

The Relation Between Light-Induced Lacrimation and the Melanopsin-Driven Postillumination Pupil Response

Shaobo Lei,¹ Herbert C. Goltz,¹⁻³ Xingqiao Chen,³ Marija Zivcevska,¹ and Agnes M. F. Wong¹⁻⁴

¹Program in Neurosciences and Mental Health, The Hospital for Sick Children, Toronto, Ontario, Canada

²Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Ontario, Canada

³The Krembil Research Institute, Toronto Western Hospital, Toronto, Ontario, Canada

⁴Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada

Correspondence: Agnes M. F. Wong, Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada; agnes.wong@sickkids.ca.

HCG and AMFW are joint senior authors.

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PURPOSE. To investigate the chromatic characteristics and intensity-response function of light-induced reflex lacrimation and its correlation with the melanopsin-driven postillumination pupil response (PIPR).

METHODS. Eleven visually normal participants completed the experiment. Lacrimation was measured in one eye by placing a calibrated filter paper strip in the conjunctival sac over a 1 minute-interval (Schirmer's test) during which participants received either no light stimulation (baseline trial) or one flash of blue or red light stimuli presented binocularly with a Ganzfeld stimulator, while the pupil response was recorded simultaneously from the fellow eye by using an eye tracker. Light stimulation trials were presented in alternating fashion at seven incremental intensity steps (0.1, 1, 3.16, 10, 31.6, 100, and 400 cd/m²). Postillumination pupil response was defined as the mean pupil constriction from 10 to 30 seconds post illumination.

RESULTS. The amount of lacrimation in response to 10 to 400 cd/m² blue light was significantly greater than baseline and increased monotonically with increasing light intensity. Red light did not induce significant reflex lacrimation until the brightest stimulation at 400 cd/m². There was a positive linear correlation between PIPR and lacrimation in response to blue light ($r = 0.74$, $P < 0.001$) but not to red light ($r = 0.13$, $P = 0.25$).

CONCLUSIONS. The chromatic characteristics and intensity-response of light-induced lacrimation are highly consistent with the features of melanopsin phototransduction. This finding is the first in vivo evidence in humans, supporting the hypothesis that light-induced reflex lacrimation is mediated primarily by melanopsin photoactivity, and provides new insight into the putative mechanisms of photophobia.

Keywords: lacrimation, melanopsin, photophobia, pupil light reflex, postillumination pupil response

Ophthalmic trigeminal nociceptive sensation can evoke lacrimation, a protective autonomic reflex that serves to lubricate the eye and dilute or flush away noxious stimulants.¹ Intriguingly, reflex lacrimation can also be induced by bright light stimulation to the eye,² a prominent symptom in some patients with photophobia,^{3,4} which is a sensory state in which light causes discomfort or pain in the eye or head.⁴ Commonly seen in a wide range of ocular and neurologic disorders, including migraine, posttraumatic brain injury syndrome, corneal abrasion, keratitis, and uveitis,⁵ photophobia is clinically important, but it is a subjective complaint that is often difficult to substantiate. An objective index may be useful in diagnosing and monitoring the underlying etiologies of photophobia. Some studies^{2,6,7} have used light-induced lacrimation in rodent models as a surrogate measure for light sensitivity. However, to the best of our knowledge, light-induced lacrimation has not been studied in humans.

The mechanisms by which light causes pain have been a topic of active research. There is a growing body of evidence^{2,4,6,8,9} suggesting that melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) may play a

critical role in the afferent limb for photophobia. Intrinsically photosensitive RGCs are a third subset of photoreceptors that mediate the light irradiance detection pathway that gives rise to a range of nonimage-forming photoresponses including the pupillary light reflex and circadian photoentrainment.¹⁰⁻¹² It has long been observed that photophobia is present in some patients with severe outer retinal dystrophies who have no light perception.⁹ Additionally, light aversion is present in newborn rats whose melanopsin system is already functional when rods/cones are not.¹³ Multiple studies^{4,8,9,14-16} have shown that ipRGCs converge on the trigeminal nociceptive pathway. Most recently, a small subset of ophthalmic trigeminal ganglion cells have been found to contain melanopsin and are intrinsically photosensitive as well.¹⁶ Collectively, this evidence strongly suggests that photophobia may be a result of melanopsin photoactivity superimposed on the trigeminal nociceptive sensory pathway.

As an initial step to evaluate the utility of light-induced lacrimation as an objective index for patients with photophobia, we sought to determine whether this reflex is indeed a melanopsin-driven response from two well-established unique



TABLE. Melanopic Illuminance of Chromatic Light Stimuli (Melanopic lux)

Stimulus Color	0.1 cd/m ²	1.0 cd/m ²	3.16 cd/m ²	10 cd/m ²	31.6 cd/m ²	100 cd/m ²	400 cd/m ²
Blue	0.95	9.48	29.96	94.82	299.63	948.21	3792.85
Red	<0.01	<0.01	0.01	0.02	0.06	0.20	0.80

photosensory properties of melanopsin: first, it has a narrow spectral sensitivity peaking at approximately 470 nm (blue light),¹⁷ and second, once melanopsin is activated, it drives a prolonged depolarizing after-potential of the ipRGCs, leading to a sustained pupil constriction after the offset of light stimulation,^{12,18} that is, the postillumination pupil response (PIPR). We hypothesized that if light-induced reflex lacrimation is melanopsin driven, the amount of tear production would be positively correlated with the PIPR induced by increasing blue light stimulation, but not with that induced by melanopsin-silent red light stimulation.

METHODS

Participants

All potential participants underwent an eye examination, including visual acuity, refractive error, color vision, ocular motility, slit-lamp, a nondilated fundus examination, and a standard 5-minute Schirmer's test I, which involved placing a 5 × 35-mm narrow filter paper strip into the inferior fornix of the eye without using anesthetic—a commonly used clinical test to measure both basal and reflex lacrimation—to screen for dry eye.¹⁹ Those with less than 10 mm of tear production on screening Schirmer's test I were excluded. A total of 11 participants with normal eye examinations were included in the study (six males, mean age 25 years, age range 18–42 years). Informed consent was obtained from each participant. The study was approved by the Research Ethics Board at The Hospital for Sick Children, Toronto, Canada. All the procedures adhered to the guidelines of the Declaration of Helsinki.

Experimental Conditions and Procedure

The experiment comprised 15 trials of 1-minute anesthetized Schirmer's test II on the right eye while the pupil response was simultaneously recorded from the left eye. Each session began with 10 minutes of dark adaptation in a quiet darkened room. Participants wore a spectacle frame-mounted eye tracker (Arrington Research, Scottsdale, AZ, USA), which used near-infrared (940 nm) illuminating diodes and miniature infrared cameras to record the changes in pupil diameter at a sampling rate of 60 Hz. Participants were seated in front of the Ganzfeld stimulator (Espion V5 system with the ColorDome LED full-field stimulator; Diagnosys LLC, Lowell, MA, USA). This stimulator is equipped with multiple narrow spectral band-width light-emitting diodes (LEDs). The light sources selected for this study were blue light (peak wavelength: 470 nm, full width at half maximum: 31 nm) and red light (peak wavelength: 635 nm, full width at half maximum: 22 nm), with specifications of LED light sources supplied by Diagnosys. For each trial, the right eye of the participant was anesthetized by instilling topical 0.5% proparacaine eye drops (Alcon, Fort Worth, TX, USA). Thirty seconds after instillation of the eye drops, excess fluid in the conjunctival sac was removed by gentle blotting of the eye with tissue paper before placing the Schirmer's strip into the inferior fornix. Immediately after the placement of the Schirmer's strip, participants were instructed to position their head on the chin rest of the Ganzfeld bowl with both eyes open for 1 minute. At 20 seconds after trial

onset, the participant received either no light stimulation (baseline trial), or one flash of blue or red light stimuli of 400-ms duration. The Schirmer's strip was removed after 1 minute, and the length of the moisture on the strip was measured in millimeters, providing the index for tear production. Because the eye was anesthetized, the Schirmer's strip would not induce reflex tearing, so the measurement in the baseline trial represented basal tear production, and the tear production above the baseline level in red and blue light conditions was attributable to light stimulation. The baseline trial was presented first, then red and blue light stimulation trials were presented in alternating fashion at seven incremental steps of intensity (0.1, 1, 3.16, 10, 31.6, 100, and 400 cd/m²); each condition was presented once, making an experiment 15 trials in total. We calculated melanopic illuminance for both chromatic conditions across all intensity steps by using the method proposed by Lucas and colleagues.²⁰ The values are reported in the Table.

Data Processing and Analysis

The left eye pupil diameter data from the eye tracker were analyzed offline by using a custom-written script (MatLab; MathWorks, Inc., Natick, MA, USA). A median (window width of 0.5 second) and low-pass (fourth-order, zero-phase Butterworth) filter with a cutoff frequency of 5 Hz were applied to remove eye blink artifacts. The filtered data were inspected visually in a graphical user interface to ensure data quality and detect artifacts. The data were then normalized to the baseline pupil diameter, calculated from the mean pupil diameter during a 50-second period before the onset of each stimulus (i.e., normalized pupil diameter = absolute pupil diameter/baseline pupil diameter). Postillumination pupil response was defined as the mean pupil constriction over a 20-second interval from 10 to 30 seconds after the offset of light stimulation. This PIPR represents melanopsin photoactivity, as validated in the literature.^{21–24}

Statistical analyses were performed with Sigmaplot 11.0 software (Systat Software, Inc., San Jose, CA, USA). The dataset passed the equal variance test (Levene's test, $P > 0.05$) and normality test (Shapiro-Wilk test, $P > 0.05$) within each combination of factors. Differences in tear production and PIPR in response to different stimulation conditions were then compared by using 2-way repeated measures ANOVA with two factors: stimulation wavelength (two levels: blue and red) and stimulation intensity (seven levels: 0.1, 1, 3.16, 10, 31.6, 100, and 400 cd/m²). Post hoc Tukey's tests were performed to adjust for multiple comparisons. Pearson's correlation coefficient was used to investigate the correlation between the amount of tear production and PIPR. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Light-Induced Lacrimation

Mean tear production in 11 participants in response to the chromatic and intensity conditions is shown in Figure 1. Tear production was significantly greater with blue light stimulation than red ($F_{1,10} = 47.12$, $P < 0.001$) and increased as light

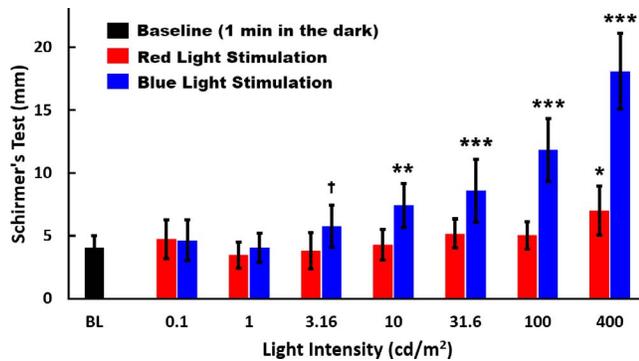


FIGURE 1. Mean tear production measured by 1-minute anesthetized Schirmer's test from 11 visually normal participants, including baseline and seven intensity steps using both blue and red light in 15 tested conditions. Error bars represent 95% confidence intervals. In the baseline condition (BL), no light stimulation was presented. In all other conditions, one flash of red or blue light stimulation was presented at 20 seconds after trial onset for 400 ms. Asterisks indicate significantly greater tear production than baseline (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). †Significantly greater tear production in blue light than red light condition with the same intensity step ($P < 0.05$).

intensity increased ($F_{7,70} = 35.46$, $P < 0.001$). There was also a statistically significant interaction between stimulation wavelength and intensity ($F_{7,70} = 18.92$, $P < 0.001$). Low-intensity (0.1–3.16 cd/m^2) red and blue light stimulation did not induce more tear production above baseline. However, when the intensity reached 10 cd/m^2 and above, blue light induced significantly higher amounts of tearing than red light. Comparisons of stimulus intensity among the blue light conditions showed that 10 to 400 cd/m^2 blue light stimulation induced statistically significant increases in tear production over baseline (post hoc pairwise comparison, $P < 0.01$). In contrast, red light stimulation did not induce tear production significantly above baseline until the highest intensity was reached at 400 cd/m^2 .

Postillumination Pupil Response

The mean pupil response is summarized in Figures 2 and 3, while the PIPR is plotted as a response-intensity function in Figure 4. Similar to light-induced lacrimation, PIPR was significantly greater with blue light stimulation than red ($F_{1,10} = 85.03$, $P < 0.001$) and increased as light intensity

increased ($F_{7,70} = 19.41$, $P < 0.001$). There was also a statistically significant interaction between stimulation wavelength and stimulus intensity ($F_{7,70} = 21.22$, $P < 0.001$). Again, similar to light-induced lacrimation, low-intensity (0.1–3.16 cd/m^2) blue light stimulation induced minimal PIPR, whereas higher-intensity blue light (≥ 10 cd/m^2) induced steadily increasing PIPR, which was significantly greater than that of baseline and red light conditions. Red light did not induce statistically significant PIPR at any intensity steps. These PIPR results are consistent with our previous report.²²

Correlation Between Light-Induced Lacrimation and PIPR

Pearson product-moment correlation analysis on all individual trials demonstrated a moderate linear correlation between the PIPR and amount of tear production in response to blue light (Pearson's $r = 0.74$, $P < 0.001$, $n = 77$; Fig. 5A); in contrast, no significant correlation was found between the PIPR and amount of tear production in response to red light (Pearson's $r = 0.13$, $P = 0.25$, $n = 77$; Fig. 5B). Pearson correlation analysis on mean PIPR and mean tear production across 11 participants revealed a strong positive linear correlation within the blue light conditions (Pearson's $r = 0.98$, $P < 0.001$, $n = 7$; Fig. 5C), but again, no significant correlation within red light conditions (Pearson's $r = 0.70$, $P = 0.08$, $n = 7$; Fig. 5D).

DISCUSSION

We designed a novel "Schirmer's test + chromatic pupillometry" experimental paradigm to study the chromatic characteristics and response kinetics of light-induced lacrimation by using both melanopsin-activating blue light and melanopsin-silent red light stimulation. The results demonstrated that the blue light-induced reflex lacrimation emerged at approximately 10 cd/m^2 and increased monotonically with increasing stimulus intensity up to 400 cd/m^2 . In contrast, red light induced no or minimal reflex lacrimation in all tested intensity steps. The chromatic properties and kinetics of these responses strikingly mimic those of the PIPR, an in vivo index of melanopsin photoactivity,²² and correspond closely to the observed dynamic range of melanopsin in vitro.^{10,11} It is somewhat surprising that 400 cd/m^2 red light induced a small but statistically significant increase of tear production, but the 400 cd/m^2 red PIPR is not significantly greater than baseline. We can speculate with two possible explanations for this

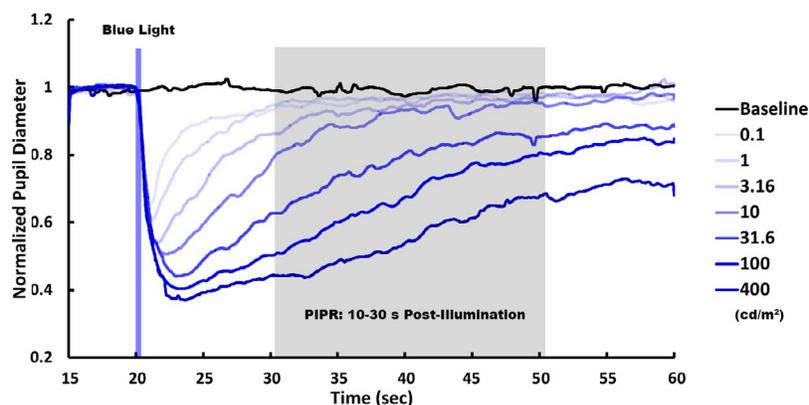


FIGURE 2. Mean pupillary response time courses recorded simultaneously from the left eyes of 11 visually normal participants in baseline and blue light conditions. The x-axis represents the actual time course of the 1-minute Schirmer's test. The light stimuli were presented in 20 seconds after trial onset for a duration of 400 ms. Pupil diameter data (y-axis) were normalized to the mean pupil diameter over the 5-second period before the onset of light stimuli. The gray area represents the time interval over which the PIPR index was computed.

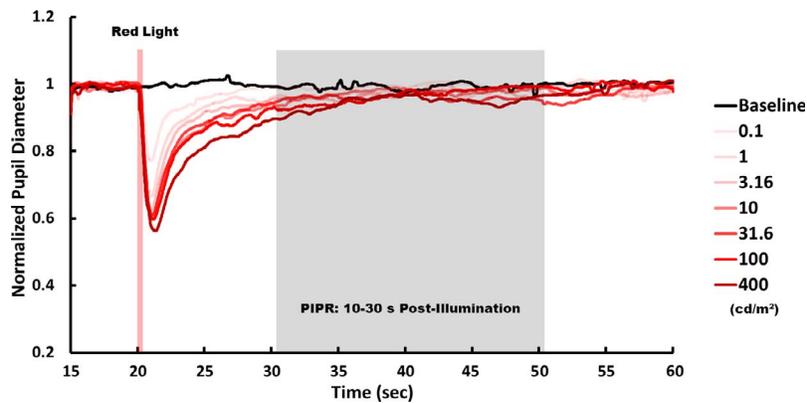


FIGURE 3. Mean pupillary response time courses recorded simultaneously from the left eyes of 11 visually normal participants in baseline and red light conditions. The x-axis represents the actual time course of the 1-minute Schirmer's test. The light stimuli were presented in 20 seconds after trial onset with a duration of 400 ms. Pupil diameter data (y-axis) were normalized to the mean pupil diameter over the 5-second period before the onset of light stimuli. The gray area represents the time interval over which the PIPR index was computed.

phenomenon. First, in high-intensity red light conditions, a strong cone-driven extrinsic ipRGC activity may enhance the minimal melanopsin activation in ipRGCs,²⁵ causing amplified PIPR and subsequent tear production. This is supported by the fact the 400 cd/m² red light stimulation caused noticeably more sustained pupil response than the red light conditions of lower intensity (Fig. 3). The PIPR changes were not statically significant because the PIPR index was computed later in the course of pupil redilation (10–30 seconds post illumination), while the tear production measure is cumulative and reflects all the reflex lacrimation over a 1-minute interval. Secondly, there may be a secondary pathway in which the cone response to bright light mediates tear production independently of melanopsin and ipRGCs. There is no clear evidence in literature to support these hypotheses, and the exact mechanism for this phenomenon remains to be further elucidated. In the bigger picture, our results demonstrate a clear linear correlation between light-induced lacrimation and PIPR in melanopsin-activating bright blue light conditions, but not in melanopsin-silent red light conditions. These findings suggest that melanopsin phototransduction exerts a substantial influence over light-induced reflex lacrimation.

Our findings are consistent with the latest developments in our understanding of the neural circuits that link melanopsin

phototransduction to ocular pain and reflex lacrimation. In a series of experiments on rats, Okamoto and colleagues^{2,6} and Katagiri and colleagues⁷ have demonstrated that bright light stimulation activates an area of the spinal trigeminal nucleus that subserves corneal nociception. This nociceptive photoresponse is attenuated by selective lesioning of the olivary pretectal nucleus (OPN), superior salivatory nucleus (SSN), and trigeminal ganglion, and by intraocular injection of vasoconstrictive agents.² Furthermore, when OPN and SSN are blocked pharmacologically, light-induced lacrimation is also reduced.² Based on these findings, a model of light-induced pain and lacrimation has been proposed^{2,4,6,8}: the OPN relays retinal photic signals to the SSN, which sends parasympathetic innervation to the iris, ciliary body, and choroid of the eye via the pterygopalatine ganglion and causes vessel dilations. The dilations of ocular vessels subsequently trigger trigeminal nociception, causing eye pain/discomfort and lacrimation.

Okamoto and coworkers^{2,6} and Katagiri and coworkers,⁷ however, have not investigated the source of the photic signal upstream of the OPN. Because the OPN is also the pupillary motion center²⁶ (in addition to its role in mediating ocular pain and lacrimation), information about the source of photic input into the OPN comes from another line of research that investigates the melanopsin-driven pupillary light response. It is now established that the OPN is heavily innervated by ipRGCs,^{27,28} which integrate melanopsin-driven intrinsic photoactivity as well as extrinsic synaptic input from rods and cones.²⁹ Among these three components of retinal photic signals, rod photoactivity is responsible for dim light transduction and saturates early,³⁰ cone photoactivity adapts rapidly and is easily fatigued,³⁰ while melanopsin photoactivity has a unique “photon-counting” ability that enables sustained coding of ambient light irradiance,¹¹ making the melanopsin component more suitable than rods and cones for detecting high irradiance light exposure that is potentially harmful to the eyes. With the development of chromatic pupillometry, the photoactivity of rods and cones as well as melanopsin-driven intrinsic photoactivity can now be differentiated by recording the pupillary response to light of different wavelengths (blue and red) and intensity.²⁴ Using the PIPR in response to bright blue light as a unique index of melanopsin-driven photoactivity and correlating it with reflex lacrimation production, we provided the first in vivo evidence that light-induced reflex lacrimation is driven by melanopsin photoactivity, and provide a putative neural circuitry that links melanopsin phototransduction to ocular pain and reflex lacrimation. Our results are also consistent with reports of decreased photosensitivity in

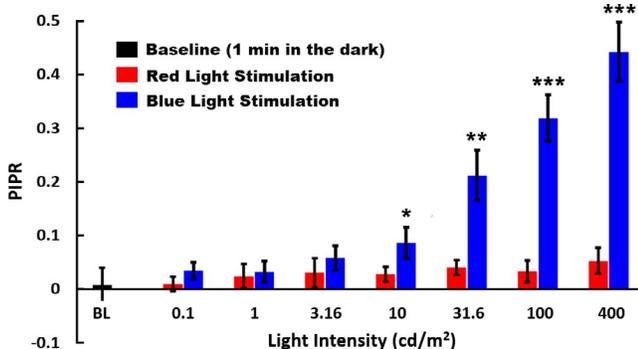


FIGURE 4. Mean PIPR measured by simultaneous chromatic pupillometry recording from 11 visually normal participants in the same viewing conditions as in Figure 1. Postillumination pupil response was defined as mean normalized pupil constriction during a 20-second interval from 10 to 30 seconds after the offset of light stimulation. Error bars represent 95% confidence intervals. Asterisks indicate significantly greater PIPR than BL and the red light conditions with the same intensity step (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

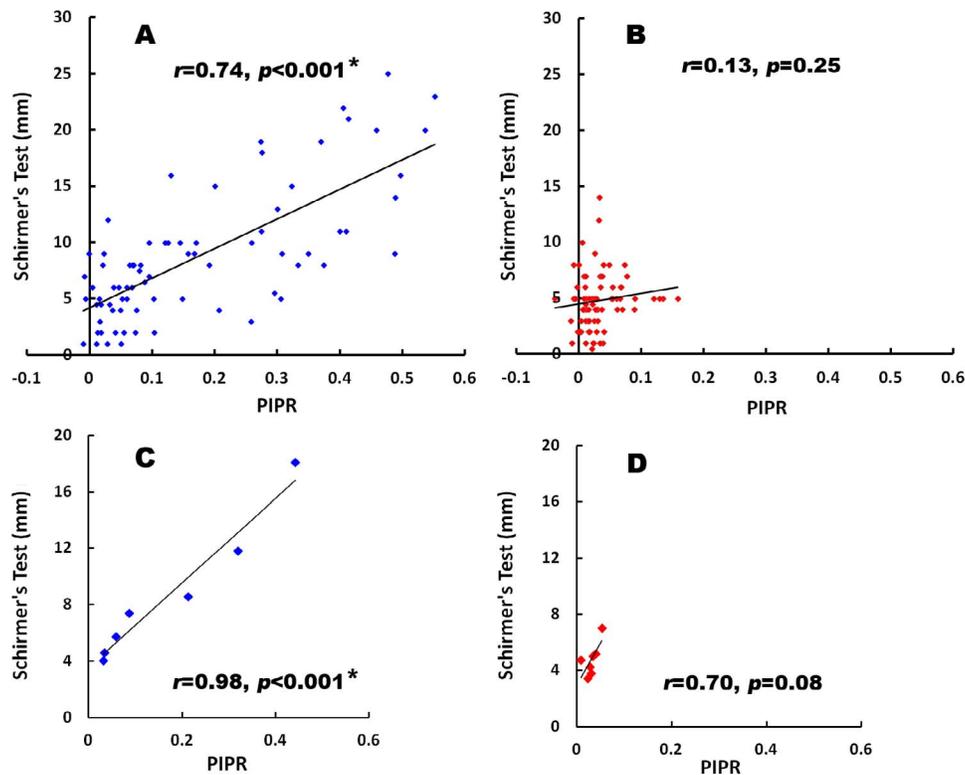


FIGURE 5. Scatter plots of tear production as a function of PIPR with best linear fits. (A) Raw data from individual blue light trials, demonstrating a positive linear relation between tear production and PIPR (Pearson's $r = 0.74$, $P < 0.001$, $n = 77$, *statistically significant). (B) Raw data from individual red light trials, showing no significant relation between tear production and PIPR (Pearson's $r = 0.13$, $P = 0.25$, $n = 77$). (C) Strong linear relation between the mean tear production and mean blue light PIPR across all participants (Pearson's $r = 0.98$, $P < 0.001$, $n = 7$). (D) No significant relation between mean tear production and mean red light PIPR (Pearson's $r = 0.70$, $P = 0.08$, $n = 7$).

patients with benign essential blepharospasm who use rose-colored tinted lenses that block out bright blue light.³¹

The recent discovery of melanopsin-containing intrinsically photosensitive trigeminal ganglion cells (which we refer to here as ipTGCs) by Matynia and coauthors¹⁶ has provided another neural circuit whereby melanopsin photoactivity is integrated with the trigeminal nociceptive sensory input. These cells are localized predominantly in the ophthalmic (V1) area of the trigeminal ganglion. Melanopsin mRNA expression is also found in the tissue of the cornea, the choroid, and iris to which the ipTGCs project, suggesting that melanopsin may be expressed in axons of ipTGCs. Based on cellular morphology, ipTGCs appear to be nociceptive C-type fibers and/or mechanoreceptor A δ -type fibers.¹⁶ In a nitroglycerin-induced migraine animal model, after the optic nerve is crushed, light aversion is still present,¹⁶ indicating that melanopsin activation in ipTGCs may cause eye pain/discomfort, and presumably reflex lacrimation as well.

Putting the findings of the present study and the preexisting evidence together, it is reasonable to conclude that the light-induced lacrimation, a phenomenon closely associated with photophobia, reflects a physiological outcome of melanopsin photoactivity superimposed on the trigeminal nociceptive sensory pathway. The relaying of light irradiance information likely involves both ipRGCs in the retina and ipTGCs innervating the ocular tissues. The relative contributions from these two pathways in physiological and pathologic conditions, however, remain to be elucidated. The present study also suggests that light-induced lacrimation may provide an objective, noninvasive, and convenient new approach to investigate the underlying mechanisms of photophobia in both research and clinical settings. Further studies are warranted to

assess light-induced lacrimation in patients with photophobia from different etiologies.

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