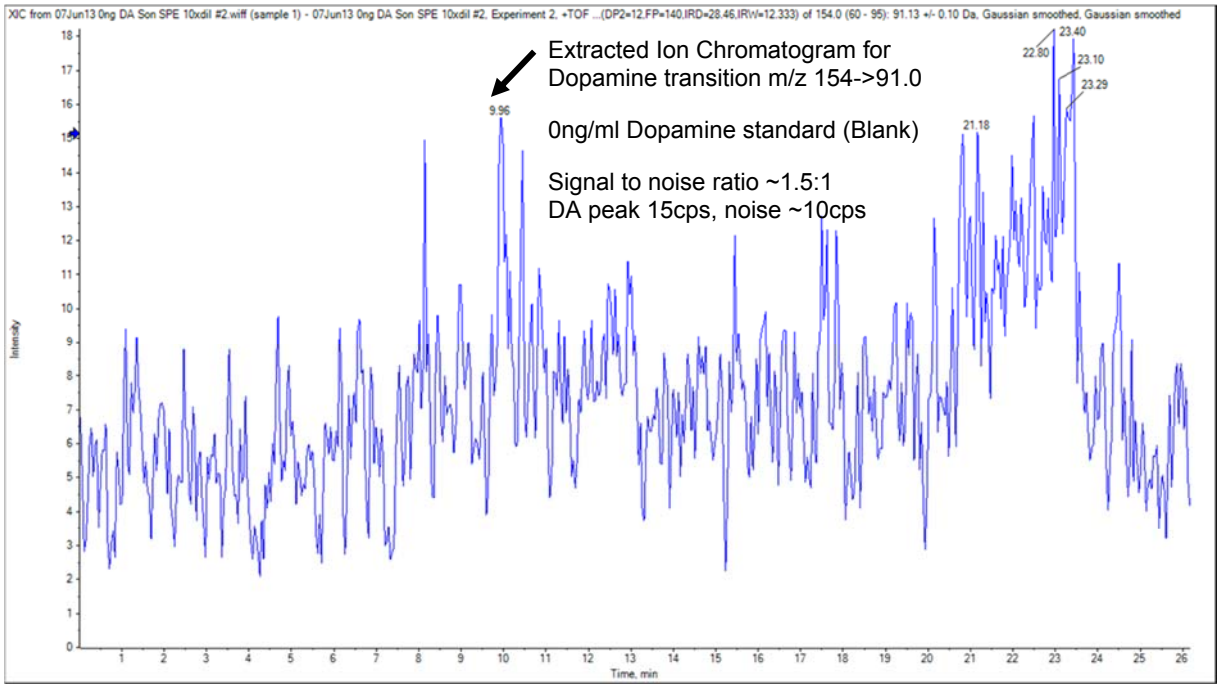

Figure A1.4. An example of the Extracted Ion Chromatogram for the dopamine transition m/z 154 – 91 for the 0 ng/ml blank standard. A small, but not significant peak is visible at the transition point at 9.96 minutes with an associated SNR of approximately 1.5:1.

The HPLC - MS/MS, or product ion scan assay monitored the dopamine transition from m/z 154.0 to 91.0. The QSTAR HPLC – MS/MS instrument used for the assays was able to collect a Full-scan Product Ion spectrum during the MS/MS assay. Figure A1.5 demonstrates the MS spectrum for a 64 ng/ml dopamine standard with the small residual peak of the dopamine precursor indicated at m/z 154.

Figure A1.6 demonstrates the MS/MS Product Ion spectrum for a 16 mg/ml dopamine standard with the fragmentation ion used for quantitation analysis at 91 m/z.

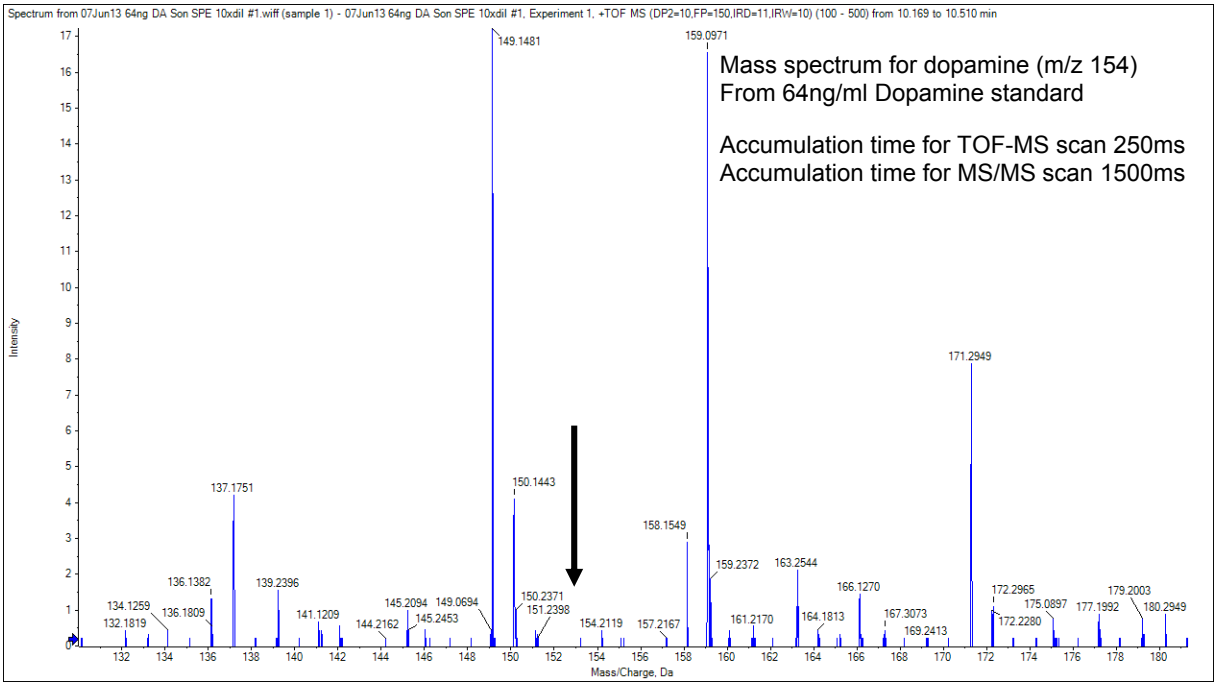

Figure A1.5. An example of the MS spectrum for the 64 ng/ml dopamine standard. Only a small peak is still present for the intact dopamine precursor at m/z 154. The MS scan accumulates data for 250 ms per cycle, whereas the MS/MS quantification scan accumulates data for 1500 ms (see Figure A1.6 below.

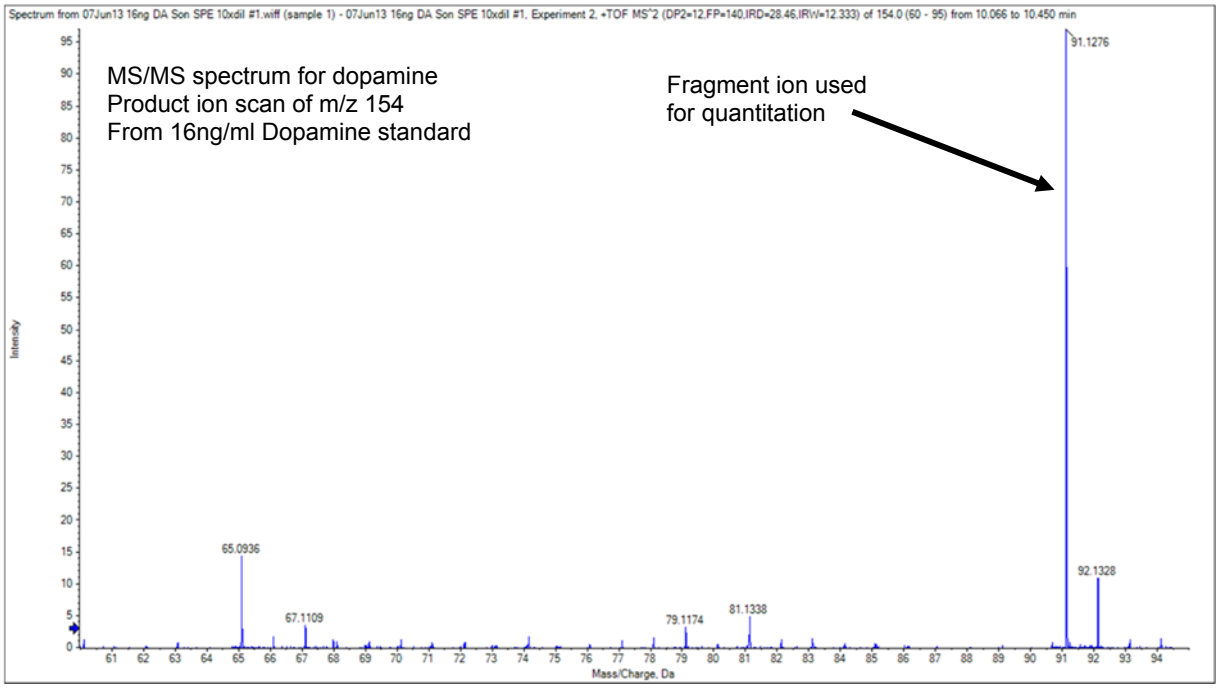

Figure A1.6. An example of the MS/MS spectrum scan of the fragmentation ion at m/z 91 used for quantitation for the 16 ng/ml dopamine standard.

Figure A1.7 illustrates representative HPLC – MS/MS scans obtained for four retinal samples. The SNR values achieved ranged between approximately 18:1 and 45:1 which were at least two-fold greater than the SNR obtained for the lowest concentration (2 ng/ml) calibration standard shown in Figure A1.3 above. Therefore the retinal dopamine concentration values measured were well above the detection limit of the HPLC – MS/MS methodology used.

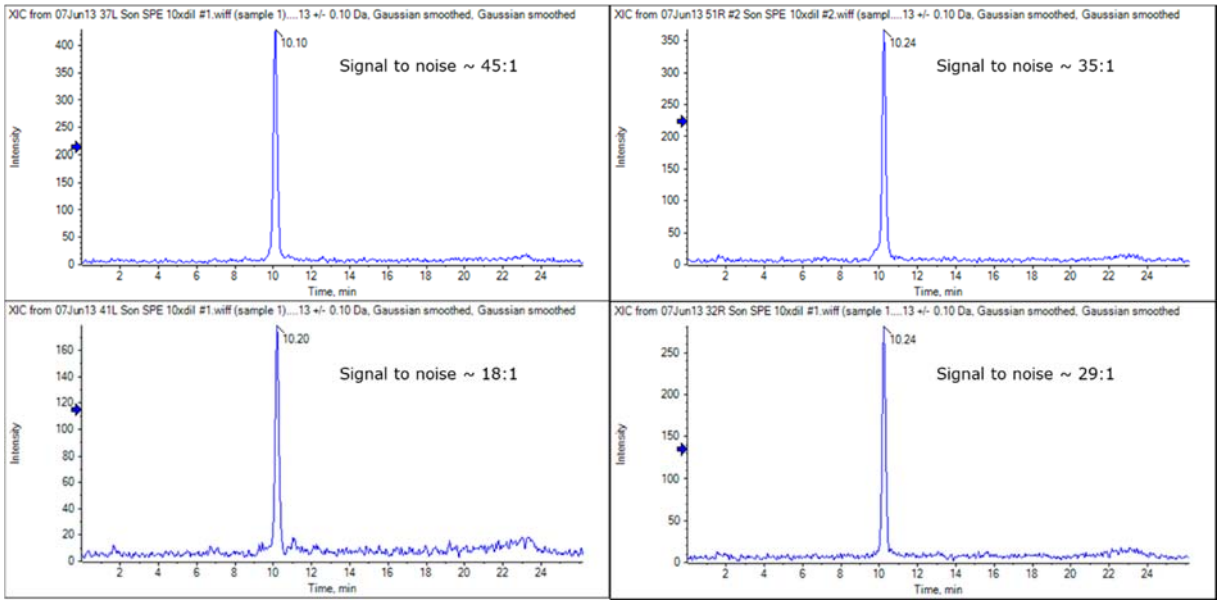

Figure A1.7. Representative Extracted Ion Chromatograms for the dopamine transition m/z 154 – 91 for four retinal samples showing SNR values between approximately 18:1 to 45:1. The SNR values obtained were at least two-fold better than the SNR obtained for the lowest concentration (2 ng/ml) calibration standard.

6.2 Appendix A2: Standard Intensity Illuminance Calculations

A2.1. Calculation of Chick and Human Photopic and Melanopic Illuminance Values for the white LED and fluorescent sources at 300 lux nominal illuminance

Spectral irradiance measurements were made using a Spectrascan PR655 spectrophotometer (PhotoResearch, USA, http://www.photoresearch.com) at a nominal 300 lux illuminance on the RS-3 reflectance accessory, as measured with a standard lux meter (Lutron LX-105) at chick eye level.

Calculation of the human photopic illuminance involved the conversion of irradiance (W/m^2) values to the equivalent photometric quantity, illuminance ($lumens/m^2$ or lux). The conversion was made by convolving the radiometric measurement with the CIE 1924 human photopic spectral efficiency function $V(\lambda)$ and multiplying by the and the photopic constant k_m (683 lm/W) (Wyszecki and Stiles 1982).

For discrete measurement values (5 nm steps in this study), the following equation can be used to convert between equivalent radiometric (R: radiant flux, radiance, irradiance) and photometric (L: luminous flux, luminance, illuminance) quantities:

Equation A2.1 $$L = k_m \sum R_\lambda V_\lambda$$

To calculate equivalent chicken photometric illuminance values, the chicken $V(\lambda)$ function values (Prescott and Wathes 1999) (Figure 4.26) were substituted into equation A2.1 (Lewis and Morris 2000; Saunders, Jarvis et al. 2008). Using this approach, a comparison could be made between a human photometric illuminance (h-lux) and chicken photometric illuminance (c-lux).

Lucas *et al* (Lucas, Peirson et al. 2014) provides method using a melanopic spectral efficiency function $N_z(\lambda)$, based on an opsin:vitamin A-based photopigment with a maximum sensitivity (λ_{max}) at 480 nm, which can be used in to calculate melanopic illuminance (m-lux) for humans and chicks. For chick calculations, the melanopic function was corrected for the spectral absorption properties of the ocular media, using an interpolation of transmission data for the visually similar galliform, the domestic turkey (Hart, Partridge et al. 1999; Hart 2001). A comparison could then be made between the calculated values for human melanopic illuminance (h-m-lux) and chicken melanopic illuminance (c-m-lux).

The various illuminance values were calculated for spectral irradiance measurements in 5 nm steps between 380 nm and 695 nm. The lower limit (380 nm) was set by the lowest wavelength measured

by the Spectrascan PR655 spectrophotometer (Photoresearch, USA), while the upper limit of 695 nm was set by the upper wavelength limit of the chicken V(λ) interpolated from the data of Prescott and Wathes (Prescott and Wathes 1999). Although this upper limit is lower than would be used typically for photometric calculations in humans, it was applied to both the human and chick illuminance calculations for comparative purposes. Furthermore, Figure 4.26 illustrates that the human V(λ) function approaches a value of zero above 695 nm, and so this upper limit to the calculation only introduces a small difference between the calculated human illuminance value and a measured value. As the irradiance measurements were made in discrete 5 nm steps, the final illuminance calculation based on Equation A2.1 was multiplied by a factor of 5.

Figure A2.1 illustrates the Excel spreadsheet used to calculate photopic and melanopic illuminance values for a QTX SL-Q8 LED source at a nominal illuminance of 300 lux. Photopic human illuminance (h-lux), chicken illuminance (c-lux) values were calculated using the CIE 1924 V(λ) function and Chick V(λ) function of Prescott and Wathes (1999). Chick and Human melanopic (m-lux) calculations used the melanopic response function $N_Z(\lambda)$ and methods of Lucas *et al* (2014) modified for chick and human media transmission characteristics respectively.

Figure A2.2 illustrates the Excel spreadsheet used to calculate photopic and melanopic illuminance values for a Philips TL5 HE 28W/840 fluorescent source at a nominal illuminance of 300 lux. Photopic human illuminance (h-lux), chicken illuminance (c-lux) values were calculated using the CIE 1924 V(λ) function and Chick v(λ) function of Prescott and Wathes (1999). Chick and Human melanopic (m-lux) calculations used the melanopic response function NZ(λ) and methods of Lucas *et al* (2014) modified for chick and human media transmission characteristics respectively.

	Prescott (1999)	CIE 1924	Lucas et al (2014)	Lucas et al (2014)	SLQ8 W300 lux				
	Chick	Human	Chick (M)	Human (M)	Irrad. (W/m^2)	Chick	Human	Chick (M)	Human
wavelength (λ)	Int. C-V(λ)	H-V(λ)	C-Nz(λ)	H-Nz(λ)	W300 S(λ)	V(λ)*S(λ)	V(λ)*S(λ)	C-Nz(λ)*S(λ)	H-Nz(λ)
380	0.203	0.000039	0.001378	0.000010	0.00000683	0.00000139	0.00000000	0.00000001	0.00000000
385	0.199	0.000064	0.001441	0.000019	0.00000686	0.00000136	0.00000000	0.00000001	0.00000000
390	0.194	0.000120	0.001541	0.000035	0.00001150	0.00000223	0.00000000	0.00000002	0.00000000
395	0.190	0.000217	0.001685	0.000067	0.00001342	0.00000255	0.00000000	0.00000002	0.00000000
400	0.185	0.000396	0.001884	0.000130	0.00001752	0.00000324	0.00000001	0.00000003	0.00000000
405	0.181	0.000640	0.002126	0.000260	0.00002960	0.00000535	0.00000002	0.00000006	0.00000001
410	0.176	0.001210	0.002432	0.000526	0.00005920	0.00001042	0.00000007	0.00000014	0.00000003
415	0.172	0.002180	0.002805	0.000906	0.00013757	0.00002360	0.00000030	0.00000039	0.00000012
420	0.227	0.004000	0.003242	0.001565	0.00032578	0.00007407	0.00000130	0.00000106	0.00000051
425	0.283	0.007300	0.003741	0.002134	0.00071456	0.00020232	0.00000522	0.00000267	0.00000152
430	0.339	0.011600	0.004317	0.002895	0.00135528	0.00045934	0.00001572	0.00000585	0.00000392
435	0.395	0.016840	0.004945	0.003658	0.00232258	0.00091675	0.00003911	0.00001149	0.00000849
440	0.450	0.023000	0.005614	0.004580	0.00377934	0.00170259	0.00008692	0.00002122	0.00001731
445	0.506	0.029800	0.006310	0.005406	0.00596588	0.00302046	0.00017778	0.00003765	0.00003225
450	0.562	0.038000	0.007017	0.006315	0.00772518	0.00434214	0.00029356	0.00005421	0.00004879
455	0.603	0.048000	0.007662	0.007182	0.00758302	0.00457368	0.00036398	0.00005810	0.00005446
460	0.644	0.060000	0.008272	0.008076	0.00585279	0.00377047	0.00035117	0.00004841	0.00004727
465	0.685	0.073900	0.008827	0.008956	0.00416182	0.00285206	0.00030756	0.00003674	0.00003727
470	0.726	0.090980	0.009305	0.009812	0.00303446	0.00220412	0.00027608	0.00002824	0.00002977
475	0.767	0.112600	0.009683	0.010467	0.00221152	0.00169719	0.00024902	0.00002141	0.00002315
480	0.809	0.139020	0.009937	0.011013	0.00169772	0.00137261	0.00023602	0.00001687	0.00001870
485	0.850	0.169300	0.010046	0.011299	0.00149658	0.00127145	0.00025337	0.00001504	0.00001691
490	0.818	0.208020	0.009994	0.011406	0.00148550	0.00121511	0.00030901	0.00001485	0.00001694
495	0.768	0.258600	0.009769	0.011315	0.00164808	0.00126608	0.00042619	0.00001610	0.00001865
500	0.718	0.323000	0.009372	0.011017	0.00202884	0.00145762	0.00065532	0.00001901	0.00002235
505	0.669	0.407300	0.008796	0.010519	0.00254500	0.00170181	0.00103658	0.00002239	0.00002677
510	0.658	0.503000	0.008089	0.009842	0.00309918	0.00203775	0.00155889	0.00002507	0.00003050
515	0.704	0.608200	0.007286	0.008956	0.00362932	0.00255587	0.00220736	0.00002644	0.00003250
520	0.751	0.710000	0.006425	0.007980	0.00409664	0.00307633	0.00290861	0.00002632	0.00003269
525	0.798	0.793200	0.005545	0.006951	0.00446106	0.00355839	0.00353851	0.00002474	0.00003101
530	0.844	0.862000	0.004680	0.005923	0.00471710	0.00398298	0.00406614	0.00002207	0.00002794
535	0.891	0.914850	0.003860	0.004933	0.00489774	0.00436430	0.00448070	0.00001890	0.00002416
540	0.938	0.954000	0.003108	0.004011	0.00502027	0.00470799	0.00478933	0.00001560	0.00002014
545	0.976	0.980300	0.002440	0.003184	0.00507446	0.00495227	0.00497449	0.00001238	0.00001616
550	0.980	0.994950	0.001865	0.002460	0.00508938	0.00498598	0.00506368	0.00000949	0.00001252
555	0.983	*1.000000*	0.001386	0.001848	0.00507838	0.00499432	0.00507838	0.00000704	0.00000939
560	0.987	0.995000	0.001003	0.001352	0.00504540	0.00498086	0.00502017	0.00000506	0.00000682
565	0.991	0.978600	0.000708	0.000962	0.00498414	0.00493913	0.00487748	0.00000353	0.00000479
570	0.995	0.952000	0.000488	0.000670	0.00488361	0.00485788	0.00464919	0.00000239	0.00000327
575	0.998	0.915400	0.000331	0.000456	0.00478072	0.00477352	0.00437627	0.00000158	0.00000218
580	0.940	0.870000	0.000221	0.000307	0.00466212	0.00438072	0.00405605	0.00000103	0.00000143
585	0.839	0.816300	0.000146	0.000204	0.00451447	0.00378783	0.00368516	0.00000066	0.00000092
590	0.738	0.757000	0.000096	0.000134	0.00432597	0.00319449	0.00327476	0.00000041	0.00000058
595	0.638	0.694900	0.000062	0.000088	0.00413512	0.00263757	0.00287350	0.00000026	0.00000036
600	0.537	0.631000	0.000041	0.000058	0.00393327	0.00211314	0.00248190	0.00000016	0.00000023
605	0.556	0.566800	0.000027	0.000038	0.00370315	0.00206043	0.00209895	0.00000010	0.00000014
610	0.576	0.503000	0.000017	0.000025	0.00344633	0.00198354	0.00173350	0.00000006	0.00000009
615	0.595	0.441200	0.000011	0.000016	0.00319657	0.00190102	0.00141033	0.00000004	0.00000005
620	0.614	0.381000	0.000008	0.000011	0.00295655	0.00181491	0.00112645	0.00000002	0.00000003
625	0.633	0.321000	0.000005	0.000007	0.00271567	0.00171905	0.00087173	0.00000001	0.00000002
630	0.652	0.265000	0.000003	0.000005	0.00247259	0.00161254	0.00065524	0.00000001	0.00000001
635	0.628	0.217000	0.000002	0.000003	0.00225307	0.00141566	0.00048892	0.00000001	0.00000001
640	0.540	0.175000	0.000001	0.000002	0.00204361	0.00110354	0.00035763	0.00000000	0.00000000
645	0.452	0.138200	0.000001	0.000001	0.00184097	0.00083150	0.00025442	0.00000000	0.00000000
650	0.363	0.107000	0.000001	0.000001	0.00164117	0.00059629	0.00017560	0.00000000	0.00000000
655	0.275	0.081600	0.000000	0.000001	0.00147482	0.00040558	0.00012035	0.00000000	0.00000000
660	0.239	0.061000	0.000000	0.000000	0.00131947	0.00031478	0.00008049	0.00000000	0.00000000
665	0.215	0.044580	0.000000	0.000000	0.00116812	0.00025126	0.00005207	0.00000000	0.00000000
670	0.192	0.032000	0.000000	0.000000	0.00103421	0.00019819	0.00003309	0.00000000	0.00000000
675	0.168	0.023200	0.000000	0.000000	0.00091986	0.00015469	0.00002134	0.00000000	0.00000000
680	0.145	0.017000	0.000000	0.000000	0.00081179	0.00011747	0.00001380	0.00000000	0.00000000
685	0.121	0.011920	0.000000	0.000000	0.00070961	0.00008603	0.00000846	0.00000000	0.00000000
690	0.098	0.008210	0.000000	0.000000	0.00062690	0.00006130	0.00000515	0.00000000	0.00000000
695	0.074	0.005723	0.000000	0.000000	0.00055174	0.00004100	0.00000316	0.00000000	0.00000000
					ΣS(λ)	C-ΣV(λ)*S(λ)	H-ΣV(λ)*S(λ)	C-ΣNz(λ)*S(λ)	H-ΣNz(λ)*S(λ)
					0.1775	0.1257	0.0886	0.00063537	0.00068316
					Km	683	683	72983.404	72983.25
					5nm steps	5	5	5	5
						429.3	302.4	231.9	249.3
						c-lux	h-lux	c-m-lux	h-m-lux
					SLQ8 W300 lux				

Figure A2.1. Excel spreadsheet used to calculate photopic and melanopic illuminance values for a QTX SL-Q8 LED-based lamp, producing a nominal 300 lux white illuminance at chick eye level in the experimental pen. Photopic human illuminance (h-lux), chicken illuminance (c-lux) values were calculated using the CIE 1924 V(l) function and Chick v(l) function of Prescott and Wathes (1999). Chick and Human melanopic (m-lux) calculations used the melanopic response function $N_Z(\lambda)$ of Lucas *et al* (2014) modified for chick and human media transmission characteristics respectively.

	Prescott (1999)	CIE 1924	Lucas et al (2014)	Lucas et al (2014)	Philips TL5 28W FL300				
	Chick	Human	Chick (M)	Human (M)	Irrad. (W/m^2)	Chick	Human	Chick (M)	Human
wavelength (λ)	Int. C-V(λ)	H-V(λ)	C-Nz(λ)	H-Nz(λ)	FL300 S(λ)	V(λ)*S(λ)	V(λ)*S(λ)	C-Nz(λ)*S(λ)	H-Nz(λ)
380	0.203	0.000039	0.001378	0.000010	0.00002727	0.00000554	0.00000000	0.00000004	0.00000000
385	0.199	0.000064	0.001441	0.000019	0.00003469	0.00000689	0.00000000	0.00000005	0.00000000
390	0.194	0.000120	0.001541	0.000035	0.00003555	0.00000690	0.00000000	0.00000005	0.00000000
395	0.190	0.000217	0.001685	0.000067	0.00009451	0.00001792	0.00000002	0.00000016	0.00000001
400	0.185	0.000396	0.001884	0.000130	0.00096510	0.00017864	0.00000038	0.00000182	0.00000013
405	0.181	0.000640	0.002126	0.000260	0.00177775	0.00032104	0.00000114	0.00000378	0.00000046
410	0.176	0.001210	0.002432	0.000526	0.00092394	0.00016268	0.00000112	0.00000225	0.00000049
415	0.172	0.002180	0.002805	0.000906	0.00051758	0.00008879	0.00000113	0.00000145	0.00000047
420	0.227	0.004000	0.003242	0.001565	0.00065848	0.00014970	0.00000263	0.00000213	0.00000103
425	0.283	0.007300	0.003741	0.002134	0.00103775	0.00029382	0.00000758	0.00000388	0.00000221
430	0.339	0.011600	0.004317	0.002895	0.00336653	0.00114099	0.00003905	0.00001453	0.00000975
435	0.395	0.016840	0.004945	0.003658	0.00713613	0.00281671	0.00012017	0.00003529	0.00002610
440	0.450	0.023000	0.005614	0.004580	0.00428827	0.00193187	0.00009863	0.00002407	0.00001964
445	0.506	0.029800	0.006310	0.005406	0.00146909	0.00074378	0.00004378	0.00000927	0.00000794
450	0.562	0.038000	0.007017	0.006315	0.00178442	0.00100298	0.00006781	0.00001252	0.00001127
455	0.603	0.048000	0.007662	0.007182	0.00174303	0.00105131	0.00008367	0.00001335	0.00001252
460	0.644	0.060000	0.008272	0.008076	0.00164965	0.00106274	0.00009898	0.00001365	0.00001332
465	0.685	0.073900	0.008827	0.008956	0.00151040	0.00103506	0.00011162	0.00001333	0.00001353
470	0.726	0.090980	0.009305	0.009812	0.00133518	0.00096982	0.00012147	0.00001242	0.00001310
475	0.767	0.112600	0.009683	0.010467	0.00122569	0.00094064	0.00013801	0.00001187	0.00001283
480	0.809	0.139020	0.009937	0.011013	0.00181458	0.00146710	0.00025226	0.00001803	0.00001998
485	0.850	0.169300	0.010046	0.011299	0.00373378	0.00317212	0.00063213	0.00003751	0.00004219
490	0.818	0.208020	0.009994	0.011406	0.00407307	0.00333168	0.00084728	0.00004071	0.00004646
495	0.768	0.258600	0.009769	0.011315	0.00272133	0.00209056	0.00070373	0.00002658	0.00003079
500	0.718	0.323000	0.009372	0.011017	0.00134869	0.00096896	0.00043563	0.00001264	0.00001486
505	0.669	0.407300	0.008796	0.010519	0.00073647	0.00049246	0.00029996	0.00000648	0.00000775
510	0.658	0.503000	0.008089	0.009842	0.00052119	0.00034269	0.00026216	0.00000422	0.00000513
515	0.704	0.608200	0.007286	0.008956	0.00043268	0.00030470	0.00026315	0.00000315	0.00000388
520	0.751	0.710000	0.006425	0.007980	0.00035123	0.00026375	0.00024937	0.00000226	0.00000280
525	0.798	0.793200	0.005545	0.006951	0.00032617	0.00026017	0.00025872	0.00000181	0.00000227
530	0.844	0.862000	0.004680	0.005923	0.00061434	0.00051873	0.00052956	0.00000287	0.00000364
535	0.891	0.914850	0.003860	0.004933	0.00312188	0.00278186	0.00285605	0.00001205	0.00001540
540	0.938	0.954000	0.003108	0.004011	0.01267947	0.01189078	0.01209621	0.00003941	0.00005086
545	0.976	0.980300	0.002440	0.003184	0.01900035	0.01854286	0.01862605	0.00004636	0.00006049
550	0.980	0.994950	0.001865	0.002460	0.01066414	0.01044748	0.01061028	0.00001989	0.00002624
555	0.983	*1.000000*	0.001386	0.001848	0.00316319	0.00311083	0.00316319	0.00000439	0.00000585
560	0.987	0.995000	0.001003	0.001352	0.00079294	0.00078280	0.00078897	0.00000080	0.00000107
565	0.991	0.978600	0.000708	0.000962	0.00040919	0.00040550	0.00040044	0.00000029	0.00000039
570	0.995	0.952000	0.000488	0.000670	0.00044925	0.00044688	0.00042768	0.00000022	0.00000030
575	0.998	0.915400	0.000331	0.000456	0.00149021	0.00148797	0.00136414	0.00000049	0.00000068
580	0.940	0.870000	0.000221	0.000307	0.00335208	0.00314975	0.00291631	0.00000074	0.00000103
585	0.839	0.816300	0.000146	0.000204	0.00443122	0.00371798	0.00361720	0.00000065	0.00000090
590	0.738	0.757000	0.000096	0.000134	0.00389715	0.00287782	0.00295014	0.00000037	0.00000052
595	0.638	0.694900	0.000062	0.000088	0.00286827	0.00182951	0.00199316	0.00000018	0.00000025
600	0.537	0.631000	0.000041	0.000058	0.00203387	0.00109269	0.00128337	0.00000008	0.00000012
605	0.556	0.566800	0.000027	0.000038	0.00440192	0.00244923	0.00249501	0.00000012	0.00000017
610	0.576	0.503000	0.000017	0.000025	0.01378217	0.00793236	0.00693243	0.00000024	0.00000034
615	0.595	0.441200	0.000011	0.000016	0.01185951	0.00705292	0.00523242	0.00000014	0.00000019
620	0.614	0.381000	0.000008	0.000011	0.00482234	0.00296024	0.00183731	0.00000004	0.00000005
625	0.633	0.321000	0.000005	0.000007	0.00392542	0.00248484	0.00126006	0.00000002	0.00000003
630	0.652	0.265000	0.000003	0.000005	0.00327432	0.00213540	0.00086770	0.00000001	0.00000002
635	0.628	0.217000	0.000002	0.000003	0.00155422	0.00097656	0.00033727	0.00000000	0.00000000
640	0.540	0.175000	0.000001	0.000002	0.00059439	0.00032097	0.00010402	0.00000000	0.00000000
645	0.452	0.138200	0.000001	0.000001	0.00066476	0.00030025	0.00009187	0.00000000	0.00000000
650	0.363	0.107000	0.000001	0.000001	0.00097012	0.00035248	0.00010380	0.00000000	0.00000000
655	0.275	0.081600	0.000000	0.000001	0.00084642	0.00023277	0.00006907	0.00000000	0.00000000
660	0.239	0.061000	0.000000	0.000000	0.00070215	0.00016751	0.00004283	0.00000000	0.00000000
665	0.215	0.044580	0.000000	0.000000	0.00061347	0.00013196	0.00002735	0.00000000	0.00000000
670	0.192	0.032000	0.000000	0.000000	0.00044516	0.00008531	0.00001425	0.00000000	0.00000000
675	0.168	0.023200	0.000000	0.000000	0.00037628	0.00006328	0.00000873	0.00000000	0.00000000
680	0.145	0.017000	0.000000	0.000000	0.00040527	0.00005864	0.00000689	0.00000000	0.00000000
685	0.121	0.011920	0.000000	0.000000	0.00055001	0.00006669	0.00000656	0.00000000	0.00000000
690	0.098	0.008210	0.000000	0.000000	0.00051616	0.00005047	0.00000424	0.00000000	0.00000000
695	0.074	0.005723	0.000000	0.000000	0.00029533	0.00002195	0.00000169	0.00000000	0.00000000
					Σ S(λ)	C-Σ V(λ)*S(λ)	H-Σ V(λ)*S(λ)	C-Σ Nz(λ)*S(λ)	H-Σ Nz(λ)*S(λ)
					0.1672	0.1175	0.0882	0.00045867	0.00048945
					Km	683	683	72983.40	72983.25
					5nm steps	5	5	5	5
						401.4	301.2	167.4	178.6
						c-lux	h-lux	c-m-lux	h-m-lux
					Philips TL5 28W FL300				

Figure A2.2. Excel spreadsheet used to calculate photopic and melanopic illuminance values for a Philips TL5 HE 28W/840 fluorescent lamp, producing a nominal 300 lux white illuminance at chick eye level in the experimental pen. Photopic human illuminance (h-lux), chicken illuminance (c-lux) values were calculated using the CIE 1924 V(l) function and Chick v(l) function of Prescott and Wathes (1999). Chick and Human melanopic (m-lux) calculations used the melanopic response function $N_Z(\lambda)$ of Lucas *et al* (2014) modified for chick and human media transmission characteristics respectively.

6.3 Appendix A3: High Intensity Illuminance Calculations

A3.1. Calculation of Chick Melanopic Illuminance Values for the QTX SL-Q8 LED-based white (W10KP), Blue (B10KP), Green (G10KP) and Red (R10KP) High Intensity Illumination Conditions

The high intensity lighting conditions W10KP, B10KP, G10KP and R10KP were equated for irradiance within the limits of the equipment design (Section 4.2.1). However, due to the differing spectral irradiance distributions of each lighting condition (Figure 4.5), melanopic illuminance (m-lux) values were calculated to investigate whether each high intensity lighting condition would have provided a differential stimulus to a putative melanopsin-based eye growth control mechanism.

Spectral irradiance measurements were made using a Spectrascan PR655 spectrophotometer (PhotoResearch, USA, http://www.photoresearch.com) for each high intensity lighting condition. The chick melanopic illuminance values were calculated using the method of Lucas *et al* (Lucas, Peirson et al. 2014) as described in Appendix A2.

Figure A3.1 illustrates the Excel spreadsheet used to calculate melanopic illuminance values for a QTX SL-Q8 LED-based lamp from irradiance values measured under the W10KP, B10KP, G10KP and R10KP conditions at chick eye level in the experimental pen. Chick melanopic (c-m-lux) calculations used the melanopic response function NZ(λ) and methods of Lucas *et al* (2014) modified for chick media transmission characteristics.

High Intensity	Lucas et al (2014)	W10KP		B10KP		G10KP		R10KP	
	Chick (M)	Irrad. (W/m^2)	Chick (M)	Irrad. (W/m^2)	Chick (M)	Irrad. (W/m^2)	Chick (M)	Irrad. (W/m^2)	Chick (M)
wavelength (λ)	C-Nz(λ)	S(λ)	C-Nz(λ)*S(λ)	S(λ)	C-Nz(λ)*S(λ)	S(λ)	C-Nz(λ)*S(λ)	S(λ)	C-Nz(λ)*S(λ)
380	0.001 378	0.00024234	0.00000033	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
385	0.001 441	0.00035908	0.00000052	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
390	0.001 541	0.00041390	0.00000064	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
395	0.001 685	0.00050454	0.00000085	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
400	0.001 884	0.00078697	0.00000148	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
405	0.002 126	0.00156114	0.00000332	0.00022375	0.00000048	0.00000000	0.00000000	0.00000000	0.00000000
410	0.002 432	0.00346062	0.00000842	0.00111746	0.00000272	0.00000000	0.00000000	0.00000000	0.00000000
415	0.002 805	0.00778487	0.00002183	0.00293370	0.00000823	0.00000000	0.00000000	0.00000000	0.00000000
420	0.003 242	0.01667557	0.00005406	0.00736075	0.00002386	0.00000000	0.00000000	0.00000000	0.00000000
425	0.003 741	0.03259324	0.00012195	0.01907732	0.00007138	0.00000000	0.00000000	0.00000000	0.00000000
430	0.004 317	0.05566902	0.00024034	0.04430902	0.00019130	0.00000000	0.00000000	0.00000000	0.00000000
435	0.004 945	0.08633882	0.00042697	0.09314037	0.00046061	0.00000458	0.00000002	0.00000000	0.00000000
440	0.005 614	0.12610353	0.00070796	0.18233804	0.00102366	0.00000928	0.00000005	0.00000000	0.00000000
445	0.006 310	0.17989545	0.00113516	0.36162873	0.00228191	0.00016923	0.00000107	0.00000000	0.00000000
450	0.007 017	0.22698007	0.00159281	0.59957296	0.00420746	0.00058811	0.00000413	0.00000000	0.00000000
455	0.007 662	0.23313759	0.00178628	0.74510724	0.00570893	0.00136345	0.00001045	0.00000000	0.00000000
460	0.008 272	0.19499866	0.00161302	0.68832295	0.00569376	0.00269109	0.00002226	0.00000000	0.00000000
465	0.008 827	0.14535364	0.00128303	0.49527208	0.00437173	0.00540511	0.00004771	0.00000000	0.00000000
470	0.009 305	0.10799225	0.00100485	0.32864201	0.00305797	0.01030914	0.00009593	0.00000000	0.00000000
475	0.009 683	0.08057400	0.00078017	0.20812266	0.00201517	0.01922105	0.00018611	0.00000000	0.00000000
480	0.009 937	0.06261194	0.00062218	0.12692034	0.00126121	0.03389778	0.00033684	0.00000000	0.00000000
485	0.010 046	0.05412179	0.00054373	0.07891681	0.00079284	0.06056991	0.00060851	0.00000000	0.00000000
490	0.009 994	0.05219756	0.00052165	0.05006128	0.00050030	0.10436371	0.00104298	0.00000000	0.00000000
495	0.009 769	0.05583396	0.00054542	0.03154709	0.00030817	0.17369866	0.00169681	0.00000000	0.00000000
500	0.009 372	0.06562787	0.00061506	0.01930195	0.00018090	0.28132962	0.00263661	0.00000000	0.00000000
505	0.008 796	0.07989070	0.00070274	0.01357796	0.00011944	0.40157408	0.00353236	0.00000000	0.00000000
510	0.008 089	0.09570862	0.00077423	0.01019918	0.00008251	0.49998447	0.00404461	0.00000000	0.00000000
515	0.007 286	0.11124380	0.00081052	0.00814929	0.00005938	0.54019686	0.00393586	0.00000000	0.00000000
520	0.006 425	0.12525530	0.00080475	0.00707487	0.00004546	0.50925217	0.00327188	0.00000000	0.00000000
525	0.005 545	0.13672997	0.00075818	0.00640335	0.00003551	0.42018802	0.00232999	0.00000000	0.00000000
530	0.004 680	0.14507875	0.00067891	0.00601458	0.00002815	0.33740705	0.00157892	0.00000000	0.00000000
535	0.003 860	0.15133052	0.00058412	0.00574126	0.00002216	0.26398018	0.00101894	0.00000000	0.00000000
540	0.003 108	0.15588583	0.00048452	0.00576168	0.00001791	0.20024512	0.00062240	0.00010342	0.00000032
545	0.002 440	0.15839910	0.00038650	0.00570513	0.00001392	0.14587200	0.00035593	0.00023526	0.00000057
550	0.001 865	0.15920021	0.00029693	0.00534542	0.00000997	0.10430088	0.00019454	0.00003269	0.00000006
555	0.001 386	0.15903527	0.00022047	0.00506111	0.00000702	0.07542964	0.00010457	0.00000000	0.00000000
560	0.001 003	0.15814777	0.00015864	0.00487889	0.00000489	0.05416106	0.00005433	0.00000000	0.00000000
565	0.000 708	0.15622355	0.00011058	0.00470611	0.00000333	0.03886778	0.00002751	0.00014668	0.00000010
570	0.000 488	0.15308981	0.00007478	0.00456473	0.00000223	0.02718891	0.00001328	0.00067026	0.00000033
575	0.000 331	0.14979114	0.00004957	0.00440451	0.00000146	0.01959568	0.00000648	0.00158572	0.00000052
580	0.000 221	0.14605264	0.00003225	0.00419088	0.00000093	0.01422827	0.00000314	0.00300148	0.00000066
585	0.000 146	0.14134811	0.00002061	0.00407229	0.00000059	0.01040731	0.00000152	0.00596667	0.00000087
590	0.000 096	0.13540264	0.00001294	0.00376520	0.00000036	0.00750998	0.00000072	0.01168358	0.00000112
595	0.000 062	0.12945718	0.00000808	0.00344633	0.00000022	0.00548444	0.00000034	0.02150499	0.00000134
600	0.000 041	0.12321326	0.00000502	0.00316358	0.00000013	0.00406836	0.00000017	0.03813893	0.00000155
605	0.000 027	0.11616824	0.00000309	0.00290354	0.00000008	0.00314199	0.00000008	0.06943705	0.00000185
610	0.000 017	0.10838495	0.00000189	0.00281707	0.00000005	0.00247353	0.00000004	0.12649623	0.00000220
615	0.000 011	0.10076658	0.00000115	0.00240450	0.00000003	0.00180414	0.00000002	0.21934600	0.00000251
620	0.000 008	0.09327389	0.00000070	0.00192831	0.00000001	0.00112281	0.00000001	0.36536723	0.00000276
625	0.000 005	0.08578904	0.00000043	0.00167400	0.00000001	0.00073827	0.00000000	0.59965150	0.00000300
630	0.000 003	0.07822566	0.00000026	0.00137900	0.00000000	0.00038184	0.00000000	0.86550878	0.00000287
635	0.000 002	0.07151050	0.00000016	0.00108943	0.00000000	0.00012716	0.00000000	0.90328643	0.00000200
640	0.000 001	0.06525088	0.00000010	0.00077283	0.00000000	0.00000000	0.00000000	0.60695570	0.00000091
645	0.000 001	0.05907765	0.00000006	0.00069853	0.00000000	0.00000000	0.00000000	0.28447121	0.00000029
650	0.000 001	0.05293584	0.00000004	0.00046023	0.00000000	0.00000000	0.00000000	0.11072543	0.00000008
655	0.000 000	0.04773650	0.00000002	0.00020592	0.00000000	0.00000000	0.00000000	0.04567090	0.00000002
660	0.000 000	0.04294557	0.00000001	0.00009503	0.00000000	0.00000000	0.00000000	0.01876159	0.00000001
665	0.000 000	0.03819391	0.00000001	0.00000290	0.00000000	0.00000000	0.00000000	0.00859147	0.00000000
670	0.000 000	0.03391349	0.00000001	0.00000000	0.00000000	0.00000000	0.00000000	0.00418146	0.00000000
675	0.000 000	0.03021034	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00223626	0.00000000
680	0.000 000	0.02689832	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00131853	0.00000000
685	0.000 000	0.02342607	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00114063	0.00000000
690	0.000 000	0.02079577	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00078697	0.00000000
695	0.000 000	0.01838460	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000	0.00084611	0.00000000
		ΣS(λ)	**C-ΣNz(λ)*S(λ)**	**ΣS(λ)**	**C-ΣNz(λ)*S(λ)**	**ΣS(λ)**	**C-ΣNz(λ)*S(λ)**	**ΣS(λ)**	**C-ΣNz(λ)*S(λ)**
		5.67619038	**0.02061431**	**4.21657415**	**0.03261830**	**4.38335271**	**0.02778712**	**4.31784916**	**0.00002595**
		Km	**72983.404**	**Km**	**72983.404**	**Km**	**72983.404**	**Km**	**72983.404**
		5nm steps	5		5		5		5
			7522.5		**11903.0**		**10140.0**		**9.5**
			c-m-lux		**c-m-lux**		**c-m-lux**		**c-m-lux**
		W10KP		**B10KP**		**G10KP**		**R10KP**	

Figure A3.1. Excel spreadsheet used to calculate melanopic illuminance values for a QTX SL-Q8 LED-based lamp from irradiance values measured under the W10KP, B10KP, G10KP and R10KP conditions at chick eye level in the experimental pen. Chick melanopic (c-m-lux) calculations used the melanopic response function $N_Z(\lambda)$ and methods of Lucas *et al* (2014) modified for chick media transmission characteristics.

6.4 Appendix A4: Time-course of form-deprivation myopia development in the Cobb chick

A4.1. Introduction

As reported in Chapter 4, the investigation into the effect of periodic high intensity illumination on the development of form-deprivation myopia (FDM) in the Cobb-strain chick found no significant difference in the refractive outcome between chicks raised for 4 days under 300 lux constant daily illumination (W300C) and those raised under 10,000 lux periodic white LED illumination (W10KP) (Figure 4.7). Under the 10,000 lux condition chicks were exposed to 15 minute periods of high intensity white LED-based illumination interspersed with 15 minutes of 300 lux illumination at a 50% duty cycle for 12 hours per day (see Section 4.2). The degree of FDM induced after 4 days of exposure to the W300C white LED-based illumination condition was found to be significantly different to that produced in a previous experiment from the same laboratory (Backhouse, Collins et al. 2013) where Cobb chicks were raised under 300 lux fluorescent lighting (Figure 4.9). However, the degree of FDM induced in the W300C cohort in this study was noted to be consistent with levels previously reported in the literature (Karouta and Ashby 2015) where chicks were also raised under a similar illumination level of white LED lighting (Figure 4.23; Table 4.10).

One possible explanation for these findings is that white LED illumination influences the time-course of development of FDM in chicks under both standard intensity (300 - 500 lux) and high intensity (10,000 - 15,000 Lux) conditions when compared to results previously published using a combination of fluorescent and halogen lighting (Backhouse, Collins et al. 2013; Lan, Feldkaemper et al. 2014). The mechanisms by which white LED-based lighting might influence FDM development are discussed extensively in Sections 4.4.2 and 4.4.3, and the case is made for the importance of considering the spectral energy distribution of lighting sources when designing illumination-dependent animal experiments such as reported in this thesis (Section 4.4.6)

Therefore, an ancillary experiment was undertaken with the aim of investigating the time-course of development of FDM in the Cobb chick under both standard (300 lux) and high intensity (15,000 lux) white LED illumination conditions.

A4.2. Methods

Chicks

Twenty-four one-day post-hatch Cobb strain chicks (*Gallus gallus domesticus*) were obtained from a local commercial hatchery. The chicks were transferred to the open-topped plastic pens used for the experiments (see Section 4.2) and allowed to acclimatise to the conditions for two days. Six chicks were kept together per pen for the duration of the experiment. Chick feeding and care protocols were as described in Section 2.1.1.

Experimental Protocol

On day 4 post-hatch, monocular translucent goggle-style diffusers were attached around one eye of each chick following as described in Section 2.1.2. The 24 chicks were divided into two equal cohorts (n = 12) and the experimental lighting cycles commenced (Figure A4.1). Cohort 1 (300) was exposed to 12 hours daily of 300 lux white LED illumination in a 12:12 hour light:dark cycle. Cohort 2 (15K) was exposed to a daily illumination cycle of 3 hours 300 lux: 6 hours 15,000 lux: 6 hours 300 lux in a 12:12 hour light:dark cycle. Lighting was provided by QTX SL-Q8 stage lights (AVSL Group Ltd, UK, http://www.avsl-qtx.com) as described in Section 4.2.1.

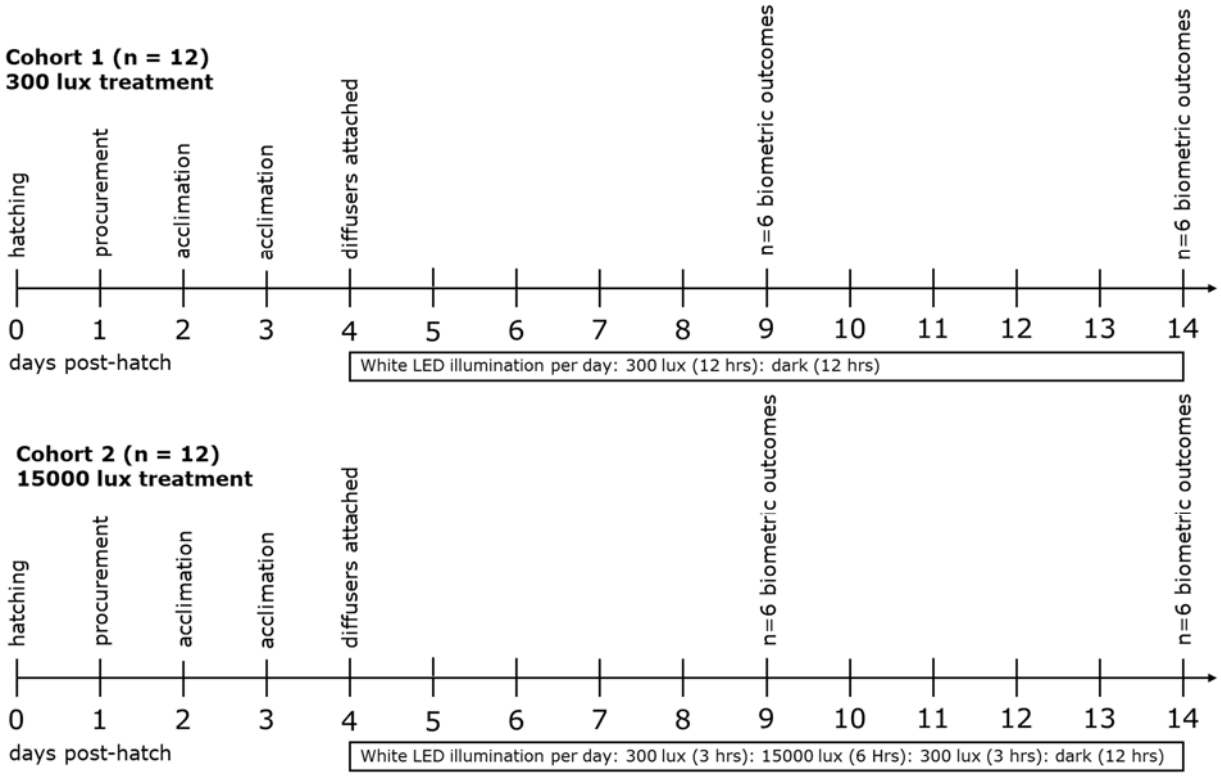

Figure A4.1. Experimental protocol showing the two lighting cohorts (300 lux and 15000 lux, n = 12 each) and the time-line of outcome measurements. For each cohort, outcome measurements were made on 6 chicks at day 9 post-hatch (5 days FD) and day 14 post-hatch (10 days FD).

The chicks were monitored at least twice a day (morning and late afternoon) to check that the diffusers were still securely attached. The diffusers were worn continuously, except for brief periods where they were removed for cleaning or replacement. This procedure was usually undertaken during the morning check, and the diffuser was removed and replaced as quickly as possible. The diffusers were left in place until after the chick was readied for ocular measurements following anaesthesia (see Section 2.3.2).

Outcome Measurements

Refractive and biometric outcome measurements were made on 6 chicks from each lighting cohort on day 9 post-hatch (5 days FD) and on day 14 post-hatch (10 days FD).

Refraction was measured in chicks using an IR optometer approximately 10 minutes after induction of anaesthesia as described in Section 2.4.2. Refractions were measured two times consecutively at the 0^o and 90^o meridians. The mean sphere refraction was calculated as the average of each pair of measurements. No correction for small eye artefact was made as is common practice in chick myopia experiments (Wallman and Adams 1987; Backhouse, Collins et al. 2013). The primary refractive outcome measurement, relative refraction, was calculated by subtracting the mean sphere of the contralateral non-deprived eye from the mean sphere of the deprived eye.

Corneal curvature was measured in anaesthetised chicks using the infra-red videokeratometer described in Section 2.5. Four images were analysed for each eye and the average of the radii from the 0^o and 90^o meridians were used to determine the corneal radius of curvature of each eye. The primary outcome measurement, relative corneal radius (R), was calculated by subtracting the average corneal radius of the contralateral non-deprived eye from the average corneal radius of the deprived eye.

Axial biometry measurements were measured using the A-scan ultrasound system described in Section 2.6. Measurements were made of anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD) and total axial length (AXL) of both the form-deprived and non-deprived eye. Relative biometry values for ACD, LT, VCD and AXL were calculated by subtracting the respective mean biometric value of the contralateral non-deprived eye from the mean biometric value of the deprived eye.

All results are presented as paired-eye or relative measurements. Paired-eye comparisons are a means of controlling for individual variation in growth rates between animals which might influence

biometric parameters. The non-occluded eye effectively acts as an internal control eye for comparison of refractive outcome with the occluded eye (Wallman and Adams 1987). Statistical analysis was conducted as described in Section 2.7.

A4.3. Results

Refractive Outcome – Relative Mean Sphere Refraction

The average relative mean-sphere refraction for each illumination group by days of form-deprivation is illustrated in Figure A4.2.

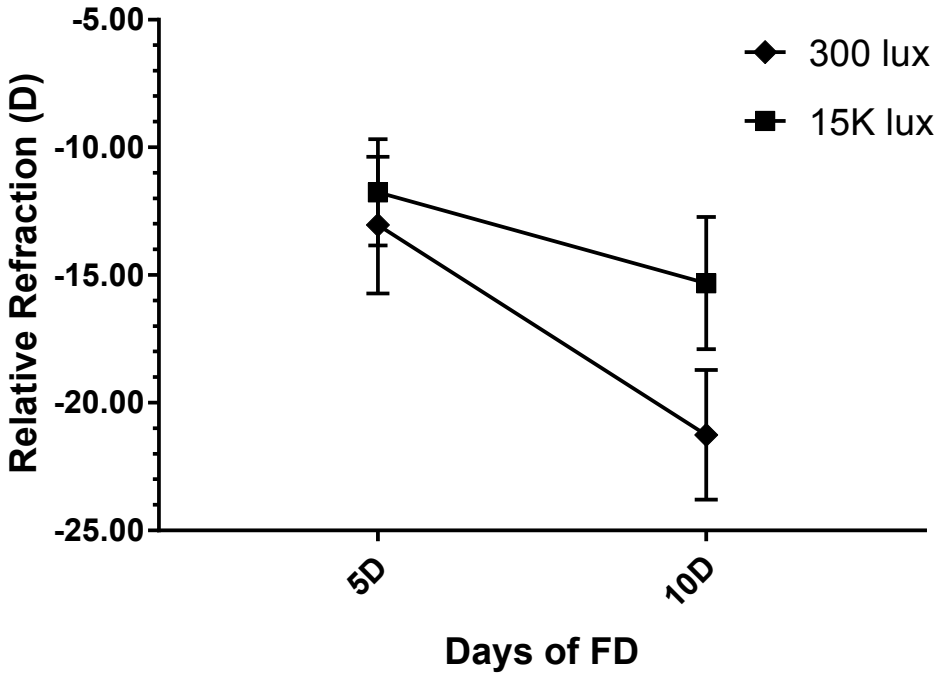

Figure A4.2. Relative mean sphere refractions (deprived eye - non-deprived eye) for the 2 illumination conditions by days of form-deprivation: White 300 lux (300, 5 days FD, n = 6; 10 days FD, n = 6), and White 15,000 lux (15K, 5 days FD, n = 5; 10 days FD n = 6. Two-way ANOVA analysis of relative mean sphere refraction found (i) a statistically significant effect of days of FD ($F_{(1, 19)}$ = 5.389, p = 0.032), and (ii) no significant effect of illumination condition ($F_{(1, 19)}$ = 2.031, p = 0.170).

All treatment groups exhibited a relative myopic refraction on average irrespective of lighting condition or days of form-deprivation (Figure A4.2; Table A4.1). Inspection of Figure A4.2 demonstrates the apparent trend for relative refraction to become more myopic with increasing time of form-deprivation as would be expected. Two-way ANOVA analysis of relative mean sphere refraction confirmed the statistically significant effect of days of FD on relative refraction ($F_{(1, 19)}$ = 5.389, p = 0.032), however illumination condition did not demonstrate an overall significant effect on relative refraction ($F_{(1, 19)}$ = 2.031, p = 0.170). This is perhaps not unexpected as Figure A4.2 illustrates that at 5 days of FD the refractive outcomes under each illumination condition were initially similar at -13.04 ± 2.68 D (300 lux)

and -11.76 ± 2.08 D (15K lux), with an apparent increasing difference in refraction developing by 10 days of FD of -21.25 ± 2.54 D (300 lux) and -15.31 ± 2.59 D (15K lux) respectively. Therefore, pair-wise post-hoc analysis was undertaken using Sidak's multiple comparisons test to assess the statistical significance of these trends as summarised in Table A4.1 below.

Relative Refraction (D)	300 Lux	15K Lux	post-hoc SIdak
5 Days FD	-13.04 ± 2.68	-11.76 ± 2.08	$p = 0.927$
10 Days FD	-21.25 ± 2.54	-15.31 ± 2.59	$p = 0.201$
post-hoc Sidak	$p = 0.059$	$p = 0.571$	

Table A4.1 Refractive outcome measures (mean ± 1 SEM) for each of the 2 illumination conditions and by days of form-deprivation. Post-hoc analysis by Sidak's multiple comparisons test comparing the relative refraction measures by row (days FD) and column (illumination condition) did not reveal any statistically significant effects. However, the difference in relative refraction between 5 days and 10 days of FD under the 300 lux condition approached statistical significance ($p = 0.059$).

Post-hoc analysis using Sidak's multiple comparisons tests showed no significant effects on relative refraction by days of FD or illumination condition. However, the difference in relative refraction between 5 and 10 days of FD under the 300 lux condition approached significance ($p = 0.059$). In comparison, the difference under the 15,000 lux condition, did not approach statistical significance ($p = 0.571$). Post-hoc power analysis (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) shows that the statistical power of the analysis for relative refraction was relatively low at 52%, and that a sample size of 11 animals per group would be required for the variation in relative refraction by days of FD under the 300 lux condition to reach statistical significance.

Although a trend for an increasing difference in relative refraction between 5 and 10 days of FD is seen in Figure A4.2, the values of -21.25 ± 2.54 D (300 lux) and -15.31 ± 2.59 D (15K lux) were not found to be significantly different ($p = 0.201$) following post-hoc analysis. Again, the small sample size of 6 per group resulted in a relatively low statistical power for this comparison of 31% (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010), and that a sample size of 19 animals per group would be required for the variation in relative refraction at 10 days FD by illumination condition to reach statistical significance.

The relationships between the underlying biometric components, including vitreous chamber depth (VCD), and these relative refraction trends are considered in the following sections.

Relative Vitreous Chamber Depth

The average relative vitreous chamber depth (VCD) for each illumination group and by days of form-deprivation is illustrated in Figure A4.3.

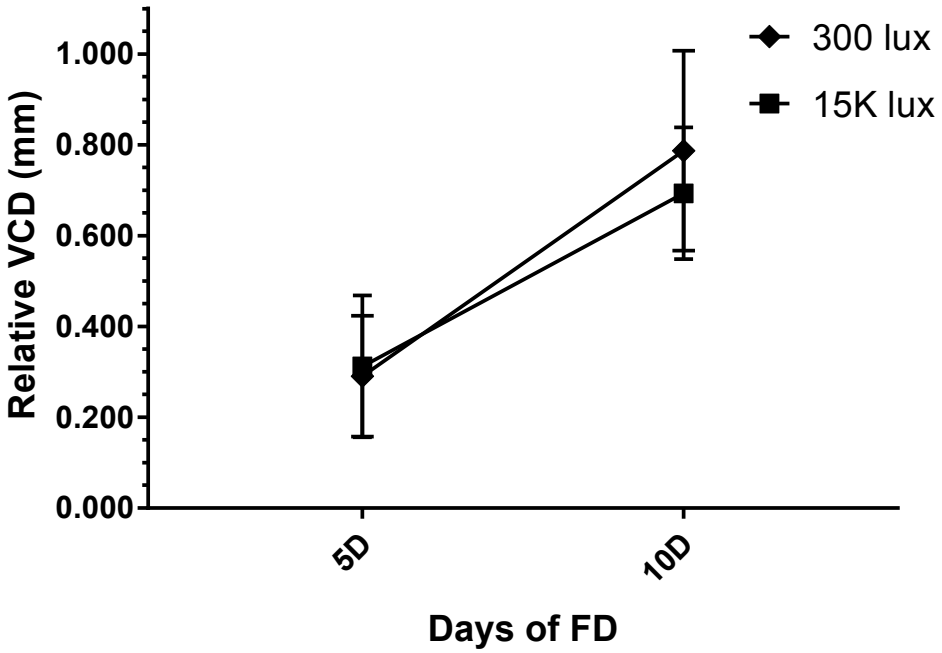

Figure A4.3. Relative vitreous chamber depth (deprived eye - non-deprived eye) for the 2 illumination conditions by days of form deprivation: White 300 lux (300, 5 days FD, n = 6; 10 days FD, n = 6), and White 15,000 lux (15K, 5 days FD, n = 5; 10 days FD, n = 6. Two-way ANOVA analysis of relative vitreous chamber depth found (i) a statistically significant effect of days of FD ($F_{(1, 19)} = 6.734$, $p = 0.018$), and (ii) no significant effect of illumination condition ($F_{(1, 19)} = 0.046$, $p = 0.833$).

Figure A4.3 illustrates the trend for relative VCD to increase with increasing time of form-deprivation as would be expected. Two-way ANOVA analysis of relative VCD confirmed the statistically significant effect of days of FD on relative refraction ($F_{(1, 19)} = 6.734$, $p = 0.018$), conversely illumination condition did not demonstrate an overall significant effect on relative refraction ($F_{(1, 19)} = 0.046$, $p = 0.833$). However, a small apparent trend is evident at 5 days FD for the 300 lux group to exhibit on average a greater relative VCD than the 15K illumination group. The relative VCD values obtained for the two illumination groups and by days of FD are summarised in Table A4.2 below. The table also includes the results of pair-wise post-hoc analysis using Sidak's multiple comparisons test to assess the statistical significance of these trends.

Relative VCD (D)	300 Lux	15K Lux	post-hoc SIdak
5 Days FD	+0.291 ± 0.133	+0.312 ± 0.156	p = 0.995
10 Days FD	+0.787 ± 0.220	+0.693 ± 0.145	p = 0.905
post-hoc Sidak	p = 0.091	p = 0.253	

Table A4.2 Relative vitreous chamber depth (mean ± 1 SEM) for each of the 2 illumination conditions and by days of form-deprivation. Post-hoc analysis by Sidak's multiple comparisons test comparing the relative VCD measures by row (days FD) and column (illumination condition) did not reveal any statistically significant effects. However, the difference in relative VCD between 5 days and 10 days of FD under the 300 lux condition approached statistical significance (p = 0.091).

Post-hoc analysis using Sidak's multiple comparisons tests showed no significant effects on relative VCD by days of FD or illumination condition. However, the difference in relative refraction between 5 and 10 days of FD under the 300 lux condition approached significance (p = 0.091). In comparison, the difference under the 15,000 lux condition, did not approach statistical significance (p = 0.253). Post-hoc power analysis (G*Power, University of Dusseldorf)(Faul, Erdfelder et al. 2007; Prajapati, Dunne et al. 2010) shows that the statistical power of the analysis for relative refraction was relatively low at 41%, and that a sample size of 14 animals per group would be required for the variation in relative VCD by days of FD under the 300 lux condition to reach statistical significance.

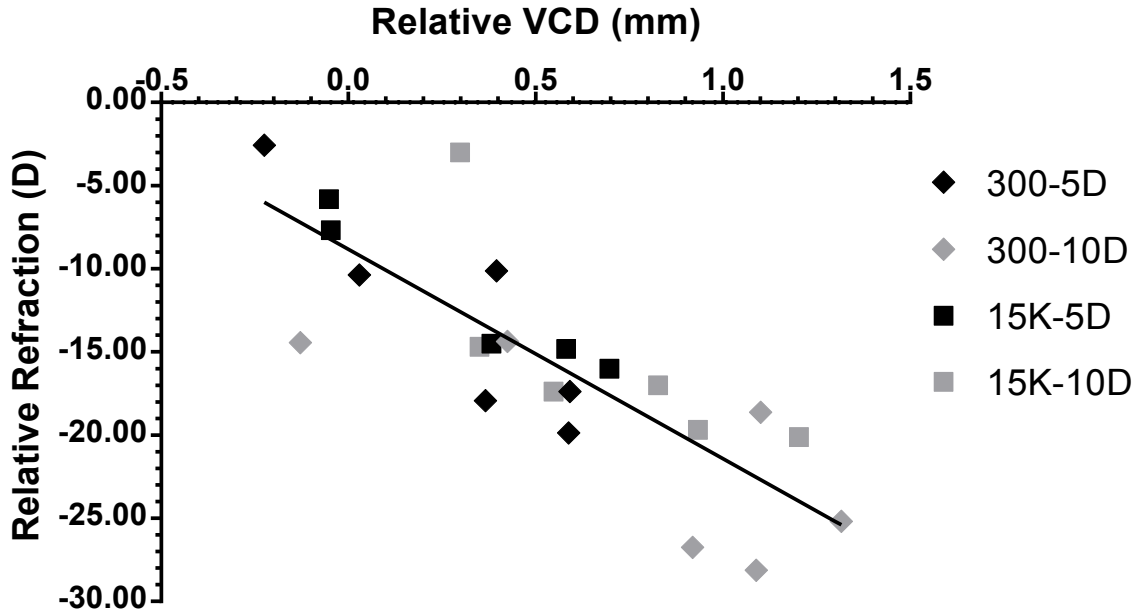

Figure A4.4. Correlation between relative mean sphere refraction and relative vitreous chamber depth (deprived eye - non-deprived eye) for all chicks: Linear regression revealed a strong correlation (R^2 = 0.671, p < 0.0001). The linear regression line is for the pooled data of all chicks.

Although no significant effect of days of FD or illumination condition on relative VCD was found, linear regression of this variable against relative mean sphere refraction (pooled data)(Figure A4.4), revealed

that increased relative VCD was strongly correlated with increased relative myopic refraction (R^2 = 0.671, $F_{1,21}$ = 42.73, p < 0.0001).

Additional Biometric Outcome Measurements

A summary of additional biometric outcome measures obtained for each treatment condition: relative corneal radius (R); anterior chamber depth (ACD); lens thickness (LT) and axial length (AXL); are summarised in Table A4.3 below. Table A4.3 also incorporates the results of analysis using two-way ANOVA with post-hoc Sidak comparison tests for statistical significance for each outcome measure by days of FD or illumination condition.

Relative R (mm)	**300 Lux**	**15K Lux**	**post-hoc SIdak**
5 Days FD	-0.175 ± 0.074	+0.113 ± 0.077	p = 0.497
10 Days FD	+0.133 ± 0.197	-0.015 ± 0.269	p = 0.813
post-hoc Sidak	p = 0.416	p = 0.871	
Relative ACD (mm)	**300 Lux**	**15K Lux**	**post-hoc SIdak**
5 Days FD	+0.114 ± 0.044	+0.145 ± 0.046	p = 0.942
10 Days FD	+0.359 ± 0.083	+0.433 ± 0.082	p = 0.693
post-hoc Sidak	p = **0.036**	p = **0.019**	
Relative LT (mm)	**300 Lux**	**15K Lux**	**post-hoc SIdak**
5 Days FD	+0.001 ± 0.043	+0.049 ± 0.046	p = 0.584
10 Days FD	+0.011 ± 0.023	-0.024 ± 0.024	p = 0.719
post-hoc Sidak	p = 0.974	p = 0.298	
Relative AXL (mm)	**300 Lux**	**15K Lux**	**post-hoc SIdak**
5 Days FD	+0.407 ± 0.163	+0.507 ± 0.125	p = 0.910
10 Days FD	+1.157 ± 0.243	+1.102 ± 0.131	p = 0.969
post-hoc Sidak	p = **0.012**	p = 0.062	

Table A4.3 Additional relative biometric outcome measures (mean ± 1 SEM) for each of the illumination conditions and by days of form-deprivation. Statistically significant (p <0.05) values in **bold** from post-hoc analysis by Sidak's multiple comparisons test comparing outcome measures by row (days FD) and column (illumination condition). A statistically significant effect of days of FD on relative ACD was found for both the 300 lux (p = 0.036) and 15K lux (p = 0.019) illumination conditions. However, for AXL, a statistically significant effect was only found for the 300 lux (p = 0.012) illumination condition.

A statistically significant increase in relative ACD depth between 5 days of FD and 10 days of FD was found for both illumination conditions. However, no significant difference was found in relative ACD by illumination condition when compared at each time point. Therefore, while relative ACD increased with time indicating that the ACD of the deprived eyes was increasing at a faster rate than in the non-deprived eyes, the intensity of the lighting condition did not have a differential effect on ACD growth.

Days of FD was found to have a statistically significant effect on relative AXL under the 300 lux, but not under the 15,000 lux illumination condition. Therefore, chicks raised under the 15,000 lux (15K) illumination condition demonstrated a smaller (non-significant) increase in relative AXL between 5 and 10 days of FD when compared with chicks in the 300 lux cohort. Although this effect is not reflected in a statistically significant difference in relative refraction at 10 days of FD between the two lighting conditions, it is consistent with the apparent trends in relative refraction illustrated in Figure A4.2.

A4.4. Discussion

This ancillary investigation into the time-course of development of form-deprivation myopia (FDM) in Cobb chicks revealed an apparent trend for less relative myopia to develop between 5 and 10 days of deprivation with exposure to the 15,000 lux lighting condition when compared to the 300 lux lighting condition. Although this difference did not quite reach statistical significance, the degree of myopia developed was strongly correlated to an increase in relative vitreous chamber depth under both lighting conditions. Moreover, relative axial length (AXL) did increase significantly between 5 and 10 days of deprivation under the 300 lux lighting condition, but not under the 15,000 lux lighting condition (Table A4.3). Therefore, the 15,000 lux lighting condition, where the high intensity illumination was provided by white LED light sources for 6 out of 12 hours per day, was sufficient to suppress axial growth induced by form-deprivation, when compared to the 300 lux lighting condition. This finding validates both the Cobb-strain chick as a suitable form-deprivation model of myopia development, and the white LED-based lighting system as capable of suppressing the axial elongation associated with FDM at sufficient intensity levels and duration of exposure.

Interestingly, the degrees of relative myopia induced under each lighting condition were not significantly different following 5 days of form-deprivation. This is consistent with the results described in Chapter 4 (Figure 4.7, Table 4.2) where it was shown that 4 days of daily exposure of chicks to periodic high intensity white LED light (10,000 lux) did not produce a significant difference in the degree of form-deprivation myopia developed when compared to chicks raised under continuous daily exposure to 300 lux white LED illumination. Therefore, the hypothesis advanced in Section 4.4.1, that

some aspect of white LED-based lighting such as spectral energy distribution may slow the development of FDM under 300 lux illumination, is consistent with the findings of this time-course experiment.

Karouta and Ashby (Karouta and Ashby 2015) have demonstrated a strong negative logarithmic relationship between the development of FDM in chicks and intensity of the illumination under which they were raised. While 40,000 lux illumination for 6 hours per day was shown to be sufficient to nearly completely abolish the development of FDM after 8 days, 10,000 lux illumination was sufficient to reduce the degree of FDM developed by nearly 60% compared to chicks raised under 500 lux lighting. Therefore the 15,000 lux lighting condition used in this experiment would have been expected to be sufficient to suppress the development of FDM, and the lack of statistical significance in the difference found in relative refraction is most likely due to the small sample size employed.

In conclusion, the Cobb chick is a suitable model for the investigation of the effect of high intensity light on the development of form-deprivation myopia. The increase in myopic refraction between 5 and 10 days of form-deprivation was strongly correlated with an increase vitreous chamber depth as expected. Furthermore, white LED-based high intensity lighting reduced the rate of development of form-deprivation myopia by reducing the rate of overall axial elongation in the Cobb chick.

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