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Pupillary Responses to High-Irradiance Blue Light Correlate with Glaucoma Severity

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Purpose: To evaluate whether a chromatic pupillometry test can be used to detect impaired function of intrinsically photosensitive retinal ganglion cells (ipRGCs) in patients with primary open-angle glaucoma (POAG) and to determine if pupillary responses correlate with optic nerve damage and visual loss.

Design: Cross-sectional study.

Participants: One hundred sixty-one healthy controls recruited from a community polyclinic (55 men; 151 ethnic Chinese) and 40 POAG patients recruited from a glaucoma clinic (22 men; 35 ethnic Chinese) 50 years of age or older.

Methods: Subjects underwent monocular exposure to narrowband blue light (469 nm) or red light (631 nm) using a modified Ganzfeld dome. Each light stimulus was increased gradually over 2 minutes to activate sequentially the rods, cones, and ipRGCs that mediate the pupillary light reflex. Pupil diameter was recorded using an infrared pupillography system.

Main Outcome Measures: Pupillary responses to blue light and red light were compared between control subjects and those with POAG by constructing dose-response curves across a wide range of corneal irradiances (7−14 log photons/cm^2 per second). In patients with POAG, pupillary responses were evaluated relative to standard automated perimetry testing (Humphrey Visual Field [HVF]; Carl Zeiss Meditec, Dublin, CA) and scanning laser ophthalmoscopy parameters (Heidelberg Retinal Tomography [HRT]; Heidelberg Engineering, Heidelberg, Germany).

Results: The pupillary light reflex was reduced in patients with POAG only at higher irradiance levels, corresponding to the range of activation of ipRGCs. Pupillary responses to high-irradiance blue light associated more strongly with disease severity compared with responses to red light, with a significant linear correlation observed between pupil diameter and HVF mean deviation ($r = -0.44$; $P = 0.005$) as well as HRT linear cup-to-disc ratio ($r = 0.61$; $P < 0.001$) and several other optic nerve head parameters.

Conclusions: In glaucomatous eyes, reduced pupillary responses to high-irradiance blue light were associated with greater visual field loss and optic disc cupping. In POAG, a short chromatic pupillometry test that evaluates the function of ipRGCs can be used to estimate the degree of damage to retinal ganglion cells that mediate image-forming vision. This approach could prove useful in detecting glaucoma. *Ophthalmology 2015;122:1777-1785 © 2015 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/).*

The pupillary light reflex (PLR) is often used to assess the integrity of the visual system. Until recently, however, the photoreceptor pathways that drive pupillary light responses were not well characterized. The afferent limb of the PLR is thought to be mediated solely by retinal ganglion cells (RGCs) that contain the short wavelength-sensitive photopigment melanopsin.[1,2] Although melanopsin-containing RGCs are intrinsically photosensitive, they are also activated extrinsically by rod and cone photoreceptors located in the outer retina.[3−5] Genetic ablation of melanopsin RGCs in mice eliminates pupillary responses to light, indicating that these cells serve as a necessary conduit for light information to reach the olivary pretectal nucleus in the midbrain.[6]

Growing evidence indicates that measuring pupillary responses to different wavelengths and irradiances of light (i.e., chromatic pupillometry) can be used to assess inner versus outer retinal degeneration, because intrinsically photosensitive RGCs (ipRGCs) and visual photoreceptors differ in their response properties. In the absence of rod−cone input, melanopsin cells are preferentially sensitive to blue light (λ_{max}, approximately 480 nm), respond sluggishly to light onset and offset, and are less sensitive to light than rods and cones.[3,4,7−9] By comparison, rods are most sensitive to bluish-green light (λ_{max}, approximately 500 nm), the photopic visual system is most sensitive to green light (λ_{max}, 555 nm), and rod−cone photoreceptors are capable of driving fast pupillary responses. The wavelength and irradiance of light exposure therefore can be manipulated to stimulate preferentially rods, cones, or the intrinsic melanopsin response, thus providing a window onto the status of each photoreceptor cell type. For example, blue-light stimuli can be used to activate rods preferentially at low irradiances and melanopsin at high irradiances, whereas

http://dx.doi.org/10.1016/j.ophtha.2015.06.002
ISSN 0161-6420/15

red-light stimuli can be used to target preferentially the activation of middle- and long-wavelength–sensitive cones.[10,11] As such, chromatic pupillometry using blue-light and red-light stimuli could be used to detect photoreceptor dysfunction associated with different types of retinal diseases.

Recent studies suggest that melanopsin-containing RGCs are damaged in glaucoma, similar to nonmelanopsin RGCs that mediate image-forming vision.[12–19] Glaucoma is a major cause of blindness, and hence early detection and treatment are important for slowing the progression of the disease. Patients often seek treatment late in the disease, however, because it is asymptomatic initially. The pupillary light reflex is impaired in severe glaucoma,[14,16] but there is limited evidence regarding whether reduced pupillary light responses correlate with glaucoma severity, as measured by visual field (VF) testing and anatomic correlates of optic nerve damage. To address this limitation, we developed and tested a pupillometry-based protocol for evaluating photoreceptor dysfunction in which the light stimulus (blue or red) is increased gradually over time to assess sustained pupillary responses across a wide range of irradiances. The aim of our study was to determine if this chromatic pupillometry test can be used to detect loss of function of ipRGCs in patients with primary open-angle glaucoma (POAG) and if the degree of impairment in the PLR correlates with disease severity.

Methods

Subjects

Three hundred subjects 50 years of age or older were recruited to undergo a chromatic pupillometry test. Subjects were recruited over a 2-month period (August–September 2013) from a larger study involving more than 2000 volunteers who underwent a standardized ophthalmic examination at a community polyclinic. At the time of enrollment, subjects were either visiting the polyclinic for minor health issues (nonocular) or accompanying another patient at the clinic. The aim of the larger study was to evaluate the incidence of ocular abnormalities in older patients seeking outpatient medical care. The eye examination consisted of tests for visual acuity, intraocular pressure measurement by Goldmann applanation tonometry, automated refraction to assess refractive error, a slit-lamp examination, iris and fundus photography, and an examination by a study ophthalmologist. The chromatic pupillometry test was performed before examination by the ophthalmologist, and hence the researcher performing the pupillary recording was not aware of the ophthalmic status of subjects at the time of testing.

Forty-eight individuals diagnosed with POAG were recruited over a 5-month period (October 2013–February 2014) from glaucoma clinics at the Singapore National Eye Centre. Patients with POAG were defined by the following criteria: the presence of glaucomatous optic neuropathy (defined as a loss of neuroretinal rim with a vertical cup-to-disc ratio of >0.7 or an intereye asymmetry of >0.2, notching attributable to glaucoma, or both) with compatible VF loss (defined below), open angles on gonioscopy, and absence of secondary causes of glaucomatous optic neuropathy. Intraocular pressure before the start of glaucoma treatment was assessed by Goldmann applanation tonometry. In addition to the ophthalmic tests described for the polyclinic study, the glaucoma patients underwent standard automated perimetry (Humphrey Visual Field [HVF] Analyzer II model 750; Carl Zeiss Meditec, Dublin, CA) and Heidelberg Retinal Tomography (HRT 3; Heidelberg Engineering, Heidelberg, Germany), performed either on the day of chromatic pupillometry testing or within the preceding 3 months. Humphrey Visual Field testing was performed with near refractive correction using the 24-2 Swedish interactive thresholding algorithm with stimulus size III. Repeat testing was performed if false-positive or false-negative responses exceeded 33% or if the fixation loss rate was more than 20%. Patients who could not achieve these reliability criteria were ineligible for the study. A glaucomatous VF defect was defined by the presence of a glaucoma hemifield test result outside normal limits and the presence of at least 3 contiguous, nonedge test points within the same hemifield on the pattern deviation probability plot at $P < 0.05$, with at least 1 point at $P < 0.01$, excluding points directly above and below the blind spot. Glaucoma severity was graded according to the Hodapp-Parrish-Anderson scale,[20] in which subjects with mean deviation less than −6 dB were classified as having early VF loss, mean deviation between −6 dB and −12 dB as moderate VF loss, and mean deviation more than (i.e., more negative than) −12 dB as severe VF loss. For HRT, global and segmental disc and cup areas were analyzed using the standard reference plane. In most cases, the POAG disease severity was known at the time that the pupillometry test was performed.

Control subjects and POAG patients were excluded from chromatic pupillometry testing if they had undergone previous intraocular surgery. Additional exclusionary criteria for individuals with POAG included significant nuclear sclerosis of more than grade 2 severity on slit-lamp examination; severe retinal or ocular comorbid conditions including, but not limited to, diabetic retinopathy and age-related macular degeneration; and clinically significant pupillary abnormalities (except for relative afferent pupillary defects). Because the ophthalmic health of subjects recruited from the polyclinic was determined after chromatic pupillometry testing, data from participants who failed to meet the above criteria were excluded post hoc from the analyses. Therefore, the number of subjects with normal ocular health was not determined a priori, whereas the sample size of the group with POAG was determined before study recruitment. Demographic information was collected using interviewer-administered questionnaires. The study was approved by the SingHealth Centralized Institutional Review Board, and all participants provided written informed consent. Research procedures adhered to ethical principles outlined in the Declaration of Helsinki.

Chromatic Pupillometry

Before each light exposure, participants were seated with their head position fixed by a chinrest for at least 1 minute in a dark environment. To measure the direct PLR, light was administered to one eye using a modified Ganzfeld dome (Labsphere, Inc, North Sutton, NH), with the other eye covered by a patch. Subjects were exposed to a blue-light stimulus (469 nm) or red-light stimulus (631 nm), with the order of exposure randomized and counterbalanced. Narrow-bandwidth light was provided using light-emitting diodes (Nichia Corporation, Tokushima, Japan) that were controlled using a function generator (Keithley Instruments, Inc, Cleveland, OH). Current was applied to the light-emitting diodes over a 2-minute period using a logarithmically increasing function in 14 000 steps. Hence, the light stimulus was perceived as increasing gradually in intensity over time, ranging from 6.8 log photons/cm^2 per second to 13.8 log photons/cm^2 per second at the level of the cornea. The maximum light level corresponded to 12.27 lux and 28.30 μW/cm^2 for the blue-light stimulus and 20.43 lux and 21.67 μW/cm^2 for the red-light stimulus. Light levels were calibrated using a portable radiometer (ILT1700 radiometer;