Outdoor Light and the Prevention of Myopia

Dissertation

zur Erlangung des Grades eines

Doktors der Naturwissenschaften

der Mathematisch-Naturwissenschaftlichen Fakultät

und

der Medizinischen Fakultät

der Eberhard-Karls-Universität Tübingen

vorgelegt

von

Weizhong Lan

aus Guangzhou, China

Juli – 2015

Tag der mündlichen Prüfung: 06/10/2015

Dekan der Math.-Nat. Fakultät: Prof. Dr. W. Rosenstiel

Dekan der Medizinischen Fakultät: Prof. Dr. I. B. Autenrieth

1. Berichterstatter: Prof. Dr. / PD Dr. / Dr. Frank Schaeffel

2. Berichterstatter: Prof. Dr. / PD Dr. / Dr. Ulrich Schraermeyer

Prüfungskommission: Prof. Frank Schaeffel

Prof. Ulrich Schraermeyer

Prof. Thomas Euler

Prof. Uwe Ilg

I hereby declare that I have produced the work entitled " **Outdoor light and the prevention of myopia** ", submitted for the award of a doctorate, on my own (without external help), have used only the sources and aids indicated and have marked passages included from other works, whether verbatim or in content, as such. I swear upon oath that these statements are true and that I have not concealed anything. I am aware that making a false declaration under oath is punishable by a term of imprisonment of up to three years or by a fine.

Tübingen, ...

Date Signature

Acknowledgments

It is my great pleasure to have the opportunity to study and do research in the Center for Ophthalmology, University of Tuebingen, Germany. I would like to express my gratitude to everyone who has given me any help and support during my stay here.

First of all, I express my deepest gratitude to my supervisor, Prof. Frank Schaeffel, whose invaluable assistance, support and guidance, stimulating suggestions, valuable advice in science discussion and encouragement helped me through all the time of research and writing of this thesis. I am extraordinarily fortunate to have Prof. Frank Schaeffel not only as my supervisor, but also as my excellent mentor and outstanding advisor. The knowledge and wisdom he has imparted upon me will, without any doubt, forever remain a major contributor behind my every single step forward in the future.

I gratefully acknowledge Dr. Marita Feldkaemper for her constructive advice throughout my research and critical help in preparing this thesis. My special thanks also go to Dr. Ute Mathis and Ms. Sandra Bernhard-Kurz for their generous help during the whole immunostaining process. I am also gratitude to Gabi Kleine for helping me to deal with administration and daily life matters during my stay.

I am very thankful to the German Academic Exchange Service (Deutsche Akademische Austauschdienst, DAAD) for giving me the opportunity to do my research in Germany and providing financial support during my stay abroad.

I am also grateful to my colleagues and friends, Yun Chen, Ulrich Wildenmann, Felix Maier, and Klaus Graef, for their great friendship and the happy time shared with me in Tuebingen.

I am deeply and forever indebted to my parents for their endless love, support, caring and encouragement throughout my entire life. I am also very grateful to my parents-in-law for continuous support and understanding.

Last but not least, I wish to express my deepest love and gratitude to my beloved family, my wife and my adorable son, for always believing in me and for never failing to provide me all the support. Without their patient love and stimulating support, I believe this work can’t have been completed.

Table of Contents

1. Summary

The management of myopia is facing an unprecedented challenge, as the prevalence of myopia has ascended rapidly in the last decades. Although there is still no clinically acceptable and satisfactory management available, growing evidence has suggested that outdoor exposure, mediated by outdoor light, is a simple and useful option to prevent the development of myopia. In my thesis, I have investigated the roles of spectral properties of indoor light and of high light intensity on experimental myopia, the dose-effect response and the temporal function of the exposure, and the possible molecular mechanism underlying the light-mediated protective effect against myopia.

I found that with the same spectral property (i.e. spectral composition and distribution), high-level light intensity prevented myopia development compared with low-level intensity. However, there was no significant difference in the protection effect between the sunlight-like and fluorescent lighting presented at the same level of intensity, indicating that high level of light intensity is the primary reason for the outdoor light to display a powerful inhibitor against myopia. Furthermore, the dose-effect response of bright light exposure was found to be not linear, but rather saturate at approximately 5 hours of treatment, suggesting that additional protection could not be obtained through simply increasing the exposure duration. By contrast, frequent

and short episodes of exposure were found to enhance the protection effect than a single continuous exposure even though the light intensity and total dose of exposure was the same. With regard to the underlying mechanism, I found that wearing diffusers dramatically suppressed dopamine release from the retina and also the expression of ZENK protein in the retinal glucagonergic amacrine cells (GACs). Bright light, no matter presented continuously or intermittently, could significantly reduce the suppression of dopamine release. But no any difference in the rescue effect of dopamine release was detected between these two exposure patterns. Neither continuous nor intermittent bright light could rescue the suppression of ZENK induced by wearing diffusers.

As a whole, my doctoral thesis has extended our knowledge of the mechanism underlying outdoor light against myopia: high level of intensity is the major reason for the protective effect of sunlight, which depends not only on the total duration but also the temporal property of the exposure. Findings in the thesis provide further evidence for a role of dopamine in the signaling cascade of the bright-light-mediated protection, but a role of ZENK in glucagon amacrine cells, representing an important element in the retinal circuitry for the detection of the sign of defocus, is unlikely. The reason why intermittent bright light had a more prominent suppressive effect on myopia than continuous bright light exposure is still unclear and needs further investigation.

2. Synopsis

Myopia and its Epidemiology

Myopia is a refractive error, in which the parallel light rays are not focused on, but rather in front of the retina. The mismatch between focal length and axial length is either due to the excessive optical power of the refracting ocular components (cornea, crystalline lens) or an increased axial length of the eyeball. The two conditions are therefore called refractive myopia and axial myopia, respectively. Refractive myopia usually exists in keratoconus (increased curvature of the cornea) or in early cataract (elevated refractive index of the crystalline lens). However, most of the so-called "school myopia", developing during the school years, is axial.

The prevalence of school myopia has ascended dramatically in the past decades, approaching approximately 30-50% of young adults in Europe (Jobke, Kasten et al. 2008, Wolfram, Hohn et al. 2014) and around 80% in urban East Asia (Wu, Seet et al. 2001, He, Zeng et al. 2004). But in some extreme cases, the prevalence has reached "epidemic" level. In Shanghai China, for instance, myopia has been found to affect 95.5% university students in Shanghai (Sun, Zhou et al. 2012). In Seoul, Korea, it seems hopeless to recruit young males without myopia for military service, as the prevalence of myopia was also up to 96.5% in the 19-year-old males (Jung, Lee et al. 2012). Myopia is far from being a simple issue of correction of vision, but rather is,

especially high myopia (greater than 6 Diopter), a high risk for irreversible blind-threatening ocular disorders, such as retinal detachment and glaucoma. Therefore, this disorder has been considered to be one of the leading causes (only secondary to cataract) of blindness (Bourne, Stevens et al. 2013).

Outdoor activity, outdoor time, light and the prevention of myopia

Although research about myopia can be dated back to more than one century ago, its exact pathogenesis is still not clear. Therefore, no clinically acceptable and satisfactory therapies are available to prevent or slow the development of myopia in children. However, recent findings both in human and animal studies give rise to some hope.

In a study investigating the association between the degree of refractive error and life style, Rose et al. (Rose, Morgan et al. 2008) revealed that primary school students who combined high levels of near work with low levels of outdoor activity had the most myopic refractions, while those combined low levels of near work but high levels of outdoor activity were least myopic. After adjusting for confounders such as near work, parental myopia, and ethnicity, the lowest odds ratios for myopia was linked to the students reporting the highest levels of outdoor activity. A number of researchers reported similar association between outdoor activity and refractive error (Wu, Tsai et al. 2010, Guo, Liu et al. 2013, Lin, Vasudevan et al. 2014), suggesting

that outdoor activity demonstrates a protective effect against myopia. This assumption gained further support from other indirect evidence. For instance, progressing myopes were reported to experience much less outdoor exposure than stable myopes, as assessed by an objective light meter (Schmid, Leyden et al. 2013, McKnight, Sherwin et al. 2014). Meanwhile, myopia progression is slower in the summer, when daylight hours are longer and average light intensity is higher, than in the winter (Fulk, Cyert et al. 2002, Deng, Gwiazda et al. 2010, Donovan, Sankaridurg et al. 2012). In addition, three recent clinical trials reported that an increase of about one hour outdoors everyday could inhibit, despite with a small magnitude, the development of school myopia (Yi and Li 2011, Morgan, Xiang et al. 2012, Wu, Tsai et al. 2013).

Many factors could contribute to the protective effect of outdoor activity. The most straightforward notion is activity or sport. Nevertheless, Rose et al. in a retrospective study has already observed that no associations existed between indoor sport and myopia, and therefore postulated that the protective effect of staying outdoors was not the engagement in sports activities *per se*, but rather the higher levels of total time spent outdoors (Rose, Morgan et al. 2008). In support of this postulation, a prospective study showed that time spent outdoors was predictive of incident myopia independently of physical activity level and concluded that the previously reported link between outdoor activity and that incident myopia is due mainly to visual

information related to time outdoors rather than physical activity (Guggenheim, Northstone et al. 2012).

Since it appears the time spent outdoor was protective against myopia, differences in visual experience between outdoors and indoors must explain the effect. Candidates are the average viewing distances, the amount of accommodation, the imposed refractive errors over the visual field (which is known to control further eye growth), physical activity and the related optical flow fields, and also the ambient illuminance (review by Flitcroft (Flitcroft 2012)). Among these candidates, the possible role of bright light (as presented outdoors) was particularly studied.

Light intensity outdoors is surprisingly higher than indoors. Outdoor illuminance at noon in a clear summer day can exceed 100,000 lux. Even in the shade of trees it is still as high as 15 000 to 20 000 lux. By contrast, the typical indoor illuminance in an office is only approximately 300 to 500 lux. Ashby and colleagues (Ashby, Ohlendorf et al. 2009) were the first to observe that bright outdoor light significantly inhibits the development of experimental myopia. They fitted chicks with frosted googles, with the googles removed for 15 minutes daily under one of the three lighting conditions: standard indoor illuminance (500 lux), intense indoor illuminance (15 000 lux) and outdoor sunlight (average 30 000lux). They found that the myopia induced was remarkably less in turn, compared with a control group that continuously wore frosted googles. They further revealed that even with frosted googles continuously in place,

an increase of indoor illuminance from 500 lux to 15 000 lux significantly reduced the development of myopia (~ 60%). To test whether potentially different physical activity of the animals played a role, an automated video surveillance technique was developed to track the activity of the birds. It turned out that physical activity did not differ between high and low illumination levels, leaving ambient illuminance as possible factor. These findings were later confirmed in tree shrews (Siegwart, Ward et al. 2012) and rhesus monkeys (Smith, Hung et al. 2012). Bright light also slows down the progression of myopia induced by negative spectacle lenses, which was confirmed in tree shrews (Siegwart, Ward et al. 2012, Norton and Siegwart 2013). In rhesus monkeys, lens-induced myopia was not suppressed by bright light, which was shown in a study (Smith, Hung et al. 2013). However, another study showed that bright light slowed the development of lens-induced myopia (-3D) to some extent (Wang, Ding et al. 2015), although the number of animals in that study was low and the effects were small. Taken together, most of the experiments indicate an inhibitory effect of bright light on the development and progression of myopia. However, the underlying mechanisms are not clear.

In addition to differences in intensity, sunlight may also differ from indoor light in its spectral composition. Specifically, the spectrum of ground-level sunlight during a typical day includes a continuous distribution of wavelengths from approximately 300 nm to about 1200 nm, while florescent lights, the most common source of

artificial indoor lighting, has a discrete spectrum between 400~700 nm, with peaks in the blue, green and red, and lacks ultraviolet and infrared components. An influence of spectral properties of light on ocular growth has been suggested since long. For instance, long wavelengths of monochromatic light were found to accelerate ocular elongation while short wavelengths inhibited ocular elongation (Kroger and Wagner 1996, Kroger and Wagner 1996, Rucker and Wallman 2009, Liu, Qian et al. 2011, Wang, Zhou et al. 2011, Rucker and Wallman 2012, Qian, Dai et al. 2013, Qian, Liu et al. 2013). In addition, it was shown that that circulating levels of vitamin D, which can only be produced in the skin with UV light (UVB), were lower in myopes than non-myopes (Mutti and Marks 2011, Choi, Han et al. 2014, Yazar, Hewitt et al. 2014) and that the polymorphisms within the vitamin D receptor (VDR) (Mutti, Cooper et al. 2011) were correlated with low to moderate amounts of myopia. These data suggest that the UV spectrum might play some role in the suppressive effect of sunlight on myopia. Indeed, when Ashby and his colleagues (Ashby, Ohlendorf et al. 2009) revealed the protection effect of bright light, they observed a stronger effect provided by sunlight compared with artificial bright light. But they could not determine whether it was related to the UV component of sunlight or just to its higher intensity. Although outdoor exposure is a simple and economical option to treat myopia, it seems difficult to implement in practice, especially in some Asian countries where education is very intensive and the available time to increase outdoor activity is limited (Ngo, Pan et al. 2014). Thus, it was of great importance to

investigate whether the spectral characteristic might be the reason for the stronger protection of sunlight. If this was the case, then modulating spectral property of artificial lighting as sunlight, rather than increasing outdoor time, might be a more feasible strategy to prevent human myopia.

Unresolved questions

While it can be concluded from previous work that outdoor light is a powerful inhibitor of myopia development, there is still much to be learned. I used two widely studied animal models of myopia, the chicken and the guinea pig, for which a lot of basic information on the biological mechanisms of myopia have been well established, to study the following questions:

1) Which exposure parameters have the greatest inhibitory effect on the development of myopia? In particular, the dose-effect response and the temporal profiles of bright light exposure are unknown.
2) What is the biochemical and neural mechanism underlying the protective effect of bright light against myopia?
3) Does the spectral composition of sunlight play a role?

Experiments

Accordingly, I have tried to tackle these questions through the experiments described below.

Experiment I: Dose-effect response function of bright light exposure

Eight-day-old chickens wore frosted diffusers over one eye to induce deprivation myopia. A reference group was kept under office-like illuminance (500 lux) at a 10:14 light:dark cycle. Another four groups were exposed to continuous bright light (15 000 lux or 15K lux) for 1 hour, 2 hours, 5 hours or 10 hours, respectively, with a 500 lux background illuminance. I found that, compared with the reference group, exposure to continuous bright light for 1 or 2 hours every day had no significant protective effect against deprivation myopia. Inhibition of myopia became significant after 5 hours of bright light exposure with suppression by approximately 70%, consistent with previous studies (Ashby, Ohlendorf et al. 2009, Ashby and Schaeffel 2010, Siegwart, Ward et al. 2012, Smith, Hung et al. 2012). Nevertheless, an additional protective effect was not observed when the exposure duration was further increased to 10 hours (Lan, Feldkaemper et al. 2014). These findings might have important implications regarding the question "*Should children be exposed to bright light outdoors for longer periods of time for greater myopia inhibition*?'', because excessively long exposure to bright light outdoors may have no additional benefit but

may rather increase the risk of side effects, such as skin cancer (Gandini, Autier et al. 2011) and retinal light damage (in the case of over-exposure to sunlight) (Youssef, Sheibani et al. 2011). According to these findings, I assume that there might be also an upper limit for the protective effect of bright light exposure in children. Further studies in monkeys and later in children will finally optimize the light exposure regimens.

Experiment II. Temporal-dose function of bright light exposure

It was previously found that intermittent treatment with diffusers (Napper, Brennan et al. 1997, Smith, Hung et al. 2002) or spectacle lenses (Winawer and Wallman 2002, Kee, Hung et al. 2007, Zhu and Wallman 2009) have different effects than continuous treatment. In particular, periodic interruption of positive lens wear, which induces hyperopia by inhibiting axial eye growth, has differently powerful effects, depending on the temporal patterns of interruption - even if the total exposure time is the same (Zhu and Wallman 2009). I studied whether something similar may apply to the exposure to bright light. Chickens were exposed to repeated cycles of bright light with 50% duty cycle and periods of either 60 minutes, 30 minutes, 15 minutes, 7 minutes or 1 minute. The background illuminance was kept constant at 500 lux. It was found that, with a matched total amount of bright light exposure (5 hours a day), bright light provided in pulses with low temporal frequency suppressed myopia more than continuous bright light. Especially, cycles of 1:1 minutes of bright light fully inhibited

the development of deprivation myopia (Lan, Feldkaemper et al. 2014). The mechanism by which intermittent bright light provided enhanced protection was unknown. I speculated that intermittent bright light might stimulate more release of dopamine (an assumed "STOP" signal for ocular growth) than continuous bright light. Evidence to support this speculation was that flickering light (1-20 Hz) was reported to stimulate more dopamine release from the retina than steady light (Kramer 1971, Kirsch and Wagner 1989, Umino, Lee et al. 1991), although the temporal frequency used in the current study was much lower (1:1 minute cycles, equivalent to 0.007 Hz) than in previous studies (Kramer 1971, Kirsch and Wagner 1989, Umino, Lee et al. 1991). The question as whether the results are directly applicable to children awaits further testing. Furthermore, compliance must be considered. Exposure to alternating illuminances between 500 lux and 15 000 lux with short cycles may be uncomfortable. Perhaps smaller steps in illuminance may already have a satisfactory effect.

Experiment III. The role of dopamine in light-mediated suppression of myopia

I had hypothesized the enhanced protective effect of intermittent bright light (0.007Hz or 1:1 minute) against the development of experimentally induced deprivation myopia was due to a higher level of dopamine release (Lan, Feldkaemper et al. 2014). To test this hypothesis, the difference of vitreal DOPAC content, a sensitive index of dopamine release (Ohngemach, Hagel et al. 1997, Feldkaemper, Diether et al. 1999, Megaw, Morgan et al. 2001, Cohen, Peleg et al. 2012), in the deprived eye and the

contralateral eye was compared among the groups of chickens exposed to 500 lux, continuously 15K lux and intermittent 15K lux (1:1 minute). I chose the time point immediate prior to the end of the light phase (10 hours after the light onset) for DOPAC measurements. This was because the DOPAC content in the vitreous accumulates over the day and it was more likely, if any, to detect potential difference among groups at this time point.

In line with published studies (Iuvone, Tigges et al. 1989, Stone, Lin et al. 1989, Bartmann, Schaeffel et al. 1994, Ohngemach, Hagel et al. 1997), I found that wearing diffusers suppressed dopamine release significantly. In addition, I observed that exposure to bright light during diffusers wear returned DOPAC levels towards control levels, albeit not completely (Lan, Yang et al.). Diffusers suppress dopamine release via both the degradation of retinal image quality and the decrease of retinal illuminance (~0.5 log unit in the current case) (Feldkaemper, Diether et al. 1999), the current findings indicate that a 1.5 log unit increase of ambient illuminance (ie., from 500 to 15K lux) can not reserve the suppression of dopamine release induced by the degradation of retinal image. But the reduction of suppression does provide a further support for the the assumption that dopamine plays an important role in bright-light-mediated myopia inhibition (Rose, Morgan et al. 2008, Ashby and Schaeffel 2010, Norton and Siegwart 2013), in addition to the previous finding that a dopamine-antagonist (spiperone) abolished the beneficial effect of bright light (Ashby and

Schaeffel 2010). Nevertheless, the exact role of dopamine in the process is still unclear and appears to be very complicated. On one hand, retinal dopamine synthesis and release are suppressed in response to form-deprivation (Iuvone, Tigges et al. 1989, Stone, Lin et al. 1989, Ohngemach, Hagel et al. 1997) and negative lenses (Guo, Sivak et al. 1995, Ohngemach, Hagel et al. 1997), both of which promote eye growth. On the other hand, it is also suppressed by positive lenses, which inhibit eye growth (Ohngemach, Hagel et al. 1997). These obviously "paradoxical" findings imply that dopamine might not simply mediate an inhibitory signal for ocular growth, but rather represents an index of the gain of the ocular growth to the modulating stimulus: if the retinal dopamine level is high, then the gain is low and the retina is insensitive to growth-modulating stimuli; by contrast, if the dopamine level is low, then the gain is high and the retina becomes susceptible to growth-modulating stimuli. Thus, bright light might protect against deprivation myopia by reducing the gain of the ocular growth via antagonizing the suppression of dopamine release by diffusers.

Surprisingly, I did not find a difference in dopamine release between continuous and intermittent bright light exposure, even though the effects on myopia were different (Lan, Yang et al.). Therefore, my initial hypothesis, that intermittent bright light might stimulate dopamine release more than continuous bright light, was not confirmed at this stage but it could also be that potential differences were below

detection limit of the EC-HPLC device or that they were visible only earlier in the diurnal cycle.

Experiment IV. The role of ZENK in the mechanism of light-mediated protection

ZENK (also known as Egr-1, Zif268, NGFI-A and Krox-24 in other species) is a member of the immediate early gene family and it is well established that the expression of ZENK in the retinal glucagonergic amacrine cells (GACs) in chicks demonstrates bi-directional regulation in response to imposed defocus of both signs. That is, the expression of ZENK is upregulated by stimuli that inhibit ocular growth, such as plus lenses and termination of deprivation of sharp vision. Conversely, visual stimuli that stimulate ocular growth, such as treatment with minus lenses or diffusers, down-regulate the expression of ZENK (Fischer, McGuire et al. 1999, Bitzer and Schaeffel 2002, Bitzer and Schaeffel 2006, Ashby, McCarthy et al. 2007). Given that atropine, which is known to inhibit myopia development in animal models (McBrien, Moghaddam et al. 1993, Schwahn, Kaymak et al. 2000, Schmid and Wildsoet 2004, Bitzer, Kovacs et al. 2006, Diether, Schaeffel et al. 2007, Barathi, Beuerman et al. 2009) and children (Shih, Chen et al. 1999, Shih, Hsiao et al. 2001, Chua, Balakrishnan et al. 2006, Chia, Chua et al. 2012, Lin, Lan et al. 2013), stimulates dopamine release from the retina at least *in vitro* (Schwahn, Kaymak et al 2000) and enhances ZENK expression *in vivo* (Bitzer, Kovacs et al 2006), it could be assumed that a common mechanism drives dopamine and ZENK production. Nevertheless, up

to now the mechanism remains unknown. In particular, it was not known whether the inhibition of myopia by bright light involves regulation of the transcription factor ZENK in glucagon amacrine cells (GACs). Therefore, in my experiments, the numbers of ZENK-positive GACs were counted after double immunostaining for glucagon and ZENK, and the difference between the deprived eyes and the contralateral undeprived eyes was determined in groups of chickens exposed to 500 lux, continuous 15K lux or intermittent 15K lux (1:1 minute cycles). I have chosen the first hour after light onset and immediately prior to the end of light phase (10 hours after light onset) to measure the level of ZENK, as previous work has shown that changes of ZENK protein are very rapid (as soon as 40 minutes reported by Fischer et al.(Fischer, McGuire et al. 1999)). I found that the expression of ZENK in GACs was significantly suppressed by wearing diffusers, which was in line with previous reports (Fischer, McGuire et al. 1999, Bitzer and Schaeffel 2006). In addition, I found that bright light, no matter if given continuously or intermittently, could not rescue the suppression, neither after one hour nor after ten hours of treatment with the diffusers. Apparently, the light-mediated protection effect against deprivation myopia does not involve changes in ZENK expression in GACs.

Experiment V. The role of spectrum property in light-mediated protection

As mentioned earlier, other than by intensity, sunlight differs from indoor light also by its spectral properties. In the current experiment, two types of commercial lighting

with distinct spectral properties (broad spectrum of halogen light, BS and fluorescent light, FL) were applied to represent the sunlight and the indoor light environments. Guinea pigs were randomly treated with these two types of light at both high (10,000 lux) and low (500 lux) intensities. It was found that under the same intensity, similar changes in refraction occurred between animals treated with BS and FL. In contrast, under the same type of light, no matter BS of FL, high intensity lighting enhanced hyperopic shifts (animals reared without lenses) or retarded the development of experimentally induced myopia (animals reared with lenses), compared to low intensity lighting (Li, Lan et al. 2014). These findings further support the results from other animal models that bright light suppresses myopia development (Ashby, Ohlendorf et al. 2009, Ashby and Schaeffel 2010, Cohen, Peleg et al. 2012, Siegwart 2012, Smith, Hung et al. 2012). More importantly, these findings suggest that the protective effect of sunlight is primarily due to the high light intensity rather than the distinct spectral property compared with standard indoor light.

Conclusions

My experiments showed that the protective effect of sunlight against the development of myopia is primarily due to the level of intensity rather than due to its spectral composition, compared with standard indoor light. The protection of bright light depended not only on the total duration but also on the temporal properties of the exposure. The effects of continuous bright light saturated at approximately 5 hours. Low frequency episodes of exposure (1:1 minute cycles, 15000 lux : 500 lux) enhanced the suppressive effect of bright light and could fully block the development of myopia in the chicken model. My experiments also confirmed a role of dopamine in light-mediated suppression of myopia but did not support a role of the glucagon/ZENK system, known to be involved in the sign of defocus-dependent growth responses of the eye. It could rather be that modulation of dopamine release by bright light reduces the gain of emmetropization.

References

Ashby, R., C. S. McCarthy, R. Maleszka, P. Megaw and I. G. Morgan (2007). "A muscarinic cholinergic antagonist and a dopamine agonist rapidly increase ZENK mRNA expression in the form-deprived chicken retina." Exp Eye Res **85**(1): 15-22.

Ashby, R., A. Ohlendorf and F. Schaeffel (2009). "The effect of ambient illuminance on the development of deprivation myopia in chicks." Invest Ophthalmol Vis Sci **50**(11): 5348-5354.

Ashby, R. S. and F. Schaeffel (2010). "The effect of bright light on lens compensation in chicks." Invest Ophthalmol Vis Sci **51**(10): 5247-5253.

Barathi, V. A., R. W. Beuerman and F. Schaeffel (2009). "Effects of unilateral topical atropine on binocular pupil responses and eye growth in mice." Vision Res **49**(3): 383-387.

Bartmann, M., F. Schaeffel, G. Hagel and E. Zrenner (1994). "Constant light affects retinal dopamine levels and blocks deprivation myopia but not lens-induced refractive errors in chickens." Vis Neurosci **11**(2): 199-208.

Bitzer, M., B. Kovacs, M. Feldkaemper and F. Schaeffel (2006). "Effects of muscarinic antagonists on ZENK expression in the chicken retina." Exp Eye Res **82**(3): 379-388.

Bitzer, M. and F. Schaeffel (2002). "Defocus-induced changes in ZENK expression in the chicken retina." Invest Ophthalmol Vis Sci **43**(1): 246-252.

Bitzer, M. and F. Schaeffel (2006). "ZENK expression of retinal glucagon amacrine cells in chicks: the effect of defocus presented in vivo, in vitro and under anesthesia." Vision Res **46**(6-7): 848-859.

Bourne, R. R., G. A. Stevens, R. A. White, J. L. Smith, S. R. Flaxman, H. Price, J. B. Jonas, J. Keeffe, J. Leasher, K. Naidoo, K. Pesudovs, S. Resnikoff, H. R. Taylor and G. Vision Loss Expert (2013). "Causes of vision loss worldwide, 1990-2010: a systematic analysis." Lancet Glob Health **1**(6): e339-349.

Chia, A., W. H. Chua, Y. B. Cheung, W. L. Wong, A. Lingham, A. Fong and D. Tan (2012). "Atropine for the treatment of childhood myopia: safety and efficacy of 0.5%, 0.1%, and 0.01% doses (Atropine for the Treatment of Myopia 2)." Ophthalmology **119**(2): 347-354.

Choi, J. A., K. Han, Y. M. Park and T. Y. La (2014). "Low Serum 25-Hydroxyvitamin D Is Associated with Myopia in Korean Adolescents." Invest Ophthalmol Vis Sci.

Chua, W. H., V. Balakrishnan, Y. H. Chan, L. Tong, Y. Ling, B. L. Quah and D. Tan (2006). "Atropine for the treatment of childhood myopia." Ophthalmology **113**(12): 2285-2291.

Cohen, Y., E. Peleg, M. Belkin, U. Polat and A. S. Solomon (2012). "Ambient illuminance, retinal dopamine release and refractive development in chicks." Exp Eye Res **103**: 33-40.

Deng, L., J. Gwiazda and F. Thorn (2010). "Children's refractions and visual activities in the school year and summer." Optom Vis Sci **87**(6): 406-413.

Diether, S., F. Schaeffel, G. N. Lambrou, C. Fritsch and A. U. Trendelenburg (2007). "Effects of intravitreally and intraperitoneally injected atropine on two types of experimental myopia in chicken." Exp Eye Res **84**(2): 266-274.

Donovan, L., P. Sankaridurg, A. Ho, X. Chen, Z. Lin, V. Thomas, E. L. Smith, 3rd, J. Ge and B. Holden (2012). "Myopia progression in Chinese children is slower in summer than in winter." Optom Vis Sci **89**(8): 1196-1202.

Feldkaemper, M., S. Diether, G. Kleine and F. Schaeffel (1999). "Interactions of spatial and luminance information in the retina of chickens during myopia development." Exp Eye Res **68**(1): 105-115.

Fischer, A. J., J. J. McGuire, F. Schaeffel and W. K. Stell (1999). "Light- and focus-dependent expression of the transcription factor ZENK in the chick retina." Nat Neurosci **2**(8): 706-712.

Flitcroft, D. I. (2012). "The complex interactions of retinal, optical and environmental factors in myopia aetiology." Prog Retin Eye Res **31**(6): 622-660.

Fulk, G. W., L. A. Cyert and D. A. Parker (2002). "Seasonal variation in myopia progression and ocular elongation." Optom Vis Sci **79**(1): 46-51.

Gandini, S., P. Autier and M. Boniol (2011). "Reviews on sun exposure and artificial light and melanoma." Prog Biophys Mol Biol **107**(3): 362-366.

Guggenheim, J. A., K. Northstone, G. McMahon, A. R. Ness, K. Deere, C. Mattocks, B. S. Pourcain and C. Williams (2012). "Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study." Invest Ophthalmol Vis Sci **53**(6): 2856-2865.

Guo, S. S., J. G. Sivak, M. G. Callender and B. Diehl-Jones (1995). "Retinal dopamine and lens-induced refractive errors in chicks." Curr Eye Res **14**(5): 385-389.

Guo, Y., L. J. Liu, L. Xu, Y. Y. Lv, P. Tang, Y. Feng, M. Meng and J. B. Jonas (2013). "Outdoor activity and myopia among primary students in rural and urban regions of Beijing." Ophthalmology **120**(2): 277-283.

He, M., J. Zeng, Y. Liu, J. Xu, G. P. Pokharel and L. B. Ellwein (2004). "Refractive error and visual impairment in urban children in southern china." Invest Ophthalmol Vis Sci **45**(3): 793-799.

Iuvone, P. M., M. Tigges, A. Fernandes and J. Tigges (1989). "Dopamine synthesis and metabolism in rhesus monkey retina: development, aging, and the effects of monocular visual deprivation." Vis Neurosci **2**(5): 465-471.

Jobke, S., E. Kasten and C. Vorwerk (2008). "The prevalence rates of refractive errors among children, adolescents, and adults in Germany." Clin Ophthalmol **2**(3): 601-607.

Jung, S. K., J. H. Lee, H. Kakizaki and D. Jee (2012). "Prevalence of myopia and its association with body stature and educational level in 19-year-old male conscripts in seoul, South Korea." Invest Ophthalmol Vis Sci **53**(9): 5579-5583.

Kee, C. S., L. F. Hung, Y. Qiao-Grider, R. Ramamirtham, J. Winawer, J. Wallman and E. L. Smith (2007). "Temporal constraints on experimental emmetropization in infant monkeys." Investigative Ophthalmology and Visual Science **48**: 957-962.

Kirsch, M. and H. J. Wagner (1989). "Release pattern of endogenous dopamine in teleost retinae during light adaptation and pharmacological stimulation." Vision Res **29**(2): 147-154.

Kramer, S. G. (1971). "Dopamine: A retinal neurotransmitter. I. Retinal uptake, storage, and light-stimulated release of H3-dopamine in vivo." Invest Ophthalmol **10**(6): 438-452.

Kroger, R. H. and H. J. Wagner (1996). "Horizontal cell spinule dynamics in fish are affected by rearing in monochromatic light." Vision Res **36**(24): 3879-3889.

Kroger, R. H. H. and H. J. Wagner (1996). "The eye of the blue acara (Aequidens pulcher, Cichlidae) grows to compensate for defocus due to chromatic aberration." Journal of Comparative Physiology a-Sensory Neural and Behavioral Physiology **179**(6): 837-842.

Lan, W., M. Feldkaemper and F. Schaeffel (2014). "Intermittent episodes of bright light suppress myopia in the chicken more than continuous bright light." PLoS One **9**(10): e110906.

Lan, W., Z. Yang, M. Feldkaemper and F. Schaeffel "Changes in dopamine and ZENK during suppression of myopia in chicks by bright light." Under review.

Li, W., W. Lan, S. Yang, Y. Liao, Q. Xu, L. Lin and Z. Yang (2014). "The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs." Invest Ophthalmol Vis Sci **55**(10): 6324-6332.

Lin, L., W. Lan, Y. Liao, F. Zhao, C. Chen and Z. Yang (2013). "Treatment outcomes of myopic anisometropia with 1% atropine: a pilot study." Optom Vis Sci **90**(12): 1486-1492.

Lin, Z., B. Vasudevan, V. Jhanji, G. Y. Mao, T. Y. Gao, F. H. Wang, S. S. Rong, K. J. Ciuffreda and Y. B. Liang (2014). "Near work, outdoor activity, and their association with refractive error." Optom Vis Sci **91**(4): 376-382.

Liu, R., Y. F. Qian, J. C. He, M. Hu, X. T. Zhou, J. H. Dai, X. M. Qu and R. Y. Chu (2011). "Effects of different monochromatic lights on refractive development and eye growth in guinea pigs." Exp Eye Res **92**(6): 447-453.

McBrien, N. A., H. O. Moghaddam and A. P. Reeder (1993). "Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism." Invest Ophthalmol Vis Sci **34**(1): 205-215.

McKnight, C. M., J. C. Sherwin, S. Yazar, H. Forward, A. X. Tan, A. W. Hewitt, C. E. Pennell, I. L. McAllister, T. L. Young, M. T. Coroneo and D. A. Mackey (2014). "Myopia in young adults is inversely related to an objective marker of ocular sun exposure: the Western Australian Raine cohort study." Am J Ophthalmol **158**(5): 1079-1085.

Megaw, P., I. Morgan and M. Boelen (2001). "Vitreal dihydroxyphenylacetic acid (DOPAC) as an index of retinal dopamine release." J Neurochem **76**(6): 1636-1644.

Morgan, I. G., F. Xiang, K. A. Rose, Q. Chen and M. He (2012). "Two Year Results from the Guangzhou Outdoor Activity Longitudinal Study (GOALS)." ARVO Meeting Abstracts. Invest. Ophthalmol. Vis. Sci. **53**(6): 2735.

Mutti, D. O., M. E. Cooper, E. Dragan, L. A. Jones-Jordan, M. D. Bailey, M. L. Marazita, J. C. Murray and K. Zadnik (2011). "Vitamin D receptor (VDR) and group-specific component (GC, vitamin D-binding protein) polymorphisms in myopia." Invest Ophthalmol Vis Sci **52**(6): 3818-3824.

Mutti, D. O. and A. R. Marks (2011). "Blood levels of vitamin D in teens and young adults with myopia." Optom Vis Sci **88**(3): 377-382.

Napper, G. A., N. A. Brennan, M. Barrington, M. A. Squires, G. A. Vessey and A. J. Vingrys (1997). "The effect of an interrupted daily period of normal

visual stimulation on form deprivation myopia in chicks." Vision Res **37**(12): 1557-1564.

Ngo, C. S., C. W. Pan, E. A. Finkelstein, C. F. Lee, I. B. Wong, J. Ong, M. Ang, T. Y. Wong and S. M. Saw (2014). "A cluster randomised controlled trial evaluating an incentive-based outdoor physical activity programme to increase outdoor time and prevent myopia in children." Ophthalmic Physiol Opt **34**(3): 362-368.

Norton, T. T. and J. T. Siegwart, Jr. (2013). "Light levels, refractive development, and myopia--a speculative review." Exp Eye Res **114**: 48-57.

Ohngemach, S., G. Hagel and F. Schaeffel (1997). "Concentrations of biogenic amines in fundal layers in chickens with normal visual experience, deprivation, and after reserpine application." Vis Neurosci **14**(3): 493-505.

Qian, Y. F., J. H. Dai, R. Liu, M. J. Chen, X. T. Zhou and R. Y. Chu (2013). "Effects of the chromatic defocus caused by interchange of two monochromatic lights on refraction and ocular dimension in guinea pigs." PLoS One **8**(5): e63229.

Qian, Y. F., R. Liu, J. H. Dai, M. J. Chen, X. T. Zhou and R. Y. Chu (2013). "Transfer from blue light or green light to white light partially reverses changes in ocular refraction and anatomy of developing guinea pigs." J Vis **13**(11).

Rose, K. A., I. G. Morgan, J. Ip, A. Kifley, S. Huynh, W. Smith and P. Mitchell (2008). "Outdoor activity reduces the prevalence of myopia in children." Ophthalmology **115**(8): 1279-1285.

Rucker, F. J. and J. Wallman (2009). "Chick eyes compensate for chromatic simulations of hyperopic and myopic defocus: evidence that the eye uses

longitudinal chromatic aberration to guide eye-growth." Vision Res **49**(14): 1775-1783.

Rucker, F. J. and J. Wallman (2012). "Chicks use changes in luminance and chromatic contrast as indicators of the sign of defocus." J Vis **12**(6).

Schmid, K. L., K. Leyden, Y. H. Chiu, S. R. Lind, D. J. Vos, M. Kimlin and J. M. Wood (2013). "Assessment of daily light and ultraviolet exposure in young adults." Optom Vis Sci **90**(2): 148-155.

Schmid, K. L. and C. F. Wildsoet (2004). "Inhibitory effects of apomorphine and atropine and their combination on myopia in chicks." Optom Vis Sci **81**(2): 137-147.

Schwahn, H. N., H. Kaymak and F. Schaeffel (2000). "Effects of atropine on refractive development, dopamine release, and slow retinal potentials in the chick." Vis Neurosci **17**(2): 165-176.

Shih, Y. F., C. H. Chen, A. C. Chou, T. C. Ho, L. L. Lin and P. T. Hung (1999). "Effects of different concentrations of atropine on controlling myopia in myopic children." J Ocul Pharmacol Ther **15**(1): 85-90.

Shih, Y. F., C. K. Hsiao, C. J. Chen, C. W. Chang, P. T. Hung and L. L. Lin (2001). "An intervention trial on efficacy of atropine and multi-focal glasses in controlling myopic progression." Acta Ophthalmol Scand **79**(3): 233-236.

Siegwart, J. T., A. H. Ward and T. T. Norton (2012). "Moderately elevated fluorescent light levels slow form deprivation and minus lens-induced myopia development in tree shrews." ARVO Meeting Abstracts. Invest. Ophthalmol. Vis. Sci. **53**: 3457.

Siegwart, J. T. J. W. A. H. N. T. T. (2012). "Moderately Elevated Fluorescent Light Levels Slow Form Deprivation and Minus Lens-Induced Myopia Development in Tree Shrews." Invest. Ophthalmol. Vis. Sci. **53**(6): 3457-.

Smith, E. L., 3rd, L. F. Hung, B. Arumugam and J. Huang (2013). "Negative lens-induced myopia in infant monkeys: effects of high ambient lighting." Invest Ophthalmol Vis Sci **54**(4): 2959-2969.

Smith, E. L., 3rd, L. F. Hung and J. Huang (2012). "Protective effects of high ambient lighting on the development of form-deprivation myopia in rhesus monkeys." Invest Ophthalmol Vis Sci **53**(1): 421-428.

Smith, E. L., L. F. Hung, C. S. Kee and Y. Qiao (2002). "Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in monkeys." Investigative Ophthalmology and Visual Science **43**: 291-299.

Stone, R. A., T. Lin, A. M. Laties and P. M. Iuvone (1989). "Retinal dopamine and form-deprivation myopia." Proc Natl Acad Sci U S A **86**(2): 704-706.

Sun, J., J. Zhou, P. Zhao, J. Lian, H. Zhu, Y. Zhou, Y. Sun, Y. Wang, L. Zhao, Y. Wei, L. Wang, B. Cun, S. Ge and X. Fan (2012). "High prevalence of myopia and high myopia in 5060 Chinese university students in Shanghai." Invest Ophthalmol Vis Sci **53**(12): 7504-7509.

Umino, O., Y. Lee and J. E. Dowling (1991). "Effects of light stimuli on the release of dopamine from interplexiform cells in the white perch retina." Vis Neurosci **7**(5): 451-458.

Wang, F., J. Zhou, Y. Lu and R. Chu (2011). "Effects of 530 nm green light on refractive status, melatonin, MT1 receptor, and melanopsin in the guinea pig." Curr Eye Res **36**(2): 103-111.

Wang, Y., H. Ding, W. K. Stell, L. Liu, S. Li, H. Liu and X. Zhong (2015). "Exposure to sunlight reduces the risk of myopia in rhesus monkeys." PLoS One **10**(6): e0127863.

Winawer, J. and J. Wallman (2002). "Temporal constraints on lens compensation in chicks." Vision Research **42**: 2651-2668.

Wolfram, C., R. Hohn, U. Kottler, P. Wild, M. Blettner, J. Buhren, N. Pfeiffer and A. Mirshahi (2014). "Prevalence of refractive errors in the European adult population: the Gutenberg Health Study (GHS)." Br J Ophthalmol **55**(4): 2041-2047.

Wu, H. M., B. Seet, E. P. Yap, S. M. Saw, T. H. Lim and K. S. Chia (2001). "Does education explain ethnic differences in myopia prevalence? A population-based study of young adult males in Singapore." Optom Vis Sci **78**(4): 234-239.

Wu, P. C., C. L. Tsai, C. H. Hu and Y. H. Yang (2010). "Effects of outdoor activities on myopia among rural school children in Taiwan." Ophthalmic Epidemiol **17**(5): 338-342.

Wu, P. C., C. L. Tsai, H. L. Wu, Y. H. Yang and H. K. Kuo (2013). "Outdoor activity during class recess reduces myopia onset and progression in school children." Ophthalmology **120**(5): 1080-1085.

Yazar, S., A. W. Hewitt, L. J. Black, C. M. McKnight, J. A. Mountain, J. C. Sherwin, W. H. Oddy, M. T. Coroneo, R. M. Lucas and D. A. Mackey (2014). "Myopia is associated with lower vitamin d status in young adults." Invest Ophthalmol Vis Sci **55**(7): 4552-4559.

Yi, J. H. and R. R. Li (2011). "[Influence of near-work and outdoor activities on myopia progression in school children]." Zhongguo Dang Dai Er Ke Za Zhi **13**(1): 32-35.

Youssef, P. N., N. Sheibani and D. M. Albert (2011). "Retinal light toxicity." Eye (Lond) **25**(1): 1-14.

Zhu, X. and J. Wallman (2009). "Temporal properties of compensation for positive and negative spectacle lenses in chicks." Investigative Ophthalmology and Visual Science **50**: 37-46.

List of papers

1. Lan W, Feldkaemper M, Schaeffel F. Intermittent episodes of bright light suppress myopia in the chicken more than continuous bright light. ***PLoS One***. 2014;9(10):e110906.
2. Li W*, Lan W*, Yang S, et al. The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs. ***Investigative ophthalmology & visual science.*** 2014;55(10):6324-6332 (* Co-first author)
3. Lan W, Yang Z, Feldkaemper M, Schaeffel F. Roles of dopamine and ZENK in the suppression of myopia by bright light in chicks (under review)

Description of personal contribution

1. **Lan W,** Feldkaemper M, Schaeffel F. Intermittent episodes of bright light suppress myopia in the chicken more than continuous bright light. ***PLoS One.*** 2014;9(10):e110906.

   Personal contribution: The idea was provided by my supervisor (Frank Schaeffel). I performed all the experiments (experimental set-up, light treatment of chicks, measurement of refractive error and ocular biometry), analyzed the data, wrote the draft of the manuscript and revised the manuscript following the comments by my supervisors (Frank Schaeffel and Marita Feldkaemper) until it was published.

2. Li W*, **Lan W***, Yang S, et al. The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs. ***Investigative ophthalmology & visual science.*** 2014;55(10):6324-6332. (* Co-first author)

   Personal contribution: I developed the idea, designed the experiments and wrote the paper. Wentao Li performed most of the experiments (experimental set-up, measurement of refractive error and ocular biometry), analyzed the data and drafted the first version of the manuscript.

3. **Lan W**, Yang Z, Feldkaemper M, Schaeffel F. Roles of dopamine and ZENK in the suppression of myopia by bright light in chicks (under review)

   Personal contribution: The idea was provided by my supervisor (Frank Schaeffel). I performed most of the experiments (experimental set-up, light treatment of chicks, preparation of vitreous body and retina, fixation and sectioning of the retina, cell counting with microscopy), analyzed the data, wrote the draft of the manuscript and revised the manuscript, following the comments of my supervisors (Frank Schaeffel, Marita Feldkaemper and Yang Z).

Curriculum Vitae

Education

Institution/Location	Years	Degree	Field of Study
Sun Yat-Sen University, China	1998-2003	Bachelor	Clinical Medicine
Sun Yat-Sen University, China	2003-2006	Master	Ophthalmology
University of Tuebingen, Germany	2012-2013	Dr. med.	Ophthalmology
University of Tuebingen, Germany	2013-2015	Ph.D Candidate	Neuroscience

Professional experience

Institution/Location	Years	Title
Zhongshan Ophthalmic Center, China	2006-2010	Resident/Teaching Assistant
Zhongshan Ophthalmic Center, China	2011-Present	Lecturer of Ophthalmology

Meetings attended during Ph.D study

Intermittent episodes of bright light enhance the protective effect against myopia. **Lan W**, Feldkaemper M, Schaeffel F. ***International Myopia Conference***. 2013, U.S. Poster.

Awards received during Ph.D study

Invitation to participation to the ***64th Lindau Nobel Laureates Meetings***, 2014, Germany.

Funding through the German Academic Exchange Service (DAAD) throughout my PhD time.

Publications during Ph.D study

1) **Lan W**, Yang Z, Feldkaemper M, Schaeffel F. Roles of dopamine and ZENK in the suppression of myopia by bright light in chicks *(under review)*
2) **Lan W**, Feldkaemper M, Schaeffel F. Intermittent episodes of bright light suppress myopia in the chicken more than continuous bright light. ***PLoS One***. 2014;9(10):e110906.
3) Li W*, **Lan W***, Yang S, et al. The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs. ***Investigative ophthalmology & visual science.*** 2014;55(10):6324-6332. (* Co-first author)
4) Chen Y, **Lan W,** & Schaeffel F. Size of the foveal blue scotoma related to the shape of the foveal pit but not to macular pigment. ***Vision Res****,* 2015, 106, 81-89.
5) **Lan W,** Lin L, Yang X, & Yu M. Automatic noninvasive tear breakup time (TBUT) and conventional fluorescent TBUT. ***Optom Vis Sci****,* 2014, 91(12), 1412-1418.
6) **Lan W,** Zhao F, Lin L, Li Z, Zeng J, Yang Z, & Morgan I. G. Refractive errors in 3-6 year-old Chinese children: a very low prevalence of myopia? ***PLoS One****,* 2013, 8(10), e78003.
7) Lin L, **Lan W***, Liao Y, Zhao F, Chen C, & Yang Z. Treatment outcomes of myopic anisometropia with 1% atropine: a pilot study. ***Optom Vis Sci****,* 2013, 90(12), 1486-1492. (*Co-first author)

OPEN ACCESS Freely available online

PLOS ONE

Intermittent Episodes of Bright Light Suppress Myopia in the Chicken More than Continuous Bright Light

Weizhong Lan[1,2,3]*, Marita Feldkaemper[1], Frank Schaeffel[1]

1 Section of Neurobiology of the Eye, Center for Ophthalmology, University of Tuebingen, Tuebingen, Germany, **2** Zhongshan Ophthalmic Center, State Key Laboratory of Ophthalmology, Sun Yat-sen University, Guangzhou, China, **3** Graduate School of Cellular & Molecular Neuroscience, University of Tuebingen, Tuebingen, Germany

Abstract

Purpose: Bright light has been shown a powerful inhibitor of myopia development in animal models. We studied which temporal patterns of bright light are the most potent in suppressing deprivation myopia in chickens.

Methods: Eight-day-old chickens wore diffusers over one eye to induce deprivation myopia. A reference group (n = 8) was kept under office-like illuminance (500 lux) at a 10:14 light:dark cycle. Episodes of bright light (15 000 lux) were superimposed on this background as follows. Paradigm I: exposure to constant bright light for either 1 hour (n = 5), 2 hours (n = 5), 5 hours (n = 4) or 10 hours (n = 4). Paradigm II: exposure to repeated cycles of bright light with 50% duty cycle and either 60 minutes (n = 7), 30 minutes (n = 8), 15 minutes (n = 6), 7 minutes (n = 7) or 1 minute (n = 7) periods, provided for 10 hours. Refraction and axial length were measured prior to and immediately after the 5-day experiment. Relative changes were analyzed by paired t-tests, and differences among groups were tested by one-way ANOVA.

Results: Compared with the reference group, exposure to continuous bright light for 1 or 2 hours every day had no significant protective effect against deprivation myopia. Inhibition of myopia became significant after 5 hours of bright light exposure but extending the duration to 10 hours did not offer an additional benefit. In comparison, repeated cycles of 1:1 or 7:7 minutes of bright light enhanced the protective effect against myopia and could fully suppress its development.

Conclusions: The protective effect of bright light depends on the exposure duration and, to the intermittent form, the frequency cycle. Compared to the saturation effect of continuous bright light, low frequency cycles of bright light (1:1 min) provided the strongest inhibition effect. However, our quantitative results probably might not be directly translated into humans, but rather need further amendments in clinical studies.

Citation: Lan W, Feldkaemper M, Schaeffel F (2014) Intermittent Episodes of Bright Light Suppress Myopia in the Chicken More than Continuous Bright Light. PLoS ONE 9(10): e110906. doi:10.1371/journal.pone.0110906

Editor: Eric James Warrant, Lund University, Sweden

Received March 21, 2014; **Accepted** September 19, 2014; **Published** October 31, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by grants from National Natural Science Foundation of China, Beijing, China (grant no.: 81170871, 81200714), (http://www.nsfc.gov.cn/nsfc/cen/xxgk/slzz.html) and a scholarship from German Academic Exchange Service (DAAD), (https://www.daad.de) awarded to WL. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: brucelarm@gmail.com

Introduction

Nearsightedness (myopia) arises from a mismatch between the focal power of the optical components (cornea and crystalline lens) and the axial length. It is the most commonly found disorder in the development of the juvenile eye and steadily rises in prevalence, currently affecting 30–50% of young adults in Europe [1,2] and around 80% in Asia [3,4]. Recent studies have shown that outdoor exposure seems to be a promising approach to reduce the development of myopia – children who spend more time outdoors appear to be less likely to become myopic [5–10].

A number of possible factors can be suggested for the protective effect of outdoor exposure, such as light intensity, physical activity, viewing distance, variations in accommodative requirement, which have been systematically discussed by a recent review [11]. Rose et al. were the first to suggest that light intensity might be an important factor [8,12] and this assumption has gained accumulating experimental evidence in animals. Specifically, with the urbanization of modern world, humans tend to spend more time indoors with illuminances typically ranging from 100 lux to 500 lux. Compared with the outdoor illuminance (as high as 150 000 lux on a sunny summer day), the indoor illuminance is very much lower. Cohen et al., observed that chickens raised at low light (50 lux) for extended periods (90 days) developed significant myopia (Mean: −2.41D), as compared to those reared under standard (500 lux, Mean: +0.03D) or high light level (10 000 lux, Mean: +1.1D). [12] Furthermore, exposure to artificial bright light (15 000 lux to 25 000 lux) has been shown to suppress deprivation myopia that is induced by covering the eye with frosted diffusers in chickens [13], tree shrews [14] and monkeys [15]. Bright light also slows down the development of myopia induced by wearing negative lenses in chickens [16] and tree shrews [14,17], although no significant effects were observed in monkeys in the only study done so far[18]. Overall, there is now convincing evidence to

support the speculation proposed by Norton and Siegwart that "ambient illuminance levels produce a continuum of effects on normal refractive development and the response to myopiagenic stimuli such that low light levels favor myopia development and elevated levels are protective" [17].

There are currently three trials on prevention of myopia with outdoor exposure [19–21]. All three trials reported statistically significant reduction in the incidence rate and the progression rate of myopia with increasing time outdoors. If the protective effect of the outdoor exposure is attributable to light intensity, given the fact that bright light was found to inhibit myopia in several different species, it is likely that a simple myopia therapy in children might be to increase ambient illuminance in classrooms. However, there is still much to be learned about the exposure parameters that have the greatest inhibitory effect on the development of myopia [15]. In particular, the dose-response function has not been explored and nor have the optimum temporal patterns for exposure to bright light been determined. In the current study, we have tackled some of these questions, using the chicken model of myopia.

Materials and Methods

Animals

One-day-old male white leghorn chickens were obtained from a local hatchery in Kirchberg, Germany. They were raised in a temperature-controlled room under 500 lux ambient illuminance with a 10/14 hour light/dark cycle (light on at 8AM and off at 6PM). Chickens had free access to food and water. All experiments were conducted at the University of Tuebingen. This study was carried out in strict accordance with the ARVO Statement and the guide of the regional council of Tuebingen for the care and use of laboratory animals. The protocol was approved by the Regional Council of Tuebingen (Reference number: AK 3/12). All efforts were made to minimize suffering during the study and chickens were sacrificed via ether after the experiments.

Experimental Paradigms

From the day 8 post-hatching, frosted diffusers were placed over chickens' right eyes to induce monocular deprivation myopia, a common model for human myopia [22–26]. Chickens were then randomly assigned to one of the following two experimental paradigms. In both paradigms, an illuminance of 500 lux served as constant background.

Paradigm I. Chickens were exposed to constant bright light (approximately 15 000 lux) for either 5 hours (from 10AM to 3PM, $n = 4$) or 10 hours (the entire light phase, $n = 4$) per day. To determine the minimum duration with a significant effect on myopia, exposure durations of 1 hour (from 12:30 AM to 1:30 PM, $n = 5$) or 2 hours (from 12AM to 2 PM, $n = 5$) were also tested.

Paradigm II. Chickens were exposed to intermittent bright light (approximately 15 000 lux) with a 50% duty cycle and either 60 minutes ($n = 7$), 30 minutes ($n = 8$), 15 minutes ($n = 6$), 7 minutes ($n = 7$) or 1 minute ($n = 7$) cycle length over a period for 10 hours. Thus, the total daily duration of exposure to bright light was 5 hours in all cases.

In addition, two control groups with four animals in each were kept under background illuminance without further interventions, except for wearing frosted diffusers over their right eyes. Two different batches were used to evaluate inter-batch variability but it turned out to be negligible (see Results).

All treatments were continued for 5 consecutive days. Details about the spectral energy distribution of the two light sources have been described previously [13]. The emission spectra of the lamps were similar to the spectrum of the sun over the visible range of wavelengths. Air conditioners were applied to match the environmental temperature in the groups of the two paradigms (range 25–27°C).

Measurement of Ocular Parameters

Ocular parameters were measured both prior to and immediately after the 5-day treatment period. Refractions were determined by automated infrared photoretinoscopy without cycloplegia [27], and ocular biometry was performed by A-scan ultrasonography with a probe of 10 MHz [28].

Statistics

Data are presented as the mean ± one standard error of the mean (SEM). Relative changes between deprived eyes and non-deprived eyes within a group were compared with paired t-tests. Comparisons among groups were assessed by one-way ANOVA, with post-hoc protected Fisher Least Significant Difference (LSD) pairwise multiple comparisons. If necessary, the absolute changes of ocular parameters over time or between two individual groups were tested with paired t-tests and unpaired t-tests respectively. All analyses were performed with commercially available software (SPSS 16.0; SPSS, Chicago, IL). Tests of significance were two-tailed, and the level of significance was set at 0.05.

Results

Refractive errors

As expected, after 5 days of diffuser wear, the covered eyes developed significant myopia in both reference groups (group 1: -10.84 ± 1.09D, $t = -9.985$, $P = 0.002$ and group 2: -10.75 ± 1.81D, $t = -5.927$, $P = 0.010$). There was no significant difference between the groups ($t = -0.043$, $P = 0.967$). Therefore, the data of both groups were pooled to generate a reliable reference group against which the effects of bright light exposure could be tested.

Varying durations of continuous bright light (paradigm 1) inhibited deprivation myopia to different extents ($F = 3.817$, $P = 0.017$; Figure 1). Post-hoc analysis revealed that exposure for 1 or 2 hours did not provide significant protection against myopia development ($P = 0.099$ and $P = 0.309$, respectively). Significant inhibition of myopia was observed only when exposure duration was extended to 5 hours or more ($P = 0.004$ and $P = 0.007$ for 5 and 10 hours, respectively). Nevertheless, there was no significant difference between groups reared with 5 or 10 hours of bright light ($P = 0.796$).

In paradigm 2, bright light was applied as a temporal square wave function (changing repeatedly between 500 and 15000 lux). It was found that the protective effect of bright light was enhanced when the cycles were in the range of minutes (Figure 1). Still, no difference was found when bright light was applied continuously for 5 hours or in episodes of 60:60 minutes for 10 hours (-3.23 ± 1.19D vs -2.70 ± 0.73D, $t = -0.404$, $P = 0.696$). However, significant difference was detected among the groups exposed to repeated cycles of bright light (Paradigm 2, $F = 3.023$, $P = 0.033$). Post-hoc tests revealed that myopia inhibition was maximal when chickens were kept at 7:7 minutes and 1:1 minutes cycles (7:7 minutes vs 30:30 minutes: $P = 0.038$; 1:1 minutes vs 60:60, 30:30, 15:15 minutes: $P = 0.041$, 0.006, 0.022, respectively). For 1:1 minute cycles, the degree of induced myopia was significantly lower than that after 5 hours of continuous bright light (-3.23 ± 1.19D vs -0.47 ± 0.38D; $t = -2.749$, $P = 0.023$).

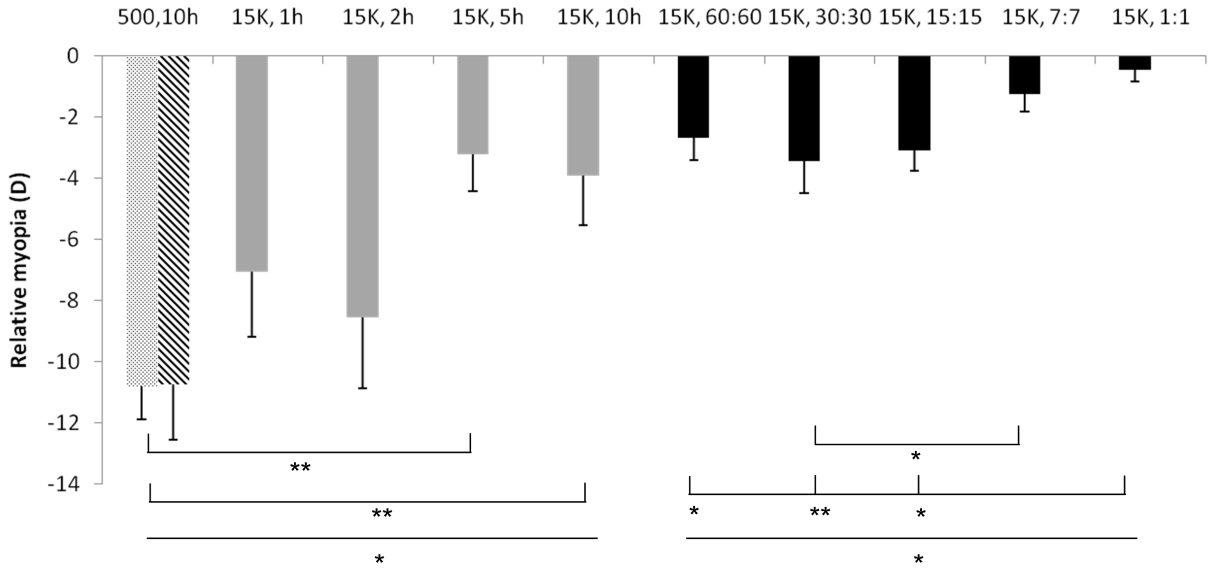

Figure 1. Myopia induced by diffusers over one eye when chickens were kept under constant bright light of 15 000 lux for 1, 2, 5, or 10 hours (''15k, time in hours''; filled gray bars) or under cycles of bright light, changing from 500 to 15 000 lux at different frequencies (''15k, half cycle duration; black bars). Patterned gray bars show the amount of myopia that developed in two batches of chickens wearing monocular diffusers under regular laboratory illumination of 500 lux (''500,10 h''). Because there was no difference between both groups, their data were pooled and provided the reference for the bright light treatment groups. An inhibitory effect of constant bright light was observed only when the exposure lasted for 5 hours or more (P = 0.004 and P = 0.007 for 5 and 10 hours, respectively). No additional benefit was observed when the bright light exposure was extended to 10 hours, compared with those exposed to 5 hours (P = 0.796). When bright light was provided as a temporal square wave function, its protective effect against myopia was enhanced. Chickens kept under 7:7 or 1:1 minute cycles developed the least myopia, compared with other cycles (P = 0.033 for differences among groups reared under cycles of bright light; Post-hoc pairwise comparison: 7:7 minutes vs 30:30 minutes, P = 0.038; 1:1 minutes vs 60:60, 30:30, 15:15 minutes: P = 0.041, 0.006, 0.022, respectively). *<0.05, **<0.01.
doi:10.1371/journal.pone.0110906.g001

Interocular differences in the refractive errors in chicks kept under different light cycles are shown in Table 1. Under 60:60 to 15:15 minute cycles, significant myopia developed in the deprived eyes (all P<0.05). However, there was no longer a significant interocular difference in the refractive errors when the animals were under 7:7 and 1:1 minute cycles (P = 0.066 and 0.256, respectively).

Ocular biometry

In the two reference groups, vitreous chamber depth (VCD) increased about linearly with the amount of myopia with about 0.1 mm per diopter of myopia ($R^2 = 0.811$, P = 0.005, Figure 2). There was no significant difference between both groups (t = −0.750, P = 0.487). The ratio of vitreous chamber elongation to increase of myopia was similar among the different groups raised in continuous bright light ($R^2 = 0.716$, P<0.001) or in intermittent bright light ($R^2 = 0.759$, P<0.001).

Interestingly, the changes in vitreous chamber depth due to exposure to the different light regimens were often not as significant as the refractive errors (Paradigm I: F = 1.639, P = 0.204; Paradigm II: F = 2.075, P = 0.109, significances refer to the differences among groups in Paradigm I and II, respectively). However, consistent with previous studies[13,16], if the data from chickens exposed to 5 or 10 hours of constant bright light was compared to those of the reference group separately, statistical significance was detected (0.45±0.20 mm vs 0.93±0.09 mm, t = −2.590, P = 0.029; 0.56±0.13 mm vs 0.93±0.09 mm, t = −2.451, P = 0.037, respectively). More importantly, it is clear that repeated cycles of bright light generated generally shorter vitreous chambers than constant bright light.

Table 1. Interocular differences in myopia and the depth of the vitreous chamber of the eyes (VCD) with monocular diffusers under different temporal cycles of bright light.

Group	N	Relative myopia		Relative VCD elongation	
		Mean±SEM	P	Mean±SEM	P
60:60	7	−2.70±0.73D	0.010*	0.24±0.07	0.012*
30:30	8	−3.45±1.04D	0.013*	0.34±0.09	0.007*
15:15	6	−3.09±0.66D	0.005*	0.28±0.08	0.017*
7:7	7	−1.27±0.56D	0.066	0.20±0.07	0.032*
1:1	7	−0.47±0.38D	0.256	0.05±0.06	0.428

*significant myopic shifts in deprived eyes compared to non-deprived fellow eyes.
doi:10.1371/journal.pone.0110906.t001

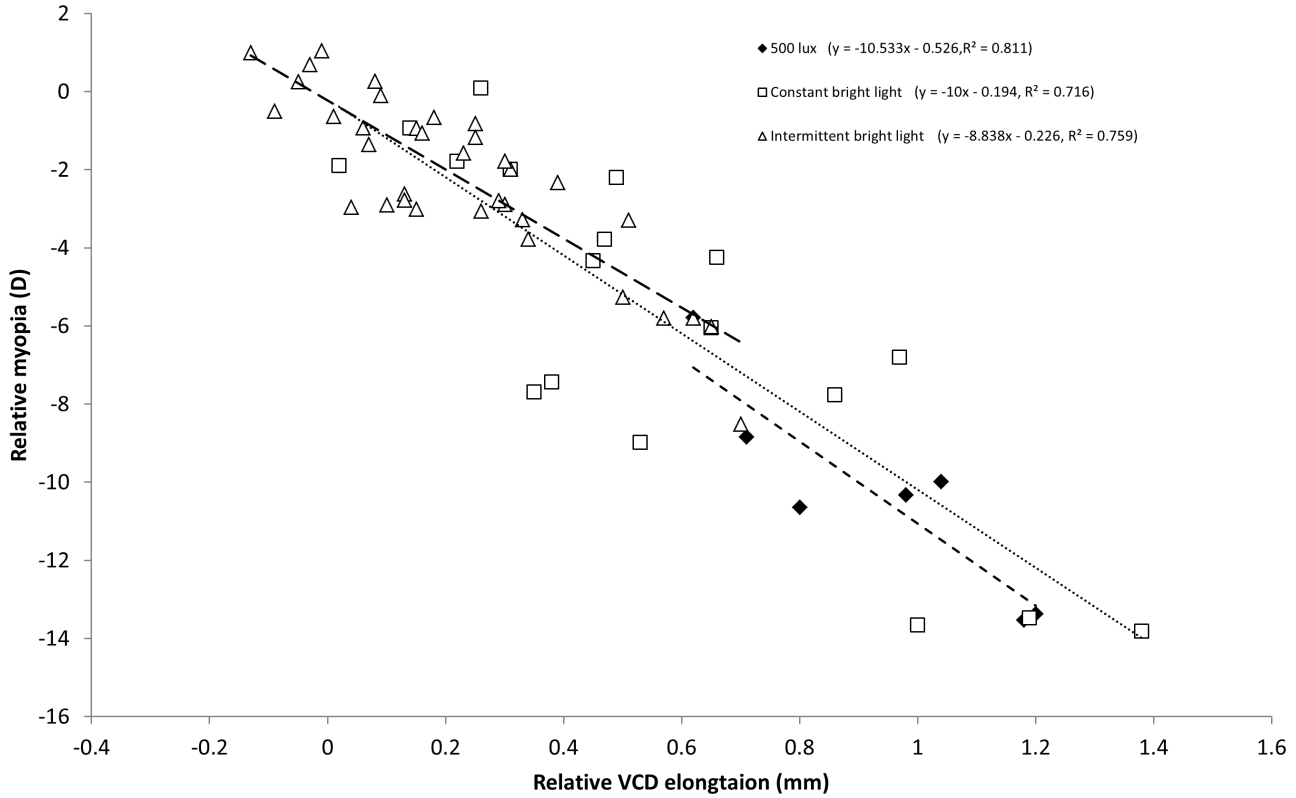

Figure 2. Correlation between vitreous chamber depth and the amount of myopia in chickens under different light regimens. Equations for the linear regression, and R^2 values are provided for each light regimen. Long dash line represents the data for intermittent bright light, dotted line for constant bright light and short dash line for standard illuminance, respectively. Note that one diopter of myopia was equivalent to about 0.1 mm of axial elongation across groups (data from one single animal were excluded from the plot because of apparent measurement error, data: −13.9D vs 0.25 mm).
doi:10.1371/journal.pone.0110906.g002

Exposure to 7:7 or 1:1 min bright light cycles inhibited axial eye growth more than constant bright light (all $P<0.05$, except for the comparison between the 7:7 minute cycle and the 5 h constant bright light exposure, $P = 0.180$ and a borderline significance between the 7:7 minute cycle and the 1 h constant bright light exposure, $P = 0.075$). Similar to refractive error, vitreous chamber elongation was almost completely suppressed in the diffusertreated eyes (Figure 3; Table 1) exposed to a 1:1 min bright light cycle.

No significant changes were detected in anterior chamber depth and lens thickness (LT) between the deprived eyes and the non-deprived eyes, regardless of treatment group (all $P>0.05$). There were also no differences in these two parameters among groups ($F = 0.478$, $P = 0.752$ and $F = 0.363$, $P = 0.833$ for ACD; $F = 0.024$, $P = 0.999$ and $F = 0.258$, $P = 0.903$ for LT, respectively).

Discussion

Our data show that exposure to continuous bright light of 15 000 lux for 1 or 2 hours every day is not sufficient to provide significant protection against deprivation myopia in the chicken model of myopia. Inhibition of myopia was significant after 5 hours of bright light exposure but, extending the duration to 10 hours, did not offer additional benefit. However, repeated cycles of 1:1 minutes of bright to standard laboratory light (15 000 versus 500 lux) enhanced the protective effect against myopia and could finally suppress its development completely.

Should children be exposed to continuous bright light for longer periods of time?

In the first paradigm, we found that 5 hours of 15 000 lux inhibited deprivation myopia by approximately 70%, similar to what was found in different animal models in previous studies with an ambient illuminance of 15 000 to 25 000 lux for 5 to 6 hours [13–16]. Short term bright light exposure for 1 or 2 hours generated only a trend towards inhibition of deprivation myopia. This result is consistent with a recent study in chickens in which bright light of 10 000 lux was provided for 2 hours a day but no significant effects were found, no matter at which time of the day it was applied [29]. In comparison, human studies show that children appear to be more "sensitive" to bright light exposure, as Jones et al. observed a marked reduction in the risk of myopia when the amount of time outdoors increased from 0–5 hours per week (approximately 1 hour per day) to>14 hours per week (approximately 2 hours per day) [9]. The three outdoor clinical trials also suggest that significant protection from myopia is also achieved with only 1–2 hours of outdoor exposure per day [19–21]. We speculate that the discrepancy between human data and animal studies might be due to, other than species differences, several critical differences in the "treatment protocols", such as differences in the visual environment, in the procedures to induce myopia, in the shape of the dioptric space, and the durations of the intervention, and their relation to the life span of humans and chicks. Finally, sample size could be another essential factor to consider. It is noted that the sample size of these clinical trials is much higher than the number of chickens in the current study

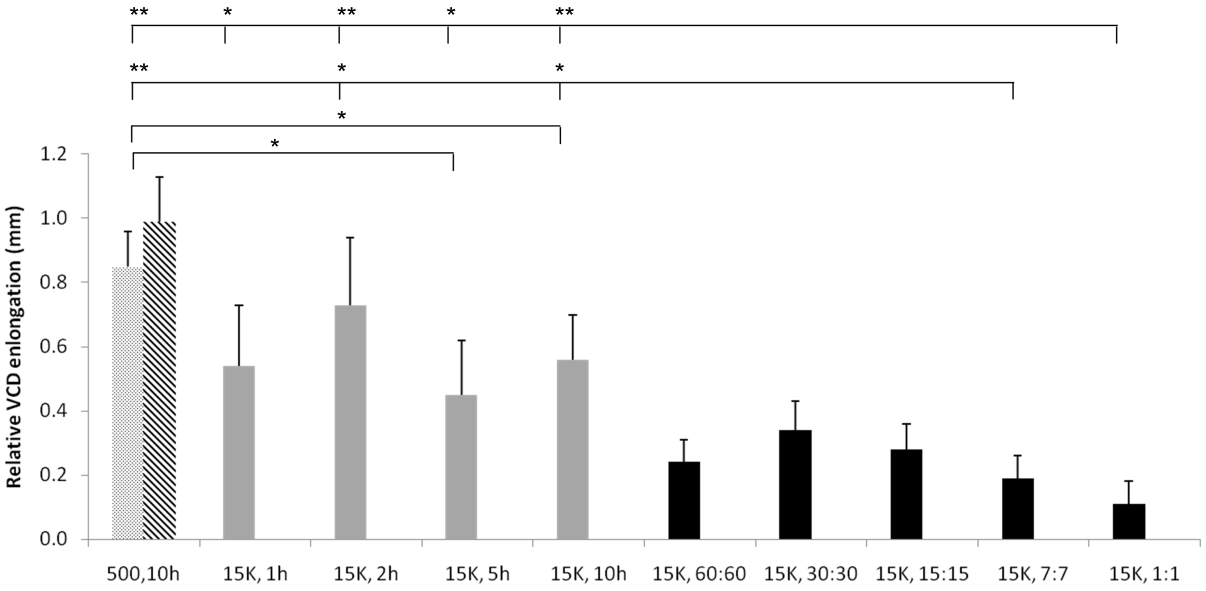

Figure 3. Relative increase in vitreous chamber depth (VCD) in eyes with monocular diffusers (bars grey-scale coded as in Figure 1). Although there was no significant difference among treatment groups for either paradigm (Paradigm I: $F = 1.639$, $P = 0.204$ and Paradigm II: $F = 2.075$, $P = 0.109$), the increase of VCD in chickens reared under constant bright light for 5 or 10 hours was signifcantly supressed compared with those under standard illuminance ($P = 0.029$ and 0.037, respectively). In comparison with constant bright light, this effect was further enhanced in chickens exposed to cycles of bright light at a frequency of 7:7 or 1:1 minutes (all $P<0.05$, except for the comparison between the 7:7 minute cycle and the 5 h constant bright light exposure, $P = 0.180$ and a borderline significance between the 7:7 minute cycle and the 1 h constant bright light exposure, $P = 0.075$).* <0.05, ** <0.01. Abbreviations as in Figure 1.
doi:10.1371/journal.pone.0110906.g003

(e.g., 1903 kids were enrolled in the trial launched in Guangzhou [21]). However, in a normal animal study, like the current one, ethical limitations usually prevent the usage of larger numbers of experimental animals.

We also found that no additional benefit was achieved when the exposure duration was extended to the entire light phase (10 hours). Therefore, we assume that there might be a plateau for the protective effect from certain level of dose for continuous bright light exposure, at least in the case of 15 000 lux. If this finding was applicable to children, then the treatment effect for this strategy might have an upper limit. Certainly, it is important to know where the optimal treatment exposure duration is located in children, as the prolonged exposure to bright light would increase the energy consumption (in the case of using artificial lighting). Additional benefit in terms of a complete inhibition of deprivation myopia development in chicks was demonstrated when illuminance level was further increased to 40 000 lux (Ashby, unpublished data), perhaps another approach to enhance the suppressive effect of bright light on myopia. Since our eyes were developed in the course of evolution to operate optimally at day light, there is no reason to assume that 15 000 lux indoors are deleterious to our retina. But a longer exposition to bright light outdoors might also increase the risk of potential side effects, such as skin cancer [30] and retinal light damage (in the case of overexposing to sunlight). [31]

Should children be exposed to cycles of bright light at low frequencies to have a larger effect on myopia?

In the second paradigm, we replaced the continuous bright light regimens with intermittent ones. Interestingly, providing bright light in pulses with low temporal frequency further suppressed the development of deprivation myopia. Inhibition appears to be frequency-dependent. When the temporal frequency reached 0.001 Hz (7:7 min cycles of bright to standard light), the differences in refractive error between eyes with normal visual experience and eyes with diffusers were no longer significant, indicating that deprivation myopia was completely suppressed. Even though our findings might be applicable to children, compliance must be considered. In particular, exposure to alternating illuminance between 500 lux and 15 000 lux with short cycles may be less comfortable than constant bright light. Future studies should test whether one really needs 15 000 lux provided at low frequency cycles to fully suppress myopia development. If low frequency flicker at lower light intensity (e.g. 2 000 lux) would have a similar effect, feasibility would be greatly improved.

Possible mechanisms by which intermittent bright light could inhibit deprivation myopia

As reviewed by French et al., [11] two factors are currently discussed that might be important for the suppression of human myopia by bright light. One is that UV exposure is important since it triggers vitamin D production in the skin; the other is that dopamine release from the retina is stimulated by bright light and has an inhibitory effect on axial eye growth. In favor of the first mechanism, Vitamin D was lower in myopes than non-myopes [32,33]. On the other hand, evidence against this hypothesis is that feeding tree shrews with a sufficient dose of Vitamin D3 supplements [34] or rearing chickens under bright UV light [35] did not prevent experimental myopia. Furthermore, our finding that deprivation myopia was significantly inhibited by light that was free of UV (cut-off at around 400 nm) also weakens this hypothesis.

By contrast, there is more evidence supporting the hypothesis that dopamine release is stimulated and inhibits axial eye growth. In the first place, dopamine release is known to be almost linearly

related to the logarithm of the ambient lighting level [12,36–39]. Furthermore, it has been speculated by Norton and Siegwart [17] that as illuminance levels rise, activation of intrinsically photoresponsive retinal ganglion cells (ipRGCs) might provide an additional way to stimulate the dopamine release, given the finding that ipRGCs synapse directly on dopaminergic amacrine cells in the retina [40]. In parallel, it is known since 1989 that the synthesis and release of dopamine is reduced during the development of deprivation myopia [41–43]. Dopamine agonists injected into the vitreous can inhibit deprivation myopia in different species, including chickens [44–47], rabbits [48] and rhesus monkeys [49]. On the contrary, spiperone, a dopamine antagonist, was found to block the beneficial effects of bright light on deprivation myopia [16]. In summary, the second hypothesis appears more likely that high illuminances stimulate dopamine release from the retina and that dopamine has an inhibitory effect on axial eye growth [11,17].

Since low frequency bright flicker light inhibited myopia more than continuous bright light in the present study, one could assume that dopamine release is further stimulated. It was found already in 1987 that flickering light with 10 Hz inhibits deprivation myopia in chickens [50]. Several studies found that flickering light can stimulate the release of dopamine from the retina [51–53]. Flickering light results in a strong stimulation of both ON and OFF pathways. Retinal ON-pathway neurons, including dopamine-releasing neurons, respond to the onset of light with a pronounced depolarizing transient that decays to a relatively low plateau level. It is possible therefore that flickering stimuli produces repeated ON-transients which might result in a greater overall release of dopamine than a steady light stimulus [9,54]. However, there is also evidence that steady light causes more dopamine release [37,55,56]. Dong and McReynolds [54] speculated that the inconsistency across these studies might be due to the fact that the light responses of retinal neurons often change dramatically with light intensity or the state of adaptation. For example, most of the studies that reported a larger effect of flickering light were done in light-adapted retinas and with bright light pulses, while those that reported a weaker effect of flickering light used dark-adapted retinas and relatively dim stimuli. In the current study, we used illuminance where the chickens were light adapted and one would assume that dopamine release is enhanced by the flickering light. Nevertheless, it should be pointed out that the current "flickering light" was in a much lower frequency band (0.007 Hz) than flickering light in other studies (1–20 Hz). Thus, it is not clear to what extent the findings from flickering light can be applied to the current light treatments. Further work needs to demonstrate that dopamine release is actually enhanced when flicker frequencies are very low.

We did not consider the effects of light on pupil constrictions, which would add transient components to the retinal illumination when a temporal square wave pattern of light was applied. However, even if the pupil constricts by 50% at the onset of each light pulse it would temporarily reduce retinal luminance by only about 0.3 log units, followed by partial recovery when the retina has adapted to the new illuminance. Thus, the magnitude of such effects would be small compared to the amplitudes of the flicker light itself. Furthermore, Ashby et al. have studied the impact of changes in pupil size on the protective effect against myopia by using artificial pupils and found no effects [13].

Potential role of changes in corneal radius of curvature

A limitation in the current study was that corneal radius of curvature was not measured even though it is known that exposing chicks to continuous light can flatten their cornea severely [57]. On the other hand, no relative changes in corneal radius of curvature were found by Ashby et al. [13] when animals were reared under 50, 500 or 15 000 lux. Also Backhouse et al. [29] did not find relative changes in chickens kept at 2,000 lux for 10 hours or at 10,000 lux for 2 hours. Interestingly, Cohen et al. [12] found that corneal radius of curvature responded differently under continuous light and under normal diurnal cycles. Under continuous light, the brighter the lighting is, the flatter the cornea became. But under normal diurnal cycles, the opposite change was observed. All effects were rather small, less than 2D between 10,000 lux and 500 lux for a period of 30 days. In the current study, potential changes in corneal radius of curvature had only a minor effect since changes in refractive state could be explained to 70% to 80% by the changes in vitreous chamber depth (Figure 2).

Summary

Temporal properties of bright light exposure modulate the impact on deprivation myopia in chickens. For continuous bright light, no significant inhibition occurs below two hours of exposure while the inhibitory effects level off between five and ten hours. With the same total light dose, intermittent bright light provides a stronger effect than continuous light. Deprivation myopia in chickens is completely inhibited by 1:1 minute square wave light cycles (0.007 Hz), presented in total for five hours a day. However, it should be pointed out that these quantitative data were found in chickens. Although the previous finding that bright light inhibits experimental myopia is an across-species' phenomenon and therefore might be applicable to humans, the exact protocolprobably might not be directly translated into human values. Thus, further amendments are required in clinical studies.

Supporting Information

Table S1 Summary of refractive error and ocular biometry data of all groups.
(XLS)

Acknowledgments

Part of the material has been presented at the International Myopia Conference in August 2013.

Author Contributions

Conceived and designed the experiments: WL FS. Performed the experiments: WL. Analyzed the data: WL FS. Contributed reagents/materials/analysis tools: MF FS. Wrote the paper: WL MF FS.

References

1. Jobke S, Kasten E, Vorwerk C (2008) The prevalence rates of refractive errors among children, adolescents, and adults in Germany. Clin Ophthalmol 2: 601–607.
2. Wolfram C, Hohn R, Kottler U, Wild P, Blettner M, et al. (2014) Prevalence of refractive errors in the European adult population: the Gutenberg Health Study (GHS). The British journal of ophthalmology 55: 2041–2047.
3. Wu HM, Seet B, Yap EP, Saw SM, Lim TH, et al. (2001) Does education explain ethnic differences in myopia prevalence? A population-based study of young adult males in Singapore. Optometry and vision science 78: 234–239.
4. He M, Zeng J, Liu Y, Xu J, Pokharel GP, et al. (2004) Refractive error and visual impairment in urban children in southern china. Investigative ophthalmology & visual science 45: 793–799.

5. Dirani M, Tong L, Gazzard G, Zhang X, Chia A, et al. (2009) Outdoor activity and myopia in Singapore teenage children. The British journal of ophthalmology 93: 997–1000.
6. Guggenheim JA, Northstone K, McMahon G, Ness AR, Deere K, et al. (2012) Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study. Investigative ophthalmology & visual science 53: 2856–2865.
7. Onal S, Toker E, Akingol Z, Arslan G, Ertan S, et al. (2007) Refractive errors of medical students in Turkey: one year follow-up of refraction and biometry. Optometry and vision science 84: 175–180.
8. Rose KA, Morgan IG, Ip J, Kifley A, Huynh S, et al. (2008) Outdoor activity reduces the prevalence of myopia in children. Ophthalmology 115: 1279–1285.
9. Jones LA, Sinnott LT, Mutti DO, Mitchell GL, Moeschberger ML, et al. (2007) Parental history of myopia, sports and outdoor activities, and future myopia. Investigative ophthalmology & visual science 48: 3524–3532.
10. Jones-Jordan LA, Mitchell GL, Cotter SA, Kleinstein RN, Manny RE, et al. (2011) Visual activity before and after the onset of juvenile myopia. Investigative ophthalmology & visual science 52: 1841–1850.
11. French AN, Ashby RS, Morgan IG, Rose KA (2013) Time outdoors and the prevention of myopia. Experimental eye research 114: 58–68.
12. Cohen Y, Peleg E, Belkin M, Polat U, Solomon AS (2012) Ambient illuminance, retinal dopamine release and refractive development in chicks. Experimental eye research 103: 33–40.
13. Ashby R, Ohlendorf A, Schaeffel F (2009) The effect of ambient illuminance on the development of deprivation myopia in chicks. Investigative ophthalmology & visual science 50: 5348–5354.
14. Siegwart JT, Ward AH, Norton TT (2012) Moderately elevated fluorescent light levels slow form deprivation and minus lens-induced myopia development in tree shrews. ARVO Meeting Abstracts Invest Ophthalmol Vis Sci 53: 3457.
15. Smith EL, 3rd, Hung LF, Huang J (2012) Protective effects of high ambient lighting on the development of form-deprivation myopia in rhesus monkeys. Investigative ophthalmology & visual science 53: 421–428.
16. Ashby RS, Schaeffel F (2010) The effect of bright light on lens compensation in chicks. Investigative ophthalmology & visual science 51: 5247–5253.
17. Norton TT, Siegwart JT, Jr. (2013) Light levels, refractive development, and myopia—a speculative review. Experimental eye research 114: 48–57.
18. Smith EL, 3rd, Hung LF, Arumugam B, Huang J (2013) Negative lens-induced myopia in infant monkeys: effects of high ambient lighting. Investigative ophthalmology & visual science 54: 2959–2969.
19. Wu PC, Tsai CL, Wu HL, Yang YH, Kuo HK (2013) Outdoor activity during class recess reduces myopia onset and progression in school children. Ophthalmology 120: 1080–1085.
20. Yi JH, Li RR (2011) [Influence of near-work and outdoor activities on myopia progression in school children]. Zhongguo dang dai er ke za zhi 13: 32–35.
21. Morgan IG, Xiang F, Rose KA, Chen Q, He M (2012) Two Year Results from the Guangzhou Outdoor Activity Longitudinal Study (GOALS). ARVO Meeting Abstracts Invest Ophthalmol Vis Sci 53: 2735.
22. Smith EL, 3rd, Harwerth RS, Crawford ML, von Noorden GK (1987) Observations on the effects of form deprivation on the refractive status of the monkey. Investigative ophthalmology & visual science 28: 1236–1245.
23. Wallman J, Gottlieb MD, Rajaram V, Fugate-Wentzek LA (1987) Local retinal regions control local eye growth and myopia. Science 237: 73–77.
24. Wallman J, Turkel J, Trachtman J (1978) Extreme myopia produced by modest change in early visual experience. Science 201: 1249–1251.
25. Zhu X, McBrien NA, Smith EL, 3rd, Troilo D, Wallman J (2013) Eyes in various species can shorten to compensate for myopic defocus. Investigative ophthalmology & visual science 54: 2634–2644.
26. Morgan IG, Ashby RS, Nickla DL (2013) Form deprivation and lens-induced myopia: are they different? Ophthalmic & physiological optics 33: 355–361.
27. Seidemann A, Schaeffel F (2002) Effects of longitudinal chromatic aberration on accommodation and emmetropization. Vision research 42: 2409–2417.
28. Schaeffel F, Howland HC (1991) Properties of the feedback loops controlling eye growth and refractive state in the chicken. Vision research 31: 717–734.
29. Backhouse S, Collins AV, Phillips JR (2013) Influence of periodic vs continuous daily bright light exposure on development of experimental myopia in the chick. Ophthalmic & physiological optics 33: 563–572.
30. Gandini S, Autier P, Boniol M (2011) Reviews on sun exposure and artificial light and melanoma. Progress in biophysics and molecular biology 107: 362–366.
31. Youssef PN, Sheibani N, Albert DM (2011) Retinal light toxicity. Eye 25: 1–14.
32. Mutti DO, Marks AR (2011) Blood levels of vitamin D in teens and young adults with myopia. Optometry and vision science 88: 377–382.
33. Choi JA, Han K, Park YM, La TY (2014) Low Serum 25-Hydroxyvitamin D Is Associated with Myopia in Korean Adolescents.Invest Ophthalmol Vis Sci 55: 2041–7.
34. Siegwart JrJT, Herman CK, Norton TT (2011) Vitamin D3 Supplement Did Not Affect the Development of Myopia Produced with Form Deprivation or a Minus Lens in Tree Shrews. ARVO E-Abstract: #6298.
35. Hammond DS, Wildsoet CF (2012) Compensation to positive as well as negative lenses can occur in chicks reared in bright UV lighting. Vision Res 67: 44–50.
36. Iuvone PM, Galli CL, Garrison-Gund CK, Neff NH (1978) Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. Science 202: 901–902.
37. Brainard GC, Morgan WW (1987) Light-induced stimulation of retinal dopamine: a dose-response relationship. Brain research 424: 199–203.
38. Megaw PL, Boelen MG, Morgan IG, Boelen MK (2006) Diurnal patterns of dopamine release in chicken retina. Neurochemistry international 48: 17–23.
39. Besharse JC, Iuvone PM (1992) Is dopamine a light-adaptive or a dark-adaptive modulator in retina? Neurochemistry international 20: 193–199.
40. Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, et al. (2008) Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. Proceedings of the National Academy of Sciences of the United States of America 105: 14181–14186.
41. Iuvone PM, Tigges M, Fernandes A, Tigges J (1989) Dopamine synthesis and metabolism in rhesus monkey retina: development, aging, and the effects of monocular visual deprivation. Visual neuroscience 2: 465–471.
42. Stone RA, Lin T, Laties AM, Iuvone PM (1989) Retinal dopamine and form-deprivation myopia. Proceedings of the National Academy of Sciences of the United States of America 86: 704–706.
43. Weiss S, Schaeffel F (1993) Diurnal growth rhythms in the chicken eye: relation to myopia development and retinal dopamine levels. Journal of comparative physiology A 172: 263–270.
44. Rohrer B, Spira AW, Stell WK (1993) Apomorphine blocks form-deprivation myopia in chickens by a dopamine D2-receptor mechanism acting in retina or pigmented epithelium. Visual neuroscience 10: 447–453.
45. Nickla DL, Totonelly K, Dhillon B (2010) Dopaminergic agonists that result in ocular growth inhibition also elicit transient increases in choroidal thickness in chicks. Experimental eye research 91: 715–720.
46. Schmid KL, Wildsoet CF (2004) Inhibitory effects of apomorphine and atropine and their combination on myopia in chicks. Optometry and vision science 81: 137–147.
47. Schaeffel F, Bartmann M, Hagel G, Zrenner E (1995) Studies on the role of the retinal dopamine/melatonin system in experimental refractive errors in chickens. Vision research 35: 1247–1264.
48. Gao Q, Liu Q, Ma P, Zhong X, Wu J, et al. (2006) Effects of direct intravitreal dopamine injections on the development of lid-suture induced myopia in rabbits. Graefe's archive for clinical and experimental ophthalmology 244: 1329–1335.
49. Iuvone PM, Tigges M, Stone RA, Lambert S, Laties AM (1991) Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. Investigative ophthalmology & visual science 32: 1674–1677.
50. Gottlieb M.D WJ (1987) Retinal activity modulates eye growth: evidence from rearing in stroboscopic illumination. Society of Neuroscience Abstracts 13: 1297.
51. Kirsch M, Wagner HJ (1989) Release pattern of endogenous dopamine in teleost retinae during light adaptation and pharmacological stimulation. Vision research 29: 147–154.
52. Umino O, Lee Y, Dowling JE (1991) Effects of light stimuli on the release of dopamine from interplexiform cells in the white perch retina. Visual neuroscience 7: 451–458.
53. Kramer SG (1971) Dopamine: A retinal neurotransmitter. I. Retinal uptake, storage, and light-stimulated release of H3-dopamine in vivo. Investigative ophthalmology & visual science 10: 438–452.
54. Dong CJ, McReynolds JS (1992) Comparison of the effects of flickering and steady light on dopamine release and horizontal cell coupling in the mudpuppy retina. Journal of neurophysiology 67: 364–372.
55. Boatright JH, Hoel MJ, Iuvone PM (1989) Stimulation of endogenous dopamine release and metabolism in amphibian retina by light- and K+-evoked depolarization. Brain research 482: 164–168.
56. Dong CJ, McReynolds JS (1991) The relationship between light, dopamine release and horizontal cell coupling in the mudpuppy retina. The Journal of physiology 440: 291–309.
57. Li T, Troilo D, Glasser A, Howland HC (1995) Constant light produces severe corneal flattening and hyperopia in chickens. Vision research 35: 1203–1209.

The Effect of Spectral Property and Intensity of Light on Natural Refractive Development and Compensation to Negative Lenses in Guinea Pigs

Wentao Li,[1] Weizhong Lan,[1–3] Shiqi Yang,[1] Yunru Liao,[1] Qinglin Xu,[1,4] Lixia Lin,[1] and Zhikuan Yang[1,4]

[1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China
[2]Section of Neurobiology of the Eye, Center for Ophthalmology, University of Tuebingen, Tuebingen, Germany
[3]Graduate School of Cellular and Molecular Neuroscience, University of Tuebingen, Tuebingen, Germany
[4]Aier Eye Hospital Group, Changsha, China

Correspondence: Zhikuan Yang, Zhongshan Ophthalmic Center, 54 S. Xianlie Road, Guangzhou 510060, China; and Aier Eye Hosiptal, 388 Furong Middle Road, Changsha 410015, China; Zhikuangyang@gmail.com.

WLi and WLa contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Submitted: December 19, 2013
Accepted: August 21, 2014

Citation: Li W, Lan W, Yang S, et al. The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs. *Invest Ophthalmol Vis Sci*. 2014;55:6324–6332. DOI:10.1167/iovs.13-13802

Purpose. To investigate the effect of spectral composition and light intensity on refractive development in guinea pigs.

Methods. One-week-old guinea pigs were randomly assigned to groups exposed to broad-spectrum Solux halogen light (BS) or spiked-spectrum fluorescent light (FL) at both high (Hi, 10,000 lux) and low (Lo, 500 lux) intensities under a 12:12 light/dark cycle. Half of the animals in each group were used as controls (n = 24, 20, 22, and 20, respectively), and half were fitted with binocular −4-diopter (D) lenses (L, lenses; n = 22, 20, 24, and 22, respectively). Refractive error, corneal curvature, and axial dimensions were determined by cycloplegic retinoscopy, photokeratometry, and A-scan ultrasonography, respectively.

Results. Guinea pigs exposed to FL and BS showed similar changes in refraction under both high (HiFL: 2.26 ± 0.55 D versus HiBS: 2.17 ± 0.65 D, $P > 0.05$)- and low-intensity lighting (LoFL: 1.39 ± 0.88 D versus LoBS: 1.40 ± 0.93 D, $P > 0.05$). This was also true for the groups wearing lenses (HiFL-L: −1.81 ± 0.73 D versus HiBS-L: −1.45 ± 0.99 D, $P > 0.05$; LoFL-L: −2.58 ± 0.65 D versus LoBS-L: −2.29 ± 0.50 D, $P > 0.05$). Nevertheless, animals under high-intensity lighting exhibited a significantly larger hyperopic shift compared with those under low-intensity lighting (HiFL versus LoFL: $P < 0.01$; HiBS versus LoBS: $P < 0.05$). Similarly, a significantly smaller myopic shift was observed with brighter light in the lens condition (HiFL-L versus LoFL-L: $P < 0.05$; HiBS-L versus LoBS-L: $P < 0.05$).

Conclusions. In guinea pigs, spectrally spiked light and broad-spectrum light have similar effects on natural refractive development and negative lens compensation. As found in other species, effects of light intensity on refractive development were also observed in guinea pigs in both illuminants.

Keywords: guinea pigs, spectral property, light intensity, lens-induced myopia

From increasing evidence, outdoor exposure is considered to be a strong protective factor against myopia. First, a series of epidemiological studies observed that children who spent more time outdoors were less likely to become myopic.[1–4] A comparison of children of Chinese ethnicity growing up in Singapore and Sydney suggested that differences in time outdoors were the main explanation for the large differences in the prevalence of myopia in the two groups.[5] Further, it was reported that indoor sports did not provide protection against myopia,[1,5,6] indicating that physical sport is not the primary reason for the beneficial effect of outdoor exposure. In addition, myopia progression was found to be slower in the summer, when daylight hours are longer and average light intensity is higher than in the winter.[7–10] With these data taken together, it seems very likely that the quantity of time spent outdoors is associated with the risk of myopia development.[11,12]

Outdoor and indoor visual experiences are fundamentally different. Therefore, many factors might contribute to the protective effect demonstrated by outdoor exposure (see Ref. 11 for review). One of the many potential factors is the distinct difference in lighting between outdoor and indoor environments. In the first place, sunlight provides much higher illumination than most indoor lighting. In Guangzhou, for instance, illumination outdoors ranges from 13,000 to 18,000 lux in the shade to over 100,000 lux in direct sunlight at noon on a clear sunny day. In contrast, indoor illumination provided by artificial lighting is usually in the range of 300 to 600 lux. Recent findings in animals indicate that significant differences in light intensity might be an important factor contributing to the protective effect of outdoor exposure against myopia. Chickens raised under high illumination (10,000 lux) were found to develop relative hyperopia compared to those raised under medium illumination (500 lux), while chickens under low illumination (50 lux) became relatively myopic.[13] Moreover, simply increasing the ambient light intensity from 500 to 15,000 lux has been shown to significantly inhibit the development of deprivation myopia in chickens,[13–15] tree shrews (Siegwart JT,

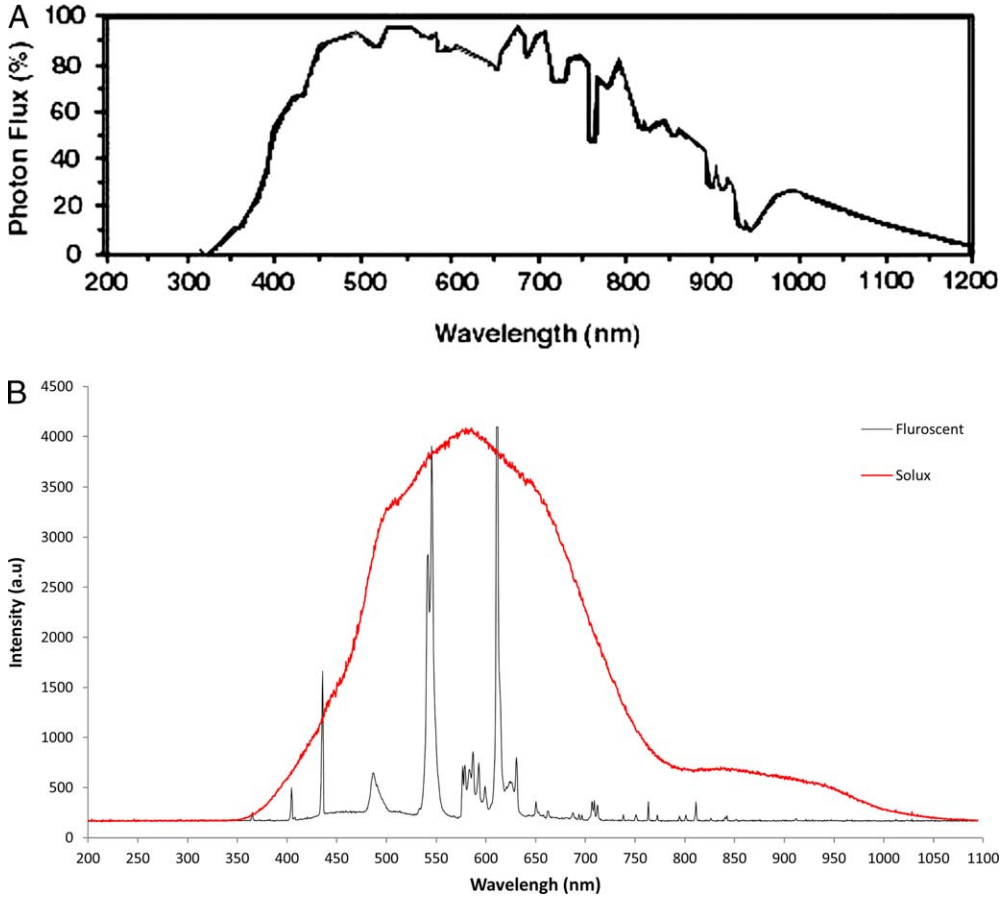

Figure 1. The spectrum of ground-level sunlight on a clear summer day (**A**) and the spectrum of Solux halogen lamp and fluorescent lamp (**B**). The spectrum of ground-level sunlight includes continuous radiation ranging from approximately 300 to 1200 nm. Adapted with permission from Smith KC, ed., *What is photobiology?* Photobiological Sciences Online. American Society for Photobiology, 2014. http://www.photobiology.info/introduction.html. Copyright August 22, 2014 Dr. Kendric C. Smith. Similarly, the Solux halogen lamp emits continuous radiation from approximately 350 to 1050 nm, which resembles the ground-level sunlight in terms of wavelength and spectrum distribution (except at radiation levels between 300 and 350 nm). By contrast, the fluorescent lamp emits only discrete rays, peaking at approximately 440, 550, and 620 nm, and the spectrum does not extend into UV and infrared regions.

et al. *IOVS* 2012;53:ARVO E-Abstract 3457), and rhesus monkeys.[16] Bright light also slowed down the development of lens-induced myopia in chickens[15] and tree shrews (Siegwart JT, et al. *IOVS* 2012;53:ARVO E-Abstract 3457), but this was not seen in rhesus monkeys.[17] In addition to light intensity, sunlight differs from indoor light in spectral composition. The spectrum of sunlight on earth during a typical day includes a continuous distribution of wavelengths from approximately 300 nm to approximately 1200 nm (Fig. 1A), as the stratospheric ozone layer filters out radiation lower than 295 nm, and radiation above 1200 nm is strongly absorbed by atmospheric water. In contrast, florescent lights, the most common source of artificial indoor lighting, emit only a spiked distribution of wavelengths from 400 to 700 nm, with peaks in the blue, green and red, and lack ultraviolet and infrared wavelengths.

As proposed previously,[16] in addition to absolute intensity, the spectral composition and distribution of light could also be critical for the protective effect from myopia observed with bright light treatment. The influence of spectral property on ocular growth has been investigated in animals by comparing the effect of different monochromatic lighting conditions.[18–25] In general, long wavelengths accelerate ocular elongation while short wavelengths inhibit ocular elongation. However, monochromatic light illumination exists only in laboratories, whereas daily illumination provided by sunlight or artificial indoor light usually consists of polychromatic spectra. In the present study, we have used two types of commercial lighting with distinct spectral properties to replicate real-world lighting environments to investigate if spectral differences are likely to have a role in the development of myopia.

Materials and Methods

Lighting

Two commercial lamps, a Solux halogen lamp (4100K; Eiko Ltd., Shawnee, KS, USA) and a fluorescent lamp (CFL23/PAR38, 4100K; Eiko Ltd.), were used as the lighting sources in the experiment. Figure 1B shows the spectrum profile of these two lamps measured with a fluorospectrophotometer (HR2000; Ocean Optics, Inc., Osaka, Japan; detection limit is 200–1100 nm) by the Department of Physics of Sun Yat-sen University in Guangzhou, China. It is noted that the Solux halogen lamp emits continuous wavelengths ranging from approximately 350 to 1050 nm (Fig. 1B). As shown in Figure 1A, the spectrum emitted by this lamp mimics the spectral composition of natural light very well except at wavelengths between 300 and 350 nm. In contrast, the fluorescent lamp emits only a discontinuous spectrum, with pronounced peaks at approximately 440, 550, and 620 nm. The spectrum does not extend into the UV and

infrared regions (Fig. 1B). To achieve the intensity of illumination needed in this study for the low (500 lux)- and high-intensity (10,000 lux) fluorescent lighting, we set three 9-W fluorescent lamps at a height of approximately 1 m above the cage and six 23-W fluorescent lamps at a height of approximately 50 cm, respectively. For the low (500 lux) and high (10,000 lux) Solux halogen lighting, we set one 50-W Solux halogen lamp at approximately 1 m above the cage and six 50-W Solux halogen lamps at a height of approximately 50 cm, respectively.

Animals and Experimental Design

The pigmented guinea pig (*Cavia porcellus*) is one of the most common mammalian models in myopia research.[23,26–33] More importantly, it has a unique wavelength-related optical system. The guinea pig has two cone types: M cones and S cones. The M-cone pigment has peak sensitivity at approximately 530 nm,[34] and the S-cone pigment has peak sensitivity at approximately 430 nm[35] (a more recent study[34] showed that the peak sensitivity for the S cone is approximately 400 nm). Thus, the S pigment is violet sensitive. Unlike what is observed in primates, the numbers of S cones in the guinea pig retina are unexpectedly high: Although the dorsal retina is dominated by M cones, having only approximately 5% S cones, all cones in the ventral retina are labeled strongly for the S pigment.[34] Furthermore, wavelengths longer than 280 nm are readily transmitted by the guinea pig cornea[36]; and although the crystal lens absorbs wavelengths shorter than 350 nm, it has a steep slope of increasing transmission for longer wavelengths including near UV (especially from 380 to 400 nm).[37,38] Consequently, the optical components of the guinea pig eye, in combination with the abundance of S pigment in the ventral retina, allows the guinea pig to have UV vision for at least wavelengths between 380 nm and 400 nm. The major difference between ground-level sunlight and the solar halogen light is the inclusion of wavelengths between 300 and 350 nm, but these wavelengths are absorbed by the crystalline lens of the guinea pig. The solar halogen light therefore reaches the guinea pig retina and stimulates the cone photoreceptors in the same way that sunlight does. The guinea pigs in the study were obtained by the Animal Experimental Centre of Zhejiang Province, China, and were raised in a temperature-controlled room with free access to food and water. In order to investigate the influence of the spectral property and light intensity on natural refractive development and refractive development affected by negative lenses, two paradigms with four different groups each were used in the experiment. Accordingly, 1-week-old guinea pigs were assigned randomly to one of the following groups.

Normal refractive development (paradigm 1): Guinea pigs were raised under one of four lighting conditions: (1) high-intensity broad-spectrum lighting (10,000 lux) of Solux halogen light (HiBS, $n = 24$); (2) high-intensity spiked-spectrum lighting (10,000 lux) of fluorescent light (HiFL, $n = 20$); (3) low-intensity broad-spectrum lighting (500 lux) of Solux light (LoBS, $n = 22$); (4) low-intensity spiked-spectrum lighting (500 lux) of fluorescent light (LoFL, $n = 20$).

Refractive development with negative lenses (paradigm 2): Guinea pigs continuously wore −4-diopter (D) lenses binocularly (L, lenses) and were raised under one of the light conditions described above: (1) high-intensity broad-spectrum lighting (10,000 lux) of Solux light with lenses (HiBS-L, $n = 22$); (2) high-intensity spiked-spectrum lighting (10,000 lux) of fluorescent light with lenses (HiFL-L, $n = 20$); (3) low-intensity broad-spectrum lighting (500 lux) of Solux light with lenses (LoBS-L, $n = 24$); (4) low-intensity spiked-spectrum lighting (500 lux) of fluorescent light with lenses (LoFL-L, $n = 22$).

The lamps for each group were switched on from 8:00 AM to 8:00 PM, giving a 12-hour light/12-hour dark cycle, for 3 weeks. The temperature was controlled to 22 ± 2°C. All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the animal experimentation ethics committee of the Zhongshan Ophthalmic Center.

Wearing of Lenses

Pieces of Velcro were modified into face masks and glued to the faces of the guinea pigs, leaving the eyes, nose, mouth, and ears exposed, as described by Howlett and McFadden.[39] Then a negative lens (−4.00 D, PMMA, diameter 18.0 mm, optical zone 12.0 mm, base curve 8.0 mm), which was already glued onto a plastic frame with Velcro, was attached to the face mask around the eye, and the optical center of the lens was aligned with the center of the pupil. The lenses were worn continuously during the experiments except when they were removed for cleaning with water-wetted gauze once a day at the commencement of the dark phase. The face masks were examined and reattached whenever necessary. In addition, whenever the lens was found to have visible scratches at its center, it was immediately replaced.

Ocular Biometry

Refractive error, corneal curvature, and axial dimensions of the eyes in each group were determined prior to the experiment and once a week for the 3 weeks of treatment.

Refractive error: Cycloplegic refractive error was measured using handheld streak retinoscopy (66 Vision-Tech Co., Ltd., Suzhou, Jiangsu Province, China) by two independent experienced optometrists from Zhongshan Ophthalmic Center, who were masked with regard to the treatment. Cycloplegia was induced by one drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Fort Worth, TX, USA), followed by five drops of 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P; Santen, Osaka, Japan) instilled 5 minutes apart. Extra attention was paid to ensure that the cornea was bathed with the drug by holding the animal horizontally for at least 1 minute after each instillation. Results from the two optometrists were averaged. Refractive error was expressed as the spherical equivalent (SE), that is, spherical error plus half of the cylinder error. No correction was made for the artifact of retinoscopy, which is relatively small in guinea pigs.[31]

Corneal curvature: The radius of the corneal curvature was measured with a custom-made infrared photokeratometer as described previously.[31,40] Readings were accepted only when the reflection of the light emitting diode (LED) rings was centered on the pupil and all six infrared lights were seen clearly from the screen. Then three readings were averaged to provide a value for each eye measured.

Axial dimensions: The axial dimension of the eye was determined by A-scan ultrasonography with a 10-MHz probe (KN-1800; Kangning Medical Device Co., Ltd., Wuxi, Jiangsu Province, China). One drop of 0.5% proparacaine hydrochloride (Alcaine, Alcon) was administered to the eye prior to the measurement. The ultrasound probe was placed in direct contact with the corneal apex, and special attention was paid to ensure that the probe was perpendicular to the corneal surface. Results from 10 readings were averaged for each eye measured.

Data Presentation and Analysis

The results are presented as mean ± standard deviation (SD) unless otherwise stated. Paired *t*-tests were used to analyze the

Investigative Ophthalmology & Visual Science

Table. Changes of Ocular Parameters With Time

Paradigms	Groups	Time Points	Refractive Error, D	Corneal Radius, mm	ACD, mm	LT, mm	VCD, mm	AL, mm
1: Without lenses	HiBS, $n = 24$	Baseline	3.68 ± 0.82	3.32 ± 0.07	1.12 ± 0.06	2.45 ± 0.12	3.42 ± 0.19	7.18 ± 0.11
		First week	4.90 ± 0.45	3.43 ± 0.11	1.11 ± 0.05	2.55 ± 0.14	3.55 ± 0.16	7.36 ± 0.12
		Second week	5.42 ± 0.39	3.52 ± 0.06	1.11 ± 0.04	2.64 ± 0.11	3.68 ± 0.13	7.50 ± 0.14
		Third week	5.84 ± 0.37	3.58 ± 0.07	1.12 ± 0.04	2.81 ± 0.09	3.82 ± 0.13	7.64 ± 0.11
		Change	2.17 ± 0.65	0.27 ± 0.07	0.00 ± 0.06	0.35 ± 0.10	0.40 ± 0.20	0.46 ± 0.14
	HiFL, $n = 20$	Baseline	3.69 ± 0.57	3.32 ± 0.07	1.10 ± 0.05	2.47 ± 0.15	3.44 ± 0.15	7.17 ± 0.10
		First week	4.81 ± 0.88	3.44 ± 0.11	1.12 ± 0.04	2.58 ± 0.10	3.57 ± 0.17	7.35 ± 0.15
		Second week	5.49 ± 0.70	3.50 ± 0.09	1.11 ± 0.05	2.67 ± 0.08	3.72 ± 0.16	7.44 ± 0.15
		Third week	5.95 ± 0.50	3.57 ± 0.09	1.12 ± 0.05	2.81 ± 0.10	3.84 ± 0.15	7.60 ± 0.18
		Change	2.26 ± 0.55	0.26 ± 0.05	0.02 ± 0.06	0.34 ± 0.12	0.40 ± 0.14	0.43 ± 0.21
	LoBS, $n = 22$	Baseline	3.69 ± 0.48	3.33 ± 0.06	1.10 ± 0.04	2.46 ± 0.14	3.42 ± 0.18	7.16 ± 0.10
		First week	4.64 ± 0.63	3.45 ± 0.07	1.12 ± 0.06	2.53 ± 0.11	3.57 ± 0.18	7.38 ± 0.11
		Second week	4.98 ± 0.55	3.51 ± 0.06	1.11 ± 0.05	2.63 ± 0.13	3.67 ± 0.25	7.49 ± 0.11
		Third week	5.10 ± 0.69	3.58 ± 0.50	1.12 ± 0.06	2.80 ± 0.11	3.86 ± 0.25	7.63 ± 0.14
		Change	1.40 ± 0.93	0.25 ± 0.06	0.03 ± 0.07	0.33 ± 0.15	0.44 ± 0.27	0.47 ± 0.18
	LoFL, $n = 20$	Baseline	3.69 ± 0.47	3.33 ± 0.07	1.10 ± 0.06	2.46 ± 0.11	3.42 ± 0.24	7.17 ± 0.08
		First week	4.56 ± 0.47	3.45 ± 0.07	1.12 ± 0.05	2.57 ± 0.19	3.56 ± 0.22	7.36 ± 0.17
		Second week	4.87 ± 0.57	3.52 ± 0.07	1.11 ± 0.06	2.63 ± 0.19	3.65 ± 0.26	7.48 ± 0.15
		Third week	5.08 ± 0.63	3.57 ± 0.06	1.10 ± 0.05	2.81 ± 0.11	3.84 ± 0.27	7.66 ± 0.10
		Change	1.39 ± 0.88	0.24 ± 0.05	0.00 ± 0.07	0.36 ± 0.13	0.42 ± 0.32	0.49 ± 0.12
2: With −4-D lenses	HiBS-L, $n = 22$	Baseline	3.69 ± 0.50	3.34 ± 0.08	1.09 ± 0.06	2.45 ± 0.11	3.46 ± 0.14	7.19 ± 0.11
		First week	3.05 ± 0.82	3.44 ± 0.06	1.11 ± 0.07	2.55 ± 0.15	3.71 ± 0.20	7.62 ± 0.11
		Second week	2.55 ± 1.03	3.52 ± 0.05	1.12 ± 0.06	2.67 ± 0.13	3.84 ± 0.17	7.69 ± 0.08
		Third week	2.24 ± 0.92	3.57 ± 0.05	1.11 ± 0.06	2.83 ± 0.11	3.98 ± 0.13	7.82 ± 0.10
		Change	−1.45 ± 0.99	0.23 ± 0.09	0.02 ± 0.04	0.38 ± 0.10	0.52 ± 0.16	0.64 ± 0.15
	HiFL-L, $n = 20$	Baseline	3.72 ± 0.61	3.32 ± 0.09	1.11 ± 0.05	2.48 ± 0.17	3.46 ± 0.17	7.19 ± 0.07
		First week	2.91 ± 0.99	3.44 ± 0.06	1.09 ± 0.05	2.54 ± 0.10	3.68 ± 0.17	7.58 ± 0.11
		Second week	2.37 ± 1.02	3.53 ± 0.07	1.10 ± 0.05	2.66 ± 0.14	3.86 ± 0.15	7.68 ± 0.08
		Third week	1.91 ± 0.93	3.59 ± 0.07	1.12 ± 0.07	2.82 ± 0.13	4.00 ± 0.14	7.80 ± 0.11
		Change	−1.81 ± 0.73	0.27 ± 0.09	0.02 ± 0.04	0.37 ± 0.16	0.54 ± 0.14	0.62 ± 0.14
	LoBS-L, $n = 24$	Baseline	3.72 ± 0.60	3.35 ± 0.05	1.09 ± 0.04	2.48 ± 0.15	3.48 ± 0.10	7.18 ± 0.12
		First week	2.73 ± 0.74	3.44 ± 0.06	1.13 ± 0.05	2.56 ± 0.14	3.72 ± 0.09	7.58 ± 0.14
		Second week	2.26 ± 0.81	3.52 ± 0.06	1.10 ± 0.05	2.69 ± 0.11	3.86 ± 0.08	7.73 ± 0.11
		Third week	1.42 ± 0.62	3.59 ± 0.06	1.09 ± 0.04	2.80 ± 0.13	4.03 ± 0.11	7.83 ± 0.10
		Change	−2.29 ± 0.50	0.24 ± 0.05	0.00 ± 0.03	0.33 ± 0.13	0.56 ± 0.15	0.65 ± 0.15
	LoFL-L, $n = 22$	Baseline	3.67 ± 0.60	3.33 ± 0.07	1.10 ± 0.06	2.48 ± 0.17	3.47 ± 0.12	7.19 ± 0.09
		First week	2.30 ± 0.58	3.43 ± 0.07	1.12 ± 0.06	2.57 ± 0.17	3.70 ± 0.16	7.56 ± 0.11
		Second week	1.84 ± 0.55	3.53 ± 0.06	1.13 ± 0.05	2.68 ± 0.11	3.85 ± 0.17	7.71 ± 0.16
		Third week	1.08 ± 0.48	3.57 ± 0.06	1.12 ± 0.06	2.82 ± 0.06	4.04 ± 0.16	7.81 ± 0.09
		Change	−2.58 ± 0.65	0.24 ± 0.07	0.02 ± 0.05	0.34 ± 0.15	0.57 ± 0.18	0.62 ± 0.12

ACD, anterior chamber depth; LT, lens thickness; VCD, vitreous chamber depth; AL, axial length. Data are presented as mean ± SD.

changes in ocular parameters between baseline and the end of the experiment for individual groups. As no interaction was found between spectral features and light intensity in either paradigm using factor analysis, the difference in changes between groups was compared by one-way ANOVA. If significant differences were detected, post hoc range tests were performed using the Duncan test. Additionally, unpaired *t*-tests were used to compare the means of independent groups with the same spectral composition but different intensities, or with different spectral features but the same light intensity. Pearson's correlation analysis was used to examine the relationship between the change of refractive error and that of axial length. All the statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). The level for statistical significance was set at two-tailed 0.05.

Results

Data on all ocular parameters at different time points are shown in the Table. At baseline, none of the parameters were significantly different between groups. In addition, there was no significant difference between the right and left eyes (data not shown) in all groups for refractive error and axial parameters. Thus, all the results were based on data from the right eyes of the guinea pigs.

Refractive Error

There was a significant hyperopic shift in refractive error in all groups reared without lenses after 3 weeks of light exposure (Table). In contrast, the eyes of animals fitted with −4-D lenses developed a myopic shift (Table). Under both rearing conditions, unpaired *t*-tests indicated significant effects of light intensity but not spectral composition on the changes in refraction.

For guinea pigs reared without lenses, at the end of the experiment, refractive error in HiFL increased by 2.26 ± 0.55 D, followed by the HiBS (2.17 ± 0.65 D), LoBS (1.40 ± 0.93 D), and LoFL (1.39 ± 0.88 D) (one-way ANOVA: $F = 8.124$, $P < 0.001$). Post hoc analysis revealed that HiBS and HiFL belonged to one subset ($P < 0.05$), while LoBS and LoFL belonged to

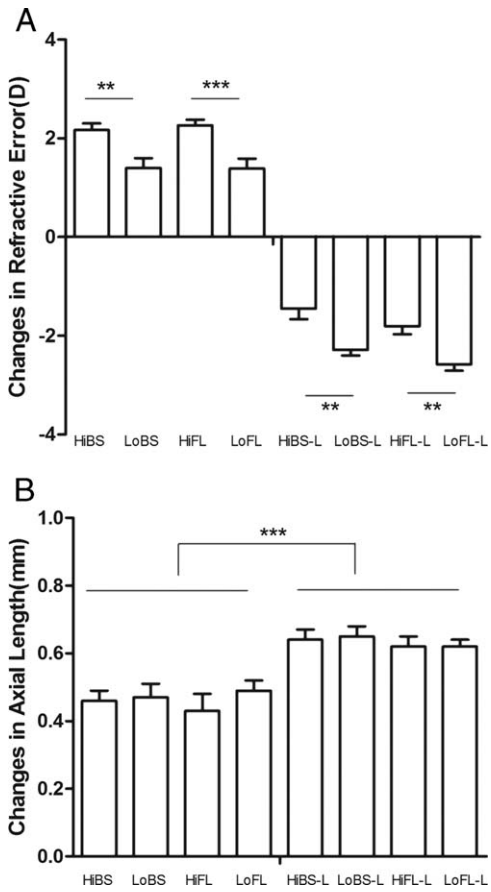

Figure 2. Comparison of the changes of refractive error (**A**) and axial length (**B**) among the groups. HiBS, high-intensity lighting of Solux halogen light; LoBS, low-intensity lighting of Solux halogen light; HiFL, high-intensity lighting of fluorescent light; LoFL, low-intensity lighting of fluorescent light; -L, with −4-D lenses; ACD, anterior chamber depth; LT, lens thickness; VCD, vitreous chamber depth; AL, axial length. Data are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *Error bars*: ±SEM.

another subset ($P < 0.05$). When comparing different intensities in the same spectrum distributions, it was found that guinea pigs exposed to HiFL exhibited a significantly increased hyperopic shift compared to those exposed to LoFL (unpaired *t*-test: $t = 3.791$, $P < 0.001$; Fig. 2A). This was also true for HiBS and LoBS (unpaired *t*-test: $t = 3.239$, $P = 0.002$). Nevertheless, when comparing different spectrum distributions at the same intensity, there was no significant difference between HiBS and HiFL or between LoBS and LoFL (unpaired *t*-test: $t = 0.521$, $P = 0.605$ and $t = -0.056$, $P = 0.956$, respectively; Fig. 2A).

In contrast, for guinea pigs reared with lenses, LoFL-L had the greatest myopic shift of −2.58 ± 0.65 D, followed by LoBS-L (−2.29 ± 0.50 D), HiFL-L (−1.81 ± 0.73 D), and HiBS-L (−1.45 ± 0.99 D) (one-way ANOVA, $F = 8.804$, $P < 0.001$). Post hoc analysis revealed that, similar to guinea pigs reared without lenses, HiBS-L and HiFL-L belonged to one subset ($P < 0.05$) while LoBS-L and LoFL-L belonged to another subset ($P < 0.05$). Also similarly, when comparing different intensities in the same spectrum distributions, HiFL-L exhibited a significantly lower myopic shift when compared to LoFL-L (unpaired *t*-test: $t = 3.748$, $P = 0.001$). This was also true for HiBS-L and LoBS-L (unpaired *t*-test: $t = 3.584$, $P = 0.001$). However, when comparing different spectrum distributions at the same intensity, the differences in the myopic shift between HiBS-L and HiFL-L (unpaired *t*-test: $t = -1.405$, $P = 0.168$), LoBS-L and LoFL-L (unpaired *t*-test: $t = -1.038$, $P = 0.305$) were not statistically significant (Fig. 2A).

Corneal Curvature

The radius of corneal curvature increased significantly in all groups (paired *t*-test: all $P < 0.05$; see Table), with changes ranging from 0.23 to 0.27 mm. However, there was no significant difference in the changes between groups (one-way ANOVA: $F = 0.591$, $P = 0.623$ for groups without lenses, and $F = 0.988$, $P = 0.403$ for groups with lenses). This was also the case when data from groups without lenses and with lenses were pooled (one-way ANOVA: $F = 0.872$, $P = 0.53$).

Ocular Dimensions

The axial length of all groups increased throughout the experiment (Table). However, there was no significant difference among groups in guinea pigs reared either without lenses (one-way ANOVA: $F = 0.507$, $P = 0.678$) or with lenses (one-way ANOVA: $F = 0.212$, $P = 0.888$). When the data from all groups were pooled, it was found that axial elongation in guinea pigs reared with lenses was significantly greater than in those without lenses (unpaired *t*-test, $t = -7.92$, $P < 0.001$; see Fig. 2B).

Unlike the changes in refractive error, there was no statistically significant difference in axial elongation between the high and low lighting intensity with the same spectrum distribution (unpaired *t*-test: $t = -0.307$, $P = 0.760$ for Solux lamps, and $t = -0.113$, $P = 0.910$ for fluorescent lamps). Neither was there a difference between the Solux light and the fluorescent light with the same intensity (unpaired *t*-test: $t = -0.372$, $P = 0.712$ for high intensity, and $t = -0.67$, $P = 0.506$ for standard intensity).

The anterior chamber depth did not show a significant change during the observation period (paired *t*-test: all $P > 0.05$; see Table). In contrast, the thickness of the crystalline lens increased significantly with age in all groups (paired *t*-test: all $P < 0.05$). However, the changes in the thickness of the crystalline lens between groups were not statistically significant (one-way ANOVA: $F = 0.209$, $P = 0.89$ for groups without lenses, and $F = 0.763$, $P = 0.518$ for groups with lenses).

Correlation Between Changes in Axial Length and Refractive Error

Figure 3 shows the correlation between the changes of axial length and refractive error for the guinea pigs reared both without lenses (paradigm 1) and with lenses (paradigm 2). It is noted that the decrease of refractive error (i.e., more myopia) correlated significantly with the elongation of axial length for both paradigms ($R^2 = 0.550$ and 0.667; both $P < 0.001$), indicating that the refraction shift in both paradigms was largely axial in origin. The ratio of axial length elongation to the increase of myopia was also similar (−3.561 D/mm, 95% confidence interval [CI]: −4.260, −2.862) and (−4.599 D/mm, 95% CI −5.295, −3.902), respectively; $P > 0.05$). If the corneal flattening in both paradigms is considered, this ratio would increase to approximately 10 to 11 D/mm, as a 0.25-mm increase of corneal radius is equal to approximately a 6.5-D hyperopia shift (assuming that the refractive index of the cornea in guinea pigs is 1.3375).

Discussion

In the current study, we found that high-intensity lighting provided by either broad-spectrum lighting of the Solux

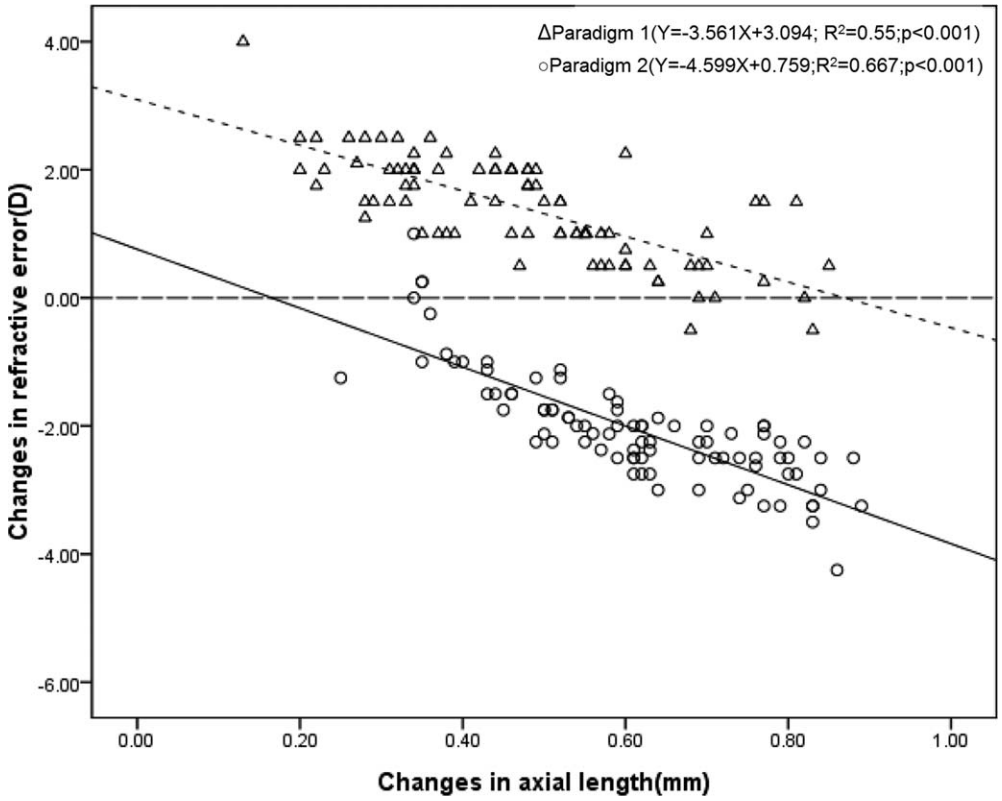

FIGURE 3. The correlations between changes of axial length and refractive error. *Triangles* represent the data from guinea pigs reared without lenses (paradigm 1), and *circles* represent the data from guinea pigs reared with lenses (paradigm 2). Both paradigms show a significant correlation between the changes of axial length and refractive error ($R^2 = 0.55$ and 0.667, respectively; both $P < 0.001$), indicating that the refraction shift in both paradigms was largely axial in origin.

halogen light or spiked-spectrum fluorescent light enhanced hyperopic shifts (guinea pigs reared without lenses) or retarded myopia development (guinea pigs reared with lenses), compared to low-intensity lighting. However, irrespective of light intensity, there was no difference in the effects of the two lamps.

In our results, one unexpected finding was the hyperopic shift in animals raised without lenses. This has not been reported in other studies.[31–33,39] There is no obvious explanation for the hyperopic shifts in normal refractive development in guinea pigs. It should also be noted that the biometric data in the present study do not match very well with those reported in previous studies[31–33,39]; however, data from these previous studies were also not consistent. In the present study, the longer the axial length, the more myopic shifts or less hyperopic the refractive error. However, actual myopia shifts were observed only in guinea pigs fitted with −4-D lenses. Apparently, the flattening of the cornea (Table) in animals reared without lenses had a greater effect than axial elongation, resulting in hyperopic shifts in 81 out of 86 animals after 3-week treatment. The different results we have obtained may be due to species differences. As for the axial length, the value found in the current study for the age-matched (1-month old) guinea pigs in the control group (LoFL) was 5.3% and 7.4% shorter, respectively, compared to the values from Zhou et al.[32] and Howlett and McFadden[31] (7.66 vs. 8.07 and 8.226 mm). The discrepancy could be related to the different ultrasound parameters used in the three studies. The frequency of ultrasound used in the current study was lower than in the experiments of Zhou et al.[32] and Howlett and McFadden[31] (10 vs. 11 MHz/20 MHz). The resolution and precision of the ultrasound used in this study were 0.01 and ±0.1 mm, respectively, while these parameters were not specified in the other two studies. These parameters may compromise the accuracy of axial length measurements and account for our failure to detect significant differences between groups, especially when the change during the experiment period was small. But the results on axial dimensions measured in the present study are still useful for the assessment of relative changes in axial components and in relation to the refractive error.

The protective effect of intensive illumination found in the present study was consistent with previous studies on other animals[13–16] (Siegwart JT, et al. *IOVS* 2012;53:ARVO E-Abstract 3457). One plausible theory for this effect is the dopamine-related pathway, as the release of dopamine from retinal dopaminergic amacrine cells is almost linear to the logarithm of the ambient lighting level,[13,41–44] and dopamine agonists inhibit experimental myopia in at least deprivation myopia.[45–48] The most convincing evidence for this hypothesis is the finding that the protective effect of bright light was abolished after a daily injection of spiperone (a dopamine D2 antagonist).[15] In addition, bright light was recently found to stimulate choroidal thickening.[49] Although there was some time delay (4 hours after the cessation of the bright light) and the magnitude was modest (+10% to +20%),[49] we speculate that choroidal thickening might also play a role in myopia inhibition by bright light exposure, as thicker choroids were linked to the inhibition of myopia.[50–52]

As mentioned previously, sunlight differs from common indoor lighting not only in illumination intensity, but also in the spectral composition and spectral distribution. In the only study comparing the myopia inhibition effect between sunlight and indoor lighting, it was shown that chicks exposed to

sunlight developed significantly less deprivation myopia than those exposed to indoor light (−1.1 ± 0.45 vs. −3.4 ± 0.6 D).[14] However, whether the greater effect of sunlight was associated with its UV component or with its stronger intensity was difficult to determine, since the halogen-quartz lamps used in that experiment were covered by UV-absorbing glass.[14] The Solux halogen lamp used in the current study emits radiation in the UV-A range (350–400 nm), which helped to clarify this puzzle. Our study showed that there was no significant difference in refractive change, at both 500 and 10,000 lux, between the UV-included Solux halogen lamps and the UV-free fluorescent lamps. Furthermore, in a study applying UV light with a high intensity (~200 lux, peaking at 390 nm with a half-band width of 25 nm), it was shown that UV did not affect emmetropization, and the chicken eyes compensated fully to the imposed negative lenses.[53] As chickens have UV cone photoreceptors,[54–56] this finding therefore showed that UV input from cone photoreceptors did not, at least at such intensity, counteract the myopigenic response induced by negative lenses. Thus, the present results indicate that inclusion of UV light in a polychromatic spectrum is unlikely to produce additional protection against myopia. It is not known if higher intensities of UV light would influence the compensation process or not, but the possible side effects of exposure to high-dose UV severely limit experimentation.

With regard to the spectral distribution, the Solux bulbs emit a smooth distribution of wavelengths, while the fluorescent light is composed of a spiked distribution. However, both of these have a broad spectral range. The brightness of the two light sources in the present study was made equivalent using a photometer, calibrated using the human L- and M-cone spectral sensitivity. The M-cone excitation was the same in the two conditions. Although the S-cone excitation was slightly different, it was unlikely to be substantially different, as both light sources have substantial energy at short wavelengths. Thus, both types of cones in guinea pigs may have been stimulated similarly, resulting in the same brightness of the two illuminants perceived by the guinea pigs, which was consistent with the similar refractive changes found in the current study. In other words, spectral distribution of polychromatic light does not seem to influence the inhibition effect against myopia by bright light, provided that the intensity is comparable.

It should also be pointed out that although the Solux halogen lamp mimics the sunlight spectrum propagated to the guinea pig retina, this lamp does not provide UV-B radiation (290–320 nm). Indeed, vitamin D_3, which has been postulated to influence scleral growth,[57] possibly by an effect on cell proliferation,[58] can be produced in the skin only with UV-B. Therefore, the lack of this range of radiation in the Solux halogen light prevents us from clarifying the issue of whether myopia is related to inadequate levels of vitamin D_3.

In conclusion, it is difficult, if not impossible, to provide a comprehensive test of the hypothesis that specific features of the spectral composition of light play an important role in the inhibition of the development of myopia by bright light, because there is effectively an infinite range of variations in spectral composition. We have therefore chosen to use two commonly used light sources in human environments with very different spectral compositions—one with a broad spectrum similar to that of sunlight, and one with a discontinuous and highly peaked distribution. There was no difference in refractive change in both natural development and compensation to negative lenses in guinea pigs reared with the two light sources. We cannot rule out the possibility that further research might find a particular pattern of spectral composition with particularly marked effects, but the current findings do not give any support to the idea that spectral composition plays an important role in the inhibition of experimental myopia. Nor do these experiments provide any support for a role of UV exposures. This supports the idea that the protective effects of bright light against the development of experimental myopia in animals depend primarily on the intensity of visible light, which also may apply to human myopia.

Acknowledgments

The authors thank Leo Peichl, PhD, for his advice and discussion of the ocular optics of guinea pigs and thank Ian Morgan, PhD, for his critique and suggested revisions of the manuscript.

Supported by funding from the National Natural Science Foundation, China (Grants 81170871 and 81200714) and the Fundamental Research Funds of the State Key Laboratory of Ophthalmology, China (Grant 2014N01).

Disclosure: **W. Li**, None; **W. Lan**, None; **S. Yang**, None; **Y. Liao**, None; **Q. Xu**, None; **L. Lin**, None; **Z. Yang**, None

References

1. Dirani M, Tong L, Gazzard G, et al. Outdoor activity and myopia in Singapore teenage children. *Br J Ophthalmol*. 2009; 93:997–1000.
2. Mutti DO, Mitchell GL, Moeschberger ML, Jones LA, Zadnik K. Parental myopia, near work, school achievement, and children's refractive error. *Invest Ophthalmol Vis Sci*. 2002;43: 3633–3640.
3. Jones LA, Sinnott LT, Mutti DO, Mitchell GL, Moeschberger ML, Zadnik K. Parental history of myopia, sports and outdoor activities, and future myopia. *Invest Ophthalmol Vis Sci*. 2007; 48:3524–3532.
4. Rose KA, Morgan IG, Smith W, Burlutsky G, Mitchell P, Saw SM. Myopia, lifestyle, and schooling in students of Chinese ethnicity in Singapore and Sydney. *Arch Ophthalmol*. 2008; 126:527–530.
5. Rose KA, Morgan IG, Ip J, et al. Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology*. 2008;115: 1279–1285.
6. Guggenheim JA, Northstone K, McMahon G, et al. Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study. *Invest Ophthalmol Vis Sci*. 2012;53:2856–2865.
7. Cui D, Trier K, Munk Ribel-Madsen S. Effect of day length on eye growth, myopia progression, and change of corneal power in myopic children. *Ophthalmology*. 2013;120:1074–1079.
8. Donovan L, Sankaridurg P, Ho A, et al. Myopia progression in Chinese children is slower in summer than in winter. *Optom Vis Sci*. 2012;89:1196–1202.
9. Fulk GW, Cyert LA, Parker DA. Seasonal variation in myopia progression and ocular elongation. *Optom Vis Sci*. 2002;79: 46–51.
10. Goss DA, Rainey BB. Relation of childhood myopia progression rates to time of year. *J Am Optom Assoc*. 1998;69:262–266.
11. French AN, Ashby RS, Morgan IG, Rose KA. Time outdoors and the prevention of myopia. *Exp Eye Res*. 2013;114:58–68.
12. Sherwin JC, Reacher MH, Keogh RH, Khawaja AP, Mackey DA, Foster PJ. The association between time spent outdoors and myopia in children and adolescents: a systematic review and meta-analysis. *Ophthalmology*. 2012;119:2141–2151.
13. Cohen Y, Peleg E, Belkin M, Polat U, Solomon AS. Ambient illuminance, retinal dopamine release and refractive development in chicks. *Exp Eye Res*. 2012;103:33–40.
14. Ashby R, Ohlendorf A, Schaeffel F. The effect of ambient illuminance on the development of deprivation myopia in chicks. *Invest Ophthalmol Vis Sci*. 2009;50:5348–5354.

15. Ashby RS, Schaeffel F. The effect of bright light on lens compensation in chicks. *Invest Ophthalmol Vis Sci*. 2010;51: 5247–5253.
16. Smith EL III, Hung LF, Huang J. Protective effects of high ambient lighting on the development of form-deprivation myopia in rhesus monkeys. *Invest Ophthalmol Vis Sci*. 2012; 53:421–428.
17. Smith EL III, Hung LF, Arumugam B, Huang J. Negative lens-induced myopia in infant monkeys: effects of high ambient lighting. *Invest Ophthalmol Vis Sci*. 2013;54:2959–2969.
18. Rucker FJ, Wallman J. Chick eyes compensate for chromatic simulations of hyperopic and myopic defocus: evidence that the eye uses longitudinal chromatic aberration to guide eye-growth. *Vision Res*. 2009;49:1775–1783.
19. Rucker FJ, Wallman J. Chicks use changes in luminance and chromatic contrast as indicators of the sign of defocus. *J Vis*. 2012;12:1–13.
20. Kroger RHH, Wagner HJ. The eye of the blue acara (Aequidens pulcher, Cichlidae) grows to compensate for defocus due to chromatic aberration. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 1996;179:837–842.
21. Kroger RH, Wagner HJ. Horizontal cell spinule dynamics in fish are affected by rearing in monochromatic light. *Vision Res*. 1996;36:3879–3889.
22. Qian YF, Liu R, Dai JH, Chen MJ, Zhou XT, Chu RY. Transfer from blue light or green light to white light partially reverses changes in ocular refraction and anatomy of developing guinea pigs. *J Vis*. 2013;13:1–9.
23. Wang F, Zhou J, Lu Y, Chu R. Effects of 530 nm green light on refractive status, melatonin, MT1 receptor, and melanopsin in the guinea pig. *Curr Eye Res*. 2011;36:103–111.
24. Liu R, Qian YF, He JC, et al. Effects of different monochromatic lights on refractive development and eye growth in guinea pigs. *Exp Eye Res*. 2011;92:447–453.
25. Qian YF, Dai JH, Liu R, Chen MJ, Zhou XT, Chu RY. Effects of the chromatic defocus caused by interchange of two monochromatic lights on refraction and ocular dimension in guinea pigs. *PLoS ONE*. 2013;8:e63229.
26. Chen BY, Wang CY, Chen WY, Ma JX. Altered TGF-beta2 and bFGF expression in scleral desmocytes from an experimentally-induced myopia guinea pig model. *Graefes Arch Clin Exp Ophthalmol*. 2013;251:1133–1144.
27. Zhi Z, Pan M, Xie R, Xiong S, Zhou X, Qu J. The effect of temporal and spatial stimuli on the refractive status of guinea pigs following natural emmetropization. *Invest Ophthalmol Vis Sci*. 2013;54:890–897.
28. Jiang L, Long K, Schaeffel F, et al. Disruption of emmetropization and high susceptibility to deprivation myopia in albino guinea pigs. *Invest Ophthalmol Vis Sci*. 2011;52:6124–6132.
29. Zeng G, Bowrey HE, Fang J, Qi Y, McFadden SA. The development of eye shape and the origin of lower field myopia in the guinea pig eye. *Vision Res*. 2013;76:77–88.
30. McFadden SA, Howlett MHC, Mertz JR. Retinoic acid signals the direction of ocular elongation in the guinea pig eye. *Vision Res*. 2004;44:643–653.
31. Howlett MHC, McFadden SA. Emmetropization and schematic eye models in developing pigmented guinea pigs. *Vision Res*. 2007;47:1178–1190.
32. Zhou X, Qu J, Xie R, et al. Normal development of refractive state and ocular dimensions in guinea pigs. *Vision Res*. 2006; 46:2815–2823.
33. Lu F, Zhou X, Jiang L, et al. Axial myopia induced by hyperopic defocus in guinea pigs: a detailed assessment on susceptibility and recovery. *Exp Eye Res*. 2009;89:101–108.
34. Parry JW, Bowmaker JK. Visual pigment coexpression in Guinea pig cones: a microspectrophotometric study. *Invest Ophthalmol Vis Sci*. 2002;43:1662–1665.
35. Jacobs GH, Deegan JF II. Spectral sensitivity, photopigments, and color vision in the guinea pig (*Cavia porcellus*). *Behav Neurosci*. 1994;108:993–1004.
36. Giblin FJ, Leverenz VR, Padgaonkar VA, et al. UVA light in vivo reaches the nucleus of the guinea pig lens and produces deleterious, oxidative effects. *Exp Eye Res* 2002;75:445–458.
37. Tafel'shtein EE, Golubinskii EP, Maramovich AS, Popov AV. Adaptive mechanisms in the ecology of the pseudotuberculosis microbe [in Russian]. *Med Parazitol (Mosk)*. 1995;30–34.
38. Cooper GF, Robson JG. The yellow colour of the lens of the grey squirrel (*sciurus carolinensis leucotis*). *J Physiol*. 1969; 203:403–410.
39. Howlett MHC, McFadden SA. Spectacle lens compensation in the pigmented guinea pig. *Vision Res*. 2009;49:219–227.
40. Howlett MHC, McFadden SA. Form-deprivation myopia in the guinea pig (*Cavia porcellus*). *Vision Res*. 2006;46:267–283.
41. Iuvone PM, Galli CL, Garrison-Gund CK, Neff NH. Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. *Science*. 1978;202:901–902.
42. Brainard GC, Morgan WW. Light-induced stimulation of retinal dopamine: a dose-response relationship. *Brain Res*. 1987;424: 199–203.
43. Megaw PL, Boelen MG, Morgan IG, Boelen MK. Diurnal patterns of dopamine release in chicken retina. *Neurochem Int*. 2006;48:17–23.
44. Besharse JC, Iuvone PM. Is dopamine a light-adaptive or a dark-adaptive modulator in retina? *Neurochem Int*. 1992;20:193–199.
45. Rohrer B, Spira AW, Stell WK. Apomorphine blocks form-deprivation myopia in chickens by a dopamine D2-receptor mechanism acting in retina or pigmented epithelium. *Vis Neurosci*. 1993;10:447–453.
46. Nickla DL, Totonelly K, Dhillon B. Dopaminergic agonists that result in ocular growth inhibition also elicit transient increases in choroidal thickness in chicks. *Exp Eye Res*. 2010;91:715–720.
47. Schmid KL, Wildsoet CF. Inhibitory effects of apomorphine and atropine and their combination on myopia in chicks. *Optom Vis Sci*. 2004;81:137–147.
48. Iuvone PM, Tigges M, Stone RA, Lambert S, Laties AM. Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. *Invest Ophthalmol Vis Sci*. 1991;32:1674–1677.
49. Lan W, Feldkaemper M, Schaeffel F. Bright light induces choroidal thickening in chickens. *Optom Vis Sci*. 2013;90: 1199–1206.
50. Zhu XY, Park TW, Winawer J, Wallman J. In a matter of minutes, the eye can know which way to grow. *Invest Ophthalmol Vis Sci*. 2005;46:2238–2241.
51. Troilo D, Nickla DL, Wildsoet CF. Choroidal thickness changes during altered eye growth and refractive state in a primate. *Invest Ophthalmol Vis Sci*. 2000;41:1249–1258.
52. Wallman J, Wildsoet C, Xu AM, et al. Moving the retina: choroidal modulation of refractive state. *Vision Res*. 1995;35: 37–50.
53. Hammond DS, Wildsoet CF. Compensation to positive as well as negative lenses can occur in chicks reared in bright UV lighting. *Vision Res*. 2012;67:44–50.
54. Wortel JF, Rugenbrink H, Nuboer JFW. The photopic spectral sensitivity of the dorsal and ventral retinae of the chicken. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 1987;160:151–154.

Investigative Ophthalmology & Visual Science

55. Chen DM, Collins JS, Goldsmith TH. The ultraviolet receptor of bird retinas. *Science*. 1984;225:337–340.

56. Bowmaker JK, Heath LA, Wilkie SE, Hunt DM. Visual pigments and oil droplets from six classes of photoreceptor in the retinas of birds. *Vision Res*. 1997;37:2183–2194.

57. Mutti DO, Marks AR. Blood levels of vitamin D in teens and young adults with myopia. *Optom Vis Sci*. 2011;88:377–382.

58. Mutti DO, Cooper ME, Dragan E, et al. Vitamin D receptor (VDR) and group-specific component (GC, vitamin D-binding protein) polymorphisms in myopia. *Invest Ophthalmol Vis Sci*. 2011;52:3818–3824.

Investigative Ophthalmology & Visual Science

Changes in dopamine and ZENK during suppression of myopia in chicks by bright light

Weizhong Lan[1,2,3*], Zhikuang Yang[4], Marita Feldkaemper[1], Frank Schaeffel[1]

[1]Section of Neurobiology of the Eye, Center for Ophthalmology, University of Tuebingen, 72076 Tuebingen, Germany
[2] State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 510060 Guangzhou, China
[3]Graduate School of Cellular & Molecular Neuroscience, University of Tuebingen, 72076 Tuebingen, Germany
[4] Aier Eye Hospital Group, 41000, Changsha, China

*To whom correspondence should be addressed. E-mail: brucelarm@gmail.com;

Acknowledgments -- This work was supported by grants from National Natural Science Foundation of China, Beijing, China (grant no.: 81170871) and from Fundamental Research Funds of the State Key Laboratory of Ophthalmology Guangzhou, China (grant no.: 2014QN01). A scholarship from German Academic Exchange Service (DAAD) was awarded to Weizhong Lan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We thank Ms Sandra Bernhard-Kurz for her help during tissue fixation, sectioning and immunostaining.

Competing Interests: The authors have declared that no competing interests exist.

Abstract

Bright light has been found to reduce the development of deprivation myopia and we have previously observed in chickens that the protective effect of bright light is enhanced when repeated episodes of bright light exposure are used rather than continuous exposure, even though the total amount of the light dose was matched. Since the mechanisms of the bright light effect on myopia are not clear, we have investigated the roles of two candidate molecules, dopamine and ZENK. In line with older studies, we found that wearing diffusers dramatically suppressed retinal dopamine release, as reflected in vitreal DOPAC content. The drop in dopamine was significantly reduced by exposure to bright light of 15 000 lux. No difference was detected between continuous and intermittent light exposure. Diffusers also suppressed the expression of ZENK protein in glucagonergic amacrine cells (GACs) but neither continuous nor intermittent bright light rescued the suppressive effect of the diffusers. In summary, while bright light compensated to some extent for the drop in dopamine during diffuser wear, it did not reduce the drop in ZENK in glucagon amacrine cells. We therefore hypothesize that ZENK in these cells is not involved in light-mediated suppression of myopia.

Introduction

A number of recent studies have shown that children who spend more time outside are less myopic (Jones, Sinnott et al. 2007, Onal, Toker et al. 2007, Rose, Morgan et al. 2008, Dirani, Tong et al. 2009, Jones-Jordan, Mitchell et al. 2011, Guggenheim, Northstone et al. 2012). Furthermore, if they were forced to stay outside longer than usual, a reduction of myopia incidence and progression was found in three recent prospective clinical trials (Yi and Li 2011, Morgan, Xiang et al. 2012, Wu, Tsai et al. 2013). The protective effect of outdoor exposure in relation to myopia does not seem to be correlated with physical activity since indoor sports did not inhibit myopia (Rose, Morgan et al. 2008, Dirani, Tong et al. 2009). It rather appears that merely the time spent outdoors seems to be important (Guggenheim, Northstone et al. 2012). Beyond, (Rose, Morgan et al. 2008) proposed that the high level of illuminance outdoors represents a key factor. Outdoor illuminance is usually hundreds of folds higher than indoors, for example there is a brightness of 50000 lux on a sunny summer day outside versus a brightness of 300 to 500 lux in a typical indoor office environment.

The link between bright light and inhibition of myopia was confirmed by experiments in animal models. Ashby et al. found that deprivation myopia in chicks, induced by translucent diffusers, could be significantly retarded (about 60%) by simply increasing ambient luminance from 500 lux to 15 000 lux (Ashby, Ohlendorf et al. 2009). Similar effects were found in tree shrews (Siegwart, Ward et al. 2012) and rhesus monkeys (Smith, Hung et al. 2012). Later, Lan et al. (Lan, Feldkaemper et al. 2014) observed that bright light given in intermittent intervals (15 000 lux with 50% duty cycle for 1:1 minute combined with a background illuminance of 500 lux) were the most potent in suppressing deprivation myopia in chicks. Ashby and Schaeffel (Ashby and Schaeffel 2010) observed in chicks that bright light also slowed the progress of a second type of experimental myopia, induced by wearing negative lenses (LIM). This result was also confirmed in tree shrews (Siegwart, Ward et al. 2012, Norton and Siegwart 2013). In rhesus monkeys, lens-induced myopia was not suppressed by bright light in a recent study (Smith, Hung et al. 2013). However, another study showed that bright light slowed the development of lens-induced myopia (-3D) to some extent (Wang, Ding et al. 2015), although the number of animals in that study was low and the effects were small. These studies provide

evidence for an inhibitory effect of bright light on the development and progression of myopia. However, the underlying mechanisms are not clear.

A number of mechanisms have been proposed (Ashby, Ohlendorf et al. 2009, Smith, Hung et al. 2012), including pupil constriction which increases the depth of focus and therefore reduces retinal image blur, higher physical activity which increases optic flow and rapid changes in local luminance on the retina, a factor which has previously been shown to inhibit myopia development (Schwahn and Schaeffel 1997). However, both factors had been ruled out in the study done by Ashby et al. (Ashby, Ohlendorf et al. 2009). Light-dependent release of dopamine from the retina (Iuvone, Galli et al. 1978, Brainard and Morgan 1987, Besharse and Iuvone 1992, Megaw, Boelen et al. 2006, Cohen, Peleg et al. 2012) remains a more likely contributor because (1) it is down-regulated during the development of deprivation myopia (Iuvone, Tigges et al. 1989, Stone, Lin et al. 1989, McBrien, Cottriall et al. 2001), (2) its agonists are known to inhibit axial eye growth and myopia in chicks (Stone, Lin et al. 1989, Rohrer, Spira et al. 1993) and monkeys (Iuvone, Tigges et al. 1991) and (3) a dopamine D2 antagonist, spiperone, was found to abolish the protective effect of bright light against deprivation myopia in chicks (Ashby and Schaeffel 2010). It is well known that diffusers or lenses, which reduce retinal image contrast and act as low pass filters for spatial frequencies, reduce retinal dopamine and its principal metabolite 3,4-dihydroxyphenlacetic acid (DOPAC) content (Stone, Lin et al. 1989, Ohngemach, Hagel et al. 1997), even if attenuation-matched neutral density filters serve as reference (Feldkaemper, Diether et al. 1999). These results indicate that dopamine metabolism is also controlled by spatial features in the retinal image and not only by retinal luminance. The central question here is then whether the inhibition of myopia by bright light is based on increasing release of dopamine from the retina, which can counteract the effects of wearing of diffusers or lenses.

Another possible candidate is the ZENK protein (also known as Egr-1, Zif268, NGFI-A and Krox-24), which is widely expressed in several subsets of bipolar cells and amacrine cells (Fischer, McGuire et al. 1999). In glucagonergic amacrine cells (GACs) in chicks, ZENK is regulated in a bi-directional way, according to the sign of imposed defocus: up-

regulation for treatments that induce hyperopia (plus lenses or recovery from induced myopia) and down regulation for treatments that induce myopia (minus lenses or diffusers) (Fischer, McGuire et al. 1999, Bitzer and Schaeffel 2002, Bitzer and Schaeffel 2006, Ashby, McCarthy et al. 2007). Changes in ZENK mRNA occur rapidly and can be detected as soon as 15 minutes after the lenses or diffusers have been applied (Simon, Feldkaemper et al. 2004). Atropine, which is known to inhibit myopia development in animal models (McBrien, Moghaddam et al. 1993, Schwahn, Kaymak et al. 2000, Schmid and Wildsoet 2004, Bitzer, Kovacs et al. 2006, Diether, Schaeffel et al. 2007, Barathi, Beuerman et al. 2009) and children (Shih, Chen et al. 1999, Shih, Hsiao et al. 2001, Chua, Balakrishnan et al. 2006, Chia, Chua et al. 2012, Lin, Lan et al. 2013), stimulates dopamine release from the retina at least *in vitro* (Schwahn, Kaymak et al 2000) and enhances ZENK expression *in vivo* (Bitzer, Kovacs et al 2006), implying that a common mechanism may drive dopamine and ZENK production. However, up to now the mechanism is unknown. The present study was performed to gain a better understanding of the possible roles of dopamine and ZENK in the mechanisms that possibly underlie the inhibition of experimental myopia by continuous bright light and by intermittent periods of bright light.

Methods

Animals and treatment

One day-old male white leghorn chickens were obtained from a local hatchery in Kirchberg, Germany. They were raised under a 10/14 hour light/dark in large chicken cages, with free access to food and water. The treatments described below were in accordance with the ARVO resolution for care and use of laboratory animals and were approved by the university commission for animal welfare.

Experiment I. Antagonistic effects of bright light and diffusers on retinal dopamine release

Vitreal DOPAC content was measured because this parameter has previously been shown to be a sensitive and robust index of retinal dopamine release (Ohngemach, Hagel et al. 1997, Megaw, Morgan et al. 2001, Megaw, Boelen et al. 2006, Cohen, Peleg et al. 2012)

and because detection of dopamine itself is limited in the vitreous by its very low concentrations (Feldkaemper, Diether et al. 1999, Cohen, Peleg et al. 2012).

Chicks aged 7 to 8 days old were monocularly occluded with translucent diffusers. They randomly allocated to one of three groups which were exposed to either of the following lighting conditions during the light phase (between 8 a.m. to 6 p.m.): 500 lux (hereafter called "standard illuminance", n = 12), continuous 15 000 lux (hereafter called "15K lux, Con", n = 11) or intermittent 15 000 lux with 50% duty cycle for 1:1 minute combined with a background illuminance of 500 lux (hereafter called "15K lux, 1:1 minute", n=12), respectively. Starting at 6:00 p.m., one chick from each group was sacrificed by an overdose of ether and eyes were immediately enucleated, the vitreous body removed with forceps and stored in a -80 °C freezer until the samples were processed.

Measurement of vitreal DOPAC concentration

The measurement of vitreal DOPAC content was performed by an analytic company (Prolytic GmbH, Frankfurt, Germany), using the same protocol as in previous studies (Bartmann, Schaeffel et al. 1994) except that the pH value of the eluent was modified to 4.05. The content of DOPAC in the vitreous body was expressed as nanogram DOPAC per 0.1 gram wet weight of vitreous body.

Experiment II. The role of ZENK in the protection of bright light against experimentally induced deprivation myopia

Seven to 14 days old chicks were monocularly treated with translucent diffusers and were raised under a 10/14 h light/dark cycle from 8 a.m. to 6 p.m. under an illuminance of 500 lux. Three groups of chicks (n = 6 for each group) were exposed to 1-hour light treatment (between 8:00 AM to 9:00 AM) of 500 lux, 15K lux, Con or 15K lux 1:1 minute, respectively. In another three groups the duration of exposure was increased to 10 hours (in these groups n = 5).

Although it has previously been described that the number of ZENK-expressing GACs glucagonergic amacrine cells does not vary over the day (Fischer, McGuire et al. 1999), only one chick for each group was treated a day ensure that the time of ZENK analysis

was the same for each chick however at the cost that the ages increased. The sequence for the groups to start the treatment every day was randomly selected.

Tissue Fixation and Sectioning

Eyecups were fixed for 30 minutes and afterwards processed as previously described (Bitzer and Schaeffel 2002). In brief, 12 µm sections were cut and thaw mounted onto silane-coated glass-slides. Sections from contralateral control and diffuser treated eyes from the same animal were placed consecutively on the same slide to ensure equal exposure.

Immunohistochemistry

Sections were dried at room temperature for 1 hour and washed once in 0.05 M PBS for 10 minutes and, after being fixed in the coverplates, washed again with 0.05 M PBS. Sections were then incubated with blocking buffer (200 µl of PBST [i.e., 0.05 M PBS plus 0.3% Triton X-100; Sigma-Aldrich, Germany] plus 20% NGS [normal goat serum; Sigma-Aldrich, Germany]) for 45 minutes. Afterwards, sections were covered with primary antibody solution (200 µl PBST plus 5% NGS and primary antibodies) and incubated for 1 hour at room temperature and then approximately 20 hours at 4°C. Primary antibodies and their working dilutions included anti-ZENK, rabbit polyclonal antibody at 1:6500 (Egr-1 [#588] X, SC-110X; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-Glucagon, mouse monoclonal antibody at 1:400 (Gordon Ohning, University of California Los Angeles, Los Angeles, CA). Subsequently, slides were washed three times in 0.05 M PBS, covered in secondary antibody solution (200 µl of 0.05 M PBS plus 1:750 Alexa Fluor® 568 orange red-conjugated goat anti-rabbit IgG (A-11036; Invitrogen, Paisley, UK) and 1:400 Oregon Green®–conjugated goat anti-mouse IgG (O6380; Invitrogen, Paisley, UK) and incubated for 1 hour at room temperature in darkness. Samples were washed three times in 0.05 M PBS and mounted under coverslips in 70% sorbitol (Caesar & Loretz GmbH, Germany) for observation under a fluorescence microscope.

Cell counts

Since ZENK staining in a particular cell was either completely absent or clearly visible, it was easy to judge whether a cell expressed ZENK or not (Fischer, McGuire et al. 1999, Bitzer and Schaeffel 2002). For the same reason, it was easy to determine the numbers of

ZENK expressing glucagon amacrine cells (Bitzer and Schaeffel 2002). The counting of the ZENK-staining GACs was performed in a masked fashion to avoid bias. The total number of GACs and ZENK-staining ones were calculated under 400X magnification for each section and at least four sections from each eye were evaluated. Then the percentage of ZENK-positive GACs was determined by dividing their number by the total number of GACs per section, expressed in percent by multiplication by 100.

Statistics

All data are expressed as mean ± 1 standard error (SE). The difference between the deprived and non-deprived eyes was compared with a one-sample *t*-test (i.e., the relative ratio vs 0), unless otherwise stated. For comparisons between two independent groups, an un-paired *t*-test was used. For comparisons among multiple groups, one-way analysis of variance (ANOVA), followed by a student's unpaired t-test with Bonferroni correction, was employed to determine whether the differences between the average levels of each group were statistically significant. Since ZENK expression was measured after two exposure times (one hour or 10 hours after light onset), a two-way ANOVA was used to reveal the potential interaction between the treatment type (light pattern) and the duration. Because normal distribution of the data is required for these parametric analyses normality of the data of each group was tested by the Shapiro-Wilk W test. If this requirement was unmet, box-cox transformations of the data (e.g. natural logarithmical transformation) were attempted. Otherwise, non-parametric analysis (Wilcoxon rank testing) was employed instead.

Given the high inter-individual variability in ocular catecholamine content (Feldkaemper, Diether et al. 1999), only relative values [i.e., (deprived eye – non-deprived eye) / non-deprived eye] were used in the comparison of the suppression of dopamine release between the groups. All analyses were performed with commercial SPSS ver. 16.0 software (SPSS, Chicago, IL). The significance level was set at two-tailed $p < 0.05$.

Results

1. Antagonistic effects of bright light and diffusers on retinal dopamine release

After 10 hours of continuous light exposure of 500 lux, all deprived eyes had a lower vitreal DOPAC content compared to their contralateral unoccluded fellow eyes (6.88 ± 0.27 vs 4.37 ± 0.24 ng/0.1g wet weight), indicating that wearing of diffusers suppresses retinal dopamine release (-36.7% ± 1.8%; one sample t-test: $p < 0.001$).

Similar to results under 500 lux, lower vitreal DOPAC content was found in deprived eyes compared to the contralateral control eyes also under 15,000 lux (7.81 ± 0.40 vs 5.56 ± 0.33 ng/0.1g wet weight for the continuous bright light group, $p < 0.001$; 8.55 ± 0.50 vs 6.05 ± 0.32 ng/0.1g wet weight for the intermittent bright light group, $p < 0.001$, respectively (Figure 1A). Only one animal kept under 15 000 lux, 1:1 minute intermittent light showed an unusually high DOPAC content in the deprived eye, compared to the non-deprived fellow eye (10.54 vs 8.65 ng/0.1 g wet weight) which might be due to contamination by retinal tissue that was attached to the sample. The data were treated as outlier since it made the distribution of the non-normal.

Although neither bright light condition completely reversed the drop of dopamine release that was induced by the diffusers, bright light at least reduced the effects of occluder wear on dopamine release, compared to 500 lux (500 lux: -36.7% ± 1.8%, 15 K, Con: -28.8 ± 2.1%, 15K, 1:1 minute, -28.9% ± 2.4%; one-way ANOVA: P=0.02, Post-*hoc* analysis: both p=0.04, compared with 500 lux). There was no difference in the rescue effect of continuous and intermittent bright light ($p = 1.00$) (Figure 1B).

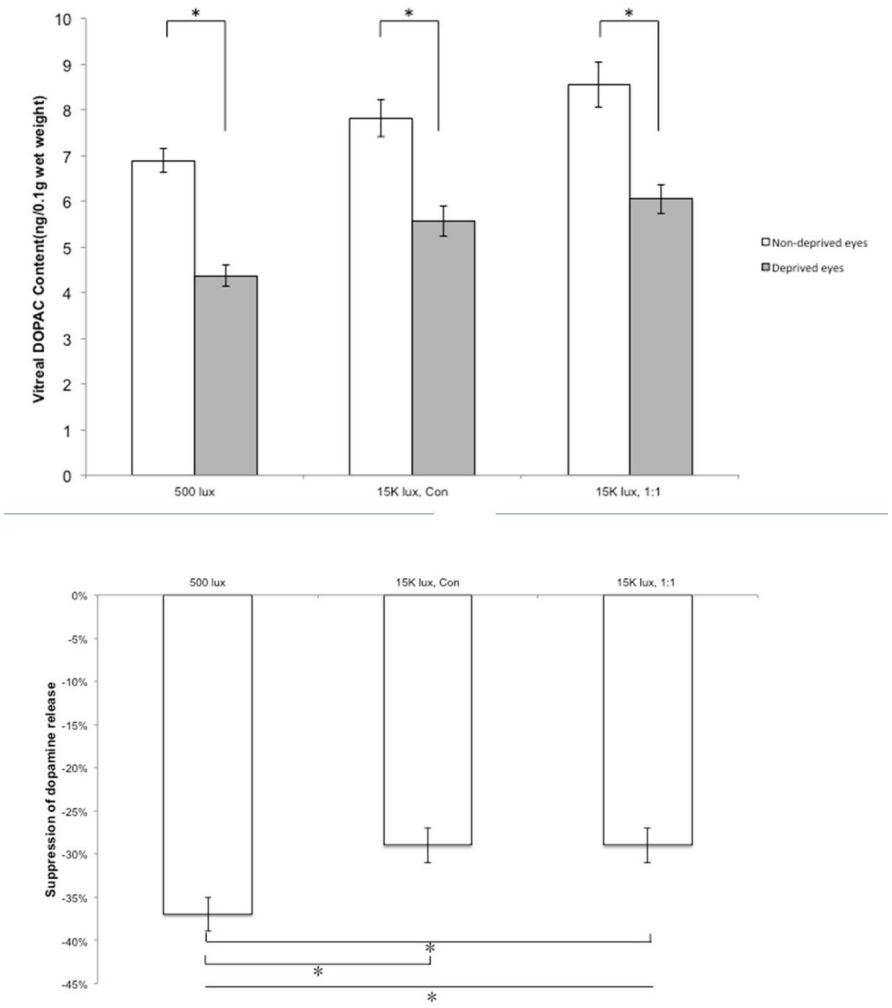

Figure 1. Vitreal DOPAC content of chicks exposed to different lighting conditions. (A) Vitreal DOPAC content in deprived eyes was significantly lower than in the contralateral non-deprived eyes (-36.7% ± 1.8%; one sample t-test: $p<0.001$) at 500 lux. This was also true for the in chicks exposed to bright light. (B) Bright light, either given continuously or in 1:1 minute intervals, significantly reduced this suppression to some extent (one-way ANOVA: $p = 0.02$). There was no difference between the two bright light regimes, continuous or intermittent (-28.8% ± 2.1% vs post -28.9% ± 2.4%; *post hoc*-analysis: $p = 1.00$).

2. Effect of diffuser wear and bright light exposure on the percentages of ZENK-positive glucagon amacrine cells

Consistent with previous studies (Fischer, McGuire et al. 1999, Bitzer and Schaeffel 2002), ZENK immuno-reactivity was observed in the nuclei of many cells in the distal inner nuclear layer (INL) and in a few nuclei in the proximal INL (Figure 2A). According to their relative positions within the INL, these were identified as bipolar and amacrine cells, respectively. By contrast, glucagon expression was exclusively detected in a few amacrine cells (Figure 2B), again in line with previous descriptions. Double

immunolabeling showed that in the amacrine layer ZENK was expressed in both GACs and other cell nuclei.

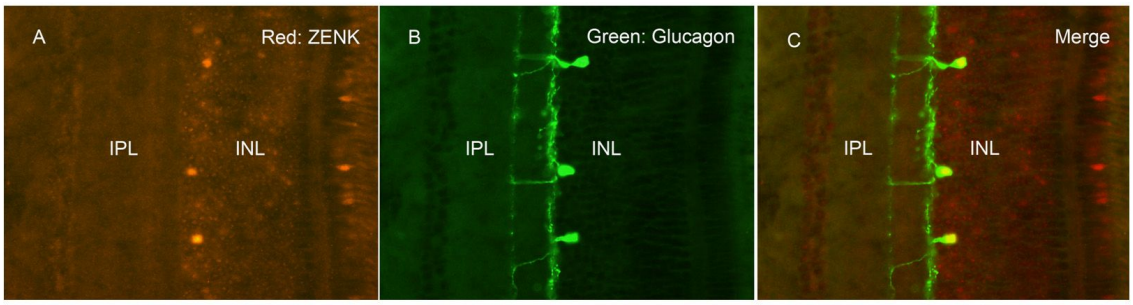

Figure 2. ZENK was detected in many nuclei in the distal inner nuclear layer (INL) and a few nuclei in the proximal INL (A). By contrast, glucagon expression was exclusively detected in amacrine cells (B). ZENK and glucagon were co-localized in the three cells shown here (B&C) although there were also ZENK-expressing cells observed with no glucagon expression.

The percentages of ZENK-positive GACs in the six groups are shown in Figure 3. Under the standard illuminance (500 lux, 1 hour), 53.0% ± 9.4% of the GACs were labeled for ZENK in the non-deprived eyes, while only 29.4% ± 9.6% of the GACs were ZENK-positive in the eyes treated with diffusers. This suggests that the expression of ZENK in GACs was significantly suppressed by the diffusers, with a mean suppression of -48.2 ±14.8% (one sample *t*-test: $p = 0.023$). Compared to the 500 lux group, one hour of treatment with bright light, given either continuously or intermittently, did NOT affect the expression of ZENK in GACs in the non-deprived eyes (one-way ANOVA: $p = 0.984$). In the occluded eyes, ZENK expression was significantly reduced to a similar extend in the bright light groups compared to chicks kept under 500 lux (-56.9% ± 5.4% and -60.8% ± 9.9% for 15K Con and 15K 1:1 minute, respectively). Obviously, bright light did not even partially rescue the suppression of ZENK that occurred during diffuser wear (one-way ANOVA: $p = 0.702$).

When the exposure to bright light was extended to 10 hours, the percentage of ZENK expression in glucagon amacrine cells was similar to that after 1 hour of treatment in the contralateral control eyes (One-way ANOVA: $p = 0.578$). This result confirms a study by Fischer et al. (Fischer, McGuire et al. 1999) showing that ZENK expression in GACs

does not vary with the duration of light exposure. However, ZENK expression in the deprived eyes was further suppressed when continuous bright light exposure was extended to 10 hours (-56.9% ± 5.4% versus -85.8% ± 5.6%; unpaired t-test: $p < 0.05$). Likewise, there was also a tendency of an increased suppression over time observed in the chicks exposed to standard illuminance (-48.2% ± 14.8% versus -79.2% ± 6.3%, unpaired t-test: $p = 0.107$), but not in the group exposed to intermittent bright light (-60.8% ± 9.9% for 1 hour versus - 69.2% ± 6.5% for 10 hours; unpaired t-test: $p = 0.515$). No matter whether bright light was provided continuously or intermittently for 10 hours, wearing diffusers reduced ZENK expression similarly as under normal laboratory lighting (One-way ANOVA: $p = 0.199$). A two-way ANOVA analysis revealed that there was no interaction between the treatment type (light pattern) and the duration ($p = 0.417$).

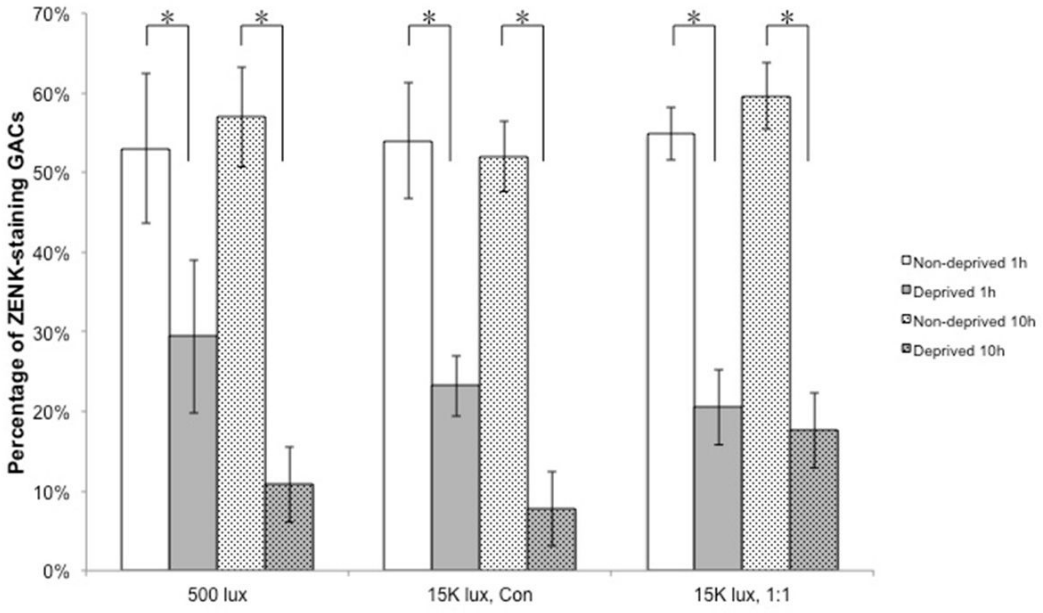

Figure 3. Percent of ZENK-immunoreactive glucagon amacrine cells under different lighting conditions. The percentage of ZENK-stained GACs in non-deprived eyes is approximately 50%, regardless of the illuminance level (one-way ANOVA: $p = 0.984$) or exposure duration (One-way ANOVA: $p = 0.578$). Form-deprivation reduced the number of double labelled cells, again regardless of the illuminance level (one-way ANOVA: $p = 0.702$). This was also the case when the exposure duration was extended to 10 hours (One-way ANOVA: $p = 0.199$).

Discussion

Because exposure to bright light represents a simple and potentially powerful intervention against myopia in children, both the underlying mechanisms and dose-

response effects are of high interest. In the current study, we have examined the possible roles of dopamine and ZENK in the mechanisms that underlie the inhibition of experimental myopia by continuous bright light and by intermittent periods of bright light. We found that exposure to bright light can partly antagonize the drop in retinal dopamine that normally occurs during induction of experimentally induced myopia but that bright light does not affect the decrease in ZENK expression in glucagon amacrine cells that occurs upon deprivation with frosted goggles.

Selection of the time points to measure ZENK expression and DOPAC

In the current study, the expression of ZENK in the retinal glucagon amacrine cells was measured after either one hour or ten hours exposure to bright light. ZENK has been found to differentially express within 40 minutes at the protein level (Fischer, McGuire et al. 1999). Even though changes in retinal ZENK mRNA levels could be detected even earlier, as soon as 15 minutes (Simon, Feldkaemper et al. 2004), they represent a global signal that originates from all ZENK-containing neurons in the retina while immunohistochemical labelling has the advantage that changes can be resolved at the cellular level. In addition, the time point prior to the dark phase (the tenth hour after light onset) was selected because the transition from light to dark actually acts as a new stimulus which has been reported to up-regulate the expression of ZENK (Brand, Burkhardt et al. 2005) and could therefore have confounded the effects of the diffusers and bright light. We measured vitreal DOPAC content at the end of the light phase because it accumulates over the day and reflects the average production over many hours (unpublished M.D. thesis by H. Kaymak in our lab, 1994). Nevertheless, we did not measure ZENK and vitreal DOPAC at a more longer-term stage, for example several days after visual deprivation. This was to avoid the potential confounding variables, such as the dilution effect of the vitreal enlargement (ie., smaller eyes in bright light groups would induce artificially higher DOPAC content)(Feldkaemper, Diether et al. 1999) and long-term readjustments of cell and network functions(Luft, Iuvone et al. 2004).

ZENK and light-mediated protection effect against deprivation myopia

Although a number of studies have proven that ZENK is a bi-directional marker of ocular growth (Fischer, McGuire et al. 1999, Bitzer and Schaeffel 2002, Bitzer and Schaeffel 2004, Bitzer, Kovacs et al. 2006, Ashby, McCarthy et al. 2007), this was the first study to test whether ZENK is involved light-mediated protection against deprivation myopia. In the present study, the percentage of ZENK-positive GACs was roughly 50% and decreased in deprived eyes and this result is in line with previous findings (Fischer, McGuire et al. 1999). Another finding of the study by Fischer et al., namely that ZENK protein expression in glucagon amacrine cells does not vary with duration of light exposure, was also confirmed in the current study. We found that high light intensities, either given continuously or in intervals, did not influence the amount of ZENK immunoreactive glucagon amacrine cells, neither after short treatment periods (1 hour) nor after longer exposition to bright light (10 hours). The observation gives rise to the hypothesis that ZENK-expressing glucagon amacrine cells are not involved in the signaling pathway(s) that underlie the inhibition of experimental myopia by bright light.

Dopamine and light-mediated protection effect against deprivation myopia

Many studies have shown that dopamine is involved in the signaling cascade that controls eye growth. Visual deprivation leads to a decrease in retinal dopamine content (Iuvone, Tigges et al. 1989, Stone, Lin et al. 1989, Bartmann, Schaeffel et al. 1994, Ohngemach, Hagel et al. 1997), a result that was confirmed in the present study. Vitreal DOPAC content was reduced in all deprived eyes not matter if chicks were kept under normal laboratory lightning or high light intensities. However, the drop in dopamine release induced by the diffusers was about 20% weaker under bright light exposure. This result supports the hypothesis by Rose et al. (Rose, Morgan et al. 2008) that dopamine might be involved in the protective effect of higher light intensities and is in good agreement with the finding that the protective effect of bright light is abolished by the administration of the dopamine D_2 receptor (Ashby and Schaeffel 2010).

Unexpectedly, no difference in the rescue effect of dopamine release was detected between 1:1 minute intermittent bright light and continuous bright light, although we had previously observed that intermittent bright light provided stronger protection against deprivation myopia (Lan, Feldkaemper et al. 2014). Indeed, many other ocular growth-

modulating stimuli, such as removal of the diffusers (Napper, Brennan et al. 1997, Smith, Hung et al. 2002), wearing of negative lens or positive lens (Winawer and Wallman 2002, Kee, Hung et al. 2007, Zhu and Wallman 2009), demonstrate similar non-linear fashion. This phenomenon is generally believed to be related to the response characteristics of avian retinal neurons which are largely transient in nature (Wallman 1990), indicating that the temporal integration of visual signals is non-linear (Napper, Brennan et al. 1997, Smith, Hung et al. 2002, Winawer and Wallman 2002, Nickla, Sharda et al. 2005, Kee, Hung et al. 2007, Zhu and Wallman 2009, Zhu 2013). We had previously hypothesized that intermittent bright light stimulates more dopamine release than continuous bright light (Lan, Feldkaemper et al. 2014) but the current study does not support this hypothesis. Therefore, the enhanced "STOP signal" induced by intermittent bright light, either in the level of retinal neural circuit or growth-related molecule(s), needs further investigation.

Conclusions

ZENK-expressing glucagon amacrine cells do not seem to be involved in the signaling pathway underlying the protective effect of bright light against myopia, no matter whether bright light was applied continuously or in intervals. In contrast there was a significant effect of bright light on dopamine release: it reduced the drop of dopamine induced by the diffuser wear. Results therefore support a role of dopamine in the suppression of myopia by bright light but do not support a role of ZENK in this process. The higher potency of intermittent periods over continuous bright to inhibit myopia cannot be explained by differences in dopamine release.

References

Ashby, R., C. S. McCarthy, R. Maleszka, P. Megaw and I. G. Morgan (2007). "A muscarinic cholinergic antagonist and a dopamine agonist rapidly increase ZENK mRNA expression in the form-deprived chicken retina." Exp Eye Res **85**(1): 15-22.

Ashby, R., A. Ohlendorf and F. Schaeffel (2009). "The effect of ambient illuminance on the development of deprivation myopia in chicks." Invest Ophthalmol Vis Sci **50**(11): 5348-5354.

Ashby, R. S. and F. Schaeffel (2010). "The effect of bright light on lens compensation in chicks." Invest Ophthalmol Vis Sci **51**(10): 5247-5253.

Barathi, V. A., R. W. Beuerman and F. Schaeffel (2009). "Effects of unilateral topical atropine on binocular pupil responses and eye growth in mice." Vision Res **49**(3): 383-387.
Bartmann, M., F. Schaeffel, G. Hagel and E. Zrenner (1994). "Constant light affects retinal dopamine levels and blocks deprivation myopia but not lens-induced refractive errors in chickens." Vis Neurosci **11**(2): 199-208.
Besharse, J. C. and P. M. Iuvone (1992). "Is dopamine a light-adaptive or a dark-adaptive modulator in retina?" Neurochem Int **20**(2): 193-199.
Bitzer, M., B. Kovacs, M. Feldkaemper and F. Schaeffel (2006). "Effects of muscarinic antagonists on ZENK expression in the chicken retina." Exp Eye Res **82**(3): 379-388.
Bitzer, M. and F. Schaeffel (2002). "Defocus-induced changes in ZENK expression in the chicken retina." Invest Ophthalmol Vis Sci **43**(1): 246-252.
Bitzer, M. and F. Schaeffel (2004). "Effects of quisqualic acid on retinal ZENK expression induced by imposed defocus in the chick eye." Optom Vis Sci **81**(2): 127-136.
Bitzer, M. and F. Schaeffel (2006). "ZENK expression of retinal glucagon amacrine cells in chicks: the effect of defocus presented in vivo, in vitro and under anesthesia." Vision Res **46**(6-7): 848-859.
Brainard, G. C. and W. W. Morgan (1987). "Light-induced stimulation of retinal dopamine: a dose-response relationship." Brain Res **424**(1): 199-203.
Brand, C., E. Burkhardt, F. Schaeffel, J. W. Choi and M. P. Feldkaemper (2005). "Regulation of Egr-1, VIP, and Shh mRNA and Egr-1 protein in the mouse retina by light and image quality." Mol Vis **11**: 309-320.
Chia, A., W. H. Chua, Y. B. Cheung, W. L. Wong, A. Lingham, A. Fong and D. Tan (2012). "Atropine for the treatment of childhood myopia: safety and efficacy of 0.5%, 0.1%, and 0.01% doses (Atropine for the Treatment of Myopia 2)." Ophthalmology **119**(2): 347-354.
Chua, W. H., V. Balakrishnan, Y. H. Chan, L. Tong, Y. Ling, B. L. Quah and D. Tan (2006). "Atropine for the treatment of childhood myopia." Ophthalmology **113**(12): 2285-2291.
Cohen, Y., E. Peleg, M. Belkin, U. Polat and A. S. Solomon (2012). "Ambient illuminance, retinal dopamine release and refractive development in chicks." Exp Eye Res **103**: 33-40.
Diether, S., F. Schaeffel, G. N. Lambrou, C. Fritsch and A. U. Trendelenburg (2007). "Effects of intravitreally and intraperitoneally injected atropine on two types of experimental myopia in chicken." Exp Eye Res **84**(2): 266-274.
Dirani, M., L. Tong, G. Gazzard, X. Zhang, A. Chia, T. L. Young, K. A. Rose, P. Mitchell and S. M. Saw (2009). "Outdoor activity and myopia in Singapore teenage children." Br J Ophthalmol **93**(8): 997-1000.
Feldkaemper, M., S. Diether, G. Kleine and F. Schaeffel (1999). "Interactions of spatial and luminance information in the retina of chickens during myopia development." Exp Eye Res **68**(1): 105-115.
Fischer, A. J., J. J. McGuire, F. Schaeffel and W. K. Stell (1999). "Light- and focus-dependent expression of the transcription factor ZENK in the chick retina." Nat Neurosci **2**(8): 706-712.
Guggenheim, J. A., K. Northstone, G. McMahon, A. R. Ness, K. Deere, C. Mattocks, B. S. Pourcain and C. Williams (2012). "Time outdoors and physical activity as predictors of incident myopia in childhood: a prospective cohort study." Invest Ophthalmol Vis Sci **53**(6): 2856-2865.
Guggenheim, J. A., K. Northstone, G. McMahon, A. R. Ness, K. Deere, C. Mattocks, B. St Pourcain and C. Williams (2012). "Time Outdoors and Physical Activity as Predictors of Incident Myopia in Childhood: A Prospective Cohort Study." Investigative Ophthalmology & Visual Science **53**(6): 2856-2865.

Iuvone, P. M., C. L. Galli, C. K. Garrison-Gund and N. H. Neff (1978). "Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons." Science **202**(4370): 901-902.
Iuvone, P. M., M. Tigges, A. Fernandes and J. Tigges (1989). "Dopamine synthesis and metabolism in rhesus monkey retina: development, aging, and the effects of monocular visual deprivation." Vis Neurosci **2**(5): 465-471.
Iuvone, P. M., M. Tigges, R. A. Stone, S. Lambert and A. M. Laties (1991). "Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia." Invest Ophthalmol Vis Sci **32**(5): 1674-1677.
Jones-Jordan, L. A., G. L. Mitchell, S. A. Cotter, R. N. Kleinstein, R. E. Manny, D. O. Mutti, J. D. Twelker, J. R. Sims, K. Zadnik and C. S. Group (2011). "Visual activity before and after the onset of juvenile myopia." Invest Ophthalmol Vis Sci **52**(3): 1841-1850.
Jones, L. A., L. T. Sinnott, D. O. Mutti, G. L. Mitchell, M. L. Moeschberger and K. Zadnik (2007). "Parental history of myopia, sports and outdoor activities, and future myopia." Invest Ophthalmol Vis Sci **48**(8): 3524-3532.
Kee, C. S., L. F. Hung, Y. Qiao-Grider, R. Ramamirtham, J. Winawer, J. Wallman and E. L. Smith (2007). "Temporal constraints on experimental emmetropization in infant monkeys." Investigative Ophthalmology and Visual Science **48**: 957-962.
Lan, W., M. Feldkaemper and F. Schaeffel (2014). "Intermittent episodes of bright light suppress myopia in the chicken more than continuous bright light." PLoS One **9**(10): e110906.
Lin, L., W. Lan, Y. Liao, F. Zhao, C. Chen and Z. Yang (2013). "Treatment outcomes of myopic anisometropia with 1% atropine: a pilot study." Optom Vis Sci **90**(12): 1486-1492.
Luft, W. A., P. M. Iuvone and W. K. Stell (2004). "Spatial, temporal, and intensive determinants of dopamine release in the chick retina." Vis Neurosci **21**(4): 627-635.
McBrien, N. A., C. L. Cottriall and R. Annies (2001). "Retinal acetylcholine content in normal and myopic eyes: a role in ocular growth control?" Vis Neurosci **18**(4): 571-580.
McBrien, N. A., H. O. Moghaddam and A. P. Reeder (1993). "Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism." Invest Ophthalmol Vis Sci **34**(1): 205-215.
Megaw, P., I. Morgan and M. Boelen (2001). "Vitreal dihydroxyphenylacetic acid (DOPAC) as an index of retinal dopamine release." J Neurochem **76**(6): 1636-1644.
Megaw, P. L., M. G. Boelen, I. G. Morgan and M. K. Boelen (2006). "Diurnal patterns of dopamine release in chicken retina." Neurochem Int **48**(1): 17-23.
Morgan, I. G., F. Xiang, K. A. Rose, Q. Chen and M. He (2012). "Two Year Results from the Guangzhou Outdoor Activity Longitudinal Study (GOALS)." ARVO Meeting Abstracts. Invest. Ophthalmol. Vis. Sci. **53**(6): 2735.
Napper, G. A., N. A. Brennan, M. Barrington, M. A. Squires, G. A. Vessey and A. J. Vingrys (1997). "The effect of an interrupted daily period of normal visual stimulation on form deprivation myopia in chicks." Vision Res **37**(12): 1557-1564.
Nickla, D. L., V. Sharda and D. Troilo (2005). "Temporal integration characteristics of the axial and choroidal responses to myopic defocus induced by prior form deprivation versus positive spectacle lens wear in chickens." Optometry and vision science : official publication of the American Academy of Optometry **82**: 318-327.
Norton, T. T. and J. T. Siegwart, Jr. (2013). "Light levels, refractive development, and myopia--a speculative review." Exp Eye Res **114**: 48-57.
Ohngemach, S., G. Hagel and F. Schaeffel (1997). "Concentrations of biogenic amines in fundal layers in chickens with normal visual experience, deprivation, and after reserpine application." Vis Neurosci **14**(3): 493-505.

Onal, S., E. Toker, Z. Akingol, G. Arslan, S. Ertan, C. Turan and O. Kaplan (2007). "Refractive errors of medical students in Turkey: one year follow-up of refraction and biometry." Optom Vis Sci **84**(3): 175-180.
Rohrer, B., A. W. Spira and W. K. Stell (1993). "Apomorphine blocks form-deprivation myopia in chickens by a dopamine D2-receptor mechanism acting in retina or pigmented epithelium." Vis Neurosci **10**(3): 447-453.
Rose, K. A., I. G. Morgan, J. Ip, A. Kifley, S. Huynh, W. Smith and P. Mitchell (2008). "Outdoor activity reduces the prevalence of myopia in children." Ophthalmology **115**(8): 1279-1285.
Schmid, K. L. and C. F. Wildsoet (2004). "Inhibitory effects of apomorphine and atropine and their combination on myopia in chicks." Optom Vis Sci **81**(2): 137-147.
Schwahn, H. N., H. Kaymak and F. Schaeffel (2000). "Effects of atropine on refractive development, dopamine release, and slow retinal potentials in the chick." Vis Neurosci **17**(2): 165-176.
Schwahn, H. N. and F. Schaeffel (1997). "Flicker parameters are different for suppression of myopia and hyperopia." Vision Res **37**(19): 2661-2673.
Shih, Y. F., C. H. Chen, A. C. Chou, T. C. Ho, L. L. Lin and P. T. Hung (1999). "Effects of different concentrations of atropine on controlling myopia in myopic children." J Ocul Pharmacol Ther **15**(1): 85-90.
Shih, Y. F., C. K. Hsiao, C. J. Chen, C. W. Chang, P. T. Hung and L. L. Lin (2001). "An intervention trial on efficacy of atropine and multi-focal glasses in controlling myopic progression." Acta Ophthalmol Scand **79**(3): 233-236.
Siegwart, J. T., A. H. Ward and T. T. Norton (2012). "Moderately elevated fluorescent light levels slow form deprivation and minus lens-induced myopia development in tree shrews." ARVO Meeting Abstracts. Invest. Ophthalmol. Vis. Sci. **53**: 3457.
Simon, P., M. Feldkaemper, M. Bitzer, S. Ohngemach and F. Schaeffel (2004). "Early transcriptional changes of retinal and choroidal TGFbeta-2, RALDH-2, and ZENK following imposed positive and negative defocus in chickens." Mol Vis **10**: 588-597.
Smith, E. L., 3rd, L. F. Hung, B. Arumugam and J. Huang (2013). "Negative lens-induced myopia in infant monkeys: effects of high ambient lighting." Invest Ophthalmol Vis Sci **54**(4): 2959-2969.
Smith, E. L., 3rd, L. F. Hung and J. Huang (2012). "Protective effects of high ambient lighting on the development of form-deprivation myopia in rhesus monkeys." Invest Ophthalmol Vis Sci **53**(1): 421-428.
Smith, E. L., L. F. Hung, C. S. Kee and Y. Qiao (2002). "Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in monkeys." Investigative Ophthalmology and Visual Science **43**: 291-299.
Stone, R. A., T. Lin, A. M. Laties and P. M. Iuvone (1989). "Retinal dopamine and form-deprivation myopia." Proc Natl Acad Sci U S A **86**(2): 704-706.
Wallman, J. (1990). "Retinal influences on sclera underlie visual deprivation myopia." Ciba Found Symp **155**: 126-134; discussion 135-141.
Wang, Y., H. Ding, W. K. Stell, L. Liu, S. Li, H. Liu and X. Zhong (2015). "Exposure to sunlight reduces the risk of myopia in rhesus monkeys." PLoS One **10**(6): e0127863.
Winawer, J. and J. Wallman (2002). "Temporal constraints on lens compensation in chicks." Vision Research **42**: 2651-2668.
Wu, P. C., C. L. Tsai, H. L. Wu, Y. H. Yang and H. K. Kuo (2013). "Outdoor activity during class recess reduces myopia onset and progression in school children." Ophthalmology **120**(5): 1080-1085.
Yi, J. H. and R. R. Li (2011). "[Influence of near-work and outdoor activities on myopia progression in school children]." Zhongguo Dang Dai Er Ke Za Zhi **13**(1): 32-35.

Zhu, X. (2013). "Temporal integration of visual signals in lens compensation (a review)." Experimental Eye Research **114**: 69-76.
Zhu, X. and J. Wallman (2009). "Temporal properties of compensation for positive and negative spectacle lenses in chicks." Investigative Ophthalmology and Visual Science **50**: 37-46.