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**Effect of bright light on the changes of choroidal
thickness in chickens as measured with OCT**

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to my family

Statement of Authorship

I herein certify that this thesis contains no material that has been extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

Work presented in this thesis is my own original work, except where otherwise acknowledged. This thesis has not been submitted for the award of any degree or diploma in any other tertiary institute.

Weizhong LAN

Abstract

Purpose. Bright light was found to be a powerful inhibitor of myopia development in some animal models. We have studied whether its effect may involve changes in choroidal thickness.

Methods. Three-day-old chickens were raised under “bright light” (15,000 lux) from 10AM to 4PM and kept under “normal” laboratory light during the remaining time of the light phase (500 lux, 8AM to 6PM, N=14) for 5 days. In contrast, a control group was kept in “normal light” (500 lux) during the entire light phase (N=14). Choroidal thickness in the posterior pole of right eyes was measured with an optical coherence tomography (OCT) in alert, hand-held animals at 10AM, 4PM and 8PM every day. Short-term effects of bright light were determined by comparing the change of choroidal thickness between the two groups immediately after the bright light was switched off, and four hours later. The long-term effect was determined by comparing the change of choroidal thickness between 10AM on day 1 and 10AM on day 5. Changes were analyzed using a repeated-measures ANOVA and independent *t*-test, respectively.

Results. In four out of the 28 chickens, the choroidal-sclera interface was not clearly visible and these animals were therefore excluded. In another three, one data point was lacking due to poor cooperation during the measurements. Thus, complete data were available only for 21 chickens (9 in normal light and 12 in bright light). Choroidal thickness initially decreased in the “bright light” group by $-5.2 \pm 4.0\%$ (mean \pm SEM), immediately after the bright light was switched off. This was different from the “normal light group” where choroidal thickness increased by $+15.4 \pm 4.7\%$ (ANOVA: $P=0.003$). However, after further four hours, choroidal thickness in the “bright light group” had also increased by $+17.8 \pm 3.5\%$ while there was little further change in the “normal light group” ($+0.6 \pm 4.0\%$; ANOVA: $P=0.004$). After 4 days of bright light exposure, the choroid was finally thicker than in the “normal light group” ($+7.6 \pm 26.0\%$ vs. $-18.6 \pm 26.9\%$; *t*-test: $P=0.036$).

Conclusions. Bright light induces transient choroidal thickening in chickens, although with some time delay, and stimulates choroidal thickening on the long term. My findings indicate that choroidal thickening is also involved in myopia inhibition by bright light. Furthermore, the diurnal cycle at the time of bright light exposure seems important which should be considered when children are enforced to enjoy outdoor activities.

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1 Introduction

Myopia is a common public health problem all over the world, with the prevalence ranging from around 15% in European-derived populations to nearly 80% in some Asian groups.¹⁻⁴ In addition to optical correction for daily activities, myopia significantly increases the risks of suffering from numerous serious complications, including retinal detachment, glaucoma, and cataract. Myopia, especially in its extreme degrees, has been ranked as a leading cause of visual impairment and blindness because of these associated diseases.⁵ On the other hand, the causative factors and the underlying mechanisms responsible for myopia are still unclear. Consequently, no fully satisfactory therapies are available to prevent the onset of myopia or myopia progression in children.⁶

In spite of that, growing evidence indicates that simply exposing to bright light may be an alternative intervention to prevent myopia. First, recent epidemic studies observed that time of outdoor activities is positively associated with lower myopic incidence and myopia progression.⁷⁻⁹ Second, this protective effect was found to rely on the time spent outdoors, rather than the engagement of physical sports.^{8, 10} Third, animal studies proved that bright light is a powerful inhibitor of myopia development: merely increased the ambient illuminance from 500 lux to 15000 lux was capable to dramatically inhibit experimental myopia in various species, including chickens,¹¹⁻¹³ tree shrew¹⁴ and monkeys.¹⁵ Furthermore, it was found that this effect was mediated, at least in part, through the retinal dopamine system.¹²

On the other hand, a multitude of studies suggest that choroid, a vascular tissue located between retina and sclera, plays an important role in the modulation of eye growth. Specifically, far prior to the response of sclera, choroid produces compensation rapidly

to the imposed ocular growth cues, either stimulatory or inhibitory, by means of modulating its thickness.¹⁶⁻¹⁹ Interestingly, increased choroidal thickness, usually seen in slowly growing eyes, could be induced by dopaminergic agonists as well.²⁰

Therefore, in the present study, we tested whether the protective effect of bright light involves changes in choroidal thickness. We were also interested to investigate whether the changes of choroidal thickness in response to bright light are similar to that induced by dopaminergic agonists. If this were true, it will deepen the understanding of the mechanism of outdoor exposure against myopia.

1.1 Definition and classification of myopia

Myopia is one type of refractive error, in which the parallel light rays are not focused on, but rather in front of the retina (Figure 1). This ocular disorder causes the images of distant objects to remain blurry on the retina. Based on the cause, myopia is classified into refractive myopia and axial myopia. For the former one, it is attributed to the ocular medias (ie., cornea, crystalline lens) being excessively refractive, such as increased curvature in keratocous and elevated refractive index in early cataract. For the latter one, it is attributed to an increase in the eye's axial length, which is the major type in school myopia.

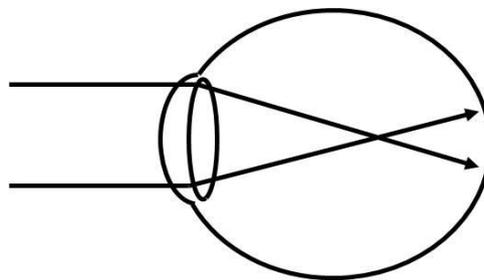


Figure 1. The optical characteristics in a myopic eye

1.2 Bright light and myopia

Although research about myopia can date back to more than one century ago, the exact pathogenesis for this ocular disorder is not well understood. Consequently, no clinically acceptable and satisfactory therapies are available to prevent or slow the progression of myopia in children.⁶ Nevertheless, accumulating evidence suggests that bright light is a powerful inhibitor against myopia.

1.2.1 Children are less likely to develop myopia when they spend more time outdoors

There is accumulating evidence showing that more time spent outdoors inhibits incident myopia and myopic progression in children. Specifically, Onal et al.²¹ noticed that the amount of outdoor activity in the early years of non-myopic adolescents was significantly higher than that of their myopic counterparts. Rose et al.⁸ reported that higher levels of total time spent outdoors, rather than engagement in sports activities *per se*, were associated with a lower degree of refraction from myopia, after adjusting for near work, parental myopia, and ethnicity. They further compared the prevalence and risk factors for myopia in children of Chinese ethnicity in Sydney and Singapore and found that increased time engaged in outdoor activities reported by Sydney Chinese was one of the major reasons for the lower prevalence of myopia in that city.⁹ It is also consistent with the observations that myopia progression is slower in the summer, when daylight hours are longer and average light intensity is higher, than in the winter.²²⁻²⁵

Apart from these retrospective studies, a recent prospective one reported that time spent outdoors was predictive of incident myopia independently of physical activity level¹⁰. In agreement with that, a two-year longitudinal clinical trial also presented evidence that

approximately one extra hour time of outdoor exposure exhibited a small, but statistically significant, protective effect against myopia progression and incidence of myopia as well.²⁶

Taken together, children appear less likely to develop myopia when they spent more outdoor time and the benefit obtained from outdoors is due mainly to the capture of information relating to time outdoors rather than physical activity.

1.2.2 The protective effect of outdoor exposure is light-driven

The exact mechanism by which outdoor exposure decreases the risk of developing myopia and its progression is yet unknown. Several hypotheses related to the specific properties of the light in the outdoor environment, the sunlight, have been put forward for that.

1.2.2.1 Ultraviolet light

Sunlight consists of a broad band spectrum, from ultraviolet (UV) to far infrared (IR), while light filament bulbs or fluorescent light sources indoors usually only contain the visual spectrum (400-780nm) and some IR, but no UV light. Reasonably, one could speculate that the protective effect of sunlight might be mediated by UV.²⁷ As a matter of fact, this hypothesis is supported by some recent observations. A photographic technique, conjunctival UV autofluorescence (UVAF) recording, was recently developed and applied as a biomarker of sunlight-induced UV damage, on the fact that excessive UV radiation (especially UV-B and UV-C) leads to many cellular responses on the ocular surface, such as inhibition of mitosis, nuclei fragmentation, eosinophilic staining, and loss of cellular adhesion.²⁸ With this technique, it was found that there was an inverse association between UVAF and myopia, indicating that the more sunlight

exposure, the lower degree the myopia is.²⁹ In agreement with this, another study group used a polysulfone UV dosimeter to measure the daily sum of UV exposure directly and found that the total amount of UV exposure was significantly lower in progressing myopes than in emmetropes and stable myopes.³⁰

Lower levels of skin-derived vitamin D resulting from decreased exposure to UV then would possibly influence refractive development,³¹ because vitamin D interacts with retinoic acid, a presumed signal molecule in the biochemical cascade that mediates the effects of vision on ocular growth.³²⁻³⁵ In support of this idea, Mutti et al. observed that myopes had 20% lower blood vitamin D than non-myopes, after adjusting for age and dietary intake.³⁶ They also revealed that polymorphisms within the vitamin D receptor were significantly associated with low to moderate amounts of myopia.³¹ Nevertheless, in a more recent experiment, feeding tree shrews with dietary supplements of sufficient vitamin D3 did not show an effect on experimental myopia produced by either form deprivation or negative lenses.³⁷

1.2.2.2 Ambient lighting levels

On the other hand, besides UV, only the light intensity between the sunlight and indoor light is significantly different. For example, at noon in a clear summer day, the outdoor illuminance levels can exceed 100,000 lux. It is still as high as 15,000 to 20,000 lux in the shade of trees. By contrast, the usual indoor illuminance level is approximately 500 to 600 lux. Thus, this enormous difference in intensity between sunlight and indoor light may be another possible mechanism for sunlight to protect against myopia.

Indeed, lines of evidence reported that, using UV-free light, merely increasing ambient light levels was able to significantly retard experimental myopia in different animals.

For instance, Ashby et al.¹¹ reared chickens, wearing translucent diffusers, under low (50 lux), normal (500 lux) or intense (15000 lux) indoor illuminance. They found that chickens reared under the first two lighting conditions developed significant deprivation myopia (approximately -8D for both groups over 4 days), while myopia development was markedly inhibited by intense light (-3D over the same time period). As the quartz-halogen lamps used in the study to produce intense illuminances didn't emit light with wavelengths in the UV range, the authors concluded that UV input was not vital for the retardation of myopia development. Subsequently, this effect was replicated in tree shrews¹⁴ and rhesus monkeys.¹⁵ Also myopia induced by negative lenses (another major type of experimental myopia), was inhibited by bright light in chickens¹² and tree shrews.¹⁴

Regarding the mechanism for the protective effect induced by bright light, a multitude of theories were discussed, including the constriction of pupil and increase of depth of focus, possible enhanced physical activity and hence faster local luminance changes on the retina, and increased release of dopamine from the retina^{11, 12, 15}.

1.3 Myopia and choroid

The choroid is a vascular layer of the eye lying between the retina and the sclera which contains supporting collagenous and elastic connective tissue (Figure 2). The main function of the choroid is to supply oxygen and nutrients to the outer retina, and, in species with avascular retinas (e.g., chickens), to the inner retina as well. Aside from that, recent studies showed that the choroid also plays an important role in the visual regulation of axial growth associated with emmetropization.³⁸

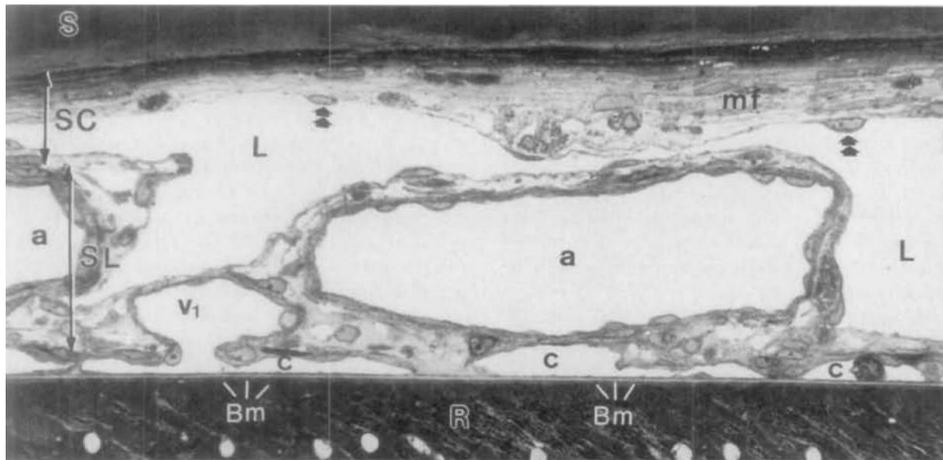


Figure 2. Light micrograph of a semithin section of the chicken choroid

S=sclera; SC=suprachoroidea (formed by the membrana fusca [mf] and the large lacunae [L]; SL=stromal layer, c=choriocapillaris; Bm=Bruch's membrane; and R=retina; a=arteriole; v1= venue. Adapted from Stefano M.E and Mugnaini E. IOVS, 1997,38:1241-60.

1.3.1 The change of choroidal thickness is an early event of the modulation of the eye growth induced by visual cues

Wallman and colleagues were the first to show that modulation of choroidal thickness can occur in response to imposed optical defocus.¹⁶ For example, when the eyes are covered with a negative lens moving the image plane behind the retina, the choroid starts thinning and the retina moves backward, probably to compensate for the imposed hyperopic defocus. By contrast, when the eyes are covered with a positive lens moving the image plane in front of the retina, the choroid thickens dramatically within hours and the retina is pushed forward, probably to compensate for the imposed myopic defocus. In another experimental paradigm in which eye elongation is slowed because the eye recovers from induced myopia, the choroid thickens significantly as well, by approximately 100 $\mu\text{m}/\text{day}$.¹⁸ By thickening or thinning, the choroid is able to change the refractive state of the eye by up to 7D.¹⁶ Similar compensatory changes for imposed

optical defocus were also found in primates, albeit the optical effects were much smaller because the eyes are larger and the similar changes in the position of the retina generate much smaller changes in refractive state.^{17, 18} Recently, choroidal thickening which is normally seen in response to eye-slowing factors (positive lens or recovery from experimental myopia) could be also induced by dopamine agonists,²⁰ indicating that this process is possibly mediated by dopaminergic system.

Besides the dramatic magnitude of visually-induced changes of choroidal thickness in chickens, the speed of the change is also rather rapid, far more rapid than responses of the sclera.¹⁹ For instance, wearing positive or negative lenses for only 10 minutes already produced significant changes in choroidal thickness in chickens within 2 hours while the sclera that ultimately controls eye size and refraction did not display any detectable shrinkage or elongation. If the lenses were worn for 2 hours, the changes of choroidal thickness could persist in darkness for up to 6 hours. Thus, it appears that the change of the choroidal thickness is an *early event* before the modulation of scleral growth when eye growth occurs. If the transient vision-induced increase in choroidal thickness was prevented by the nitric oxide (NO) synthesis inhibitor (L-NAME), the reduction in ocular growth rate was inhibited. This observation further suggests that there is a causal correlation between the changes in choroidal thickness and the regulation of axial eye growth.³⁹

1.3.2 The phase relationship between the diurnal rhythms in choroidal thickness and axial length are linked to ocular growth rates

Similar with other biological parameters (eg., blood pressure,⁴⁰ intraocular pressure⁴¹

and the axial length of the eye⁴²), choroidal thickness also undergoes a diurnal rhythm.⁴³⁻⁴⁶ It was reported that, measured at 6 hours intervals, the choroidal thickness was thinnest at around noon and thickest at around midnight. This diurnal rhythm is approximately anti-phasic to the axial elongation of the eye.⁴³ Interestingly, it was found that in eyes growing into myopia (e.g., in response to deprivation with diffusers or due to hyperopic defocus induced by negative lenses), the phase of axial length rhythms advanced to become exactly anti-phasic to the choroidal rhythms. On the contrary, in slowly growing eyes (during recovery from myopic defocus), the phase of axial length rhythm was delayed and in-phase with the choroidal rhythm.⁴³ It was further found that these phase relationships were significantly related to the ocular growth rates over the subsequent 24 hours⁴⁵ and therefore a causative correlation between phase relationship and ocular growth rate was suggested.⁴⁷

To sum up, growing evidence indicates that simply exposure to bright light may be an alternative intervention to prevent myopia. Meanwhile, a multitude of studies suggest that choroidal thickness plays an important role in the modulation of eye growth. Both of them are found to be related to the retinal dopamine system. Therefore, it is of high interest to find out whether the protective effect exerted by bright light involves changes in choroidal thickness and whether these changes may be similar to those induced by application of dopaminergic agonists, which are known to suppress experimental myopia.

2 Methods and materials

2.1 Animals

One-day-old male white leghorn chickens were obtained from a local hatchery in Kirchberg, Germany. The chickens were raised in a temperature-controlled room under a 10/14 hour light/dark cycle, with human illumination of 500 lux during the light phase, with lights on at 8AM and off at 6PM. Chickens had free access to food and water. All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the university committee for experiments involving animals.

2.2 Experimental design

From the third day post-hatching on, the experimental group (N=14) was maintained under “bright light” (15,000 lux) from 10AM to 4PM, while being kept under 500 lux for the remaining time of the light phase for 5 consecutive days. In contrast, the control group was kept in “normal light” during the whole light phase (500 lux, from 8AM to 6PM, N=14). Choroidal thickness in the posterior pole of the eye was measured in alert, hand-held animals at 10AM, 4PM and 8PM every day, using a small-animal-modified optical coherence tomography (Spectralis OCT, Heidelberg Engineering, Germany).

2.3 Lighting

For the “normal lighting”, the chickens were kept under an illuminance of 500 human lux at cage level, as measured by a radiometer (PCE-174 Datalogging Light Meter; PCE Instruments UK Ltd., Southampton, UK). Light was provided by normal ceiling-mounted triphosphor fluorescent lights (400 to 800 nm, peaking at 550 and

620 nm; Lumilux T8, 36W/21-840, Cool White, 4000K, Osram Ltd., Munich, Germany). For the “bright lighting”, the chickens were kept under two 1500 W (230 V) quartz-halogen lights (300–1000 nm, peaking at 700 nm) situated 1.5 m above the cage, which provided an illuminance of around 15,000 lux. Air conditioners were applied to match the temperature between the two groups (25-27 °C)(Figure 3). The spectral composition of the quartz-halogen lighting and the sun were very similar over the visible range of the spectrum for the chickens (360–700 nm). However, the quartz-halogen lamps contained a protective UV-absorbing cover glass which blocked wavelengths below 400 nm.



Figure 3. The bright light setting

2.4 OCT measurement and image analysis

Alert chickens were hand-held on the chin rest of a spectral-domain, small-animal-modified OCT (Spectralis OCT, Heidelberg Engineering, Germany) (Figure 4). This OCT device offers a very fast scan rate (40,000 A-scans/second), with high resolution (3.9 μm for axial resolution and 14 μm for transverse resolution) and satisfactory penetrating capability (scan depth 1.9 mm). Settings for the current study are shown in Table 1.

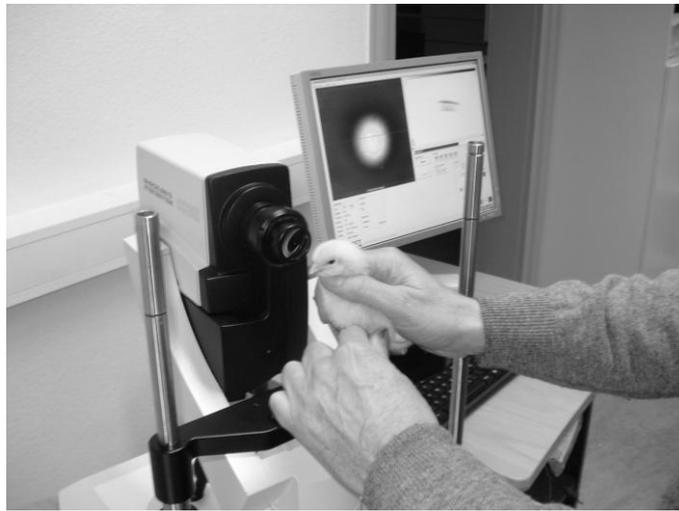


Figure 4. OCT measurements in an alert chicken
(Spectralis OCT, Heidelberg Engineering, Germany)

Table 1. Settings of Spectralis OCT

Items	Settings
Working Mode	IR + OCT
Light Source	820 nm
Intensity of IR	100%
Mode	Section
Scan mode	Line
Scan length	30°
OCT control	Small eyes
A-scans per B-scans	768
Averaging or Overlapping per image	100

During measurement, the position of the chicken head and the camera lens of the OCT were aligned by the operator, so that the laser was projected about the center of the pupil and the images of the posterior pole of the eye were captured. A representative OCT image is shown in Figure 5. Multiple pictures were taken for each eye and only those in which the pupil was nicely centered and the borders of the individual fundal layers were clearly visible were accepted for further analysis.

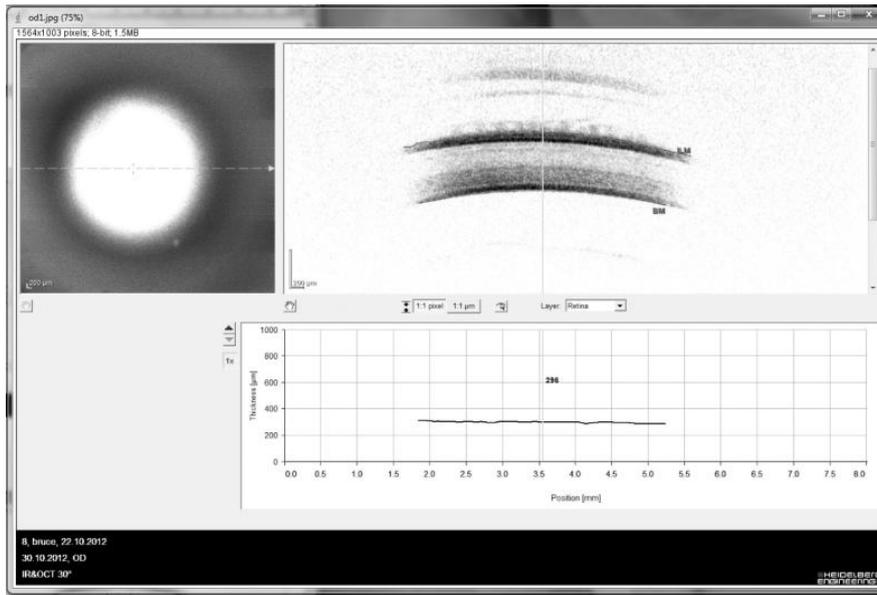


Figure 5. A representative OCT image

Each eligible image was manually processed with Image J (Ver. 1.46r, National Institutes of Health, USA). Firstly, the image was transformed into a grey mode (8 bit). Then, the choroidal thickness, defined by the distance between the inner border of sclera and the outer border RPE, was selected (the upper half of Figure 6). Thirdly, the vertical profile was checked in the profile plot options (Edit→ Options →Profile plot options). Fourthly, a plot profile of the grayness was produced (Analyze→ Plot profile) and the distance between the second peak (the inner border of sclera) and the subsequent trough (the outer border of RPE) was calculated (the lower half of Figure 6) in the units of *pixel*. Finally, the distance was transformed into the unit of μm ,

according to the scale bar in the picture. The results of the choroidal thickness were averaged from at least five eligible images for each eye.

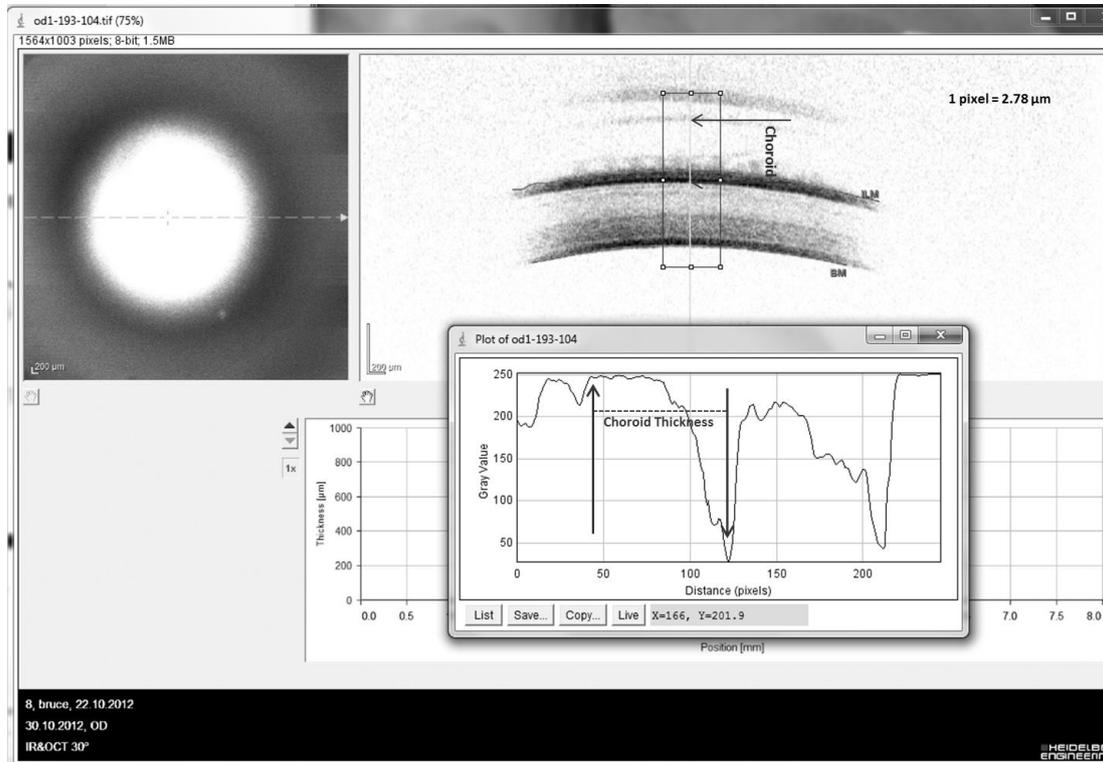


Figure 6. Individual OCT image processed in Image J.

2.5 Statistics

All analyses were performed with a commercial software (SPSS ver. 16.0; SPSS, Chicago, IL) and the data is presented as the mean \pm standard error of mean (SEM), unless otherwise indicated. The effect of bright light on choroidal thickness includes short- and long-term effects. Short-term effects were observed both immediately (4PM - 10AM) and 4 hours after the end of bright light treatment (8PM - 4PM). Long-term effects were evaluated by comparing the choroidal thickness between the same time point in the first and the last day of the experiment (10AM on day 1 and

10AM on day 5). Thus, a repeated-measures ANOVA and independent *t*-test was applied to uncover the short-term effects and the long-term effect, respectively. Tests of significance were two-tailed and the level of significance was set at 0.05.

3 Results

3.1 Validation and repeatability of OCT in measuring choroidal thickness in alert chickens

It was found that OCT is a very convenient and rapid technique to measure the choroidal thickness in alert chickens. After a couple of days' practice, one could obtain sufficient numbers of satisfactory OCT images for each eye within 10 seconds.

In order to study the reproducibility and the inter-visit repeatability of OCT in alert chickens, choroidal thickness from both eyes of 6 chickens at 4 time points (8AM, 12PM, 4PM and 8PM) on two consecutive days was compared in a pilot study. The average choroidal thickness was found to be $141.87 \pm 25.09 \mu\text{m}$ and for each single time point, the average within-individual standard deviation was $6.87 \mu\text{m}$ (4.84%), indicating a very good reproducibility of OCT scans in alert chickens. Comparing choroidal thickness on two consecutive days, a significant correlation between corresponding measurements at the same time points was found ($N=48$, $R^2=0.70$, $P<0.001$) (Figure 7).

There was no significant difference in choroidal thickness on these two consecutive days ($0.45 \pm 14.35 \mu\text{m}$, $P=0.83$), indicating a very good inter-visit repeatability.

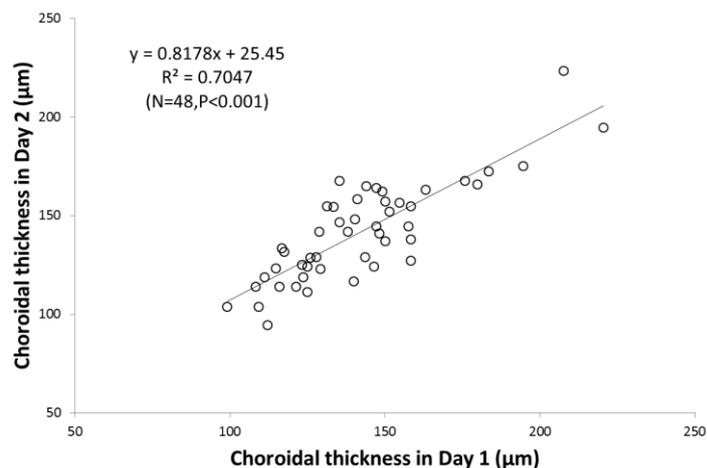


Figure 7. Inter-visit repeatability of OCT

3.2 Interocular correlation of choroidal thickness

In the pilot study, it was found that the choroidal thickness of both eyes was significantly correlated (N=48, $R^2=0.67$, $P<0.001$). Thus, the effects of bright light on choroidal thickness were only measured in the right eyes.

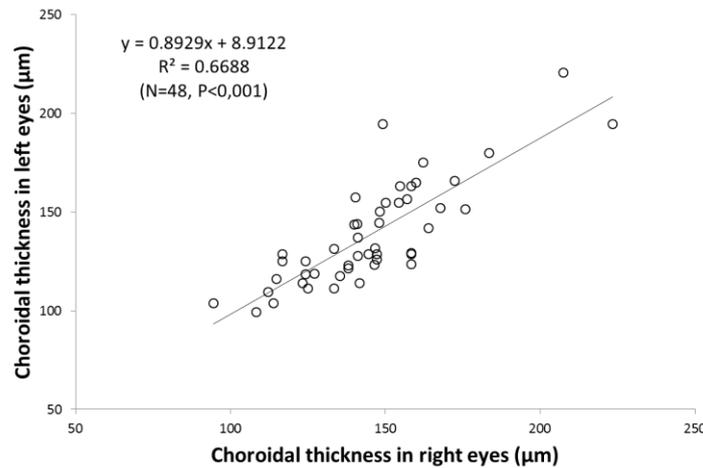


Figure 8. Interocular correlation of choroidal thickness

3.3 Short-term effects of bright light

In four out of the 28 chickens, the choroid-sclera interface was not clearly visible and these animals were therefore excluded. In another 3, one data point was lacking due to poor cooperation during the measurements. Thus, complete data were available only for 21 chickens (9 in normal light and 12 in bright light). Because the inter-subject variability of choroidal thickness was rather high (23% in the present study, similar to 24% reported previously³⁹), all changes were normalized to the individual baseline thickness of the choroids, rather than analyzing absolute average changes. Specifically, to uncover short-term effects of bright light, data collected at 4PM and 8PM were normalized to the individual choroidal thickness data at 10AM every day, respectively.

As shown in Figure 9-a, the choroidal thickness basically remained unchanged or even tended to decreased (all $P>0.05$) every day after six hours of bright light (15000 lux), compared to the value in 10AM. By contrast, the choroidal thickness showed a trend to increase ($P=0.07$, 0.03 and 0.03 for day 2, 4, 5, respectively) after the same time period in the control group (500 lux). On average, the everyday transient change in choroidal thickness in the bright light group was $-5.2\pm 4.0\%$ but $15.4\pm 4.7\%$ in the normal light group. The changes in choroidal thickness for the two levels of illuminance were significantly different (repeated-measures ANOVA: $P=0.003$) (Figure 9-b).

Surprisingly, reversed change in choroidal thickness occurred in both groups after additional four hours (8 PM – 4 PM). At 8 PM every day, the choroidal thickness significantly increased in the bright light group (all $P<0.05$), while it remained unchanged in the control group (all $P>0.05$) (Figure 10-a). On average, the transient change in choroidal thickness 4 hours after the cessation of bright light was $17.8\pm 3.5\%$ in the bright light group but only $0.6\pm 4.0\%$ in the normal light group. The changes in choroidal thickness between these two groups were significantly different (repeated measures ANOVA: $P=0.004$) (Figure 10-b).

3.4 Long term effect of bright light

To determine the long-term effects, individual choroidal thickness at 10AM on day 1 was regarded as baseline, and the relative changes between 10AM on day 1 and 10AM on day 5 were calculated accordingly. As show in Figure 11, at 10AM on day 5, choroidal thickness in the bright light group was significantly thicker than in the normal light group ($7.6\pm 26.0\%$ vs. $-18.6\pm 26.9\%$, independent t -test: $P=0.036$), indicating that choroidal thickness became actually thinner in normal light over this period of time.

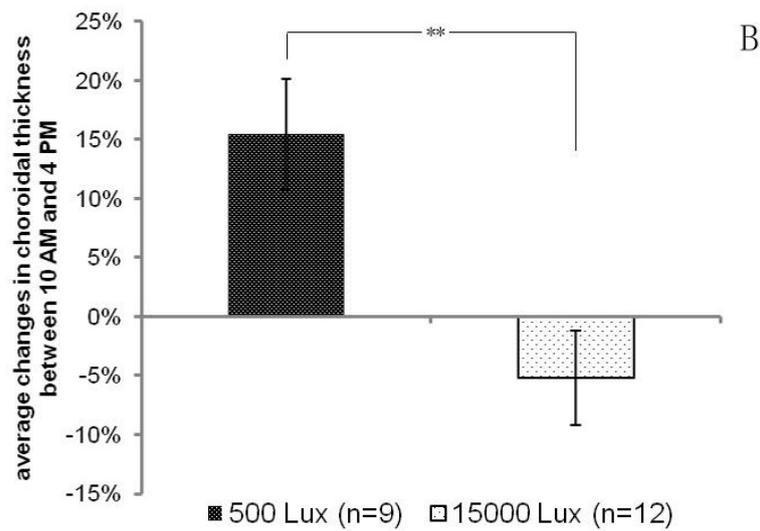
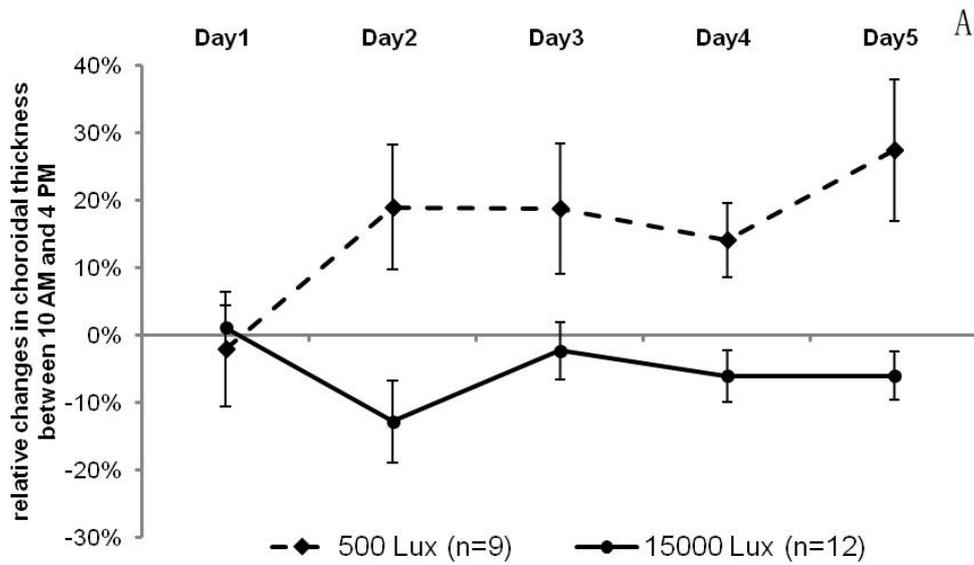


Figure 9. The relative changes of choroidal thickness after 6 hours of bright light treatment.

Relative changes for every day (A) and the mean level of changes for five days (B). Values of choroidal thickness are normalized to the individual data measured at 10AM every day. Error bars represent +/- SEM. Repeated measures ANOVA indicates significantly different pattern of change in choroidal thickness in response to these two illuminance levels (P= 0.003).

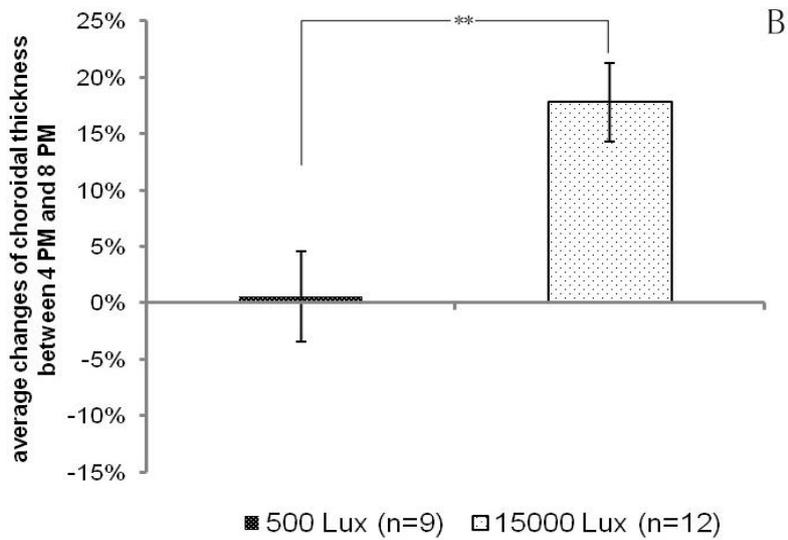
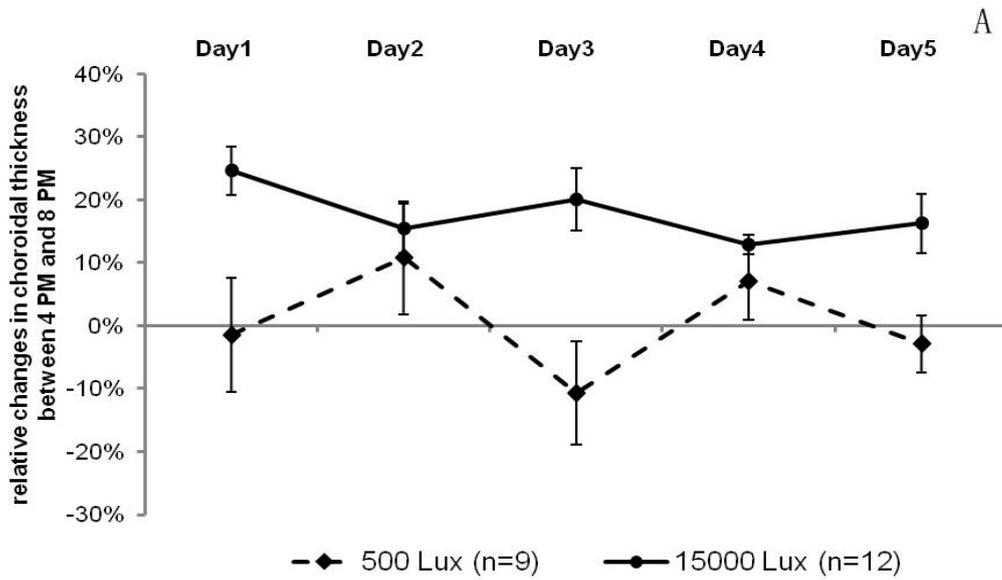


Figure 10. The relative changes of choroidal thickness after 4 hours of cessation of bright light treatment.

Relative changes for every day (A) and the mean level of changes for five days (B). Values of choroidal thickness are normalized to the individual data measured at 10AM every day. Error bars represent +/-SEM. Repeated measures ANOVA indicates significantly different pattern of change in choroidal thickness between the two groups (P= 0.004).

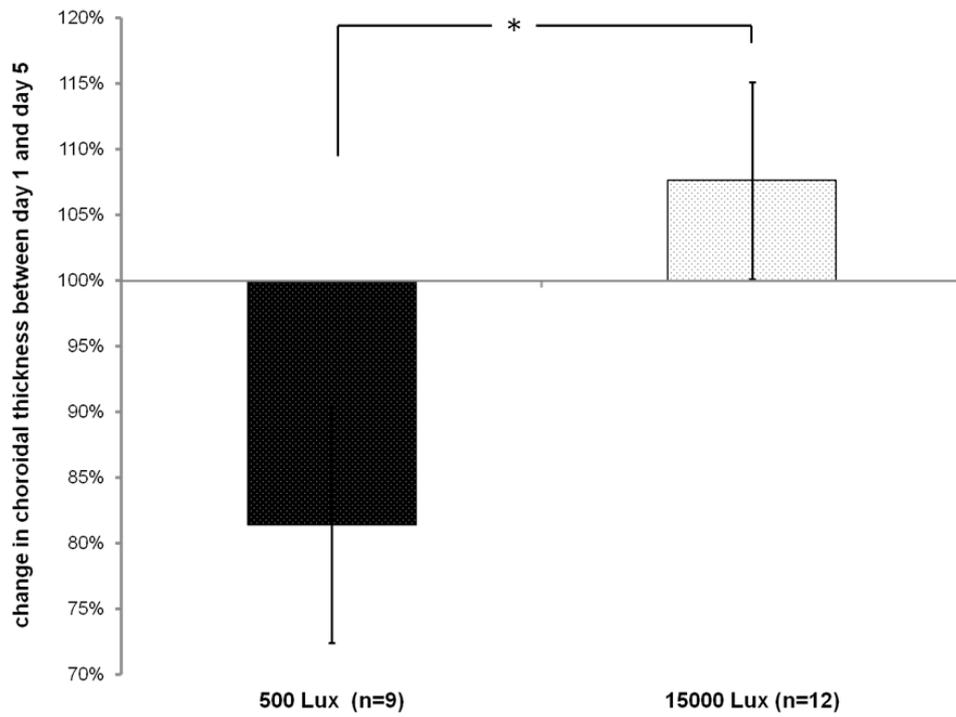


Figure 11. The long-term change of choroidal thickness over 5 days.

Choroidal thickness data are normalized to the individual data measured at 10AM on Day 1. Error bars represent +/-SEM. An independent *t*-test indicates a significant difference between the two groups in the changes of choroidal thickness between 10AM on Day 1 and 10AM on Day 5 ($P=0.036$).

4 Discussion

To the best of our knowledge, this is the first study investigating the effect of bright light on choroidal thickness, as measured with OCT. It was found that OCT is a convenient technique to measure choroidal thickness in alert chickens with good repeatability and resolution. Since it is a non-contact technique that does not require anesthesia, it is especially valuable for studies in which multiple measurements are taken within the same day. Using this technique, it is showed that bright light elicits transient increases in choroidal thickness, similar to changes observed after intravitreal application of dopamine agonists.²⁰ The effect appeared to add up over the 4 day period, so that the choroidal thickness was finally significantly thicker in the bright light group than in the normal light group.

4.1 The application of OCT in measuring choroidal thickness in alert chickens

In addition to optical variables like refractive state, the distances between individual fundal layers in the eye represent another fundamental biometric data, describing the output of the biochemical signaling cascade of ocular growth. A variety of different approaches have been used to obtain these data. Among them, A-scan ultrasonography is the most widely-used technique for the analysis of ocular biometry. Due to the limited penetration depth, a conventional A-scan (e.g., with a 10 MHz transducer) can't obtain reliable echoes from the posterior layers beyond the inner limiting membrane (ILM) of the retina and is therefore not useful for measuring choroidal thickness. Higher frequency (30 MHz) transducers can solve this problem under optimal conditions and considerable improvements have been achieved.^{18, 39, 43}

But in most cases, it also required animals to be anesthetized prior to the measurement. It is, therefore, not an ideal tool for studies on time-courses of biometric changes.

Recently, an optical low coherence interferometer (Lenstar LS 900; Haag-Streit, Switzerland), primarily designed for assessment of axial length,⁴⁸ has been shown to provide also choroidal thickness data in human eyes.^{46, 49} Still, since it is an A-scan technique, data from individual layers of the fundus, including choroidal thickness, can be obtained only in one dimension. By contrast, The optical coherence tomography (OCT) offers additional advantages. First, it is a B-scan technique, which is capable of producing a two-dimensional, cross-sectional view of the eye. Second, applying long-wavelength light as the signal source enables it to have a satisfactory penetrating capability (for instance, 1.9 mm for the current instrument). Third, it allows the simultaneous measurements with image-capturing of cornea and fundus. Consequently, through comparing the position of the cross-sectional image to a reference (e.g., the fovea or optic nerve in the primate fundus or the center of the cornea captured in synchronization with the fundus OCT image in birds), the thickness of each layers in one specific location can be accurately determined. Further, the unprecedentedly high resolution (up-to-micrometer resolution) and scanning speed enables it to produce precise and repeatable results within brief time, just before the animal changes its fixation point of interest.

In the present study, it was found that the reproducibility of the application of OCT in measuring choroidal thickness was high (average SD: 6.87 μm) and the inter-visit repeatability was also good ($R^2=0.70$, $P<0.001$). It is noted that this resolution is incomparable to that of the same technique applied in humans, which was as high as approximately 1-2 μm .^{50, 51} Nevertheless, given the fact that it is alert chickens which

were measured in the present study and the mean choroidal thickness of them is much bigger than the variance among repeated measures (coefficient of variance: 4.84%), OCT provides an invaluable methodology for research into dynamic changes of choroidal thickness related to visual manipulation in alert chickens.

It has to be noted that some authors (Guggenheim et al., 2011³⁹.) used a different definition of the choroidal boundaries in OCT images than we did. They used a higher resolution OCT (1060nm) and found an apparent, fine and continuous boundary in the middle of choroid in the OCT picture (designated as C in Figure 12-a). However, this boundary was invisible in the corresponding magnified ultrasound picture (the lower half of Figure 12-b). Accordingly, we speculate that this could be due to the different properties detected by ultrasonography and OCT respectively. On one hand, ultrasonography detects the echo reflected from the interface between two layers. Consequently, if the acoustic impedance of two layers is not very different, no specific echo will be received by the ultrasound detector and therefore the boundary might be invisible. This might be especially true if no high frequency transducer is used. On the other hand, what OCT acquires is the optical signals reflected from interfaces and, therefore, is capable of producing images with micrometer-resolution. Thus, we suspect that the acoustic impedances of choroid and cartilage sclera (the inner part of sclera of avian eyes) might be too similar to be distinguished in ultrasonography, while the interface between them is still visible in OCT. As a consequence, the middle boundary in the OCT picture by Guggenheim et al. might have represented the outer border of choroid and the space between the middle and the *so-called* outer boundary of choroid might be the cartilage sclera layer. Due to its molecule nature, the thickness of cartilage sclera layer would not manifest an obvious shift within limited time of development. If our speculation is correct, then the thickness of that space in OCT images should be

constant. In supporting this, we noted, in our study, that the thickness of the cartilaginous sclera was relative constant, regardless of treatment (normal illumination vs. bright light) and time. This is also supported by the fact that, if we use their definition, the average thickness of choroidal would increase from the current 141.87 μm to 221.64 μm , which is very close to their result³⁹ and that from another report using ultrasonography⁴³. Further, our speculation is directly confirmed by a recent report using a ultrahigh-resolution OCT (UHROCT).⁵²In Figure 13, it is clearly shown that the middle boundary in the area C in Figure 12-a is actually the choroid-sclera interface, that is, the outer border of choroid. Indeed, we found that using their definition does not have an impact on the results of the statistical analysis as mentioned above.

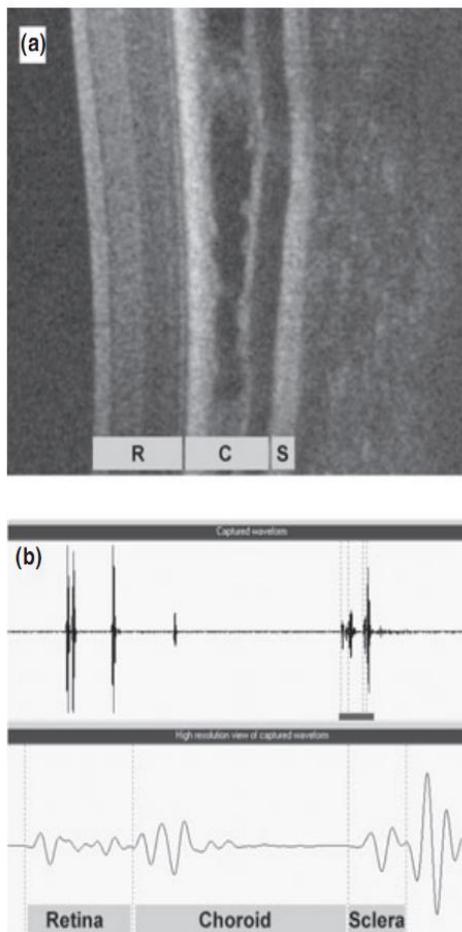


Figure 12. Detection of posterior segment layer boundaries in a chicken

The figure is adapted from Guggenheim et al. 2011.³⁹ The image in Panel a is a 1060nm optical coherence tomogram image of fundal layers of a chicken. R: Retina, C: Choroid, S: Sclera. In the upper half of Panel b, the observer is presented with a low-resolution view of the ultrasound waveforms generated from the whole eye, while a magnified view of the posterior segment region is shown in the lower half of the screen. It is noted that an apparent, fine and continuous boundary in the middle of choroid in the OCT picture. But this boundary is invisible in the corresponding magnified ultrasound waveforms.

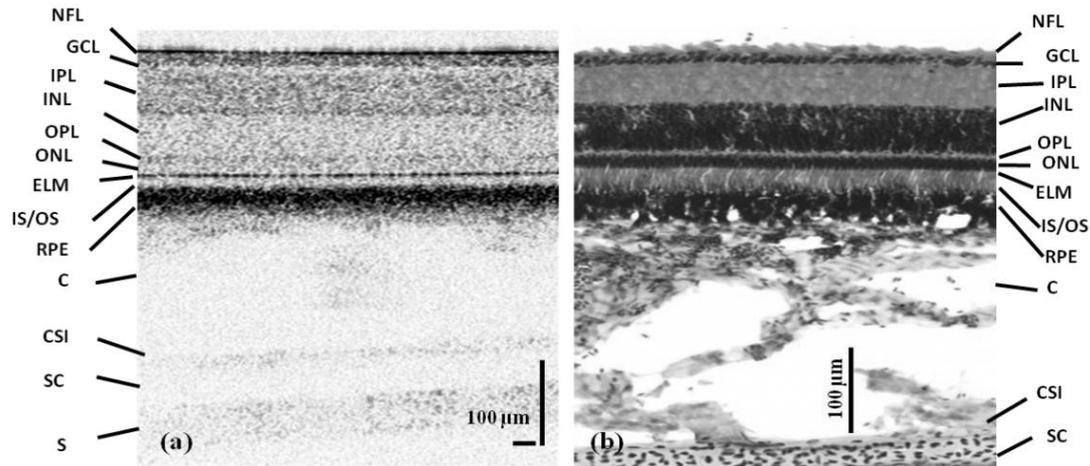


Figure 13. Chicken fundal layers as measured with an ultrahigh-resolution OCT and the corresponding histological tomogram

The figure is adapted from Moayed, et al., *Biomedical Optics Express*, 2011.⁵² NFL: Nerve Fiber Layer, GCL: Ganglion Cell Layer, IPL: Inner Plexiform Layer, INL: Inner Nuclear Layer, OPL: Outer Plexiform Layer, ONL: Outer Nuclear Layer, ELM: External Limiting Membrane, RPE: Retinal Pigment Epithelium, IS/OS: Inner segment-Outer segment junction of photoreceptor, C: Choroid, CSI: Choroid-scleral Interface, SC: Cartilaginous layer, S: Sclera. It is clearly shown that the middle boundary in the area C in Figure 12-a is actually the choroid-sclera interface.

4.2 Variability of choroidal thickness in young chickens

In the current study, we noticed a rather wide variability of choroidal thickness in the chickens of the study, with a coefficient of variance 23%. This is almost the same magnitude as reported recently in chickens with the same age (24%).³⁹ In comparison, the authors reported that the coefficients of variance for retina and sclera were much smaller (3% and 6%, respectively). They speculated that the high variability of choroidal thickness may be due to the fact that choroid thickness continuously responds to fluctuations in refractive errors during emmetropization³⁹. Specifically, chickens emmetropizing to compensate for hyperopia may show thinner-than-average choroids, and chickens emmetropizing to compensate for myopia thicker-than-average choroids⁵³. A recent study shows considerable inter-individual variability in the set-point of

emmetropization in chickens,⁵⁴ which could also relate to a large inter-individual variability in choroidal thickness. Parental choroidal thickness was another determinant of choroidal thickness in young chickens and the heritability h^2 was estimated to be at least 50%.³⁹

4.3 Mechanisms for transient change of choroidal thickness induced by bright light

The exact mechanism of choroid thickening is unknown, but several theories have been documented.^{16, 53} First, an increase in choroidal thickness might be achieved by increasing synthesis of proteoglycans, which are extremely hydrophilic and act as “sponges” in extracellular matrix.⁵³ Supporting evidence was that an increased amount of proteoglycans was found in thickened choroids in response to inhibiting eye growth stimulus (e.g., recovering from deprivation myopia¹⁶ and myopic defocus⁵⁵), while a decreased amount of proteoglycans was found in thinned choroids in response to hyperopic defocus.⁵⁵ A second way that choroid could thicken is by means of increasing permeability of choroidal capillaries, which could cause proteins to move into the extracellular matrix and consequently result in the passive fluid flow. This theory is directly supported by the finding that the amount of intravenous-injected fluorescein dextran was significantly higher in the choroids of recovering eyes than the form-deprived or normal choroids.⁵⁶ Third, because the choroid is one part of the uveoscleral outflow way for the aqueous humor and it is proved that the lacunae of the choroid are connected to the anterior chamber¹⁶, it seems possible that aqueous humor flows through this way and increases the choroidal thickness. Alternatively, it is also possible that the inflow of ions and water from retina and RPE to choroid, which happens constantly⁵⁷, exceeds the outflow from the choroid. Finally, given that the

lacunae of the choroid are always somewhat hypertonic, they tend to acquire water.⁵⁷ And the choroid is abundant of non-vascular smooth muscle. Thus, the tonus of the non-vascular smooth muscle in the choroid is also a possible contributing factor to modulate the choroidal thickness. Although direct evidence is lacking, it is theoretically possible that the choroid tends to thicken if non-vascular smooth muscles are relaxed.

Related to the present study, an increase of aqueous humor into the choroid could be a reason for choroidal thickening induced by bright light, because the passage through the ciliary muscle is necessary for the aqueous humor to reach the choroid and the constriction of the pupil (roughly 50% of its maximum diameter occurs under an illuminance of only 1000 lux⁵⁸) and the concomitant contraction of the ciliary muscle towards to the crystal lens would ease the efficacy of the diffusional barrier for this passage. But if this would be the primary reason, choroidal thickening should have occurred during the period of bright light treatment, rather than after this period. As the tonus of the non-vascular smooth muscle in the choroid is under sympathetic and parasympathetic control,⁵³ one might also consider that chickens' autonomic nervous system might be modulated under high ambient illumination, resulting in the regulation of the tonus of the non-vascular smooth muscle and consequently influence the choroidal thickness. Nevertheless, the physical activity of chickens exposed to 15 minutes of diffuser-free vision under high or normal ambient illumination was not different.¹¹ This weakens the notion that ambient illumination affects the status of autonomic nervous system and therefore the tonus of the non-vascular smooth muscle. For the same reason, the theories of increased permeability of choroidal capillaries and increased choroid blood flow seem unlikely.

Given that the choroidal thickening did not happen during the treatment of bright light,

but rather after the cessation of treatment, this “*delay*” indicates that maybe some biochemical pathways are triggered in response to bright light but that only after a certain amount of accumulation of the molecules or only when these molecules by themselves or their downstream signals reach the choroid, its thickness displays detectable changes. Proteoglycans may be one of these molecules acting in the choroid. But whether the synthesis of proteoglycans is up-regulated by increased illuminance needs further evidence.

Apart from that, retinal dopamine is another important candidate, as the synthesis and release of dopamine is light-regulated.⁵⁹⁻⁶⁵ Studies further showed that there is a dose-response relationship between the synthesis and release of retinal dopamine and the intensity of ambient light in mammals⁶⁶ and chickens.¹³ The protective effect of bright light against form-deprivation myopia has been shown to be mediated, at least in part, by the retinal dopamine system.¹² More importantly, dopamine agonists that inhibit the ocular growth also elicited a transient increase in choroidal thickness in chickens.²⁰ Nevertheless, dopamine is unlikely to be the molecule to directly mediate the choroidal thickening. It is synthesized in the retina, by a subpopulation of retinal amacrine cells, and can not pass through the RPE cells because of their tight junctions. Further, the synthesis and release of dopamine is very sensitive and rapid in response to a light stimulus.⁶⁷ Consequently, it is more likely that dopamine acts upstream of the choroidal response. In support of this notion, Sekaran et al.,⁶⁸ reported that steady or flicker light stimulation enhanced retinal NO. A similar effect was observed after application of exogenous dopamine to retinas in the darkness, while inhibition of endogenous dopaminergic activity *completely* suppressed the light-evoked NO release. Meanwhile, Nickla et al. showed that inhibiting NO synthase prevented the choroidal thickening response to myopic defocus and disinhibited ocular growth.^{69, 70} NO is a gaseous

neuroactive substance which has a diffusional radius of at least $100\mu\text{m}$ ⁷¹ and is capable of diffusing across biological membranes freely. Therefore, it is speculated that dopamine released from dopaminergic amacrine cells in response to exposure to bright light might trigger the release of NO which diffuses across the RPE and causes choroidal thickening.

4.4 Biological significance and clinical implications of choroidal thickening in response to bright light

In the current study, it was found that the choroid thickens over a 4 day period in response to an increase in ambient illuminance. Bright light also modulates the diurnal cycles in choroidal thickness. Since choroidal thickness changes represent early events in the signaling cascades for visual control of eye growth, the current findings support the notion that an increase in choroidal thickness induced by bright light may also mediate the inhibition of myopia.

Interestingly, we found that choroidal thickening did not occur during, but rather after the intervention with bright light. Indeed, many studies showed that the sensitivity of eye growth to respond to visual stimulation or pharmacological intervention varies with the time of day. For example, brief periods of normal vision inhibited deprivation myopia more if they occurred in the evening than in the morning.⁷² Stroboscopic light was more effective in suppressing myopia development during mid-night than during mid-day.⁷³ If our findings in chicken would also apply to humans, then the time for outdoor exposure should be taken into account when children are enforced to enjoy outdoor activities. That is, the protective effect of outdoor exposure might be enhanced before, rather after, the engagement of near work, or outdoor exposure should be

divided into several episodes, rather than one continuous, so that the eye-growing stimulus (i.e., near work) would be encountered by the thicken choroid.

The hypothesis already gains some support by comparing the results from two recent clinical trails finished in Guangzhou²⁶ and Taiwan⁷⁴. The study in Guangzhou showed that an additional 1 hour of outdoor activities everyday after school for a two-year period led only to a small treatment effect (3.7% less of myopia incidence). This small effect, albeit statistically significant, was attributed to the limited amount of time outdoors. By contrast, a similar amount of time (80 minutes), but being arranged in 6 separate episodes (10, 20 and 10 minutes in both the morning and afternoon), for outdoor activities every day in the latter study, was reported to have a more prominent protective effect after just one-year intervention (8.41% in the treatment group vs. 17.65% in the control group, $P < 0.001$). Therefore, it is speculated that bright light appreciated by children during outdoor exposure might induce choroidal thickening and subsequently result in a compensation for the myopicgenic factors. Nevertheless, it is also noted that frequent breaks from continued near work accompanied with the separate recess time in the latter study is also likely another important reason for this enhanced effect.

5 Zusammenfassung

Sowohl Untersuchungen an Tiermodellen, als auch epidemiologische Untersuchungen an Kindern haben gezeigt, dass helles Licht eine hemmende Wirkung auf die Entstehung von Kurzsichtigkeit (Myopie) hat. Aus Untersuchungen an Tiermodellen ist bekannt, dass die Dicke der Aderhaut eine wichtige Rolle bei der Myopieentstehung spielt: sobald das Augenlängenwachstum stimuliert wird (z.B. durch Tragen von Streulinsen oder Mattgläsern), wird die Aderhaut dünner. Wenn dagegen das Augenlängenwachstum gehemmt wird (z.B. durch Tragen von Sammellinsen oder während der Erholung von experimentell induzierter Myopie), wird die Aderhaut dicker. Ein Anzahl von Pharmaka, die die Entwicklung der Myopie beeinflussen, erzeugt ähnliche Muster der Dickenänderungen der Aderhaut. Dopamin-Agonisten bewirken dickere Aderhäute, während sie die Myopie hemmen. Es ist auch bekannt, dass helles Licht die Freisetzung von Dopamin aus der Netzhaut stimuliert. Deshalb ist es denkbar, dass helles Licht auch die Aderdicke erhöht.

Ich habe diese Frage am Modell des Haushuhns untersucht. Drei Tage alte Hühnchen wurden entweder unter hellem Licht aufgezogen (15,000 lux, etwa die Hälfte der Helligkeit im Freien) von 10:00 bis 16:00 Uhr. In der restlichen Zeit (von 8:00-10:00 und von 16:00-18:00) wurden sie unter normaler Laborbeleuchtung gehalten (500 lux). Eine zweite Gruppe von Tieren wurde von 8:00 – 18:00 unter 500 lux gehalten. Die Aderhautdicke wurde am hinteren Augenpol mittels optischer Kohärenztumographie (OCT) um 10:00, 16:00 und 20:00 Uhr gemessen. Die Kurzzeit-Wirkung von hellem Licht wurde gemessen unmittelbar nach der Phase hellen Lichts, sowie 4 Stunden später. Die Langzeit-Wirkung wurde ermittelt durch Vergleich der Aderhautdicken um 10:00 Uhr am ersten Tag und 10:00 Uhr am Tag 5. Es wurde festgestellt, dass die Aderhautdicke im hellen Licht anfangs abnahm um $-5.2 \pm 4.0\%$ (Mittelwert und

Standardfehler). Dies war bei der Gruppe in normaler Beleuchtung nicht so, wo die Aderhautdicke zunächst um $+15.4 \pm 4.7\%$ ($p=0.003$) zunahm. In der Gruppe, die in hellem Licht gehalten wurde, hatte jedoch die Aderhautdicke nach 4 weiteren Stunden wieder erheblich zugenommen, um $17.8 \pm 3.5\%$, während in der anderen Gruppe keine weitere Änderung erfolgte ($+0.6 \pm 4.0\%$, $p=0.004$). Deshalb war schliesslich die Aderhaut in der Gruppe in hellem Licht schliesslich nach 4 Tagen dicker als in der anderen Gruppe ($+7.6 \pm 26.0\%$ versus $-18.6 \pm 26.9\%$, $p=0.036$).

Zusammenfassend kann man feststellen, dass helles Licht zunächst, mit einigen Stunden Zeitverzögerung, die Aderhaut vorübergehend verdickt. Nach 4 Tagen hat sich diese Wirkung manifestiert und die Aderhaut bleibt permanent dicker. Die Ergebnisse zeigen, dass die Aderhautdicke bei der Myopiehemmung durch helles Licht eine Rolle spielt. Darüberhinaus legen die Ergebnisse auch nahe, dass die Tageszeit, bei der helles Licht einwirkt, eine Rolle spielt. Dies sollte bei der Entscheidung berücksichtigt werden, wann man Kinder ins Freie schicken sollte, um deren Myopierisiko zu verringern.

6 Publications or presentations

Lan Weizhong, Feldkaemper Marita, Schaeffel Frank. Effect of bright light on the transient changes of choroidal thickness in chickens as measured with OCT. Poster to be presented at the Annual meeting of the Association for Research in Vision and Ophthalmology, Seattle, May 2013. (LAN Weizhong was awarded the Josh Wallman Memorial Travel Grant)

7 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	May 2012-Present	Dr. Med Candidate	Ophthalmology(Myopia)

Professional Experience

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

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