The Post-Illumination Pupil Response Is Reduced in Glaucoma Patients

Laxmikanth Kankipati,*1,2 Christopher A. Girkin,2 and Paul D. Gamlin*1

**Purpose.** The post-illumination pupil response (PIPR), which is driven by the intrinsic response of melanopsin-containing, intrinsically photosensitive retinal ganglion cells, has previously been characterized in healthy eyes. The present study examined whether the PIPR is affected in patients with glaucoma compared with healthy subjects.

**Methods.** Sixteen glaucoma patients (mean age, 63.7 years) were tested by presenting a 60°, 10-second light stimulus (13 log quanta/cm²/s retinal irradiance) of either 470 nm (blue) or 623 nm (red) to one eye after dilation. The consensual pupil response of the fellow undilated eye was recorded by infrared pupillometry for 50 seconds after light offset. These pupillary responses were compared with those of 19 age-matched controls (mean age, 59 years).

**Results.** The glaucoma patients displayed a net PIPR (blue PIPR minus red PIPR) that was significantly (t-test, P < 0.001) smaller (0.6 mm, SEM 0.12; P < 0.05) than in age-matched controls (1.3 mm, SEM 0.16; P < 0.001). For the patient population, the magnitude of the net PIPR was inversely correlated with the measured visual field loss (mean deviation) of the tested eye.

**Conclusions.** This study demonstrates that there is a significant decrease in the ipRGC-mediated PIPR in glaucomatous patients when compared to age-matched controls. As the severity of the glaucomatous neuropathy increases, there is a correlated decrease in the PIPR. Therefore, this test has the potential for use as a clinical tool in evaluating patients with glaucoma.

**Materials and Methods**

**Participant Selection**

Patients with glaucoma were recruited as part of ongoing studies within the Glaucoma Service of the Eye Foundation Hospital (EFH) at University of Alabama at Birmingham. Data from age-matched controls were selected from a group of subjects with healthy eyes collected for a previous study in our laboratory who underwent a similar testing protocol. We also correlated the PIPR of the patients with their mean deviation (MD) values (a measure of central visual field loss) in the tested eye.

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TABLE 1. Demographic Characteristics of Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 16)</th>
<th>Age-Matched Controls (n = 19)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63.7</td>
<td>59</td>
<td>&gt;0.05</td>
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<tr>
<td>Sexa</td>
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<tr>
<td>Female</td>
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<td>Male</td>
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<td>Racea</td>
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<td></td>
</tr>
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<td>European ancestry</td>
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<td>9</td>
<td></td>
</tr>
<tr>
<td>African ancestry</td>
<td>5</td>
<td>10</td>
<td></td>
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<td>Baseline pupil diameter, mm</td>
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<td>4.8</td>
<td>&gt;0.05</td>
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<tr>
<td>Mean deviation, dB</td>
<td>-12.44</td>
<td>WNL</td>
<td></td>
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</tbody>
</table>

Values are rounded to 1 decimal point. WNL, within normal limits.
*a Sample size was too small to test for differences between the groups.

who were judged to have GON on fundus examination and on masked evaluation of fundus stereographs by a fellowship-trained glaucoma specialist (CAG) were included in the study. For the subjects who had cataracts removed and had intraocular lenses in place, there was no statistically significant difference in pupil size compared with those who did not and with the age-matched control group (P < 0.05). Given that the purpose of this initial study was to determine whether the PIPR is diminished by the glaucomatous process, we avoided enrolling subjects with early glaucoma.

Testing Apparatus

A novel optical system that has previously been described was used for this study.13 The viewing eye of the subject was dilated with 1% tropicamide (Mydral; Bausch & Lomb, Rochester, NY) and 2.5% phenylephrine (Neofrin; Bausch & Lomb), and the light stimulus was presented to this eye while the consensual pupil response in the undilated eye was recorded by infrared camera (Digivue EC-PC-Cam; Elyssa Corporation, Briarcliff Manor, NY) and computer.

Statistical Analysis

Data from all tests were stored and analyzed off-line. Traces showing pupillary diameter and eye position were displayed for analysis, and regions of the data were selected for further analysis by data analysis software (Excel [Microsoft, Redmond, WA], SAS [SAS Institute, Chicago, IL], and MatLab [MathWorks, Natick, MA]), and data plots were generated (SigmaPlot; SyStat Software, San Jose, CA).

As described previously,13 we defined the following measures for data analysis. Baseline pupil diameter was the average pupil diameter, over a 7-second period, before light stimulus. Sustained pupil diameter was the average pupil diameter over a period of 30 seconds, starting 10 seconds after light offset. The variables used for subsequent analyses were:

\[
\text{PIPR (mm)} = \frac{[\text{Baseline pupil diameter (mm)}] - \text{sustained pupil diameter (mm)}}{\text{Baseline pupil diameter (mm)}}
\]

PIPR change (%) = \([\text{PIPR} \times 100]/\text{Baseline pupil diameter}\)

Net PIPR (mm) = \([\text{Blue PIPR} - \text{Red PIPR}]\)

Net PIPR change (%) = \([\text{Blue PIPR change (%)} - \text{Red PIPR change (%)}]\)
Descriptive statistics (mean and standard errors) were calculated for baseline, sustained response, and PIPR. Test for normalcy was performed using the Shapiro-Wilk test. Statistical analyses were performed using linear regression models, and statistical significance was obtained with a nominal $P < 0.05$. Blue and red light responses were compared using the Student’s $t$-test and the slopes for regression were tested with the $F$-test. Relationships between baseline pupil sizes and the PIPR parameters were investigated by means of Pearson’s correlation coefficients and analysis of covariance within patient and control groups.

**RESULTS**

Sixteen persons with GON were recruited for the study (10 women and 1 man of European ancestry; 2 women and 3 men of African ancestry). Demographic characteristics of the patients and controls are shown in Table 1. The patients’ average age was 63.7 years (range, 42–88 years). There were 19 control subjects (3 women and 6 men of European ancestry; 3 women and 7 men of African ancestry) with an average age of 59 years (range, 49–80 years), whose data were collected in our previous study. There was no significant difference in the mean age between the two groups ($t$-test, $P > 0.05$). The number of subjects in the race and sex categories was not sufficient to test for differences between the two groups.

Average pupil diameters of the patients were plotted against time for both control and test stimuli (623 and 470 nm, respectively; Fig. 1A). For comparison, pupil traces for the same stimuli in the control subjects are presented in Figure 1C. PIPR values to both red and blue stimuli were plotted against the baseline pupil diameters (Fig. 1B; blue light, $R^2 = 0.335$, $P < 0.05$; red light, $R^2 = 0.034$, $P < 0.05$). PIPR values for the control subjects were also plotted as a function of baseline pupil diameter (Fig. 1D; blue light, $R^2 = 0.303$, $P < 0.05$; red light, $R^2 = 0.045$, $P < 0.05$).

Values for the baseline pupil diameter, sustained pupil diameter, and PIPR measures for red (control) and blue (test) stimuli, respectively, of the patients and age-matched controls are shown in Table 2. For the patient population, the mean response to the blue stimulus was 0.7 mm (SEM 0.14, $P < 0.05$), with a net PIPR of 0.6 mm (SEM 0.12, $P < 0.05$). For the control subjects, the mean response to the blue stimulus was

| Table 2. Pupil Measurements in Patients and Age-Matched Controls |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                          | Patients ($n = 16$)      | Age-Matched Controls ($n = 19$) |
|                          | R            | B            | B-R          | R            | B            | B-R          |
| Baseline                 | 4.6          | 4.5          | 0.1          | 4.9          | 4.8          | 0.1          |
| Sustained                | 4.5          | 3.8          | 0.7          | 4.7          | 5.3          | 1.4          |
| PIPR                     | 0.1 (0.05)   | 0.7* (0.14)  | 0.6* (0.12)  | 0.2 (0.05)   | 1.5* (0.15)  | 1.3† (0.16)  |

Baseline measure is the average pupil diameter over a 7-second period before light onset. Sustained measure is the average pupil diameter over a period of 30 seconds, starting 10 seconds after light offset. The difference between the two measures provides the PIPR value. SEM is indicated in parentheses. Values are rounded to 1 decimal point. R, red; B, blue.

* $P < 0.05$; † $P < 0.001$. 

**Figure 2.** Net PIPR plotted against baseline pupil diameters, with linear regression lines (solid line) and 95% confidence intervals (dashed lines) comparing the patients ($n = 16$) (A, B) and the age-matched controls ($n = 19$) (C, D). (A) Baseline diameter of patients plotted against absolute net PIPR values ($R^2 = 0.433$; $P < 0.05$). (B) Net PIPR change (%) of patients plotted against baseline diameter ($R^2 = 0.239$; $P = 0.055$). (C) Baseline diameter of the age-matched controls plotted net PIPR (mm) ($R^2 = 0.237$, $P < 0.05$). (D) Net PIPR change (%) of age-matched controls plotted against baseline diameter ($R^2 = 0.004$; $P > 0.05$). The offset for all the parameters was not significantly different from zero ($P > 0.05$) for patients.
1.5 mm (SEM 0.15, \( P < 0.05 \)) with a net PIPR of 1.3 mm (SEM 0.16, \( P < 0.001 \)).

For subsequent analyses, we used the net PIPR values. Figures 2A and 2B show the relationship of the net PIPR (Fig. 2A; \( R^2 = 0.433, P < 0.05 \)) and the net PIPR change (%) (Fig. 2B; \( R^2 = 0.239, P = 0.055 \)) as a function of the baseline pupil diameter for the patient population. Figures 2C and 2D show the relationship of the net PIPR (Fig. 2C; \( R^2 = 0.237, P = 0.05 \)) and the net PIPR change (%) (Fig. 2D; \( R^2 = 0.004, P = 0.055 \)) as a function of the baseline pupil diameter for the control population. It should be noted in Figures 2B and 2D that there is no significant relationship between baseline pupil diameter and net PIPR change (%), showing that this metric effectively controls for baseline pupil diameter in both the patients and the age-matched controls.

When we compared the results from our glaucoma patients with those of the age-matched controls, we found that all PIPR measures were significantly lower (\( P < 0.001 \)) in the patient population than in the control group (Table 3). When we compared the net PIPR change (%) among the patients and the controls (Fig. 3), we noted that most patients had a PIPR change of <20% (\( n = 16; \text{mean}, 13\% \)), whereas most control subjects had a PIPR change of >20% (\( n = 19; \text{mean}, 27\% \)).

We then examined whether the magnitude of central visual field loss, as measured by the MD (in dB), affected the net PIPR change (%) (Fig. 4). For this analysis we used data from all the patients whose MD values were outside the normal limits (\( n = 15 \)). We observed a tendency for baseline pupil diameter to become smaller as the visual field loss became worse (Fig. 4A; \( R^2 = 0.363, P < 0.05 \)). However, multiple regression analysis of visual field loss as a function of baseline pupil diameter and net PIPR change (%) indicated that visual field loss was correlated with the net PIPR change (%) (\( P < 0.05 \)) but not with the baseline pupil diameter (\( P > 0.05 \)). This latter observation is consistent with the observation that the mean and variance of the baseline pupil diameter in the age-matched controls (4.85 mm; SD, 0.99 mm) are very similar to those of the patient population (4.54 mm; SD, 1.07 mm). Given the results of the multiple regression analysis, we investigated the relationship between MD and the net PIPR change (%) with single linear regression. These data show that as visual field loss

<table>
<thead>
<tr>
<th></th>
<th>Patients (( n = 16 ))</th>
<th>Age-Matched Controls (( n = 19 ))</th>
<th>Difference</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline pupil, mm</td>
<td>4.5</td>
<td>4.8</td>
<td>0.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Net PIPR, mm</td>
<td>0.6</td>
<td>1.3</td>
<td>0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Net PIPR change, %</td>
<td>13.0</td>
<td>27.3</td>
<td>14.3</td>
<td>&lt;0.001</td>
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</table>

Baseline pupil diameter was similar in both populations, but all net PIPR measures were significantly lower in the patient population. Values are rounded to 1 decimal point.
increases in severity, there is a reduction in the net PIPR change (%) (Fig. 4B; \( R^2 = 0.466, P < 0.05 \)). Importantly, the intercept of the regression line with the 0 dB value was at a net PIPR change (%) of 25.5%, which was similar to the value of 27.3% for the net PIPR change (%) in the age-matched controls, who had visual fields within normal limits.

**DISCUSSION**

The results of the present study demonstrate a significantly reduced PIPR in glaucoma patients compared with those in age-matched controls without ocular disease. To our knowledge, this is the only study to show such a significant reduction in the ipRGC-mediated PIPR in human subjects with GON. Indeed, there is an ongoing debate about the classes of RGCs that are damaged in glaucoma. For example, it has been suggested that RGC with larger soma are preferentially affected in GON.22–25 Other studies in rodents suggest that both ipRGC and RGC death is topological and not related to cell size or class.27,28 Li et al.10 reported that rodent ipRGCs, which have generally large cell bodies with extensive dendritic arbors,10 are preferentially spared in glaucoma.29 In contrast to this latter observation, the present study clearly demonstrates a loss of PIPR in patients with GON, indicating that ipRGCs are significantly affected in glaucomatous injury. However, it is unclear from the present study whether the ipRGCs are lost preferentially or the PIPR reduction reflects a generalized RGC loss.

It is important to note that the PIPR response is not significantly reduced in amplitude as part of the normal aging process. We have previously shown in healthy subjects that as age increased, there was a decrease in baseline pupil diameter accompanied by an insignificant tendency for the net PIPR amplitude to decrease.15 We also showed that there was no effect of age on the net PIPR change (%) because this metric takes into account any age-related differences in baseline pupil diameter.

The loss of PIPR in the patients was correlated with their MD values as determined by central visual field measurements. As the MD values increased, we noticed a decline in PIPR. This correlation was present for both the net PIPR and the net PIPR change (%) (slope for all regression lines, \( P < 0.05 \)) thus demonstrating that MD was a determinant of PIPR independently of baseline pupil diameters.

Compared with the control subjects in our previous study,15 our age-matched sample had smaller baseline pupil diameters. This smaller baseline pupil is almost certainly attributable to the older age of this group compared with the control group in our previous study because it is well known that baseline pupil size decreases as age progresses.30–35

In conclusion, this study has demonstrated a significant reduction in the PIPR in patients with GON and has shown that reduced PIPR is correlated with the severity of visual field loss. It is clear from our results that measuring PIPR may provide an additional objective method to detect and monitor glaucoma. Furthermore, given that the PIPR is significantly associated with visual field severity, monitoring this response over time may provide an objective measurement of progressive GON. However, to be of significant use, the PIPR will have to be evaluated in larger populations of healthy subjects and in disease categories other than glaucoma. In addition, studies will be required to better define intratest variability in patients with disease and healthy subjects, the relationship of the PIPR across disease severity, and the prospective performance of the PIPR test.

In summary, a novel wide-field optical system has the potential to be used as a clinical screening tool, along with other clinical parameters, in patients with retinal disorders such as glaucoma.

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