Intrinsically Photosensitive (Melanopsin) Retinal Ganglion Cell Function in Glaucoma

Beatrix Feigl, Dietmar Mattes, Ravi Thomas, and Andrew J. Zele

PURPOSE. To determine whether glaucoma alters intrinsically photosensitive retinal ganglion cell (ipRGC) function.

METHODS. Forty-one patients (25 with glaucoma and 16 healthy age-matched control participants) were tested. Intrinsically photosensitive retinal ganglion cell function was directly measured by the sustained, postillumination pupil response (PIPR). Forty-one eyes of 41 participants were tested with 7°, 10-second, short-wavelength (488 nm; bluish) and long-wavelength (610 nm; reddish) stimuli (14.2 log photons · cm⁻² · s⁻¹) presented to the right eye in Maxwellian view, and the consensual pupil response of the left eye was measured by infrared pupillometry. The difference between PIPR amplitude (percentage baseline pupil diameter), net PIPR (percentage change) and kinetics (time in mm · s⁻¹ to the PIPR plateau) for the blue and red stimuli in patients with early and advanced (moderate/severe) glaucoma was compared to that in age-matched control participants.

RESULTS. The blue PIPR was significantly smaller between normal participants and patients with advanced glaucoma, as well as between those with early and those with advanced glaucoma (P < 0.05). The kinetics of the red and blue PIPRs were not significantly different between any groups. Normal age-matched participants and patients with early-stage glaucoma were not significantly different on any parameter, and neither was the normal and glaucoma group (advanced and early combined).

CONCLUSIONS. Persons with moderate and severe glaucoma have a dysfunctional ipRGC-mediated PIPR. Intrinsically photosensitive retinal ganglion cell function measured directly with the PIPR may become a clinical indicator of progressive changes in glaucoma. (Invest Ophthalmol Vis Sci. 2011;52:4362–4367) DOI:10.1167/iovs.10-7069

The pupillary light reflex is a fundamental diagnostic tool for the assessment of afferent and efferent defects in neuroophthalmic disorders. The recently discovered intrinsically photosensitive retinal ganglion cells (ipRGCs) directly contribute to the postillumination pupil response (PIPR) as a sustained constriction (>30 seconds) of the pupillary light reflex after offset of high retinal irradiance (half-maximum, ~13.6 log photons · cm⁻² · s⁻¹), short wavelength light (ipRGC peak spectral sensitivity, ~482 nm). In vitro recordings in macaque and human retinas showed that ipRGCs display a typical transient increase in firing rate at stimulus onset and a unique sustained firing that continues after light offset. It is this sustained, intrinsic ipRGC photoresponse that controls the PIPR. The ipRGCs are sparse (~3000; approximately 0.2% of all ganglion cells), have the longest dendrites and largest somata of all known ganglion cell types (diameters of 350 to 1200 μm, increasing with retinal eccentricity), are mainly located in the ganglion cell layer, and are at peak density in the paracentral retina (2°) for review see, Markwell et al.). The normal functional ipRGC-mediated PIPR range has been determined in a sample of healthy persons, and the PIPR magnitude was independent of age, race, or sex and shows high repeatability. However, its potential use in disease detection and management is still to be determined and may open a new view of the pupillary light reflex in the diagnosis and monitoring of a wider range of retinal and optic nerve diseases. Investigations in a few reports indicated that the pupillary light reflex can be used to differentiate outer and inner retinal function in disease by appropriate choice of stimulus wavelength and intensity. For example, outer and inner retinal contributions to the pupillary light reflex can be determined by varying the intensity of a reddish (long-wavelength) or bluish (short-wavelength) light. Indeed, with such stimuli, deficits in retinitis pigmentosa and anterior ischemic neuropathy have been identified.

In vitro evidence from flat-mounted rat retinas suggests that chronic ocular hypertension induced over 12 weeks does not produce observable ipRGC morphologic changes or cell loss, whereas non-melanopsin-labeled superior colliculus projecting retinal ganglion cells do exhibit significant loss. It was inferred that ipRGCs have a high cellular resistance to injury-induced damage. In another study using a rat model of ocular hypertension, reduced ipRGC density was found, but there was no change in soma size and dendritic morphology in the remaining ipRGCs. There is, however, anecdotal evidence showing no immunoreactivity of the ipRGC photopigment melanopsin in one patient with long-standing glaucoma. Moreover, patients with advanced glaucoma show reduced melatonin suppression, implying that ipRGC function may be affected. Recently Kankipati et al. demonstrated reduced ipRGC-mediated PIPR function in glaucomatous optic neuropathy, but did not investigate the PIPR in early stages of glaucoma, nor did they study pupil kinetics. In the present study we determined whether the PIPR (amplitude and kinetics) is dysfunctional in patients with early, moderate, or severe glaucoma compared with an age-matched control group.

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 PARTICIPANTS AND METHODS

Participants

All 41 participants underwent a comprehensive ophthalmic examination including best-corrected visual acuity, anterior eye slit lamp biomicroscopy, gonioscopy, intraocular pressure measured with Goldmann applanation tonometry and optical coherence tomography by one of the three eye specialists (BF, DM, and RT). Of these, 25 were glaucoma patients (age range, 31–78 years, mean ± SD, 60 ± 11.7; 15 men and 10 women) recruited from a glaucoma specialist practice (RT) where they had undergone testing for glaucoma, including conventional visual fields (Humphrey 30-2, Humphrey Field Analyzer, HFA; Carl Zeiss Meditec, Inc. Dublin, CA). Patient characteristics are outlined in Table 1.

Glucoma was defined by an intraocular pressure of ≥ 21 mm Hg before the beginning of the pressure-lowering therapy. Combining optical changes similar to glaucoma (neuroretinal rim thinning, loss of inferior temporal, nasal, and/or superior temporal nasal [ISTN] rule, notch, disc hemorrhage, nerve fiber layer defect), with or without characteristic visual field defects; secondary glaucoma was excluded. All glaucomatous eyes were phakic, with lens densities < 1 (for nuclear, subcapsular, and cortical cataracts) according to the Age-Related Eye Disease Study Research Group (AREDS) classification. One patient (G20) had undergone cataract surgery and intraocular lens (IOL) implantation in the right eye and one in the left eye, and one patient (G16) had no treatment.

Sixteen healthy participants without ocular disease (age range, 30–72 years, mean ± SD 58 ± 12.9) served as the age-matched normal control group and were randomly selected from a university cohort. The control participants had no morphologic or functional damage indicating glaucoma and were matched to cases by age (< 5 years).

Medical history including diabetes mellitus, hypertension, general medical history including best corrected visual acuity, anterior eye slit lamp biomicroscopy, gonioscopy, intraocular pressure measured with Goldmann applanation tonometry and optical coherence tomography by one of the three eye specialists (BF, DM, and RT). Of these, 25 were glaucoma patients (age range, 31–78 years, mean ± SD, 60 ± 11.7; 15 men and 10 women) recruited from a glaucoma specialist practice (RT) where they had undergone testing for glaucoma, including conventional visual fields (Humphrey 30-2, Humphrey Field Analyzer, HFA; Carl Zeiss Meditec, Inc. Dublin, CA). Patient characteristics are outlined in Table 1.

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Informed consent was obtained from all participants, and the study was conducted in accordance with the requirements of Queensland University of Technology Human Research Ethics Committee and the tenets of the Declaration of Helsinki.

Pupillometry

The consensual pupilary light reflex of the left eye was recorded with an infrared camera (Pixelink; IEEE-1394, PL-A741 FireWire; 480 × 640 pixels; 62 frames · sec⁻¹) through a telecentric lens (Computar 2/3 in; 55-mm and 2 × extender C-Mount; CBC, Tokyo, Japan) in response to a calibrated, monochromatic, 14.2 log photon · cm⁻² · s⁻¹, 10-second, 7.15° stimulus (488 nm, 610 nm; 10–12 nm full-width at half maximum; Edmund Optics, Barrington, NJ) presented to the right eye with a Maxwellian view optical system, controlled and analyzed with custom software (MatLab; The MathWorks, Natick, MA). The viewing distance (1.15 m) of the 6.3° × 8.9° back-lit fixation screen (5.6 cd · m⁻²) for the consensual left eye was determined by a control study, to minimize accommodation and convergence-driven pupil fluctuations.

Temple bars, head restraint, and chin rest stabilized the head position in the pupillometer.

Patients with abnormal nuclear, subcapsular, or cortical lens changes greater than 1 were excluded according to the AREDS classification. To compensate for any change in stimulus retinal irradiance due to senile miosis and/or age-related optical media changes (AREDS grade < 1), the stimulus was presented in Maxwellian view, and corneal irradiance was set to 14.2 log photons · cm⁻² · s⁻¹. This irradiance was chosen based on the retinal irradiance-PIPR functions measured by Gamlin et al. in three individuals at 493 nm between 9 and 15 log photons · cm⁻² · s⁻¹. The half-maximum PIPR constriction was at −13.6 log photons · cm⁻² · s⁻¹, with higher irradiances producing larger PIPR constrictions until a plateau of −15 log photons · cm⁻² · s⁻¹.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Duration of Glaucoma since Diagnosis</th>
<th>IOP RE/LE</th>
<th>Grading*</th>
</tr>
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<tbody>
<tr>
<td>G1</td>
<td>M</td>
<td>58</td>
<td>2 Years (POAG)</td>
<td>8/16</td>
<td>Advanced</td>
</tr>
<tr>
<td>G3</td>
<td>M</td>
<td>76</td>
<td>7 Years (POAG)</td>
<td>12/15</td>
<td>Advanced</td>
</tr>
<tr>
<td>G4</td>
<td>M</td>
<td>71</td>
<td>1 Year (ACG)</td>
<td>13/11</td>
<td>Advanced</td>
</tr>
<tr>
<td>G5</td>
<td>F</td>
<td>58</td>
<td>12 Years (POAG)</td>
<td>19/20</td>
<td>Early</td>
</tr>
<tr>
<td>G6</td>
<td>M</td>
<td>42</td>
<td>New (POAG)</td>
<td>21/17</td>
<td>Early</td>
</tr>
<tr>
<td>G7</td>
<td>M</td>
<td>77</td>
<td>New (POAG)</td>
<td>13/12</td>
<td>Early</td>
</tr>
<tr>
<td>G8</td>
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<td>60</td>
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<td>14/18</td>
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<td>F</td>
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<td>22/15</td>
<td>Early</td>
</tr>
<tr>
<td>G11</td>
<td>M</td>
<td>40</td>
<td>1 Months (POAG)</td>
<td>10/16</td>
<td>Advanced</td>
</tr>
<tr>
<td>G14</td>
<td>F</td>
<td>57</td>
<td>New (POAG)</td>
<td>18/17</td>
<td>Early</td>
</tr>
<tr>
<td>G15</td>
<td>F</td>
<td>67</td>
<td>18 Months (POAG)</td>
<td>18/21</td>
<td>Early</td>
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<td>G16</td>
<td>F</td>
<td>60</td>
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<td>18/15</td>
<td>Early</td>
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<tr>
<td>G17</td>
<td>M</td>
<td>70</td>
<td>18 Years (POAG)</td>
<td>8/16</td>
<td>Advanced</td>
</tr>
<tr>
<td>G18</td>
<td>F</td>
<td>65</td>
<td>6 Months (POAG)</td>
<td>20/16</td>
<td>Advanced</td>
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<td>G19</td>
<td>F</td>
<td>48</td>
<td>4 Years (POAG)</td>
<td>19/20</td>
<td>Early</td>
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<tr>
<td>G20</td>
<td>M</td>
<td>62</td>
<td>&gt;20 Years (POAG)</td>
<td>10/12</td>
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<td>Unknown (POAG)</td>
<td>19/15</td>
<td>Advanced</td>
</tr>
<tr>
<td>G23</td>
<td>M</td>
<td>47</td>
<td>18 Months (POAG)</td>
<td>12/10</td>
<td>Advanced</td>
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<td>G24</td>
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<td>17/17</td>
<td>Early</td>
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<td>G26</td>
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<td>18 Months (POAG)</td>
<td>10/10</td>
<td>Early</td>
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<td>M</td>
<td>71</td>
<td>1 Year (POAG)</td>
<td>18/13</td>
<td>Early</td>
</tr>
<tr>
<td>G28</td>
<td>F</td>
<td>56</td>
<td>2 Weeks (POAG)</td>
<td>17/15</td>
<td>Early</td>
</tr>
<tr>
<td>G29</td>
<td>M</td>
<td>54</td>
<td>8 Months (POAG)</td>
<td>15/15</td>
<td>Early</td>
</tr>
<tr>
<td>G30</td>
<td>M</td>
<td>78</td>
<td>1 Month (POAG)</td>
<td>24/20</td>
<td>Advanced</td>
</tr>
</tbody>
</table>

* Grading based on MD on SAP (Humphrey perimetry; Carl Zeiss Meditec, Inc., Dublin, CA) and mean sensitivity on microperimetry (MP-1; Nidek, Padova, Italy).
mediated response. Our studies indicate that the within-subject test (610 nm) was chosen as a control of nonspecific factors such as fatigue measures analysis of variance (RM-ANOVA) and one-way ANOVA. The blue PIPR, red GR, and net PIPR were analyzed by using repeated-
that did not show major deviations from normality. The blue PIPR, red dilation to the sustained PIPR after light offset.

constant (in millimeters per second) from the best-fitting exponential


tations. The kinetics of the PIPR were described by an exponential function of the form \( y = \Delta \cdot \exp(k \cdot x) + P \), where \( \Delta \) is a constant, \( k \) is the redilation velocity (global rate constant; GR) in millimeters per second, \( x \) is time in seconds, and \( P \) is the sustained plateau pupil diameter (in millimeters) of the PIPR. The prestimulus baseline pupil diameter and PIPR values were derived from the best-fitting model parameters and used for statistical analysis. The PIPR was described according to three parameters. First, to control for the influence of baseline pupil diameter on PIPR amplitude, the percentage PIPR amplitude was calculated relative to the baseline pupil diameter for the 488 nm (blue PIPR) and the red stimulus (red PIPR). Second, the net PIPR value (percentage change) was assessed by subtracting the blue PIPR percentage from the red PIPR percentage. Third, the GR constant (in millimeters per second) from the best-fitting exponential functions to the blue and red PIPR data estimated the kinetics of redilation to the sustained PIPR after light offset.

The initial data analysis included the distributions of the variables that did not show major deviations from normality. The blue PIPR, red PIPR, blue GR, red GR, and net PIPR were analyzed by using repeated-measures analysis of variance (RM-ANOVA) and one-way ANOVA. \( P \leq 0.05 \) was considered as significant. Post hoc analysis (LSD) was performed to identify significant group differences.

Microperimetry

Microperimetry was performed with a device (Microrperimeter-MP1; Nidek Technologies, Padova, Italy) that allows a 45° view of the fundus during testing. An inbuilt automated tracking system corrects for eye movements, ensuring that the tested retinal location is aligned to the reference area identified at the start of the examination. The manufacturer-customized Humphrey 10-2 program was used, and 68 Goldmann III stimuli were presented in a 10° test grid with a 4-to-2 staircase strategy. The background luminance was 1.27 cd · m\(^{-2}\), and the stimulus duration was 100 ms.

Glaucome Grade

Mean deviation (MD) of standard automated perimetry (SAP) (Humphrey 30-2) and microperimetry (MP) mean sensitivity (MS) of the right eyes were used to grade glaucoma participants according to two groups. Humphrey MD less than –6.0 dB and MS greater than 15.5 dB on microperimetry (based on our normal age-matched cohort \( n = 39 \); mean \( \pm SD \)) was assigned to the early glaucoma group. Patients with Humphrey MD greater than –6.0 dB and <15.5 dB on microperimetry formed the advanced glaucoma group where moderate and severe glaucoma cases were combined based on the MD on Humphrey. The inclusion of microperimetry in the glaucoma grading is based on a recent finding that central defects can remain undetected on SAP but are evident on microperimetry. Group 1 (early) consisted of 14 patients (mean age, 57 ± 11.6 years) with glaucomatous disc changes and/or mild defects on SAP but no central field defects on microperimetry. Group 2 (advanced) consisted of 11 patients (mean age, 63 ± 11.6 years) with moderate to severe glaucomatous field defects on SAP (MD > 6.0 dB) and/or central defects on microperimetry (MS < 15.5 dB). This group included two patients (G11 and G30) who had central defects on microperimetry and an MD < 6 dB on SAP (with no central deficits). One patient (G3) could not perform microperimetry due to advanced visual field defects on SAP and was assigned to group 2. Two further patients (G24, G27) did not complete microperimetry due to technical or ocular issues (i.e., concentration, dry eyes) and were assigned to group 1 based on SAP.

RESULTS

The pupillary light reflex (% baseline pupil diameter) of a healthy control participant and a patient with advanced glaucoma (G23) is shown in Figure 1 as a function of time (in seconds) for the 488 nm (blue; test) and 610 nm (red; control) stimuli. The pupillary light reflex is composed of the baseline pupil diameter (10-second prestimulus recording), response latency and recovery (redilation after light offset) to the PIPR. In the healthy participant (Fig. 1A), the sustained PIPR for 20 to 50 seconds after light offset with the blue light stimulus is

\( \Delta \) Exemplary pupillary light reflex of a healthy 55-year-old person with an 84.6% ipRGC-mediated, sustained PIPR compared with baseline pupil diameter, indicating normal ipRGC function (net PIPR change, 14.9%). \( \Delta \) Exemplary pupillary light reflex of a 47-year-old glaucoma patient with field defects classified as advanced glaucoma (G23). The difference in net PIPR change is <5%, indicating ipRGC dysfunction.

\[ \Delta \text{Amplitude} = \frac{\text{amplitude}_{	ext{baseline}} - \text{amplitude}_{	ext{final}}}{} \times 100 \]
significant main effect between the three groups. This analysis identified a significant PIPR percentage to examine the difference between the glaucoma and normal participants. There was no significant difference between early glaucoma and control groups. The PIPR global-rate constant estimated the resistance to injury-induced damage. Studies in patients with toxic neuropathy or hereditary mitochondrial neuropathy also indicate a robustness of ipRGCs to injury and a neuroprotective role of pituitary adenylate cyclase-activating polypeptide (PACAP) has been discussed. Melatonin has also been suggested to be neuroprotective in glaucoma because of its antioxidant and antinitridergic properties. Jean-Louis et al. hypothesized that glaucoma is consistent with intrinsically photosensitive retinal ganglion cell (ipRGC) contributions to the PIPR are altered in patients with glaucoma. It was observed that ipRGC function was reduced in advanced glaucoma patients compared with persons with early glaucoma and persons in the healthy normal control group. The PIPR global-rate constant estimated the kinetics of pupil readilization after light offset and was not significantly different between the glaucoma and control groups. Our findings of ipRGC dysfunction in humans with advanced glaucoma suggest a role of this ganglion cell subtype in the pathomechanisms of progressive disease.

Our observation that the PIPR was not reduced in early glaucoma was consistent with in vitro ocular hypertension experiments in rats that show that ipRGCs have a high cellular resistance to injury-induced damage. Studies in patients with toxic neuropathy or hereditary mitochondrial neuropathy also indicate a robustness of ipRGCs to injury and a neuroprotective role of pituitary adenylate cyclase-activating polypeptide (PACAP) has been discussed. Melatonin has also been suggested to be neuroprotective in glaucoma because of its antioxidant and antinitridergic properties. Although the neuroprotective mechanisms are still to be determined, the resistance of ipRGCs to damage may be important, given their reduced redundancy, and their non–image-forming role in signaling the environmental light levels to the central circadian clock in suprachiasmatic nucleus (SCN) for photentrainment. Jean-Louis et al. hypothesized that glaucoma

**Table 2.** ipRGC Controlled PIPR Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Blue PIPR Amplitude*</th>
<th>Red PIPR Amplitude*</th>
<th>Net PIPR†</th>
<th>Blue Global Rate (mm·s⁻¹)</th>
<th>Red Global Rate (mm·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>89.0 ± 5.8</td>
<td>97.0 ± 3.3</td>
<td>7.9 ± 5.5</td>
<td>−0.4 ± 0.2</td>
<td>−0.4 ± 0.2</td>
</tr>
<tr>
<td>Advanced</td>
<td>94.8 ± 2.6</td>
<td>97.7 ± 3.1</td>
<td>2.9 ± 2.3</td>
<td>−0.3 ± 0.2</td>
<td>−0.3 ± 0.2</td>
</tr>
<tr>
<td>Control</td>
<td>88.1 ± 3.8</td>
<td>97.4 ± 5.1</td>
<td>9.3 ± 4.9</td>
<td>−0.3 ± 0.2</td>
<td>−0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Data are expressed as the mean ± SD.

* Percentage amplitude relative to baseline pupil diameter.
† Percentage change.
would provide an opportunity to evaluate whether ganglion cell loss, in particular ipRGCs, could compromise photic input to the circadian system. Animal models in rats suggest that there may be impairment of the circadian rhythm and melatonin secretion in glaucoma. Further research is needed, to evaluate whether patients with advanced glaucoma have difficulty readjusting to changes in the light-dark environment, such as traveling across different time zones. An ipRGC resistance to injury, however, could weaken during longstanding conditions of ocular hypertension or in manifest glaucoma. The observed ipRGC dysfunction in the advanced cases may therefore indicate glaucoma progression. In this framework, dysfunction alters ipRGC irradiance signaling to the pineal gland, thereby impairing melatonin release, resulting in reduced neuroprotection. In our medical evaluation of all participants, we informally discussed sleep behavior, and none of our patients complained of any circadian disturbances. Taken together, we infer from the patient reports that photic entrainment is still possible in advanced glaucoma patients with ipRGC dysfunction. However, future studies should determine patient melatonin levels, evaluate sleep patterns with objective measures and relate these with different levels of ipRGC dysfunction or loss.

The ipRGC inputs to the pupil response may be nonlinear, so that a large level of loss or dysfunction is required before the PIPR is affected. In the absence of a complete irradiance response study of the PIPR to determine the threshold ipRGC number for a normal PIPR, a resistance to injury, as shown in rodents, may not apply in humans. It has been shown, at least in mice, that photon capture by only a few ipRGCs is required at the pupil reflex threshold, whereas only the slightest pupil constriction is present after near complete ablation of ipRGCs. However, the peak ipRGC density (~2° paracentral) is outside the most severely affected areas in glaucoma, and therefore many macular ipRGCs could remain unaffected by arcuate defects and only show deficits when glaucomatous damage has progressed to the center. To account for this latter hypothesis, patients with severe glaucoma were graded according to both the MD on SAP and mean sensitivity on microperimetry, because microperimetry is sensitive to central deficits not apparent on SAP. Indeed, those participants graded as advanced had a more impaired PIPR on average than did patients graded with early glaucoma (and no deficits on microperimetry). This result is in accordance with a recent finding that shows that as visual field loss increases in severity there is a reduction in the net PIPR change. Our data further indicate that ipRGC dysfunction in glaucoma can occur in central retinal areas before deficits on SAP. In most of the glaucoma patients, SAP central visual field performance was relatively better than in the midperipheral retina (data not shown).

Monitoring glaucoma progression is as important as early diagnosis and requires precise assessment of functional loss and structural change relative to baseline measurements. The gold standard for monitoring functional loss is SAP, and statistical programs are available to assist the ophthalmologist in the difficult task of assessing progression. While imaging techniques are likely to be useful earlier in the course of disease, the monitoring and prognostication for advanced glaucoma is far more challenging. Visual sensitivity is more variable, testing requires larger stimuli, and statistical programs to assess progression for advanced stages are not available. The "macula split" as demonstrated by the size V target on the macula program of the Humphrey field analyzer can be used as a crude measure to assess prognosis of vision and the probability of a wipeout after surgery. However, the determination of ipRGC function using the blue PIPR may have potential to monitor or determine progression in the later stages of the disease, especially if baseline measurements are available. Although our exploratory study did not have enough cases to analyze ipRGC function separately in the moderate and severe glaucoma patients, who were combined into one group, future research is planned to explore its potential in differentiating between these two groups. A further extension of this study will be to use a range of stimulus irradiance levels to study how the PIPR difference between the glaucoma patients and age-matched controls depend on irradiance, and to determine the threshold irradiance for detection of early glaucomatous change.

In summary, this exploratory study is the first demonstration that patients with advanced glaucoma have ipRGC dysfunction compared with patients with early glaucoma and normal participants as determined by direct measurement of ipRGC function with the PIPR. Future research is needed to determine how ipRGC function can assist in the assignment of prognosis, assessment of progression, and decision-making in advanced glaucoma cases.

References


