Effects of Light of Different Spectral Composition on Refractive Development and Retinal Dopamine in Chicks

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Purpose. There is ample evidence that retinal dopamine (DA) is involved in the biochemical signaling cascade that controls emmetropization, but it is unknown how its release depends on the spectral composition of ambient light. We have studied DA release, refractive development, and growth in chicken eyes that were exposed to light of different spectral bands, and had either normal vision or were covered by frosted diffusers to induce myopia.

Methods. Experiment 1: After spending the night in the dark, chicks were exposed to white room light (spectral range, 430–630 nm) or kept in the dark. Additional chicks were unilaterally exposed to blue (peak at 470 nm), red (620 nm), or UV lighting (375 nm) for 30 minutes and their fellow eyes covered with black occluders to minimize light exposure. Experiment 2: In the second experiment, chicks wore diffusers over one eye to induce deprivation myopia and were raised for 5 days in either white room light or in lighting supplied by UV, blue, or red light-emitting diodes (LEDs). Ret refractive states were recorded daily with infrared photoretinoscopy, and ocular dimensions at the start and end of the experiment with A-scan ultrasonography. DA and its metabolites were measured in retina and vitreous by high pressure liquid chromatography-electrochemical detection (HPLC-ED) in all cases.

Results. Compared to chicks kept in the dark, retinal DA and vitreal 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations were clearly elevated after 30 minutes in white light. Vitreal DOPAC was also increased in red, blue, and UV lighting, compared to the fellow eyes covered with black occluders (black occluder versus blue light: 1.31 ± 0.32 vs. 1.70 ± 0.37; red: 1.26 ± 0.35 vs. 1.64 ± 0.38; UV: 1.13 ± 0.19 vs. 1.63 ± 0.21 ng/0.1 g wet weight). Chickens developed significantly less deprivation myopia, with shorter eyes, when raised under UV and blue lighting for 5 days, compared to under red and white light. Eyes with normal vision became more hyperopic in blue and UV lighting. Vitreal DOPAC levels were lowest after 5 days of exposure to UV lighting.

Conclusions. Red, blue, and UV lighting all stimulated the release of retinal DA, but there were wavelength-dependent differences in DA release and metabolism. Less deprivation myopia developed in UV and blue lighting, compared to white and red light. The application of these findings to humans is limited by the fact that, different from chicks, humans have very low sensitivity in the near-UV region of the spectrum.

Keywords: myopia, dopamine, spectral composition, refractive development

Several studies have shown that time spent outdoors can delay the onset of myopia during childhood.1–5 It was also shown that the number of hours spent outdoors was predictive of incident myopia, independent of the physical activity level.6 Neither the longer viewing distances outdoors nor the replacement time otherwise spent with near work7 could explain the delay of myopia onset. Finally, experiments in chicks,7,8 tree shrews (Siegwart JT, et al. IOVS 2012;53:ARVO E-Abstract 3457), and rhesus monkeys9 suggested that the inhibitory effect on myopia may be due to considerably higher ambient illuminances. Whether certain spectral ranges of light are more potent in delaying and inhibiting myopia than others is a topic of ongoing research. For instance, sunlight has much more energy in the short-wavelength range than traditional artificial light bulbs, but it is not clear whether the spectral difference may have an impact on myopia development.10,11

Recently, the effects of the spectral composition of light on refractive development were studied in a number of animal models. When infant rhesus monkeys wore spectacles with red filters (transmission above 650 nm), or were raised in red light,12 they became consistently more hyperopic.13 Similarly, light from red light-emitting diodes (LEDs) represented a strong inhibitory stimulus for axial eye growth also in tree shrews,14 even in adolescent animals. In contrast, tree shrews exposed to short-wavelength light tended to become more myopic.14 Different from monkeys and tree shrews, chickens became more myopic in red and more hyperopic in blue light, which was interpreted as an attempt of the eye to compensate for longitudinal chromatic aberration.15 The effect was reversible when the light conditions were switched.16 Similarly findings were reported in fish17 and guinea pigs.18–21 Why manipulations of the spectral composition have opposite effects in chicks, guinea pigs, and fish, compared to tree shrews22 and

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rhesus monkeys, is an important open question. The lack of UV vision cannot be taken as a reason since also guinea pigs have no UV receptors.

Based on studies in humans and chickens, Torii et al. proposed that “violet” light (between 400 and 370 nm) may prevent myopia and axial elongation in children and therefore suggested that spectacle lenses and contact lenses should not incorporate UV filters. On the other hand, it was also found that lenses that cut out blue and violet light may improve low- and medium-frequency contrast sensitivity in young people and also accommodation. To date, it remains unclear how UV lighting could impact human myopia, given that the human crystalline lens transmits less than 1% of light below 400 nm.

To learn more about the effects of the spectral composition of light on myopia development and eye growth, we have studied both normal refractive development and deprivation myopia in chicks that were exposed to either near-UV illumination (peak at 375 nm), or blue (465 nm), red (620 nm), or broadband white light. Illuminances were matched to the spectral sensitivity function of the chicken. While it has been shown that white light activates DA turnover and release in retinas of several vertebrates, including chickens, it remains unknown whether certain spectral bands of light may stimulate DA metabolism and release differentially. Because DA plays an important role in light-mediated control of eye growth and myopia, this remains an important question in myopia research. In addition to DA, serotonin, a precursor of melatonin, and 5-hydroxyindoleacetic acid (HIAA), the end product of serotonin metabolism, were also measured. Serotonin modulates the response of the circadian system to light and has been implicated in the regulation of ocular growth because it was demonstrated that the pharmacologic depletion of serotonergic neurons enhances the development of FDM in chicks, although paradoxically, serotonergic antagonists have been shown to inhibit the development of lens-induced myopia in chicks.

METHODS

Animals

Seventy-seven 1-day-old male White Leghorn (strain W36) chickens were obtained from a local hatchery in Kirchberg, Germany. They were raised in temperature-controlled facilities under a 12/12-hour light/dark cycle at an illuminance of approximately 500 lux during the light phase. Lights were turned on at 8 AM and off at 8 PM. All experiments were done at the University of Tuebingen and were approved by the university commission for animal welfare. They were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Treatments

Experiment 1: In 21 chicks, 7 to 14 days old, the effects of short-term exposure to light on DA were studied. In the evening before the experiment started, chicks were moved to a dark room with no light exposure. Three of them were kept in the dark room for an additional 30 minutes ($n=3$, from 8:00 to 8:30 AM), or 2 hours ($n=3$, from 8:00 to 10:00 AM), or were kept under white room light ($430-650$ nm, 468 lux) for 30 minutes ($n=3$, from 8:00 to 8:30 AM). In these chicks both eyes were otherwise untreated. An additional 12 chicks were gently restrained by hand for 30 minutes so that the left eyes were covered with a diffuser screen that was illuminated from the back by a video projector and either a blue filter ($470$ nm, half band width [HBW] $10$ nm), a red filter ($620$ nm, HBW $15$ nm), or UV LEDs ($375$ nm, HBW $5$ nm). The right eyes, covered with a black occluder to minimize light exposure, served as references. Black occluders attenuated both UV and white light by at least 2 log units. Four chickens were used in each illumination condition, one after the other, within a time window from 8:00 to 10:00 AM. The luminance of the diffuser screen for red and for blue light was 360 cd/27.

Experiment 2: In 56 chicks, 7 days old, the effects of long-term exposure to different illumination conditions were studied. They were raised in their standard cages for 5 days under light that was provided by an arrangement of 50 equally spaced LEDs, fixed on a large plastic plate ($70 \times 50$ cm). The plate was positioned above the cage. Either UV LEDs (peak at $375$ nm, HBW 5 nm, $n=13$ chicks), blue LEDs ($465$ nm, HBW 15 nm, $n=6$ chicks), or red LEDs ($620$ nm, HBW 10 nm, $n=13$ chicks) were used. Three groups of chickens, randomly selected from the same batches, were raised under broadband white room light (range, 450-630 nm, $n=24$ chicks) and served as controls for the blue, red, or UV lighting conditions. In all chicks, the right eyes were covered with translucent plastic diffusers to induce deprivation myopia, while the uncovered fellow eyes had unobstructed vision and served as controls. Diffusers transmitted similarly in red, blue, and UV light with an attenuation of only approximately 0.1 log units. From previous studies it was known that the frosted diffusers had little effect on retinal image brightness (approximately 0.3 log units attenuation).

Matching the Subjective Brightness of the Light Sources for the Chicken

Brightness was matched using the spectral sensitivity function of the chickens as previously determined by luminance ERGs by adjusting the voltage of the LEDs, considering equivalent numbers of photoisomerizations for all three conditions. Because the chicken spectral sensitivity function is very similar to the human photopic spectral sensitivity function, except for their additional sensitivity in the near-UV spectrum, sources with no energy below 400 nm can be brightness matched simply with a photometric detector. In this case, a Minolta Candela meter (LS-100 Luminance Meter, Tokyo, Japan) was used. Illuminance provided by the blue LEDs was 435 lux, for red LEDs 453 lux, and for the white room light 468 lux. To match the brightness of the UV source, a radiometric detector was necessary (United Detector Technology, serial No. 26091; OSI, Howthorne, CA, USA). Its reading in UV lighting (3.4 relative units) was matched to the reading in the blue (3.47) and red (3.48).

Preparation of Tissue

Because retinal DA levels vary with both light levels and the diurnal cycle, preparation of retinal and vitreous samples was completed in the morning under dim red illumination before any light exposure on that day. The red light source used during preparation was spectrally broadband with a cutoff below approximately 630 nm. Animals were killed by an overdose of diethyl ether inhalation and decapitated. Eyes were immediately enucleated, cut with a razor blade perpendicular to the anterior-posterior axis, approximately 1 mm posterior to the ora serrata. The anterior segment of the eye was discarded. The vitreous was removed and immediately frozen in liquid nitrogen. From the posterior part of the eye, a biopsy punch of 8 mm was made and the retina was carefully separated under visual control of a dissecting microscope, frozen in liquid nitrogen, and stored at $-80^\circ$C. All vitreous samples were weighed and homogenized in 750 μL mobile phase (Thermo Fisher Scientific, Sunnyvale, CA, USA) while
Each retina was mixed with 350 μL mobile phase. The tissues were then disrupted using 5-mm stainless steel beads and a tissue lyser (TissuesLyser LT; Qiagen, Hilden, Germany) at 50 Hz for 5 min. Then 25 μL of the retinal homogenate was taken out for later protein determination (Pierce BCA Protein kit; Thermo Scientific, Rockford, IL, USA). The retinal and vitreous homogenates were centrifuged at 14,000 g for 10 min, the supernatant was filtered through a 0.2-μm nylon membrane sample filter (Thermo Fisher Scientific), and 25 μL was directly injected into the HPLC system.

Measurement of Biogenic Amines by High Pressure Liquid Chromatography-Electrochemical Detection (HPLC-ED)

Samples were analyzed for catecholamine and indolamine content by ion-pair reverse-phase HPLC with coulometric detection (Ultimate 3000 LC with electrochemical detection ECD3000RS; Thermo Fisher Scientific). A hypersil C18 column was used (150 × 3 mm, 3 μm) and the system was run with a test mobile phase containing 10% acetonitrile and 1% phosphate buffer (Thermo Fisher Scientific) at a flow rate of 0.4 mL/min. The potential of the first channel was set to +370 mV, the second channel to −200 mV.

Dopamine, DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid), 5-hydroxyindoleacetic acid (HIAA), and serotonin (5-HT) concentrations were determined by comparing peak areas of the samples with those of standards using Chromleon 7 chromatography data system software (Thermo Fisher Scientific). Each of the compounds in the standard solution was determined with a high correlation linearity (r² = 0.98) and good reproducibility in retention time (variance approximately 0.03%). The limit of detection was approximately 0.03%. The limit of detection was 1 pg for all analyzed metabolites.

In retina, biogenic amine content was determined as nanograms per milligram protein (ng/mg protein). In the vitreous, biogenic amines were determined relative to wet weight (ng/0.1 g wet weight).

As described by other authors, vitreous DOPAC levels are considered a sensitive measure of DA release from the retina, and the abundance of the DA metabolite DOPAC was therefore taken as a measure of DA activity.36–39 It has also been shown previously that DA is not metabolized to DOPAC in the vitreous,36 and most likely leaves it via Schlemm’s canal.

FIGURE 1. Retinal DA concentration (A) and vitreal DOPAC concentration (B) after 30 minutes of exposure to white light, and in the dark for 30 minutes or 2 hours. DA and DOPAC concentrations were much enhanced after 30 minutes of white light, but there were no differences between the 30-minute and 2-hour dark exposures (*P < 0.05; **P < 0.01; ***P < 0.001; n.s., not significant).

Measurement of Refractions and Axial Lengths

In experiment 1, no refractive or biometric data were collected. In experiment 2, refractions were measured daily using an automated version of infrared photoretinoscopy.40 A-scan ultrasonography41 was used to measure axial length before and after treatment with the diffusers.

Statistics

Statistical analyses were done using commercial software JMP 13 (SAS Institute, Cary, NC, USA). Distributions of data on biogenic amines were tested for normality. For the comparison of two independent groups, a 2-tailed unpaired t-test was used. Differences in occluded and contralateral open eyes were compared using 2-tailed paired t-tests. One-way analysis of variance (ANOVA) was used for comparisons among multiple groups, followed by a Tukey-Kramer honestly significant difference (HSD) test for post hoc analysis. Average data are expressed as mean ± SEM. The limit of significance was set to P < 0.05.

RESULTS

Experiment 1: Changes in Retinal DA and Vitreal DOPAC Concentrations Induced by Short-Term Exposure to White Light

On the day of experiment, chicks were kept in the dark until exposed to white room light for 30 minutes, or kept in the dark room for an additional 30 minutes (from 8:00 to 8:30 AM) or 2 hours (8:00 to 10:00 AM). Compared to chicks kept in the dark, retinal DA concentration was significantly increased after 30 minutes of white light exposure (white light versus “dark 30 min” and “dark 2 h”: 2.00 ± 0.16 vs. 1.30 ± 0.17 and 1.16 ± 0.21 ng/mg protein, ANOVA followed by a Tukey-Kramer HSD test, both P < 0.05, n = 6 eyes; Fig. 1A). The same was true for vitreal DOPAC concentration (1.88 ± 0.25 vs. 1.02 ± 0.21 and 1.11 ± 0.11 ng/0.1 g wet weight, both P < 0.05, n = 6 eyes; Fig.
1B). There was no difference in the amount of retinal DA or vitreal DOPAC content between chicks kept in the dark for an additional 30 minutes or 2 hours.

Changes in Retinal DA and Vitreal DOPAC Concentrations After Short-Term Exposure to UV, Blue, and Red Lighting

In uncovered eyes, retinal DA levels were elevated after short-term exposure to UV, blue, and red lighting compared to the contralateral eyes treated with black occluders (blue light: $2.45 \pm 0.11$ vs. $1.95 \pm 0.04$ ng/mg protein; red light: $2.10 \pm 0.08$ vs. $1.91 \pm 0.04$ ng/mg protein; UV lighting: $1.34 \pm 0.13$ vs. $1.01 \pm 0.22$ ng/mg protein, paired t-test, all $P < 0.05$; Fig. 2A). Retinal DOPAC concentrations were significantly higher in red light than in fellow eyes covered with black occluders (Fig. 2C). There was also a trend in the same direction in blue and UV lighting but it did not achieve statistical significance (blue light versus black occluder, paired t-test; $P = 0.06$; UV lighting versus black occluder, paired t-test; $P = 0.07$). An interesting finding was that the retinal DA level was significantly higher after short-term exposure to blue compared to UV light ($P < 0.001$, ANOVA, followed by a Tukey-Kramer HSD test, $n = 4$ chicks per group) but similar to the amount found after red light exposure ($P = 0.28$). In addition, retinal DA levels were significantly lower in the eyes covered with black occluders in the UV lighting group (Uv-Occ versus Blue-Occ, $P < 0.001$; Uv-Occ versus Red-Occ, $P < 0.001$). Different from DA, HIAA as well as HVA and 5-HT content was similar in eyes exposed to different light spectra, as well as in their fellow eyes covered with dark occluders (see Supplementary Table S1). In the vitreous, DOPAC levels were always significantly higher in eyes exposed to light, compared to the fellow eye covered with a black occluder (blue: $1.70 \pm 0.37$ vs. $1.31 \pm 0.32$ ng/0.1 g wet weight, $P < 0.01$; red: $1.64 \pm 0.38$ vs. $1.26 \pm 0.33$ ng/0.1 g wet weight, $P < 0.01$; UV: $1.63 \pm 0.21$ vs. $1.13 \pm 0.19$ ng/0.1 g wet weight, $P < 0.001$, paired t-test, $n = 4$; Fig. 2D).
Experiment 2: Effects of Red and White Light on Myopia Development and DA Metabolism

When chicks were reared in red light ($n = 13$) under a 12/12-hour light/dark cycle for 5 days (day 8 to 13), they developed $-10.53$ diopters (D) of relative myopia in their occluded eyes (baseline refraction 3.38 ± 0.04 D, after deprivation −7.15 ± 0.70 D). In white light they developed $-9.53$ D of myopia (baseline refraction 2.65 ± 0.22 D, after deprivation −7.98 ± 0.85 D; Fig. 3A). Obviously, there was little difference in the amount of induced myopia. Also the increase in axial length in the deprived eyes was similar in red and white light (from 7.72 ± 0.06 to 8.95 ± 0.08 mm and from 7.78 ± 0.06 to 8.93 ± 0.08 mm) while their fellow control eyes grew from 7.80 ± 0.04 to 8.37 ± 0.06 mm and 7.83 ± 0.05 to 8.41 ± 0.06 mm, respectively (Fig. 3B). Refractions in control eyes in red light changed only slightly during the 5 days, becoming less hyperopic (in red light from 3.39 ± 0.05 to 2.64 ± 0.06 D, in white light from 2.66 ± 0.23 to 2.70 ± 0.12 D, Fig. 3A). As expected, differences in refraction and axial length between control and deprived eyes were highly significant in all cases ($P < 0.001$, paired $t$-test).

Both in red and white light, retinal DOPAC levels were reduced in deprived eyes, relative to their contralateral eyes, from 0.68 ± 0.14 to 0.51 ± 0.11 ng/mg protein (red light) and 0.63 ± 0.13 to 0.51 ± 0.10 ng/mg protein (white light) (Fig. 3C). In the vitreous, DA, DOPAC, and HVA levels were significantly reduced in deprived eyes relative to their fellows (red light: DA from 0.60 ± 0.08 to 0.32 ± 0.06 ng/0.1 g wet weight; white light: DA from 0.41 ± 0.05 to 0.24 ± 0.03 ng/0.1 g wet weight, paired $t$-test: $P < 0.01$ in both cases) (Fig. 3D). For HIAA and 5-HT, no differences were found between control and deprived eyes (see Supplementary Table S2).
Effects of Blue Light on Myopia Development and DA Metabolism

Six chicks were exposed to blue lighting and 5 chicks to white lighting, with their treatments as described above for red and white light. After 5 days, deprived eyes developed $-4.99 \pm 0.05$ D relative myopia in blue light (from $3.48 \pm 0.05$ to $-1.51 \pm 0.51$ D). At the same time, the animals in white light developed $-7.5 \pm 0.02$ D relative myopia (from $3.40 \pm 0.02$ to $-4.10 \pm 0.85$ D; Fig. 4A). This difference in induced deprivation myopia in blue and white light was significant ($P < 0.05$, unpaired t-test). Control eyes became approximately 1 D more hyperopic under blue lighting (from $3.53 \pm 0.06$ to $4.50 \pm 0.10$ D), while their hyperopia was reduced in white light (from $3.46 \pm 0.05$ to $2.68 \pm 0.17$ D, Fig. 4A). This difference in refraction in the control eyes was highly significant (day 5; unpaired t-test, $P < 0.001$). On the other hand, changes in axial length over the 5 days of exposure to blue and white light were not significantly different: Axial length increased from $7.90 \pm 0.06$ to $8.75 \pm 0.08$ mm in deprived eyes in blue light, and from $7.95 \pm 0.05$ to $8.89 \pm 0.08$ mm in white light (Fig. 4B, unpaired t-test, n.s.). Control eyes grew over the treatment period from $7.87 \pm 0.04$ to $8.37 \pm 0.06$ mm in blue light and from $7.97 \pm 0.05$ to $8.47 \pm 0.06$ mm in white light (Fig. 4B, unpaired t-test, n.s.).

After 5 days in blue light, retinal DOPAC and DA levels were significantly reduced in the deprived eyes relative to their fellow eyes, from $0.44 \pm 0.09$ to $0.17 \pm 0.02$ ng/mg protein (DOPAC) and $3.25 \pm 0.24$ to $2.4 \pm 0.24$ ng/mg protein (DA) ($P < 0.05$ in both cases, paired t-test, Fig. 4C). In white light, only retinal DOPAC concentrations declined from $0.34 \pm 0.05$ to $0.25 \pm 0.03$ ng/mg protein ($P < 0.05$, paired t-test). In the vitreous, DA, DOPAC, and HVA levels were reduced in diffuser-covered eyes in blue light (DA from $0.32 \pm 0.03$ in control eyes to $0.22 \pm 0.04$ ng/0.1 g wet weight, $P < 0.01$; DOPAC: from $2.34 \pm 0.14$ to $1.20 \pm 0.19$ ng/0.1 g wet weight, $P < 0.001$;
HVA: from 1.62 ± 0.07 to 1.02 ± 0.11 ng/0.1 g wet weight, \( P < 0.01 \); paired \( t \)-test, Fig. 4D). Vitreal DOPAC and HVA levels were also lower in the diffuser-covered eye in white light whereas DA levels did not change. For HIAA and 5-HT, no differences were found between the two eyes (see Supplementary Table S2).

**Effects of UV Lighting on Myopia Development and DA Metabolism**

When chicks (\( n = 13 \)) were exposed to UV lighting for 5 days, they developed significantly less deprivation myopia than chicks exposed to white light (change in refraction day 0 to 5: \(-5.07 \) vs. \(-7.88 \) D; \( P < 0.01 \), unpaired \( t \)-test). At the same time, control eyes became approximately 1 D more hyperopic in UV lighting (from 3.31 ± 0.04 to 4.31 ± 0.10 D), with little change in white light (from 3.37 ± 0.03 to 3.02 ± 0.07 D, Fig. 5A). The difference in refraction in the control eyes was highly significant (day 5: unpaired \( t \)-test, \( P < 0.001 \)). Deprived eyes grew considerably less in UV lighting (from 7.82 ± 0.03 to 8.90 ± 0.05 mm) than in white light (from 7.80 ± 0.03 to 9.02 ± 0.08 mm, Fig. 5B, unpaired \( t \)-test, \( P < 0.05 \)). Control eyes grew from 7.82 ± 0.03 to 8.57 ± 0.04 mm in white light from 7.82 ± 0.03 to 8.49 ± 0.07 mm; these changes were not significantly different (Fig. 5B, unpaired \( t \)-test, n.s.). Anterior chamber depth was also significantly shorter in the deprived eyes of animals kept under UV lighting compared to animals in the white light group (UV: 0.96 ± 0.018 mm; white light group: 1.04 ± 0.051 mm, unpaired \( t \)-test, \( P < 0.05 \)).
Spectral Control of Myopia and Retinal Dopamine in Chicks

After 5 days of exposure to UV lighting, retinal DOPAC and DA content was reduced in deprived eyes (DOPAC from 0.58 ± 0.06 in control eyes to 0.39 ± 0.05 ng/mg protein in deprived eyes, P < 0.001; DA from 4.59 ± 0.36 to 3.86 ± 0.32 ng/mg protein, P < 0.05, paired t-test, Fig. 5C). In white light, only retinal DOPAC content was reduced (0.80 ± 0.09 to 0.58 ± 0.08 ng/mg protein, P < 0.01, paired t-test). No differences were found between deprived and control eyes for HVA and 5-HT (see Supplementary Table S2). Vitreal DA, DOPAC, and HVA levels were significantly reduced in deprived eyes, under both UV and white lighting conditions (UV: DA 0.34 ± 0.08 in control eyes to 0.08 ± 0.02 ng/0.1 g wet weight in deprived eyes; DOPAC from 2.49 ± 0.10 to 1.85 ± 0.08 ng/0.1 g wet weight; HVA from 1.88 ± 0.07 to 1.33 ± 0.11 ng/0.1 g wet weight, all P < 0.001, paired t-test, Fig. 5D).

Comparative Analysis of the Effect of Light With Different Spectral Compositions on Eye Growth and Catecholamine Metabolism

To directly compare the effects of all lighting conditions, we performed a combined analysis of all treatment groups (Figs. 3, 4, 5). As shown in Figure 6A, blue and UV lighting had an inhibitory effect on the development of deprivation myopia, since the difference in refraction between deprived and open eyes (Δ = deprivation-control eyes) was less in blue and UV lighting than in red and white light (ΔUV: −6.10 ± 0.52 Δblue: −5.96 ± 0.43 D, Δred: −9.79 ± 0.66 D, Δwhite: −8.87 ± 0.64 D; ΔUV versus Δred and Δwhite, P < 0.01 in both cases; Δblue versus Δred and Δwhite, P < 0.02 and P < 0.07, ANOVA, followed by a Tukey-Kramer HSD test, Fig. 6A). In previous studies, vitreal DOPAC content was used as an index of retinal DA release. Since it was sometimes difficult to measure the amount of vitreal DOPAC due to technical reasons (an unknown substance coeluted at a similar retention time), we also analyzed HVA and found that the vitreal HVA level was significantly correlated with vitreal DOPAC content (Fig. 6E). Furthermore, we compared the effect of light with different spectral composition on the ΔDA level in retina and ΔDOPAC and ΔHVA content in vitreous. As shown in Figure 6B, retinal DA level dropped due to diffuser wear in UV and blue lighting but the drop was lacking in red and white light, although this result was not significant after correction for multiple comparisons (ΔUV: −0.71 ± 0.30 ng/mg protein, Δblue: −0.85 ± 0.32 ng/mg protein, Δred: 0.09 ± 0.35 ng/mg protein, Δwhite: 0.16 ± 0.19 ng/mg protein, ANOVA P = 0.03, followed by a Tukey-Kramer HSD test, n.s.; Fig. 6B). On the other hand, the drop in the DA metabolites DOPAC and HVA was more pronounced with diffuser wear in the white light, compared to UV lighting. There was also a trend of higher DA level in retina and ΔDOPAC content, with blue light stimulating more DA release in deprived eyes compared to UV lighting. There was also a trend of higher vitreal DA release. Since it was sometimes difficult to measure refractions in several meridians to determine astigmatism, but the relatively small changes in spherical refractions in our study normally do not cause distortions in the optics of cornea and lens.

A combination of absolute levels of DA and its metabolites in the chicks kept under light of different spectral composition has limited value because of the high interindividual and interbatch variability of these variables. Nevertheless, we found that short-term exposure (30 minutes) to light of different spectral composition generally increased the amount of vitreal DOPAC content, with blue light stimulating more DA release in the retina than UV lighting. After long-term exposure (5 days), a trend toward a higher retinal DA release was found in UV lighting, compared to white light (P = 0.06, 1-way ANOVA followed by a Tukey-Kramer HSD test; Fig. 5C versus Figs. 4C, 5C), even though the luminances were matched to the chicken spectral sensitivity function. In line with earlier studies, retinal and vitreal catecholamine levels dropped when eyes were covered with diffusers, although it is striking that less deprivation myopia in UV and blue lighting is associated with the largest changes in retinal DA (Figs. 6A, 6B). In general, the consistent changes in retinal and vitreal catecholamine content with diffusers and light of different spectral composition emphasize a role of dopaminergic signaling in light-mediated changes in eye growth. In contrast to the results for catecholamines, diffusers and light of different wavelengths influenced neither the amount of 5-HT nor of the end product of 5-HT metabolism, HIAA.

Effects of Blue and Red Light on Refractive Development in Chicks in Previous Studies

We found that eyes with unobstructed vision became 1 D more hyperopic in blue and 0.7 D more myopic in red light over the 5 days of treatment. Also after exposure to UV lighting, eyes became approximately 1 D more hyperopic. Similar effects had previously been observed. Seidemann and Schaeffel found that chickens became more hyperopic in blue light (430 nm, HBW 15 nm) in 2 days and more myopic in red light (615 nm, HBW 15 nm). The differences approximately matched the longitudinal chromatic aberration function, which was assumed to be the reason for the changes in refraction. Also Foulds et al. studied a similar question in chickens. However, in contrast to the study by Seidemann and Schaeffel and the current study, they observed progressive hyperopia in the blue and progressive myopia in the red light, which is not explained by longitudinal chromatic aberration. Recently, Rucker et al. also investigated the role of blue light, although their work was focused on the effect of blue light deficiency, which often occurs in indoor illuminations, and on temporal sensitivity to...
FIGURE 6. (A) Difference in refraction between deprived and fellow eyes. Less myopia was induced in UV and blue lighting than in red and white light. (B) The drop in retinal DA due to diffuser wear appeared pronounced in UV and blue lighting but was not detectable in red and white light. (C) The drop of vitreal DOPAC, a measure of DA turnover, was larger with diffusers in white light, compared to UV lighting. (D) The drop of vitreal HVA, another measure of DA turnover, was also larger in white light, compared to UV lighting. (E) HVA content in the vitreous was significantly correlated with the amount of vitreal DOPAC.
blue light. They found that “at low temporal frequencies the eyes responded to the focal planes of the illuminant growing less when blue light was present” and concluded that “illuminants rich in blue light can protect against myopic eye growth when the eye is exposed to slow changes in luminance contrast as might occur with near work” (p. 6128). While we find that blue and UV lighting indeed reduces deprivation myopia development in the chicken, our light sources were not modulated in time so the statement by Rucker et al. does not directly apply.

Role of Melanopsin Signaling in the Blue Light–Triggered Changes in DA Metabolism and Inhibition of Myopia

In the current study, differences in DA metabolism cannot be explained by differences in subjective brightness. The question remains as to why UV and blue lighting stimulate larger changes in retinal DA. It could be that another blue-sensitive photopigment is involved, as two orthologues of “nonvisual” photopigments were previously located to the chicken retina: Opn3im (mammalian-like melanopsin) and Opn4-x (xenopus-like melanopsin). Both were found in a subset of ganglion cells, but Opn4-x was also located to the horizontal cells. Melanopsins drive diurnal cycles in retina and pineal organ but do not mediate spatial vision. Accordingly, they affect retinal DA and melatonin cycles. There is some probability that they also mediated the spectrally different light-dependent effects on DA metabolism and refractive development in our study, since it is known that melanopsin-containing retinal ganglion cells (mRGCs) signal to dopaminergic amacrine cells via their axon collaterals. Schaeffel et al. (Schaeffel E et al. IOVS 2016;57;ARVO E Abstract 2494) have studied whether the strength of the melanopsin input may predict myopia in humans. Melanopsin input was quantified in each subject with a red–blue pupil response paradigm. Since myopia is generally inhibited by bright light, it could be that those subjects who respond with a strong melanopsin response may also become less myopic. However, no correlation could be found. Similarly to human melanopsin, which has a peak absorption at 479 nm, also chicken melanopsins have a peak absorption at 476 to 484 nm or 475 nm. Interestingly, in our study prominent effects of UV lighting at 375 nm were found. Since the UV LEDs did not have energy above 400 nm, they should not have stimulated melanopsin, in particular because the brightness in UV was reduced to match the spectral sensitivity function of the chickens. Chicks are highly light sensitive around 370 nm due to their UV receptor but their peak sensitivity lies between 500 and 600 nm. While melanopsins could have mediated the inhibitory effect on myopia of blue light in the current study, they cannot explain the inhibition by UV radiation. Interestingly, other “opsin-like” genes have recently been identified in the neural retina and in many other tissues. Among them is opn5, also called neuropsin. Heterologously expressed chicken opn5 was shown to bind retinaldehyde to form a UV-sensitive photopigment. Chicken Opn5 is a bistable pigment with absorption maxima of 360 and 474 nm, respectively, and was localized within some types of amacrine cells and some cells in the ganglion cell layer of the retina. Yamashita et al. observed that opn5-positive amacrine cells were located in the vicinity of the dopaminergic amacrine cells and hypothesized that neuropsin-positive amacrine cells might regulate the DA release from dopaminergic amacrine cells in a UV radiation-dependent manner. They suggested that, in chicken retinas, “UV-sensitive opn5 and blue-sensitive melanopsin, may concertedly control the activity of dopaminergic amacrine neurons, resulting in retinal adaptation” (p. 22088). The inhibitory effect of UV lighting on myopia might therefore possibly be explained by neuropsin having a stimulatory effect on DA release and refractive development.

Surprisingly, Rohrer and colleagues found that emmetropization failed in chicks raised with lenses in light of 385 nm with a half bandwidth of 24 nm at 66 mW/m². It is difficult to convert radiometric energy units into photometric units as used in the current study, but the most likely explanation for the discrepancy is the much lower brightness used in the study by Rohrer et al. This explanation is also supported by a recent paper of Hammond and Wildsoet in which the authors found that the sampling by the UV cone network in chicks may have sufficient spatial resolution to detect optical defocus and to guide emmetropization, but that either a high amount of defocus or low illumination may compromise this process.

Can Our Findings Support the Idea of a Potentially Inhibitory Effect of “Violet” Light on Human Myopia?

Torii et al. have proposed that near-UV lighting (“violet” light) can inhibit myopia progression in children. Different from humans, chicks have near-UV vision and their ocular media, which transmit light down to at least 360 nm. Therefore, it remains unclear how “violet” light could stimulate retinal S-cones in humans by a significant amount (peak absorption of the S-cone photopigment at approximately 455 nm) when less than 1% of violet light is transmitted through the crystalline lens below 400 nm. Furthermore, in two mammalian models (rhesus monkey and tree shrew), red light was found to stimulate hyperopia development, while blue light tended to make eyes more myopic at least in one of the models (tree shrew). Therefore it could be that inhibition of eye growth by blue light is typical for only some animal models (chick, guinea pig) and extrapolation to humans may be difficult. Data covering the effects of exposure to near-UV lighting on refractive development are not yet available for mammalian models.

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