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Ryan G. Strickland April 9, 2018

Influence of chromatic light on lens-induced myopia in mice

by

Ryan G. Strickland

Machelle T. Pardue, PhD
Adviser

Neuroscience and Behavioral Biology

Machelle T. Pardue, PhD

Adviser

Michael Crutcher, PhD

Committee Member

Kristen Frenzel, PhD

Committee Member

Erica Landis

Committee Member

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Ryan G. Strickland

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An abstract of
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Abstract

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Importance: Across multiple populations, the prevalence of myopia, or nearsightedness, has increased in recent decades. In addition to the high cost of treatment, pathological myopia increases the risk of blindness. Therefore, investigations into how environmental factors, such as light, influence the development of myopia are essential.

Purpose: White light contains several wavelengths that are focused at different focal planes within the eye, referred to as longitudinal chromatic aberrations (LCA). To characterize the effect of LCA on refractive development, wild-type C57BL/6J mice were placed in monochromatic light both with and without hyperopic lens defocus. To determine the role of cone photoreceptors in LCA, mice with abnormal cone function were housed under monochromatic light.

Methods: Wild-type C57BL/6J and cone dysfunction ALS/LtJ/Gnat2^{cpfl3} mice were housed in one of three LED lighting conditions beginning at post-natal day 28 (P28): white (425-700 nm), green (525 nm), or violet (400 nm) light. A subset of C57BL/6J mice received a head-mounted -10 diopter (D) lens over the right eye at P28. Refractive error, corneal curvature, and ocular axial parameters were measured weekly until P56 using photorefraction, keratometry, and spectral-domain optical coherence tomography (SD-OCT), respectively.

Results: By P42, C57BL/6J mice exposed to violet light became significantly more hyperopic than mice exposed to white or green light. Lens-treated C57BL/6J mice exposed to violet light demonstrated a significantly smaller myopic shift than lens-treated mice exposed to white or green light at P56. The difference in refractive error can be attributed to a significantly elongated vitreous chamber in mice exposed to white light compared to violet light. ALS/LtJ/Gnat2^{cpfl3} mice exposed to violet, green or white light did not demonstrate any significant differences in refractive error or ocular axial parameters by P56.

Conclusions: Mice can be used as an effective model to study chromatic influence on refractive development. Mice respond to monochromatic light in a way that could be predicted by LCA, and the effect of lens defocus was altered depending on light exposure. Cones are likely responsible for the hyperopic refractive changes in response to violet light, as mice with cone dysfunction eliminated this effect.

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Introduction

For proper visual clarity, eyes must achieve emmetropia; a state where the optical power of the eye matches the axial length, and the image can be focused properly on the retina. The process by which the eye grows to achieve emmetropia is called emmetropization. Alternatively, myopia, commonly known as nearsightedness, is a condition of the eye where incoming rays of light refract and converge at a focal point in front of the retina because the eye is elongated, and the power is too great. As a result, myopia causes the light from distant objects to create blur on the retina, which requires the use of corrective eye wear or refractive surgery to alter the focal point accordingly. Furthermore, severe myopia, referred to as pathological myopia, increases the risk of developing additional ocular anomalies such as retinal detachment and glaucoma, ultimately leading to permanent vision loss (Baba et al., 2003; Xu et al., 2007). It is concerning that the prevalence of myopia has risen substantially over the past few decades; rates of myopia in young people have increased from 25 – 42% in the United States, and myopia affects roughly 80% of children in Asian countries (Vitale et al., 2009; Lougheed, 2014; Dolgin, 2015; Pan et al., 2015). Such studies reported these increases over roughly 30 years, indicating that the increase in prevalence cannot be fully attributed to genetic factors. Consequently, investigations of environmental factors have become a focus of research to understand the mechanisms underlying myopic development.

The study of environmental light and its effect on emmetropization stemmed from correlation studies on light intensity that found myopic children, when compared to children that are emmetropes, spend less time in outdoor sunlight (Read et al., 2014; Jin et al., 2015). Such

associations with time spent in bright light suggest that the intensity of the light from the sun is protective against myopic development in children (Rose et al., 2008; Read et al., 2015).

Refractive errors can be experimentally induced in animals by using lenses that alter the focal point of light; the eye then grows to compensate for the altered focal point of light. Using experimental methods that are known to induce myopia, studies involving chicks, tree shrews, and rhesus monkeys have all shown that increased light intensity can protect against experimentally induced myopia (Ashby et al., 2009; Siegwart et al., 2012; Smith et al., 2012). Such corroborating evidence supports the idea that light intensity plays a role in emmetropization and could potentially influence susceptibility to myopia.

Other than the intensity of light, the spectral composition of environmental lighting has also become a target in animal studies of myopic development since the spectral compositions of sunlight and indoor light differ greatly. The difference in the chromatic characteristics of these environmental light sources are of interest because of the theory of longitudinal chromatic aberrations (LCA). The theory of LCA postulates that as short wavelengths enter the eye, they bend to a greater degree than longer wavelengths and, therefore, come to a focal point that is relatively anterior within the eye when compared to the focal point created by a longer wavelength (Mandelman & Sivak, 1983; Howarth & Bradley, 1986). As a result, a single image falling on the retina consists of multiple monochromatic focal planes, each creating some degree of defocus that is detected by the retina (Seidemann & Schaeffel, 2002). This is important when considering the differences in environmental lighting because artificial light, when compared to sunlight, typically excludes a variety of wavelengths and, consequently, a variety of monochromatic focal planes that may be used in emmetropization (Torii et al., 2017).

Experimentally, the focal plane created by LCA while using constant monochromatic light exposure impacts refractive growth predictably; the eye will emmetropize to the focal plane defined by the wavelength of light and the degree to which the eye refracts the various wavelengths of light. For example, exposing chicks and guinea pigs to monochromatic, long wavelength light for at least one week induced myopia but short wavelength exposure induced hyperopia, or the ocular state where the axial length of the eye is too short and the focal point of light is behind the retina (Liu et al., 2011; Torii et al. 2017). Typically, these studies have found changes in the depth of the vitreous chamber, the space between the lens and the retina, as the defining characteristic of altered eye size. These results suggest that increased exposure to short wavelengths could potentially be protective against myopic development, as demonstrated specifically by violet light providing protection for lens-induced myopia in chicks (Torii et al., 2017). However, other animal models, such as tree shrews and rhesus monkeys, have demonstrated mixed results; tree shrews became hyperopic with long wavelength exposure and rhesus monkeys did not respond to the treatment consistently (Smith et al., 2015; Gawne et al., 2017). The reasons behind these inconsistencies are not well understood; the findings could be the result of different animal models and their respective retinal signaling pathways or the result of variable light intensities between the studies.

Nonetheless, it is believed that cone photoreceptors, which are responsible for detecting color in the retina, are responsible for interpreting how chromatic cues should be utilized to guide emmetropization in LCA studies (Kruger et al., 1995; Lee et al., 1999; Rucker & Kruger 2004; Rucker & Wallman, 2009). There is even evidence that suggests short and long wavelength sensitive cones affect eye growth differently (Rucker & Wallman, 2008). Possible retinal

pathways that underlie LCA-induced refractive errors can be tested with a mouse model due to various genetic models, such as those with dysfunctional cones.

In order to investigate LCA and associated retinal pathways in this study, we will use the mouse model. Currently, there are no published studies on refractive changes in mice after prolonged monochromatic exposure. The LCA created by the mouse eye is predicted by optic models to be approximately 15 diopters (D) when comparing the extremes of visible light spectrum for the mouse retina (Geng et al., 2011). In other words, the focal plane created by 400 nanometer (nm) light and the focal plane created by 550 nm light are separated by roughly 15 D. We hypothesize that short wavelength light will induce relatively hyperopic refractions in the normal mouse eye, and long wavelength light will cause relatively myopic refractions when compared to a control white light condition. Furthermore, we hypothesize that monochromatic light could alter the response to lens-induced myopia in the mouse model. We hypothesize that mice with dysfunctional cones will not demonstrate differences in refractive development when simply exposed to short and long wavelength monochromatic light.

Methods

Animals and Housing

The animals involved in this study include 37 male and female wild-type C57BL/6J (C57) (Jackson Laboratory, Bar Harbor, ME) and 15 ALS/LtJ/Gnat2^{cpfl3} (*Gnat2*$^{-/-}$) (Jackson Laboratory, Bar Harbor, ME) mice (Table 1). *Gnat2*$^{-/-}$ mice contain a missense mutation that affects the α-subunit of transducin in cone photoreceptors, effectively hindering the phototransduction cascade within cones (Chang et al., 2006). The dysfunctional cone responses to light have been demonstrated by absent photopic electroretinogram (ERG) responses (Chang et al., 2006). Animals were transferred from standard colony housing under fluorescent lights to wire-top cages in custom light boxes outfitted with light-emitting diodes (LEDs) at post-natal day 28 (P28) where they lived constantly until P58. LEDs were either white, green (peak 525 nm ± 10 nm), or violet (peak 400 nm ± 10 nm) in color (NFLS-X3-LC2; Super Bright LEDs Inc., St. Louis, MO). The choice of wavelengths was based on previous studies that have found the mouse retina to include two predominant populations of cones: UV-cones have a peak sensitivity to 360 nm light (Jacobs et al., 1991) and lack significant responses to light above 425 nm, while M-cones have a peak sensitivity to 508 nm light (Wang et al., 2011). Lights were on a 12:12 light:dark cycle with standard mouse chow and water provided *ad libitum*. Food was placed on the bottom of the cage to eliminate shadowed areas. Cages and animals were checked daily. All procedures were approved by the Atlanta Veterans Affairs Institutional Animal Care and Use Committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Light Measurements

Light intensity of the white LED light was measured using a Control Company 3251 light meter (Transcat, Rochester, NY) at 447 lux ± 16 lux. However, light meters are not calibrated to accurately determine the brightness of monochromatic light sources; to control for the light intensity of the green and violet light sources, waveforms from the white-light LEDs were sampled using an Exemplar Smart CCD Spectrometer (B&W Tek, Newark, DE) after light intensity was determined. The monochromatic light intensities were then altered until their respective peak intensities, sampled with the spectrometer, resembled the peak relative intensity of the white LED light (Figure 1). All wavelength measurements were obtained using identical capture settings and random samplings from the base of the light box (i.e., the approximate distance the animal would be from the light source).

Ocular Measurements

At P28, mice underwent baseline measurements of refractive error and ocular biometrics. In a dark room, lit only with dim red light, eyes were first dilated with 1% tropicamide. Refractive error was measured using a custom-made, automated photorefractor (Figure 2A; Schaffel et al., 2004). Refractions were measured while the mouse was awake and after the mouse was anesthetized using an injection (intraperitoneal; i.p.) of ketamine (80 mg/kg) and xylazine (16 mg/kg) (Pardue et al., 2008). Only refraction data from anesthetized mice were used in the data analysis. Mice with refractive error differences between their two eyes that was greater than 2.0 D were considered abnormal and excluded from the study. Corneal curvature was then measured with a custom-made keratometer while the animal was still anesthetized (Figure 2B; Schaffel et al., 2004). Lastly, while anesthetized, optical parameters were obtained by using a 840 nm

Bioptigen Envisu 4300 System (SD-OCT; Bioptigen, Durham, NC) (Figure 2C; Pardue et al., 2013). Images obtained from the system allow for measurement of optical parameters such as axial length using digital calipers in a custom-made MatLab program (The MathWorks, Inc., Natik, MA; Figure 2C). At the end of testing, mice received an injection (i.p.) of antisedian (1 mg/kg) (Zoetis Services LLC, Parsippany, NJ) to reverse the effects of the ketamine/xylazine as well as sterile saline drops to prevent dehydration of the cornea before the mice returned to the light boxes. Measurements from the photorefractor, keratometer, and SD-OCT were captured weekly until P56 (Figure 2E).

Lens Defocus

Mice in the lens defocus group underwent surgery at P28 after baseline measurements in order to secure a head-mounted pedestal and a clear -10 D lens (X-Cel Specialty Contact, Duluth, GA) over the animal's right eye as previously described (Figure 2D; Faulkner et al., 2007; Pardue et al., 2013). Briefly, the animals received an additional injection of ketamine/xylazine (i.p.) and a subcutaneous injection of the painkiller meloxicam (5 mg/kg). The skin of the scalp was removed to expose the skull; three screws were inserted for cyanoacrylate and dental cement to fasten to. The dental cement formed a head pedestal which held the lens frame that inserted into an aluminum cube and was complemented by a balancing bar to offset the weight of the lens. An injection of antisedan (i.p) was given to reverse the effects of ketamine/xylazine and a subcutaneous injection of Ringer's solution (0.30 mL) prevented dehydration. The cube and lens frame were easily removed for weekly measurements and cleaning to prevent eye infections.

Statistical Analyses

All analyses performed utilized the right eye unless otherwise specified. Categories of measurement that required one-way, two-way, or repeated measures analysis of variance (ANOVA) were calculated using SigmaPlot (Systat Software, Inc., Chicago, IL). However, if data failed a Shapiro-Wilk normality test, then a Kruskal-Wallis ANOVA on ranks was calculated instead. Refractive data across time was analyzed using mixed modeling in SAS (SAS Institute, Inc., Cary, NC). The covariance structure that best suited the repeated measures design in this study was the Toeplitz structure (Littell et al., 2000). All data are presented as mean±standard error of the mean (SEM). For all calculations, Holm-Sidak *post-hoc* comparisons were used to make pairwise comparisons.

Results

C57 Refractive Development

Regardless of light exposure, control C57 mice became significantly more hyperopic (greater refractive values) with increasing age from P28 to P56 (Figure 3A; Mixed model ANOVA main effect of age, $F(4,60)=7.02$, $p<0.001$). There was a significant interaction effect between age and light color (Mixed model ANOVA, $F(8,60)=3.40$, $p=0.002$) such that control mice exposed to violet light became significantly more hyperopic than control mice exposed to white light or green light at P42 (average refractive error: Violet=7.26±0.42 D; vs White=3.46±0.56 D, $p=0.001$; vs Green=4.01±0.31 D, $p=0.026$). Although this effect continued throughout the experiment, it did not reach *post-hoc* significance at the final 2 timepoints. Notably, the refractive errors of control mice exposed to green light were not significantly different than that of control mice in the white light group throughout the study.

Among -10 D lens treated animals, there were significant interactions between age and treatment (Figure 3B; Mixed model ANOVA, $F(4,60)=19.48$, $p<0.001$) such that the average refractions of lens-treated eyes across age in each light condition became more myopic than their respective control group (average refractive error of Lens vs Ctrl across age: White=-4.63±0.39 D, $p<0.001$; Green=-2.88±0.37 D, $p<0.001$; Violet=-2.55±0.37 D, $p<0.001$). In addition, each light condition had a significantly different effect on the refractive error of lens-treated eyes (Mixed model ANOVA interaction effect of treatment and light color (Mixed model ANOVA, $F(2,60)=7.94$, $p<0.001$). The lens treated eyes exposed to white light became significantly more myopic than lens treated eyes in violet light beginning at P35 (average refractive error: White=-0.70±1.0 D vs Violet=3.92±0.43 D, $p<0.001$) and this significant difference lasted throughout the study. Lens

treated C57 mice exposed to green light had refractive errors between the violet and white light groups but did not show statistical differences with the other light groups. The refractive errors of the contralateral eyes of lens defocus animals were similar to that of control animals in respective light conditions over time with the exception of eyes exposed to violet light at P42 (average refractive error: Ctrl=7.26±0.42 D vs Contralateral=3.80±0.97 D, p=0.002) (data not shown).

The myopic shifts (right eye minus left eye; OD – OS) of the animals between light conditions were also calculated at P56 (Figure 3C). The refractive shift of lens defocus animals in green light (-3.63±0.55 D; p=0.002) and violet light (-2.47±0.63 D; p<0.001) were significantly reduced compared to animals in white light (-5.72±0.47 D) (two-way ANOVA interaction of light and treatment, F(2,35)=10.45, p<0.001). In addition, the refractive shift of lens defocus animals exposed to violet light was significantly decreased when compared to lens defocus animals exposed to green light at P56 (p=0.04).

C57BL/6J Ocular Biometry

As expected, corneal curvature increased with age, indicating a flattening of the cornea. Furthermore, the degree to which the shape of the cornea changed was not significantly different between control animals and lens defocus animals within or between each light condition by the time the animals reached P56 (Figure 4A; two-way ANOVA interaction of light and treatment, F(4,52)=0.44, p=0.79). Similarly, at P56, the thickness of the cornea was not significantly different between the various groups (Figure 4B; two-way ANOVA interaction of light and treatment, F(4,45)=0.32, p=0.87).

The depth of the vitreous chamber demonstrated significant differences between C57 animals at P56 dependent on light color (Figure 4C; two-way ANOVA main effect of light, $F(2,45)=7.64$, $p=0.002$), such that the vitreous chamber in animals exposed to white light (0.593±0.003 mm) was significantly deeper than animals exposed to violet light (0.578±0.004 mm; $p<0.001$) but not green light (0.583±0.004 mm; $p=0.08$), regardless of lens treatment. Despite minor differences in retinal thickness, there were no statistically significant differences between or within groups at P56 (Figure 4D; two-way ANOVA interaction of light and treatment, $F(4,45)=1.96$, $p=0.12$). Likewise, at P56, there were no significant differences in the axial length between or within groups of the C57 mice (Figure 4E; two-way ANOVA interaction of light and treatment, $F(4,45)=0.24$, $p=0.91$).

Gnat2$^{-/-}$ Refractive Development

Regardless of light condition, the average refraction of the mice typically became more hyperopic with age (Figure 5; two-way repeated measures ANOVA interaction of age and light, $F(8,72)=4.81$, $p<0.001$). The one exception to this trend was the P35 timepoint for animals exposed to white light (-1.67±0.72 D); these animals demonstrated significantly more myopic refractions when compared to animals in the green light (2.18±0.51 D; $p<0.001$) and violet light (1.44±0.30 D; $p<0.001$) at the same timepoint. However, by the P56 timepoint, there was no significant difference in the average refraction of *Gnat2$^{-/-}$* mice between the three light groups.

Gnat2$^{-/-}$ Ocular Biometry

As predicted, corneal curvature increased with age across all three light conditions. However, no differences were found between the light exposure groups with respect to corneal curvature as demonstrated by the data at P56 (Figure 6A; one-way ANOVA, $F(2,13)=0.80$, $p=0.48$).

The depth of the anterior chamber was not significantly different between groups at P56 (Figure 6B; one-way ANOVA, $F(2,13)=1.04$, $p=0.39$). The depth of the vitreous chamber was not significantly different between groups at P56 either (Figure 6C; Kruskal-Wallis one-way ANOVA on Ranks, $p=0.08$). Lastly, the average axial length of the mice was not significantly different between the light groups at P56 (Figure 6D; one-way ANOVA, $F(2,13)=1.11$, $p=0.36$).

Discussion

This study demonstrates the effect of monochromatic light exposure on the refractive development of the mouse eye. The refraction of C57BL/6J mice exposed to violet light became significantly more hyperopic than C57BL/6J mice exposed to green or white light. Also, shorter wavelengths provided protection against lens-induced myopia.

The use of the mouse model in myopia

Mouse models of refractive development are nonexistent in the studies of chromatic influence on refractive development. The absence of the mouse model in such studies may be attributed to the lack of three cone specific opsins in the rod dominated mouse retina that is typical of the primate retina (Schaeffel & Feldkaemper, 2015). Instead mice only have two cone specific opsins; short and medium wavelength at 360 nm and 511 nm peak sensitivity, respectively (Carter-Dawsin & LaVail, 1979; Applebury et al., 2000; Nikonov et al., 2006). However, the results reported here agree with the findings from chickens with cone dominated retinas (Rucker, 2013) and guinea pigs, another rodent model (Long et al., 2009; Liu et al., 2011; Jiang et al., 2014). Importantly, only mice allow for the use of genetic mutants to study a variety of possible underlying factors to refractive development.

LCA theory and monochromatic light exposure

The findings in this study are consistent with the LCA theory that shorter wavelengths would fall in front of the retina, signaling for slowed refractive eye growth to position the retina at the focal point, thus producing a more hyperopic eye than longer wavelengths. Of the three light conditions here, violet light has the shortest wavelength and, therefore, comes to a focal point more anteriorly in the eye compared to the focal point of green light as well as the

chromatic focal points created by white light which is absent of wavelengths as low as 400 nm. The retina interprets these chromatic focal points by an undetermined mechanism that alters ocular growth accordingly; mice housed in violet light had the most hyperopia (Figure 3A).

Furthermore, when these wild-type mice were treated with -10 D lens defocus, the animals displayed myopic shifts to varying degrees based on wavelength exposure (Figure 3B; 3C). Both green and violet light were protective against the effects of lens defocus, but violet light was also significantly more protective against the lens defocus compared to green light. This finding agrees with previous studies that have found short wavelength light, such as 365 nm or 470 nm, to be protective against lens-induced myopia (Jiang et al., 2014; Torii et al., 2017).

The influence of ocular parameters

Of the measured ocular parameters in C57 mice, only vitreous chamber depth demonstrated significant differences between groups of animals; specifically, animals exposed to white light had elongated vitreous chambers compared to animals exposed to violet light, regardless of treatment, which agrees with the refractive errors detailed here (Figure 3B; 4C). Changes in the other ocular parameters were not found and thus, the optical source of the refractive error differences could not be determined from these data. In mice, it is often difficult to detect minor alterations in structures such as axial length which have previously failed to correlate with refractive changes (Chakraborty et al., 2014; Chakraborty et al., 2017). It is worth mentioning that data concerning many of the ocular structures demonstrated statistical power that was much lower than what is typically desired. For this reason, it was highly unlikely that significant differences could be detected between groups. Perhaps the easiest way to solve the issue is to increase the sample size of the groups in the study which were relatively small.

The contribution of cone photoreceptor signaling

To evaluate a possible retinal mechanism underlying the refractive changes in the mouse eye, the refractive development of the *Gnat2*$^{-/-}$ mouse was also evaluated under the three chromatic conditions; these mice lack functional cones (Chang et al., 2006). In contrast to the wild-type C57 mice, *Gnat2*$^{-/-}$ mice exposed to violet or green light had similar refractions across age. Mice exposed to white light did not demonstrate typical refractive development with myopic growth after the first week of light exposure. The reasons behind the growth pattern at P35 are unclear, and there is no published data on the refractive development of these mice under LED light exposure for comparison. However, by the final timepoint, the refractions of mice in the three light conditions were the same.

In this study, the intensity of the white light is in the range of typical mesopic light levels; a level similar to that of an indoor workplace which involves both rod and cone activation in the retina (Zele & Cao, 2015). In a normal retina, M-cones are responsible, in part, for the detection of green light; the maximum sensitivity of the M-cone opsin is approximately 511 nm (Jacobs et al., 2004; Rocha et al., 2016) while the peak wavelength of the light source is 525 nm. In the *Gnat2*$^{-/-}$ mice, green light can still be detected by rods due to the overlap in the spectral sensitivity of rhodopsin and M-opsin (Peirson et al., 2017). In addition to this overlap, it is known that rods play a vital role in the typical refractive development of the mouse eye (Park et al., 2014), and the excitation of the rods may be sufficient for maintaining typical hyperopic growth with age in *Gnat2*$^{-/-}$ mice (Figure 5).

On the other hand, violet light exposure did not cause exaggerated hyperopic growth in *Gnat2*$^{-/-}$ mice. UV-cones are not maximally sensitive to the violet light used in this study (Jacobs

et al., 1991; Jacobs et al., 2004), but their spectral sensitivity does include the range of violet light used here (Wang et al., 2011). We showed that without the response of these cones to violet light, the increased level of hyperopia is not demonstrated. However, the mice still show some degree of hyperopic development and must be using another visual cue to guide emmetropization. The mechanisms at play are not clear. It could be that rhodopsin is slightly sensitive to the wavelengths of light emitted by the violet LEDs; *Gnat2*$^{-/-}$ mice have been shown to have abnormally increased rod sensitivity to near-UV light (Wang et al., 2011). Another potential opsin that could conceivably respond to this light source is melanopsin, a non-visual opsin (Berson et al., 2002) that has also been implicated in refractive growth (Stone et al., 2013). These opsins, along with other non-vision forming opsins, may combine to guide emmetropization in the mouse eye in the absence of UV-cones. Regardless, the refraction data from *Gnat2*$^{-/-}$ mice suggests that UV-cones are necessary for the hyperopic increase seen in response to short wavelength light in the mouse eye. Furthermore, at least one study of high school students with red-green color blindness has found that the prevalence of myopia is significantly lower than that of their classmates with normal color vision (Qian et al., 2009). This is interesting since, of the three cone types, only the S-cone would be functional in these color-blind students which may lead to reduced levels of myopia in the studied population.

Analysis of the ocular parameters did not demonstrate any differences at the final P56 timepoint. In this case, such a finding is to be expected since there were no refractive differences at this point either. Much like the issue with the C57 mice, these data concerning the ocular parameters of the *Gnat2*$^{-/-}$ mice had low statistical power and made it unlikely to demonstrate significant differences.

Future Directions

Due to issues with breeding, lens defocus surgeries, and timing, the subgroups involving *Gnat2*$^{-/-}$ lens defocus were not included in the study due to small sample size. Therefore, the next step to take this study would be to collect data on an appropriate sample size of *Gnat2*$^{-/-}$ mice with -10 D lens defocus. This experiment would provide additional evidence for the role of cones in the refractive development of mice under monochromatic light. We would predict that much like the control animals, lens treated animals would respond to defocus to the same degree and there would be no differences between the groups. Unlike the C57 animals, the *Gnat2*$^{-/-}$ mice would not have cones to modulate the response. Defocused green light would likely activate rods much like white light would in these mice and cause similar changes in refractive error. Defocused violet light may activate non-vision forming opsins that are likely also activated by white light, but the lack of the UV-opsin may prevent the protective effects that were seen in the C57 mice. Also, the retinas collected from these mice can be processed in high-performance liquid chromatography (HPLC) for neurotransmitters such as dopamine (DA) or gamma-aminobutyric acid (GABA) (Blaszczyk et al., 2004; Pozdeyev et al., 2008). Both neurotransmitters have been independently assessed in chromatic pathways involving cones in the retina (Schmidt et al., 2014; Qiao et al., 2016) as well as refractive development (Stone et al., 2003; Feldkaemper & Schaeffel, 2013).

Conclusion

The mouse can be used as an effective model for the study of chromatic influence on refractive development. Mice respond to monochromatic light in a way that could be predicted by LCA, and the effect of lens defocus was altered depending on light exposure; the response is

mediated by change in the vitreous chamber depth. Cones are likely responsible for the hyperopic refractive changes that were seen in wild-type mice in response to violet light as shown by mutant mice with dysfunctional cones. These initial findings in the mouse model agree with other animal models that have undergone similar light exposure and may warrant investigations about lighting environments for children as we witness the prevalence of myopia increase worldwide.

Tables & Figures

C57BL/6J	**White**		**Green**		**Violet**	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Control	2	4	3	4	4	3
	n= 6		n= 7		n= 7	
	3	2	3	3	3	3
Lens Defocus	n= 5		n= 6		n= 6	

ALS/LtJ/Gnat2^{cpfl3}	**White**		**Green**		**Violet**	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Control	5	0	1	3	3	3
	n= 5		n= 4		n= 6	
	2	0	0	2	2	2
Lens Defocus	n= 2		n= 2		n= 4	

Table 1. A summary of animal assignment to experimental groups for C57BL/6J and ALS/LtJ/Gnat2^{cpfl3} mouse strains used in the study.

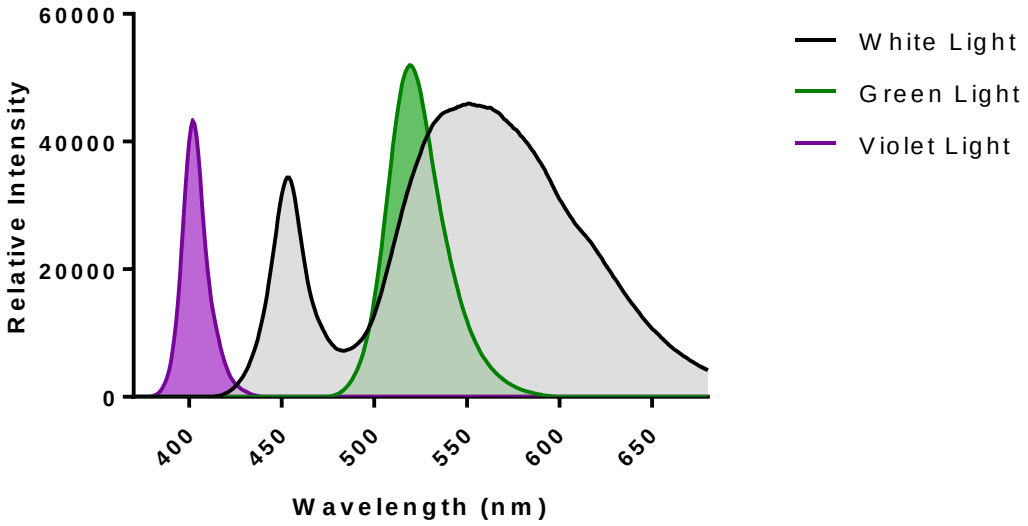


Figure 1. The relative intensity and wavelengths of the LED light boxes used in the study. White light intensity was measured at 447 lux. The green LEDs peak at 525 nm and the violet LEDs peak at 400 nm.

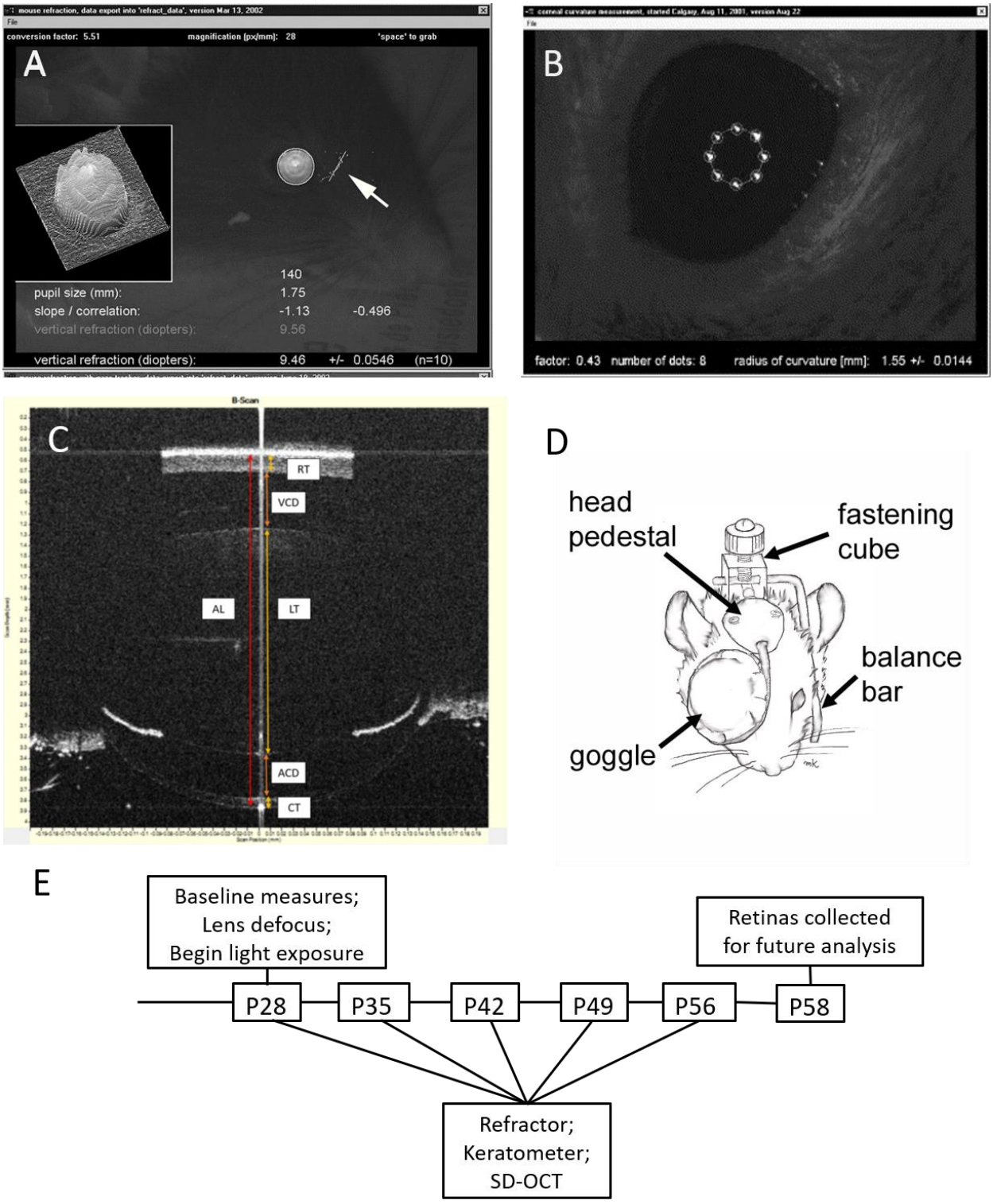
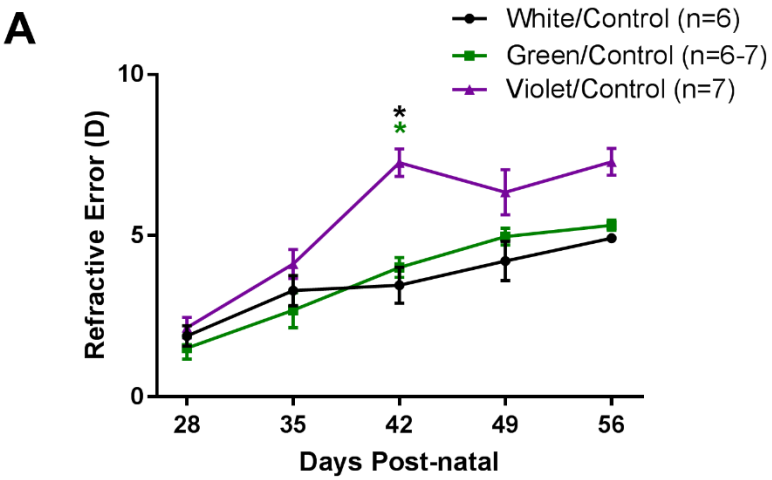
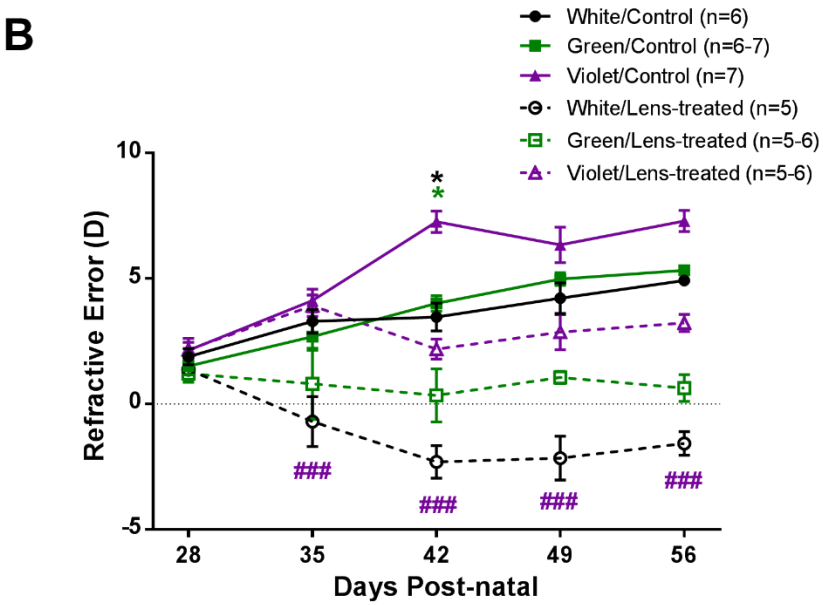


Figure 2. An overview of the timeline and equipment used throughout the study. **A)** An example image from a custom-made photorefractor detecting the brightness profile of the mouse eye which is used to determine the refractive power of the eye. **B)** An example image from a custom-

made keratometer detecting the Purkinje images on the anterior surface of the cornea that originate from the keratometer LED lights. **C)** An example image of the mouse eye in cross-section that was captured by the SD-OCT. Lines indicate the boundaries of the ocular structures that are measured. Abbreviations: Corneal Thickness (CT); Anterior Chamber Depth (ACD); Lens Thickness (LT); Vitreous Chamber Depth (VCD); Retinal Thickness (RT); Axial Length (AL). **D)** A diagram for the configuration of the head-mounted pedestal used in the lens defocus group (Faulkner et al., 2007). **E)** A timeline of the study indicates the start point for each litter of mice at P28 with measurements occurring weekly until P56. Three instruments were used throughout the experiment to capture ocular parameters.


A
White/Control (n=6)
Green/Control (n=6-7)
Violet/Control (n=7)
Refractive Error (D)
10
5
0
28
35
42
49
56
Days Post-natal
*
*


B
White/Control (n=6)
Green/Control (n=6-7)
Violet/Control (n=7)
White/Lens-treated (n=5)
Green/Lens-treated (n=5-6)
Violet/Lens-treated (n=5-6)
Refractive Error (D)
10
5
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-5
28
35
42
49
56
Days Post-natal
*
*
###
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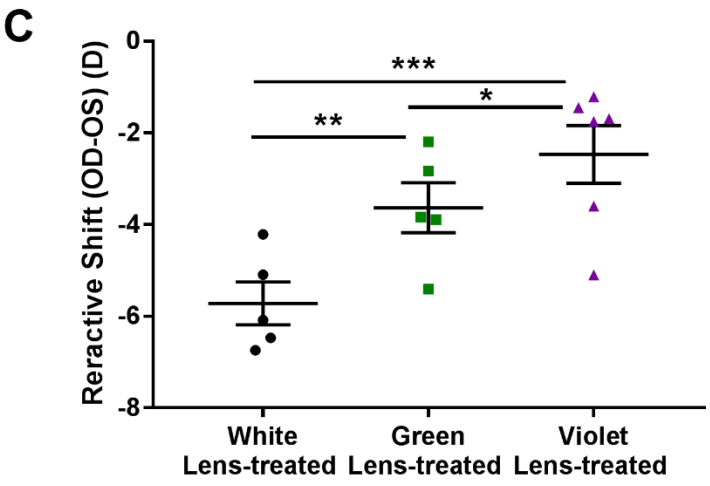
C
Reractive Shift (OD-OS) (D)
0
-2
-4
-6
-8
***
**
*
White
Lens-treated
Green
Lens-treated
Violet
Lens-treated

Figure 3. Refraction data of C57BL/6J mice housed in different light conditions with and without lens defocus. **A)** Control mice become more hyperopic with increasing age regardless of light condition (Mixed model ANOVA main effect of age, $F(4,121)=7.02$, $p<0.001$). Control mice exposed to violet light were significantly more hyperopic than white or green light groups at P42. **B)** Lens treated eyes become relatively myopic compared to controls (Mixed model ANOVA interaction effect of age and treatment $F(4, 121)=19.48$, $p<0.001$). Additionally, the magnitude of myopia was dependent on the light color (Mixed model ANOVA interaction effect of treatment and light color $F(4, 121)=7.94$, $p<0.001$). Lens-treated eyes exposed to white light developed significantly more myopia than lens-treated eyes in violet light. **C)** The myopic shift (OD-OS) of lens treated animals in the three light groups. Mice in the white light group demonstrated a significantly greater myopic shift compared to mice in green and violet light (two-way ANOVA interaction of light and treatment, $F(2,35)=10.45$, $p<0.001$). Violet light reduced the effects of lens defocus when compared to white light and green light. Data are expressed as mean±SEM. Significance level of *post-hoc* comparisons: *p<0.05; **p<0.01; ***p<0.001; ###p<0.001. Colored asterisks indicate the light treatment and a significant comparison with the marked data, always within the treatment groups. Colored number sign indicates the light treatment and a comparison with the marked data, always within the lens-treatment groups.

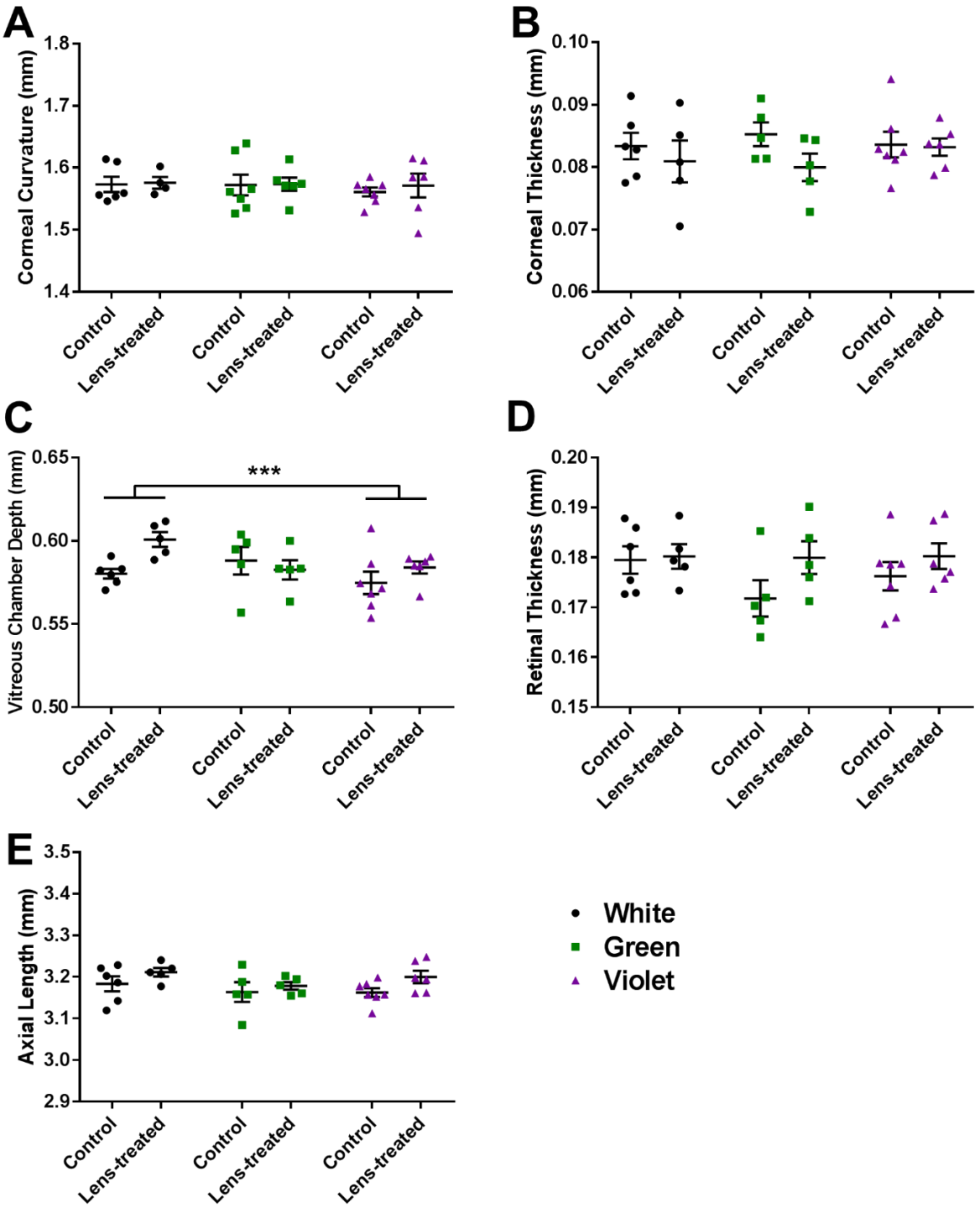

Figure 4. Ocular parameters of C57BL/6J mice that can affect the refractive power of the eye. **A)** There are no significant differences between groups with respect to the curvature of the anterior

cornea at P56 **B)** Thickness of the cornea at P56 shows no significant difference between groups. **C)** Vitreous chamber depth is significantly elongated in white light when compared to eyes in violet light at P56 (two-way ANOVA main effect of light, $F(2,45)=7.64$, $p=0.002$). **D)** Thickness of the retina shows no significant difference at P56. **E)** Despite significant changes in vitreous chamber depth, significant changes in axial length were not detected at P56. Data are expressed as mean±SEM. Significance level of *post-hoc* comparisons: ***$p<0.001$, refers to comparison between all eyes in white and violet light.

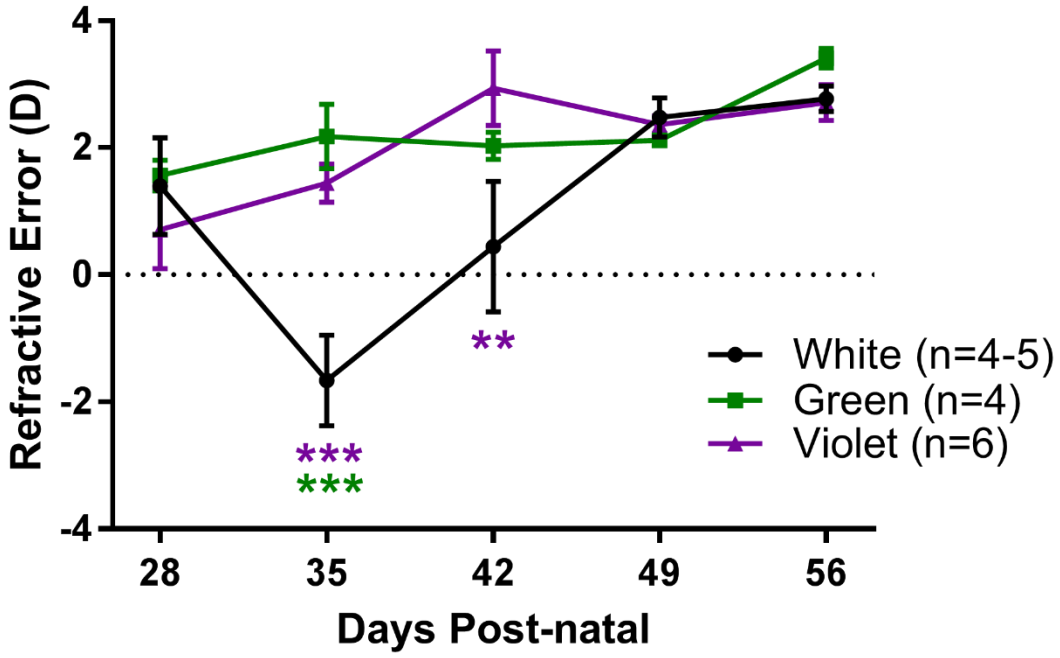

Figure 5. The refractive development of *Gnat2*$^{-/-}$ mice without lens defocus. Mice in green and violet light had a slightly hyperopic development with increasing age. However, mice exposed to white light became significantly more myopic after one week of light exposure before following the hyperopic growth trend with increasing age (two-way repeated measures ANOVA interaction of light and age, $F(8,72)=4.81$, $p<0.001$). Data are expressed as mean±SEM. Significance level of *post-hoc* comparisons: **p<0.01; ***p<0.001. Colored asterisks indicate the light treatment and a significant comparison with the marked data.

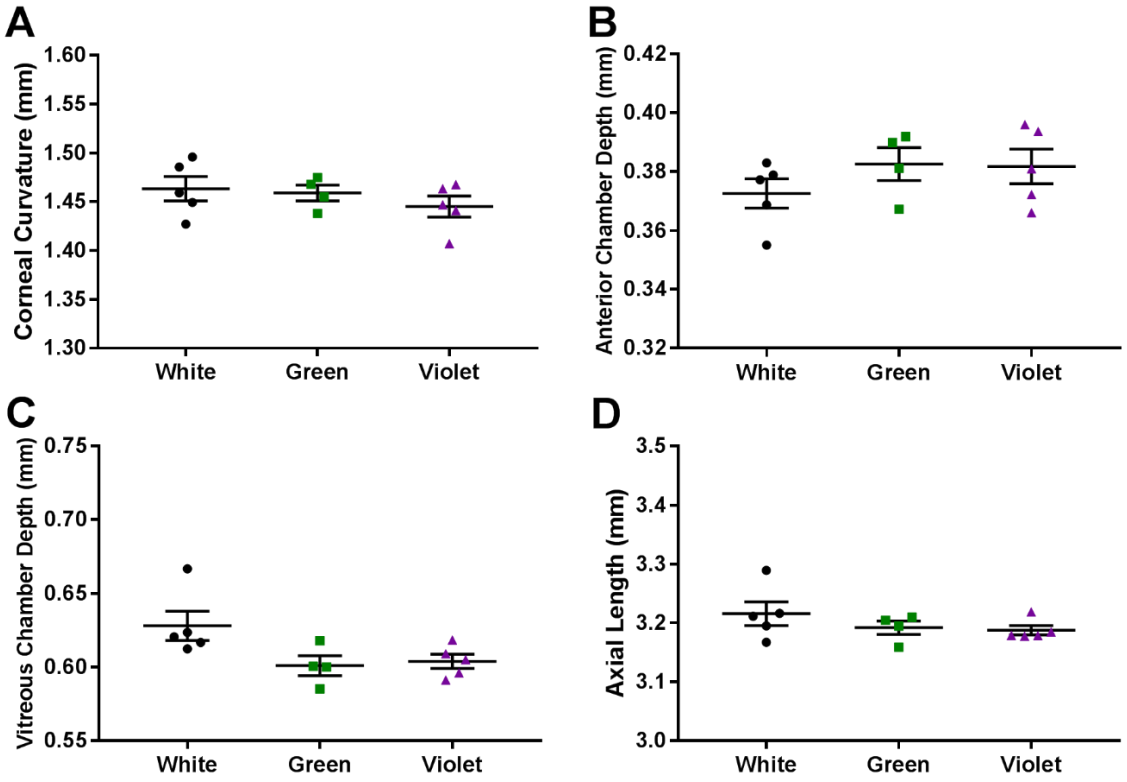

Figure 6. Ocular parameters of *Gnat2*$^{-/-}$ mice that can affect the refractive power of the eye. In *Gnat2*$^{-/-}$ mice, no differences were found between groups at P56 for **A)** curvature of the anterior cornea, **B)** depth of the anterior chamber, **C)** depth of the vitreous chamber, **D**) or axial length of the eye. Data are expressed as mean±SEM.

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