AMERICAN ACADEMY OF OPHTHALMOLOGY®

Pupillary Responses to Full-Field Chromatic Stimuli Are Reduced in Patients with Early-Stage Primary Open-Angle Glaucoma

Raymond P. Najjar, PhD,[1,2] Sourabh Sharma, MD,[1,3] Eray Atalay, MD,[1,4] Annadata V. Rukmini, MD, PhD,[1] Christopher Sun, MBBS,[3] Jing Zhan Lock, MBBS,[1] Mani Baskaran, DNB, PhD,[1,2,3] Shamira A. Perera, FRCOphth,[1,2,3] Rahat Husain, FRCOphth,[3] Ecosse Lamoureux, PhD,[1,2,5] Joshua J. Gooley, PhD,[6] Tin Aung, PhD, FRCS,[1,2,3,7] Dan Milea, MD, PhD[1,2,3]

Purpose: To evaluate the ability of chromatic pupillometry to reveal abnormal pupillary responses to light in patients with early-stage primary open-angle glaucoma (POAG) and to test whether the degree of pupillometric impairment correlates with structural hallmarks of optic nerve damage in the disease.

Design: Cross-sectional study.

Participants: Forty-six patients with early-stage POAG (63.4±8.3 years, 63% male, 87% ethnic-Chinese) and 90 age-matched healthy controls (61.4±8.6 years, 34% male, 89% ethnic-Chinese). Patients with POAG had a visual field mean deviation (VFMD) of −6 decibels or better on automated perimetry.

Methods: Each participant underwent a monocular 2-minute exposure to blue light (462 nm) followed by another 2-minute exposure to red light (638 nm) using a modified Ganzfeld dome equipped with a light-emitting diode lighting system. The light stimuli intensity was increased logarithmically to evaluate the combined extrinsic and intrinsic response of intrinsically photosensitive retinal ganglion cells (ipRGCs). Light-induced changes in horizontal pupil diameter were assessed monocularly using infrared pupillography.

Main Outcome Measures: Baseline-adjusted, light-induced pupillary constriction amplitudes were calculated, and individual irradiance-response curves were constructed for each stimulus. Pupillary constriction amplitudes were compared between groups and across light intensities using a linear mixed model analysis. The linear relationship between pupillometric parameters and different structural and functional features of glaucoma was assessed using Pearson's correlation analysis.

Results: Light-induced pupillary constriction was reduced in patients with early-stage POAG compared with controls at moderate to high irradiances (≥11 Log photons/cm^2/s) of blue ($P = 0.003$) and red ($P < 0.001$) light. Maximal pupillary constriction amplitude was correlated with retinal nerve fiber layer thickness (RNFL) thickness (blue: $r = 0.51$, $P < 0.001$; red: $r = 0.45$, $P = 0.002$) in patients with POAG but not in controls. Conversely, pupillometric parameters were not correlated with visual field scores in patients with early-stage POAG.

Conclusions: Patients with early-stage POAG exhibit reduced pupillary responses to moderate and high irradiances of blue and red lights. This wavelength-independent functional alteration correlates with structural thinning of the RNFL and could be the consequence of dysfunction or loss of melanopsin expressing ipRGCs in the early stages of the disease. *Ophthalmology 2018;125:1362-1371 © 2018 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Supplemental material available at www.aaojournal.org.

Glaucoma is the leading cause of irreversible blindness, with a global prevalence of 3.5% and an expected increase rate of 74% between 2013 and 2040.[1] Primary open-angle glaucoma (POAG) accounts for the majority of glaucoma cases worldwide.[1] It is characterized by a progressive and painless loss of retinal ganglion cells and their axons, leading to a gradual, yet irreversible, visual field (VF) loss.[2] Early detection of POAG is critical for vision preservation and consequently reduction of health economic burden,[3,4] yet even in developed countries such as Singapore, 81.6% of patients with primary glaucoma are unaware of their condition.[4]

Although OCT is efficient in detecting early structural changes associated with the onset of POAG,[5] VF testing by standard automated perimetry (SAP) remains the sole functional and clinically adopted assessor of vision loss and progression of the disease.[6] Although SAP is essential to evaluate visual declines associated with POAG, the test remains subjective and demanding in time and effort for patients and medical personnel alike,[7] and does not always

© 2018 by the American Academy of Ophthalmology
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Published by Elsevier Inc.
https://doi.org/10.1016/j.ophtha.2018.02.024
ISSN 0161-6420/18

reflect the severity of structural decrements observed in glaucoma.[8,9]

Retinal photoreception is not exclusive to rods and cones but is also dependent on intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the blue-sensitive photopigment melanopsin.[10] The ipRGCs integrate inputs from rods and cones,[11] along with their sluggish intrinsic photo-responses to moderate and high intensities of light (above 11 Log photons),[12] and convey the nervous signal to brain regions such as the suprachiasmatic nucleus for circadian photo-entrainment,[13] the olivary pretectal nuclei controlling the pupillary light response (PLR),[11] and other image-forming and nonimage-forming structures.[14] The discovery of these inner retina photoreceptors offers a potential way for glaucoma screening with animal and human histological and electrophysiological studies asserting a loss or disruption of ipRGCs in this disease.[15-17]

Pupillometry is a simple, noninvasive, and objective procedure that consists of measuring pupil size and reactivity. Pupillary light responses are dependent on the integrity of ipRGC projections and the interplay between the outer (red/green-shifted peak sensitivity) and inner (blue-shifted peak sensitivity) retinal photoreception.[18] Such interplay lays the foundation for the use of chromatic pupillometry as a tool to evaluate the integrity of visual functions and localize retinal dysfunctions in various ocular diseases,[19-21] including glaucoma.[22-25]

Whether using direct or consensual pupillometry,[26,27] concomitantly with full-field, regional, or multifocal chromatic illumination paradigms,[22-25,27,28] previous studies have shown that the PLR, and particularly the intrinsic melanopsin-mediated postillumination pupillary response (PIPR), is altered in moderate and advanced stages of glaucoma. These pupillometric alterations correlate with structural (e.g., cup-to-disc ratio) and functional (e.g., VF mean deviation [VFMD]) defects across disease severities.[24,26,27] Recently, Adhikari et al[25] reported a dysfunction in the PIPR subsequent to a 1-second high-intensity light pulse of blue light delivered in the superonasal quadrant field of patients with early-stage glaucoma and glaucoma suspects.

With the use of chromatic pupillometry and ramping-up lighting stimuli designed to sequentially test the functional integrity of retinal photoreceptors without isolating the melanopsin-mediated pupillary response, previous research from our group revealed a reduction in PLR to both blue and red lights in patients with moderate and severe POAG.[27] Using a similar light stimulation design, we evaluate the ability of chromatic pupillometry to reveal abnormal pupillary responses to light in patients with early-stage POAG and test whether the degree of pupillometric impairment correlates with structural and functional markers of glaucomatous optic nerve damage.

Methods

Participants

A total of 136 participants (46 patients with bilateral or unilateral early-stage POAG and 90 age-matched healthy controls) took part in this cross-sectional study. Patients with POAG were recruited from the glaucoma clinics at the Singapore National Eye Center, whereas control participants were recruited from the general population through local advertisement and word-of-mouth referrals. All participants underwent a comprehensive ophthalmological assessment that comprised slit-lamp and gonioscopic examinations, best-corrected visual acuity (BCVA) (logarithm of the minimum angle of resolution chart, Lighthouse International, New York, NY), color vision testing (Ishihara plates, Kanehara & Co, Tokyo, Japan), automated refraction (Canon RK 5 Auto Ref-Keratometer, Canon, Tochigiken, Japan), ocular biometry (IOLMaster, Carl Zeiss Meditec, Jena, Germany) to measure ocular axial length (AxL) and anterior chamber depth (ACD), intraocular pressure (IOP) measurement using Goldmann applanation tonometry, and peripapillary retinal nerve fiber layer (RNFL) thickness assessment via high-definition OCT (Cirrus version 6.0, Carl Zeiss Meditec, Dublin, CA). The OCT results were validated only if the recorded signal strength had a value of 6 or better. Participants also underwent SAP (Humphrey VF analyzer II model 750, Carl Zeiss Meditec). The Humphrey VF testing was performed with near refractive correction using the 24-2 Swedish Interactive Thresholding Algorithm (Fast) with stimulus size III. Repeat testing was performed if false-positive or false-negative responses exceeded 33% or if the fixation loss rate was greater than 20%. Subjects who could not achieve these reliability criteria were excluded. Patients were diagnosed as having POAG, in the absence of any other causes of secondary optic neuropathy, on the basis of abnormal optic disc cupping (vertical cup-to-disc ratio over 0.7 or an inter-eye asymmetry over 0.2) or neuroretinal rim notching, with thinning of the RNFL on high-definition OCT, compatible glaucomatous VF defect based on the criteria reported by Hodapp et al,[6] and open angles on gonioscopy. Patients with VFMD scores of −6 decibels or better were graded as having early-stage POAG.[6]

Patients and controls were excluded if they had any associated ophthalmic conditions such as myopia worse than −6 diopters, dense cataracts, retinopathies, other causes of optic neuropathy and ocular motor disorders, or pupillary abnormalities except for relative afferent pupillary defects. Patients with clinically diagnosed psychiatric or neurologic disorders, including cognitive impairment or dementia, were also excluded, as were patients who had previously undergone intraocular surgery or were taking psychotropics or other medications that could affect the PLR or alertness. Demographic information and medication and smoking history were collected using interviewer-administered questionnaires. The study was approved by the SingHealth Centralized Institutional Review Board, and written informed consents were obtained from all participants. Research procedures adhered to ethical principles outlined in the Declaration of Helsinki.

Chromatic Pupillometry

Subsequent to ophthalmic examinations, participants' PLRs were evaluated in a dedicated dark room at the Singapore Eye Research Institute clinics. Each subject underwent two 4-minute pupillary assessment blocks that consisted of 1 minute of darkness to measure baseline pupil size followed by 2 minutes of light exposure and another 1 minute of darkness to assess pupillary redilation to baseline (Fig 1). Participants were first exposed to a blue light stimulus (peak wavelength = 462 nm, full width at half maximum = 25.2 nm) then to a red light stimulus (peak wavelength = 638 nm, full width at half maximum = 15.5 nm). The order of light exposures was fixed because our intent was to evaluate the usefulness of our protocol as a standardized clinical screening procedure. Light was administered to the study eye, with the fellow eye covered by an eye patch, using a modified Ganzfeld dome (Labsphere, Inc, North Sutton, NH) equipped with narrow-bandwidth light-emitting

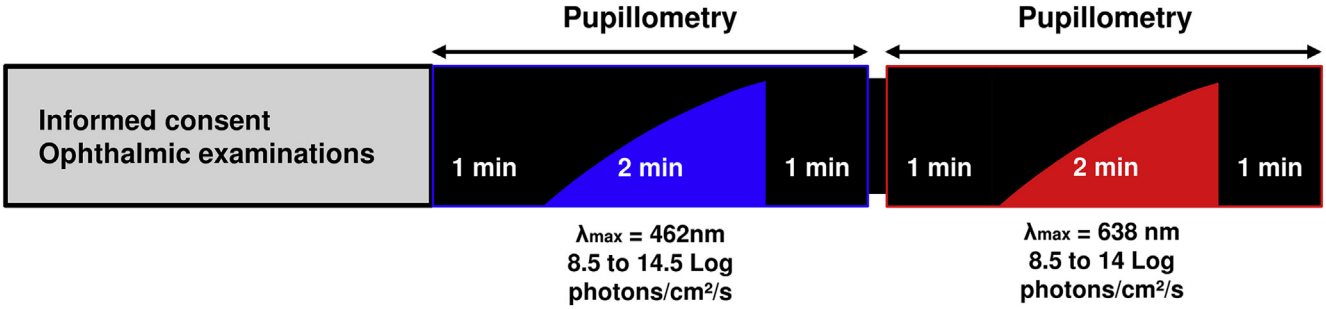


Figure 1. Chromatic pupillometry protocol. After ophthalmic examination, each participant underwent a 2-minute full-field exposure to blue light (462 nm) followed by another 2-minute exposure to red light (638 nm) using a modified Ganzfeld dome equipped with a light-emitting diode lighting system. The light stimuli intensity increased logarithmically from 8.5 to 14.5 Log photons/cm^2/s for blue light and from 8.5 to 14.0 Log photons/cm^2/s for red light. Both blue and red light stimulations were preceded and followed by 1 minute of darkness. Horizontal pupil diameter was monitored during both 4-minute blocks with a head-mounted monocular infrared pupillometer. λ_{max} = peak wavelength; min = minute(s).

diodes (Nichia Corporation, Tokushima, Japan) controlled using a function generator (Keithley Instruments Inc, Cleveland, OH). Current was applied to the light-emitting diodes over a 2-minute period (14 400 steps) using a logarithmic function, thus generating gradually increasing light stimuli ranging from 8.5 to 14.5 Log photons/cm^2/s for blue light and from 8.5 to 14 Log photons/cm^2/s for red light (Figs 1 and 2A). The 2 pupillary assessment blocks were separated by a 1-minute period of darkness, during which the patient remained seated in darkness with his/her head in the Ganzfeld dome. These increasing light regimens were intended to assess the combined extrinsic (rods- and cones-mediated) and intrinsic (melanopsin-mediated) inputs of ipRGCs to the PLR and were not intended to isolate and measure the intrinsic melanopsin-dependent response. During both blocks, patients wore a head-mounted monocular infrared eye-tracking device that recorded horizontal pupil diameter with a sampling rate of 120 frames per second (ETL-100H Pupillometry Lab, ISCAN Inc, Woburn, MA). Pupillometric traces underwent a quality-control check at the end of each session to ensure appropriate pupil detection and infrequency of blinks. In the event where artifacts affected pupillometric traces, participants underwent a third light exposure sequence. Light levels and spectra were calibrated with the sensor placed at the patient's eye position using a calibrated radiometer (ILT1700, International Light Technologies, Peabody, MA) and spectroradiometer (ILT950, International Light Technologies), respectively.

Data Analysis and Statistics

Horizontal pupil diameter measurements were processed for blink artifact removal, smoothed using a locally weighted scatterplot smoothing (span = 0.09), and then expressed as a percentage change from baseline pupil size (Fig 2B, C). Baseline pupil size was calculated as the median of horizontal pupil diameters assessed in darkness 30 seconds before each light onset. The normalization of pupil diameter to baseline accounted for baseline pupil size differences between participants and groups. Irradiance response curves were constructed for each participant for both light exposures. Pupil sizes were expressed as a function of 13 irradiances separated by an interval of 0.5 Log photons/cm^2/s ranging from 8.5 to 14.5 Log photons/cm^2/s for blue light, and 12 irradiances separated by an interval of 0.5 Log photons/cm^2/s ranging from 8.5 to 14.0 Log photons/cm^2/s for red light. Baseline-adjusted pupillary constriction amplitudes were examined using a linear mixed model with irradiance and group as within- and between-subject factors, respectively. For those comparisons in which the omnibus test reached statistical significance, pairwise multiple comparison procedures were performed using the Holm–Sidak method. Pupillary constriction threshold was defined as the irradiance at which the pupil reached 10% of constriction from baseline. Individual constriction slopes were analyzed for the log-linear portion of the irradiance response curves between 11.5 and 14 or 14.5 Log photons/cm^2/s. Individual redilation slopes were analyzed after light offset between maximal pupillary constriction and 10% of baseline-adjusted pupil size.

Demographics and ophthalmologic examination outcomes are represented as median (interquartile range) and compared between groups using a nonparametric Mann–Whitney U test or a chi-square test (Table 1). Pupil baseline, constriction thresholds, maximum constriction, and constriction and redilation slopes are presented as average (standard deviation) and were compared between groups using a Student t test (Table 2). The linear relationship between pupillary constriction parameters and different clinical features of glaucoma[29] was assessed using Pearson's correlation analysis. The threshold for significance for all statistical tests was set at $\alpha = 0.05$, and Sidak correction was applied for all post hoc pairwise comparison. Statistics were performed using SPSS 22 software (IBM Corp, Armonk, NY). Figures were plotted using Sigmaplot 12.0 (Systat Software, Inc, San Jose, CA).

Results

Participants

Forty-six patients (63.4 [8.3] years, 63% male, 87% ethnic-Chinese) with bilateral or unilateral early-stage POAG and 90 age-matched healthy controls (61.4 [8.6] years, 34% male, 89% ethnic-Chinese) were included in this study. The POAG patient group comprised a greater proportion of male participants ($\chi^2 = 8.97$, $P = 0.003$) and a higher family history of glaucoma incidence (20%) compared with controls (0%) ($\chi^2 = 15.82$, $P < 0.001$). Patients and controls were not different in terms of refractive spherical equivalent, BCVA, ACD, and IOP because patients with POAG were receiving glaucoma medications (primarily prostaglandin analogs; only 6 of 46 patients were treated with α2-adrenergic agonists). Compared with controls, patients with POAG also displayed increased cup-to-disc ratio ($U = 109$, $P < 0.001$) and AxL ($U = 1549$, $P = 0.02$), worse VFMD ($U = 866$, $P < 0.001$), pattern standard deviation (PSD) ($U = 799.5$, $P < 0.001$), and VF index (VFI) ($U = 773.5$, $P < 0.001$) scores, and an overall reduced RNFL thickness ($U = 220$, $P < 0.001$) particularly in the inferior ($U = 238$, $P < 0.001$), superior ($U = 477$, $P < 0.001$), and temporal ($U = 1159$, $P < 0.001$) quadrants. A detailed description of the demographics and clinical appraisals of all participants is provided in Table 1.

Of the 109 consenting healthy controls, 19 were excluded because of an underlying ophthalmic condition (e.g., severe

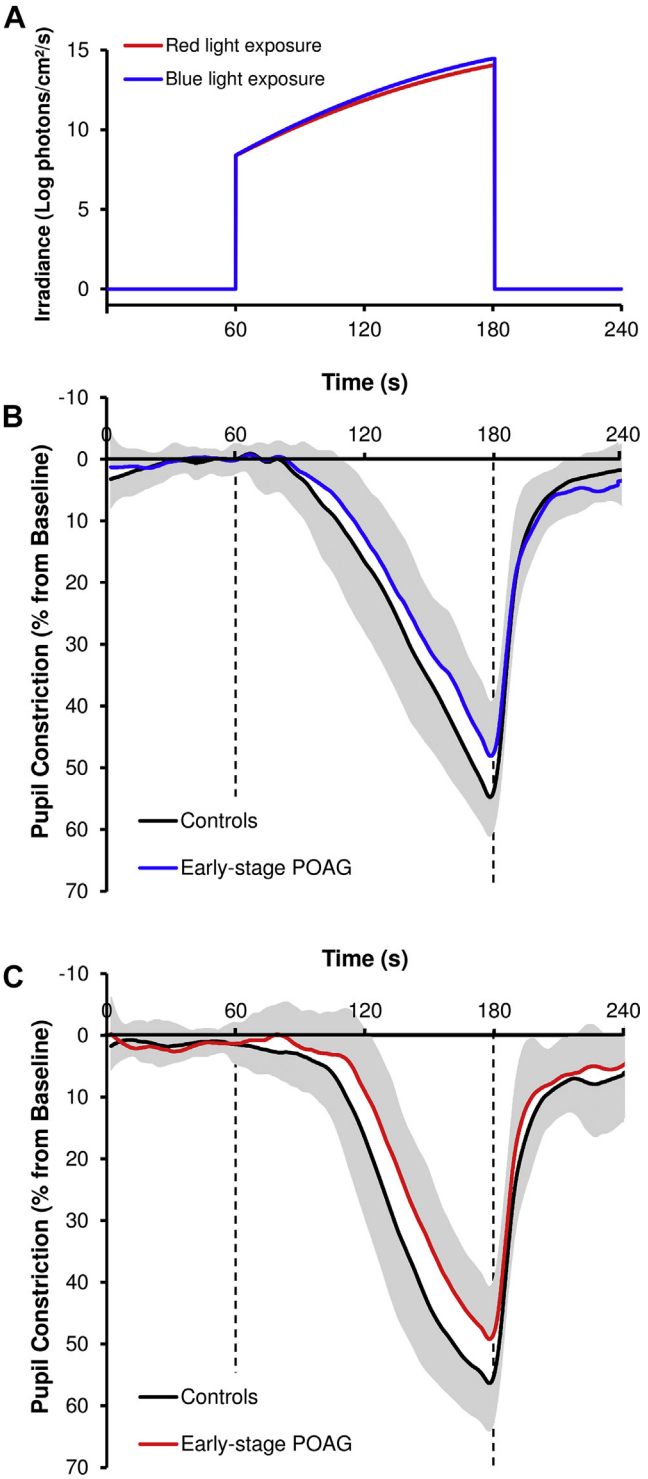


Figure 2. Average baseline-adjusted pupillary light responses (PLRs) in patients with primary open-angle glaucoma (POAG) and controls. **A,** The light stimuli intensity increased logarithmically from 8.5 to 14.5 Log photons/cm^2/s for blue light and from 8.5 to 14 Log photons/cm^2/s for red light. Both blue and red light exposures were preceded and followed by 60 seconds (1 minute) of darkness. Pupil size was normalized to baseline assessed in darkness 30 seconds before light onset. Pupil size decreased progressively as a function of the gradually increasing light exposure **(A)**. Compared with controls, patients with POAG exhibited a significant reduction in pupillary constriction to blue **(B)** and red **(C)** lights. Data are presented as average ± standard deviation. Averages are represented by full lines for controls (*black line*) and patients with POAG (*blue* or *red line*) and standard deviations as gray shaded area downwards for controls and upwards for patients with POAG. s = seconds.

cataracts, primary angle-closure suspect, or ocular hypertension). Pupillometric findings under red light exposure were discarded in 4 control participants because of an aberrant return of pupil size to 10% of baseline after 1 minute of darkness.

Deficits in Pupillary Constriction to Blue Light in Patients with Early-Stage POAG

Baseline pupil size measured in darkness before blue light onset was reduced in patients with early-stage POAG compared with controls (7.2% reduction, $t = 2.46$, $P = 0.02$) (Table 2). Baseline pupil size did not differ between light conditions (blue and red) in both groups (controls: $t = -0.91$, $P = 0.36$; POAG: $t = 0.04$, $P = 0.96$). There was a significant effect of irradiance on pupil size in both groups, with pupils displaying a gradual constriction as the intensity of blue light increased ($F(12, 134) = 1113.6$, $P < 0.001$) (Figs 2B and 3A). Pupillary constriction threshold was not significantly different in patients with POAG (12.03 [0.83] Log photons/cm^2/s) compared with controls (11.80 (0.89) Log photons/cm^2/s) ($t = -1.47$, $P = 0.14$) (Table 2). The overall amplitude of baseline-adjusted pupillary constriction was reduced in patients with early-stage POAG compared with controls ($F(1, 134) = 9.48$, $P = 0.003$) (Fig 3A). This reduction was dependent on the irradiance of the light exposure ($F(12, 134) = 5.79$, $P < 0.001$) and was particularly pronounced at moderate to high irradiance levels of blue light (above 11 Log photons/cm^2/s) (Fig 3A). To address the possibility that the observed reductions in pupillary responses in patients with POAG might be driven by differences in AxL and gender between groups (Table 1), we performed a post hoc analysis taking into account AxL measurements and gender differences as covariates. The reduction in pupillary constriction remained significant between groups after adjusting for AxL and gender differences ($F(1, 132) = 14.32$, $P < 0.001$). Maximum amplitude of pupil constriction was reduced by 6.67% under blue light ($t = 5.16$, $P < 0.001$) in patients with POAG compared with controls. The slopes of constriction and redilation were reduced in patients with POAG compared with controls (constriction slope: $t = 2.12$, $P = 0.04$; redilation slope: $t = -2.28$, $P = 0.02$; Table 2). The exclusion of the 6 patients with POAG treated with α2-adrenergic agonists, known to cause mild miosis,[30] did not affect any of our aforementioned findings.

Deficits in Pupillary Constriction to Red Light in Patients with Early-Stage POAG

Baseline pupil size measured in darkness before red light onset was reduced in patients with early-stage POAG compared with controls (9.3% reduction before red light, $t = 3.23$, $P = 0.002$; Table 2). There was a significant effect of irradiance on pupil size in both groups, with pupils displaying a gradual constriction as the intensity of red light increased ($F(11, 130) = 1011.2$, $P < 0.001$; Figs 2C and 3B). Pupillary constriction threshold was increased in patients with POAG (12.03 [0.66] Log photons/cm^2/s) compared with controls (11.59 [0.90] Log photons/cm^2/s) ($t = -2.89$, $P = 0.005$; Table 2). The amplitude of light-induced pupillary constriction from baseline was reduced in patients with early-stage POAG compared with controls ($F(1, 130) = 19.86$, $P < 0.001$). This reduction was dependent on the irradiance of the

Table 1. Demographics and Ocular Characteristics of the Study Eye in the Control and Early-Stage POAG Groups

	Controls	Early-Stage POAG
Sample size	90	46
Age (yrs)	61.4 (8.6)	63.4 (8.3)
Gender (% male)	34.4	63.0**,†
Ethnicity (% Chinese)	88.9	87.0†
Family history (% with)	0.0	20.0***,†
Spherical equivalent (D)	0.0 (3.1)	−1.2 (3.3)
BCVA (logMAR)	0.1 (0.2)	0.2 (0.1)
IOP (mmHg)	15.0 (2.8)	15.0 (2.0)
CDR	0.5 (0.2)	0.8 (0.1)***
AxL (mm)	24.1 (1.2)	24.7 (1.9)*
ACD (mm)	3.1 (0.4)	3.2 (0.5)
VFMD (dB)	−1.2 (2.3)	−3.7 (2.4)***
PSD (dB)	1.8 (0.8)	2.9 (2.4)***
VFI (%)	98.0 (2.8)	93.5 (6.5)***
RNFL-T Average (μm)	94.0 (14.0)	70.0 (16.5)***
RNFL-T-Inf (μm)	120.0 (19.0)	79.5 (20.0)***
RNFL-T-Sup (μm)	117.5 (23.8)	85.5 (26.3)***
RNFL-T-Nas (μm)	64.5 (11.0)	64.0 (11.8)
RNFL-T-Tem (μm)	69.5 (17.8)	58.0 (19.0)***

ACD = anterior chamber depth; AxL = axial length; BCVA = best-corrected visual acuity; CDR = cup-to-disc ratio; D = diopters; dB = decibels; HVF = Humphrey visual field; inf = inferior quadrant; IOP = intraocular pressure; logMAR = logarithm of minimum angle of resolution; nas = nasal quadrant; POAG = primary open-angle glaucoma; PSD = pattern standard deviation; RNFL-T = retinal nerve fiber layer thickness; sup = superior quadrant; tem = temporal quadrant; VFI = visual field index; VFMD = visual field mean deviation.
Data are represented as median (interquartile range) and compared using the Mann−Whitney *U* test. †Statistics done using a chi-square test. Difference between participant groups is indicated as * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

Table 2. Pupillometric Parameters in the Control and Early-Stage POAG Groups

	Controls	Early-Stage POAG
Blue light		
Baseline pupil size (mm)	5.52 (0.86)	5.13 (0.93)*
Constriction threshold (Log photons/cm^2/s)	11.80 (0.89)	12.03 (0.83)
Maximum pupil constriction (%)	55.21 (6.21)	48.55 (8.67)***
Constriction slope (%/Log Photons/cm^2/s)	15.60 (3.93)	14.14 (3.50)*
Redilation slope (%/s)	−2.81 (0.94)	−2.41 (1.05)*
Red light		
Baseline pupil size (mm)	5.64 (0.86)	5.12 (0.92)**
Constriction threshold (Log photons/cm^2/s)	11.59 (0.90)	12.03 (0.66)**
Maximum pupil constriction (%)	55.95 (7.71)	49.19 (8.25)***
Constriction slope (%/Log Photons/cm^2/s)	19.81 (5.34)	19.56 (4.97)
Redilation slope (%/s)	−2.38 (1.26)	−2.19 (0.96)

POAG = primary open-angle glaucoma.
Constriction slope is calculated between 11.5 and 14 (red) or 14.5 (blue) Log photons/cm^2/s. Data are represented as average (standard deviation) and compared using a Student *t* test. Difference between participant groups is indicated as * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

light exposure ($F(11, 130) = 8.41$, $P < 0.001$) and was particularly pronounced at moderate to high irradiance levels above 11.5 Log photons/cm^2/s (Fig 3B). This reduction in pupillary constriction remained significant between groups after adjusting for AxL and gender differences ($F(1, 128) = 16.64$, $P < 0.001$). Maximum amplitude of pupil constriction was reduced by 6.76% under red light ($t = 4.68$, $P < 0.001$) in patients with POAG compared with controls. The slopes of constriction and redilation were not different between groups (constriction slope: $t = 0.26$, $P = 0.80$; redilation slope: $t = 0.96$, $P = 0.34$; Table 2). Excluding the 6 patients with POAG treated with α2-adrenergic agonists did not affect our findings.

Correlations between Pupillometric Parameters and Ophthalmic Features in POAG

Average RNFL thickness was positively correlated with the amplitudes of pupil constriction under high irradiances of light (above 12.5 Log photons/cm^2/s). The highest correlation coefficients were between average RNFL thickness and maximal pupillary constriction obtained at the maximal irradiances of blue ($r = 0.51$, $P < 0.001$; Fig 4A) and red ($r = 0.45$, $P = 0.002$; Fig 4B) lights in patients with POAG, but not in controls. Considering the linear relationship between pupil constriction to maximal irradiances of blue light and RNFL thickness (linear model: y=0.40x+20.14; where *y* is pupil constriction and *x* is the average RNFL thickness), an average RNFL thickness of 83.46 μm (99% confidence interval [CI], 75.24−91.71 μm) or less would yield a higher probability of reduced pupillary constriction to blue light in patients with POAG (i.e., constriction below the lower limit of the 99% CI of the control group: 53.52%). Likewise, patients with an average RNFL thickness of 84.40 (78.07−90.68) μm or less are more likely to exhibit a reduction in pupil response to maximal irradiance of red light (linear model: y=0.34x+25.11) (Fig S5, available at www.aaojournal.org). Conversely, in patients with POAG, changes in VF scores were not correlated with pupillometric parameters, including maximal pupil constriction amplitude to blue (VFMD: $r = 0.04$, $P = 0.80$; Fig 4C; VFI: $r = 0.06$, $P = 0.68$; PSD: $r = 0.12$, $P = 0.43$) and red (VFMD: $r = 0.02$, $P = 0.86$; Fig 4D; VFI: $r = 0.08$, $P = 0.58$; PSD: $r = -0.04$, $P = 0.81$) lights, nor with changes in RNFL thickness (VFMD: $r = 0.22$, $P = 0.14$; VFI: $r = 0.136$, $P = 0.37$; PSD: $r = 0.04$, $P = 0.79$). No correlations were found with VF scores even after pupillometric parameters were log-transformed. Other ophthalmic parameters, such as cup-to-disc ratio, IOP, BCVA, ACD, AxL, and spherical equivalent, were not significantly correlated with maximal pupillary constriction to blue or red light. Maximum pupillary constriction under blue light was inversely correlated with age in both controls ($r = -0.36$, $P < 0.001$) and patients with POAG ($r = -0.32$, $P = 0.03$). This inverse correlation with age was not observed for maximal constriction to red light and could be due to subtle spectral transmittance changes in the ocular media of older patients without clinically significant cataract.[31]

Discussion

In this study, we demonstrate that early-stage POAG is associated with reduced pupillary constriction amplitudes to moderate and high irradiances of blue and red lights. This wavelength−independent alteration suggests attenuated ipRGC signaling in the early stages of the disease. In addition, we also highlight a close relationship between pupillary constriction amplitude under high irradiances of

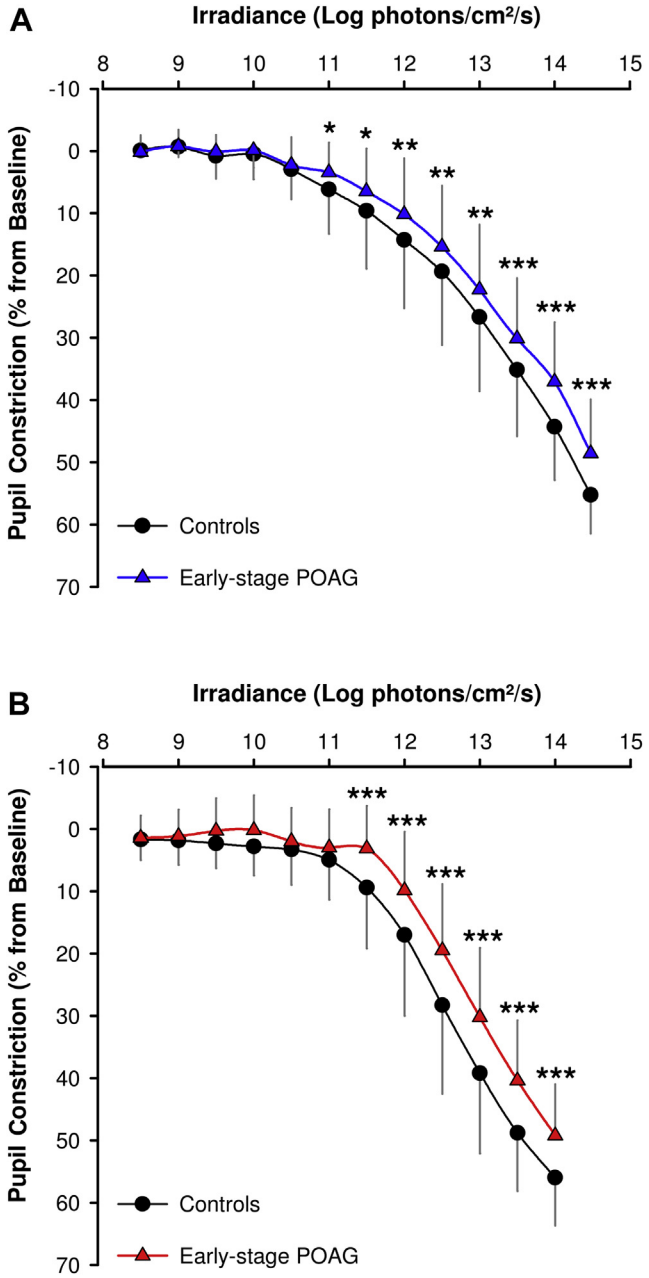


Figure 3. Irradiance response curves in patients with primary open-angle glaucoma (POAG) and controls. **A,** Baseline-adjusted pupillary constriction to ramping-up blue light was reduced in patients with early-stage POAG compared with controls ($F(1, 134) = 9.48$, $P = 0.003$). This reduction was dependent on the irradiance of the light exposure ($F(12, 134) = 5.79$, $P < 0.001$) and was particularly pronounced at moderate to high irradiance levels above 11 Log photons/cm^2/s. **B,** Baseline-adjusted pupillary constriction to ramping-up red light was reduced in patients with early-stage POAG compared with controls ($F(1, 130) = 19.86$, $P < 0.001$). This reduction was dependent on the irradiance of the light exposure ($F(11, 130) = 8.41$, $P < 0.001$) and was particularly pronounced at moderate to high irradiance levels above 11.5 Log photons/cm^2/s. Data are presented as average ± standard deviation. Averages are represented by full lines for controls (*black line*) and patients with POAG (*blue* or *red line*) and standard deviations as gray vertical bars downwards for controls and upwards for patients with POAG. Difference between groups is indicated as * for $P < 0.05$, ** for $P < 0.01$, and *** for $P < 0.001$.

light with changes in anatomic correlates of optic nerve damage (i.e., RNFL thinning) within the group of patients with early-stage POAG.

Pupillary responses to light are dependent on the functionality of photoreceptors within the inner and outer retina, as well as on the integrity of their neural circuitry.[18] We have previously demonstrated that irradiance-response curves can quickly be established for PLR using logarithmic ramping-up light paradigms.[27] In principle, this approach, combined with chromatic light stimulations, elicits a sequential activation of rods, cones, and ipRGCs, and evaluates the functional integrity of the retina across a wide range of irradiances.[27] Although such a pupillometric screening method may not allow an isolated evaluation of the melanopsin-dependent intrinsic response of ipRGCs, it is not affected by mild and moderate cataracts,[32] and may provide a more detailed insight on the origin and magnitude of underlying neuroanatomic abnormalities, and detects subtle physiologic deficits that previous studies using continuous or pulses of full-field light stimulations failed to uncover in early-stage glaucoma.[22,24]

In this study, the reduction in pupillary constriction amplitudes to moderate and high irradiances of blue light (above 11.0 Log photons/cm^2/s) corresponding to the range of activation of ipRGCs[12] is indicative of altered intrinsic photosensitivity or a degeneration of these cells in early-stage POAG. Our findings are in agreement with recent studies showing altered electrophysiologic responses of ipRGCs in patients with glaucoma[17] and with earlier reports in rodent models of chronic ocular hypertension showing a reduction of ipRGCs and their brain projections.[15,33] Recent histologic findings in glaucomatous human retinas have suggested significant loss of ipRGCs in the severe stages of the disease, but earlier alterations were not clearly documented in this article.[16] Disrupted ipRGC signaling in early-stage POAG suggests that ipRGCs may be more vulnerable in glaucoma compared with other ocular conditions.[34] The reduced slope of redilation subsequently to blue light in the POAG group was unexpected. Although we do not have a tangible explanation for this phenomenon yet, it might be due to the nature of the light exposure paradigm. Further investigations are needed to confirm and interpret this finding.

The increase in threshold and reduction in pupillary constriction amplitudes to moderate to high irradiances of red light (above 11.5 Log photons/cm^2/s) are suggestive of altered synaptic transmissions to ipRGCs or reduced outer retina photoresponsiveness in early-stage POAG. It is not clear yet if outer retina photoreception remains intact in glaucoma, but electrophysiologic studies have suggested a loss of rods and cones, in conjunction with retinal ganglion cell damage.[35,36] Furthermore, histologic reports in humans and animal models have shown cone swelling and loss,[37] and reduced dendritic branching of ipRGCs in the early stages of glaucoma.[38] Interestingly, irradiance response curves to ramping up red light in patients with POAG display a similar pattern, notably an increase in threshold and decrease in the amplitude of constriction, as those derived from investigations using pharmacologic synaptic blockade of outer retina signal in nonhuman primates.[18]

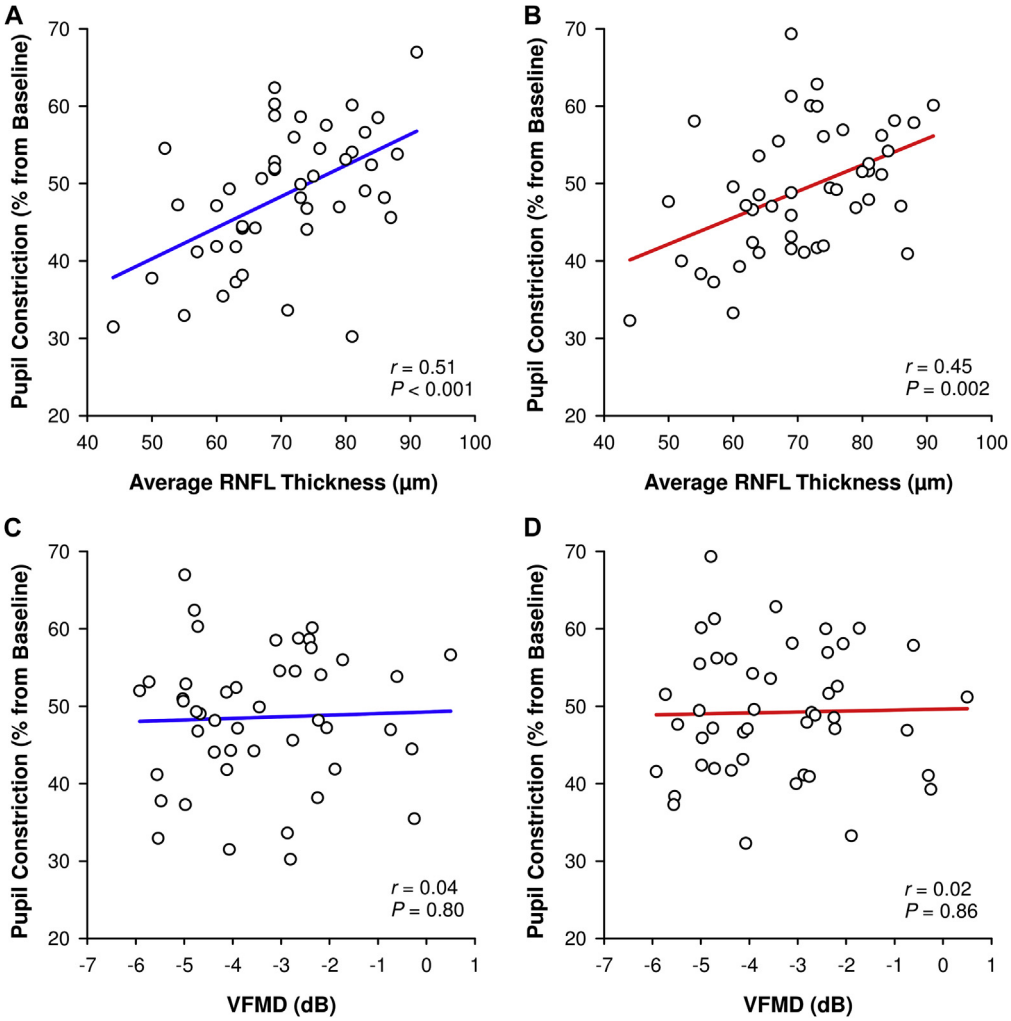


Figure 4. Correlations between pupillary responses and structural and functional features of glaucoma in patients with early-stage primary open-angle glaucoma (POAG). Decreased average retinal nerve fiber layer (RNFL) thickness in patients with early-stage POAG was associated with a decreased pupillary constriction to maximal irradiances of blue ($r = 0.51$, $P < 0.001$) **(A)** and red ($r = 0.45$, $P = 0.002$) **(B)** lights. Conversely, the severity of visual field (VF) loss was not correlated with pupillary constriction to maximal irradiances of blue ($r = 0.04$, $P = 0.80$) **(C)** and red ($r = 0.02$, $P = 0.002$) **(D)** lights. dB = decibels; VFMD = visual field mean deviation.

Although our findings support outer retina dysfunctions in early-stage POAG, given that rod- and cone-mediated PLRs are dependent on the integrity of the afferent pathways originating from the ipRGCs to the olivary pretectal nuclei,[39] reduced pupillary responses to red light could also arise from ipRGC degeneration, if present in the early stages of the disease.

Whether stemming from outer or inner retinal dysfunctions, or both, our findings are consistent with the current literature associating glaucoma to PLR impediments in moderate and advanced stages of the disease.[22-24,26-28] Until recently, however, there was no tangible evidence of altered PLR in early-stage glaucoma. In a recent study, Adhikari et al,[25] reported that the PIPR, a proxy of melanopsin photoresponsiveness, is decreased in response to a 1-second high intensity (15.5 Log photons/cm^2/s) light pulse of blue light delivered regionally in the superonasal quadrant field of patients with mild glaucoma and glaucoma suspects. However, the amplitudes of pupillary constriction to full-field light stimulation were not altered in this group of patients. Such discrepancy with our findings can be due to light paradigm differences as our long duration ramping-up stimuli are most likely to illicit different photoreceptor contribution and important photoreceptor adaptation effects compared with short duration light pulses. Notwithstanding the innovative aspect of this investigation, exemplified by regional chromatic light stimulations targeting RNFL loss patterns in the early stages of the disease, precise quadrant stimulations require the full cooperation of patients, as well as good fixation aptitudes. Furthermore, PIPR often requires the administration of a mydriatic agent to the study eye and the consensual assessment of the PLR,[40,41] which may be problematic if pupillometric screening is to take place at a wider community level. While Adhikari et al[25] have shown melanopsin dysfunction in patients with early-stage glaucoma and glaucoma suspects using quadrant field stimulations, in this study we show that a nonmydriatic full-field light

stimulation delivered in a logarithmic ramp-up fashion that tests the gradually integrated extrinsic and intrinsic ipRGCs response can be used in direct pupillometry to detect ipRGC dysfunction in early-stage POAG. Furthermore, irradiance-response curves drawn from this light regimen may allow for a broader characterization of pupillometric features in various retinal and optic nerve diseases, including other types of glaucoma.

The VF examination is clinically accepted as the functional marker of glaucoma severity and progression.[6] Although linear and nonlinear models were used to describe a relationship between pupillometric and VF variables in groups of patients with POAG with various disease severity,[25,27] in our study, VF parameters (i.e., VFMD, VFI, and PSD) did not correlate with pupillometric features or with average RNFL thickness within the group with early-stage POAG with similar magnitudes of VF loss (VFMD >-6 decibels). The absence of linear correlation between VFMD and RNFL thickness is not surprising given that patients with POAG may exhibit structural changes in the optic nerve before VF changes detectable on SAP.[42] Of note, pupillary constriction amplitudes to high irradiances of blue and red lights displayed a linear correlation with RNFL thickness. These findings, as well as previous reports in groups with patients at different stages of the disease,[27,43,44] insinuate that some nonimage-forming responses to light, such as PLR, may offer less variable, more sensitive correlates of structural glaucomatous damage compared with VF parameters. This statement is in agreement with recent reports showing PIPR deficits in glaucoma suspects (i.e., those with preperimetric glaucoma).[25]

Study Limitations

Our study has a few limitations. First, because participants were only briefly ($\sim$2 minutes) dark-adapted before blue light exposure, we assume that rhodopsin was not fully regenerated to optimally capture light and rods' contribution to the pupillary responses to light may have been suboptimal. Therefore, we cannot exclude that a reduction in pupil constriction to dimmer irradiances of blue light or an increased threshold of constriction to this wavelength of light could be observed in patients with POAG after being fully dark adapted. A dark adaptation of 30 to 45 minutes is usually required to fully regenerate rhodopsin. Such a procedure is cumbersome in a clinical setting, but could be replaced by a partial dark adaptation period of at least 5 minutes ($\sim$50% of regenerated rhodopsin).[45] Second, we acknowledge that our paradigm may not allow us to fully disentangle the disease-dependent pupil responses from direct pupillometric changes resulting from the lower retinal illumination induced by the gradual pupil constriction. In fact, we likely underestimated the pupillary response to both blue and red lights, especially in healthy controls, because participants' pupils were not dilated pharmacologically. Nevertheless, given the potential effect of antimuscarinics on the physiologic properties of retinal ganglion cells,[46] we believe that the use of such agents would generate greater uncertainty when testing retinal integrity. Conversely, adopting a Maxwellian view optical system may improve the control of retinal light exposure during the ramping-up light regimen and across age groups. Third, PIPR metrics (slope of redilation, amplitudes) were unconventional in our study. This could be due to the duration and pattern of our light exposure paradigm, in addition to the gradual constriction of the pupil and the reduction in the amount of light reaching the retina. Conventionally, optimal PIPR is induced by short bright stimulations[47] to a dilated pupil[40] and is reduced under blue background,[48] or subsequently to blue light exposure.[49] The dynamics of the light stimulation used in our study do not necessarily allow for a direct and isolated assessment of the melanopsin-based response and may have led to gradual light adaptation and to less discernible PIPRs under blue light. Although PIPR is a proxy to assess the integrity of the melanopsin-signaling pathway, melanopsin is not exclusively affected in glaucoma but rather the ipRGCs expressing the photopigment. In this study, we show that the amplitudes of pupil constriction during a nonmydriatic full-field light stimulation delivered in a logarithmic ramping-up fashion can be used to detect ipRGC dysfunction in early-stage POAG. Further experiments in melanopsin knockout animal models are required to discern the melanopsin-dependent pupillometric component in our ramping-up paradigm. Finally, given that our pool of participants included mainly ethnic Chinese individuals, it will also be important to determine whether our findings are generalizable to other ethnic groups.

In conclusion, our study shows that early-stage POAG is associated with altered pupillary responses to ramping-up full-field light stimulations. These wavelength-independent deficits in pupillary constriction to high irradiances of light correlate with subtle changes in RNFL thickness and are indicative of ipRGC dysfunction or loss early in the disease. Our findings also add to the body of evidence supporting chromatic pupillometry as an objective, functional, and fast method that does not require clinical expertise to evaluate retinal integrity in ophthalmic diseases such as glaucoma. Although our pupillometric findings show promise for detecting POAG, further improvements are required to render this approach more viable in a clinical setting. With modern technologic advancements, pupillometry-based methods can potentially be refined and adapted into reliable population-based ocular screening tools for early POAG detection.

References

1. Tham Y-C, Li X, Wong TY, et al. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology*. 2014;121:2081–2090.
2. Prum BE, Rosenberg LF, Gedde SJ, et al. Primary open-angle glaucoma preferred practice pattern® Guidelines. *Ophthalmology*. 2016;123:41–111.
3. Bramley T, Peeples P, Walt JG, et al. Impact of vision loss on costs and outcomes in Medicare beneficiaries with glaucoma. *Arch Ophthalmol*. 2008;126:849–856.
4. Chua J, Baskaran M, Ong PG, et al. Prevalence, risk factors, and visual features of undiagnosed glaucoma: the Singapore epidemiology of eye diseases study. *JAMA Ophthalmol*. 2015;133:938–946.

5. Kanamori A, Nakamura M, Escano MFT, et al. Evaluation of the glaucomatous damage on retinal nerve fiber layer thickness measured by optical coherence tomography. *Am J Ophthalmol*. 2003;135:513–520.
6. Hodapp E, Parrish RK, Anderson DR. *Clinical Decisions in Glaucoma*. St. Louis: The CV Mosby Company; 1993.
7. Glen FC, Baker H, Crabb DP. A qualitative investigation into patients' views on visual field testing for glaucoma monitoring. *BMJ Open*. 2014;4:e003996.
8. Leung CKS, Liu S, Weinreb RN, et al. Evaluation of retinal nerve fiber layer progression in glaucoma: a prospective analysis with neuroretinal rim and visual field progression. *Ophthalmology*. 2011;118:1551–1557.
9. Quigley HA, Dunkelberger GR, Green WR. Chronic human glaucoma causing selectively greater loss of large optic nerve fibers. *Ophthalmology*. 1988;95:357–363.
10. Hattar S, Liao HW, Takao M, et al. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. 2002;295:1065–1070.
11. Lucas RJ, Hattar S, Takao M, et al. Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science*. 2003;299:245–247.
12. Dacey DM, Liao H-W, Peterson BB, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. 2005;433:749–754.
13. Gooley JJ, Lu J, Chou TC, et al. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat Neurosci*. 2001;4:1165.
14. Najjar RP, Zeitzer JM. Anatomy and physiology of the circadian system. In: Miglis MG, ed. *Sleep and Neurologic Disease*. San Diego, CA: Academic Press; 2017:29–53.
15. Wang H, Lu Q, Wang N, et al. Loss of melanopsin-containing retinal ganglion cells in a rat glaucoma model. *Chin Med J (Engl)*. 2008;121:1015–1019.
16. Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of melanopsin-expressing retinal ganglion cells in severely staged glaucoma patients. *Invest Ophthalmol Vis Sci*. 2016;57:4661–4667.
17. Kuze M, Morita T, Fukuda Y, et al. Electrophysiological responses from intrinsically photosensitive retinal ganglion cells are diminished in glaucoma patients. *J Optom*. 2017;10:226–232.
18. Gamlin PDR, McDougal DH, Pokorny J, et al. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vision Res*. 2007;47:946–954.
19. Kardon R, Anderson SC, Damarjian TG, et al. Chromatic pupil responses: preferential activation of the melanopsin-mediated versus outer photoreceptor-mediated pupil light reflex. *Ophthalmology*. 2009;116:1564–1573.
20. Kardon R, Anderson SC, Damarjian TG, et al. Chromatic pupillometry in patients with retinitis pigmentosa. *Ophthalmology*. 2011;118:376–381.
21. Richter P, Wilhelm H, Peters T, et al. The diagnostic accuracy of chromatic pupillary light responses in diseases of the outer and inner retina. *Graefes Arch Clin Exp Ophthalmol*. 2017;255:519–527.
22. Feigl B, Mattes D, Thomas R, Zele AJ. Intrinsically photosensitive (melanopsin) retinal ganglion cell function in glaucoma. *Invest Ophthalmol Vis Sci*. 2011;52:4362–4367.
23. Nissen C, Sander B, Milea D, et al. Monochromatic pupillometry in unilateral glaucoma discloses no adaptive changes subserved by the ipRGCs. *Front Neurol*. 2014;5:15.
24. Kankipati L, Girkin CA, Gamlin PD. The post-illumination pupil response is reduced in glaucoma patients. *Invest Ophthalmol Vis Sci*. 2011;52:2287–2292.
25. Adhikari P, Zele AJ, Thomas R, Feigl B. Quadrant field pupillometry detects melanopsin dysfunction in glaucoma suspects and early glaucoma. *Sci Rep*. 2016;6:33373.
26. Kelbsch C, Maeda F, Strasser T, et al. Pupillary responses driven by ipRGCs and classical photoreceptors are impaired in glaucoma. *Graefes Arch Clin Exp Ophthalmol*. 2016;254:1361–1370.
27. Rukmini AV, Milea D, Baskaran M, et al. Pupillary responses to high-irradiance blue light correlate with glaucoma severity. *Ophthalmology*. 2015;122:1777–1785.
28. Carle CF, James AC, Kolic M, et al. Blue multifocal pupillographic objective perimetry in glaucoma. *Invest Ophthalmol Vis Sci*. 2015;56:6394–6403.
29. Zangwill LM, Weinreb RN, Beiser JA, et al. Baseline topographic optic disc measurements are associated with the development of primary open-angle glaucoma: the confocal scanning laser ophthalmoscopy ancillary study to the ocular hypertension treatment study. *Arch Ophthalmol*. 2005;123:1188–1197.
30. Kesler A, Shemesh G, Rothkoff L, Lazar M. Effect of brimonidine tartrate 0.2% ophthalmic solution on pupil size. *J Cataract Refract Surg*. 2004;30:1707–1710.
31. Najjar RP, Teikari P, Cornut P-L, et al. Heterochromatic flicker photometry for objective lens density quantification. *Invest Ophthalmol Vis Sci*. 2016;57:1063–1071.
32. Rukmini AV, Milea D, Aung T, Gooley JJ. Pupillary responses to short-wavelength light are preserved in aging. *Sci Rep*. 2017;7:43832.
33. Drouyer E, Dkhissi-Benyahya O, Chiquet C, et al. Glaucoma alters the circadian timing system. *PloS One*. 2008;3, e3931.
34. Nissen C, Rönnbäck C, Sander B, et al. Dissociation of pupillary post-illumination responses from visual function in confirmed OPA1 c.983A > G and c.2708_2711delTTAG autosomal dominant optic atrophy. *Front Neurol*. 2015;6:5.
35. Choi SS, Zawadzki RJ, Lim MC, et al. Evidence of outer retinal changes in glaucoma patients as revealed by ultrahigh-resolution in vivo retinal imaging. *Br J Ophthalmol*. 2011;95:131–141.
36. Fazio DT, Heckenlively JR, Martin DA, Christensen RE. The electroretinogram in advanced open-angle glaucoma. *Doc Ophthalmol*. 1986;63:45–54.
37. Nork TM, Ver Hoeve JN, Poulsen GL, et al. Swelling and loss of photoreceptors in chronic human and experimental glaucomas. *Arch Ophthalmol*. 2000;118:235–245.
38. El-Danaf RN, Huberman AD. Characteristic patterns of dendritic remodeling in early-stage glaucoma: evidence from genetically identified retinal ganglion cell types. *J Neurosci*. 2015;35:2329–2343.
39. Güler AD, Ecker JL, Lall GS, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature*. 2008;453:102–105.
40. Nissen C, Sander B, Lund-Andersen H. The effect of pupil size on stimulation of the melanopsin containing retinal ganglion cells, as evaluated by monochromatic pupillometry. *Front Neurol*. 2011;2:92.
41. van der Meijden WP, te Lindert BHW, Bijlenga D, et al. Post-illumination pupil response after blue light: Reliability of optimized melanopsin-based phototransduction assessment. *Exp Eye Res*. 2015;139:73–80.
42. Kerrigan-Baumrind LA, Quigley HA, Pease ME, et al. Number of ganglion cells in glaucoma eyes compared with threshold visual field tests in the same persons. *Invest Ophthalmol Vis Sci*. 2000;41:741–748.
43. Chang DS, Boland MV, Arora KS, et al. Symmetry of the pupillary light reflex and its relationship to retinal nerve fiber

layer thickness and visual field defect. *Invest Ophthalmol Vis Sci*. 2013;54:5596−5601.
44. Gracitelli CPB, Duque-Chica GL, Moura AL, et al. A positive association between intrinsically photosensitive retinal ganglion cells and retinal nerve fiber layer thinning in glaucoma. *Invest Ophthalmol Vis Sci*. 2014;55:7997−8005.
45. Hecht S. The dark adaptation of the human eye. *J Gen Physiol*. 1920;2:499−517.
46. Strang CE, Renna JM, Amthor FR, Keyser KT. Muscarinic acetylcholine receptor localization and activation effects on ganglion response properties. *Invest Ophthalmol Vis Sci*. 2010;51:2778−2789.
47. Adhikari P, Zele AJ, Feigl B. The post-illumination pupil response (PIPR). *Invest Ophthalmol Vis Sci*. 2015;56:3838−3849.
48. Park JC, Moura AL, Raza AS, et al. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci*. 2011;52:6624−6635.
49. Mure LS, Cornut P-L, Rieux C, et al. Melanopsin bistability: a fly's eye technology in the human retina. *PloS One*. 2009;44:e5991.

Footnotes and Financial Disclosures

Originally received: July 26, 2017.
Final revision: January 9, 2018.
Accepted: February 15, 2018.
Available online: March 22, 2018. Manuscript no. 2017-1705.

[1] Singapore Eye Research Institute, Singapore.
[2] Ophthalmology and Visual Sciences Program, Duke-NUS Medical School, Singapore.
[3] Singapore National Eye Center, Singapore.
[4] Eskisehir Osmangazi University, Faculty of Medicine, Eskisehir, Turkey.
[5] Office of Clinical Sciences, Duke-NUS Medical School, Singapore.
[6] Program in Neuroscience and Behavioral Disorders, Duke-NUS Medical School, Singapore.
[7] Department of Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

Presented at: the Association for Research in Vision and Ophthalmology, May 1−5, 2016, Seattle, Washington (oral communication); and the World Glaucoma Association meeting, World Glaucoma Congress, June 28 - July 1, 2017, Helsinki, Finland (poster presentation)

Financial Disclosure(s):
The authors have made the following disclosures: D.M. and J.J.G.: Patent application based on the pupillometry protocol used in this study (PCT/SG2015/050494): a method and system for monitoring or assessing pupillary responses.

S.A.P.: Board Member − Alcon, Novartis, Allergan; Payment for lectures − Santen, Glaukos, Ellex.

Supported by the National Medical Research Council, Singapore (NMRC/CIRG/1401/2014) to D.M.; the Singapore National Eye Centre Health Research Endowment Fund, Singapore (R1005/20/2013) to T.A.; and the Biomedical Research Council, Singapore (01-TCRP-2010 and TCR0101674) to T.A. The funding organizations had no role in the design and conduct of the research.

HUMAN SUBJECTS: Human subjects, human-derived materials, or human medical records were part of this study protocol. Study protocol was approved by the SingHealth Centralized Institutional Review Board. Written informed consent was obtained from all participants. All studies adhered to the tenets of the Declaration of Helsinki.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Najjar, Sharma, Baskaran, Gooley, Aung, Milea

Data collection: Najjar, Sharma, Atalay, Rukmini, Sun, Lock, Baskaran, Perera, Husain, Aung, Milea

Analysis and interpretation: Najjar, Sharma, Atalay, Rukmini, Lamoureux, Milea

Obtained funding: Milea, Aung

Overall responsibility: Najjar, Atalay, Baskaran, Perera, Husain, Lamoureux, Gooley, Aung, Milea

Abbreviations and Acronyms:
ACD = anterior chamber depth; **AxL** = axial length; **BCVA** = best-corrected visual acuity; **IOP** = intraocular pressure; **ipRGC** = intrinsically photosensitive retinal ganglion cell; **PIPR** = postillumination pupillary response; **PLR** = pupillary light response; **POAG** = primary open-angle glaucoma; **PSD** = pattern standard deviation; **RNFL** = retinal nerve fiber layer; **SAP** = standard automated perimetry; **VF** = visual field; **VFI** = visual field index; **VFMD** = visual field mean deviation.

Correspondence:
Dan Milea, MD, PhD, Singapore National Eye Centre, 11 Third Hospital Avenue, Singapore, 168751. E-mail: dan.milea@snec.com.sg.