Blue Multifocal Pupillographic Objective Perimetry in Glaucoma

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Purpose. This study investigated multifocal pupillographic objective perimetry (mfPOP) stimuli that target the intrinsic photosensitivity of melanopsin retinal ganglion cells. The diagnostic potential for glaucoma is compared between stimuli biased toward either cone input to these cells or their melanopsin response.

Methods. Nineteen glaucoma patients and 24 normal subjects were tested using mfPOP stimulus protocols with either 33-ms yellow or 750-ms blue stimuli. Subjects’ color discrimination was assessed using the Farnsworth 100-hue test. Pupillary responses were measured, and mixed-effects regression was used to quantify results. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) analysis.

Results. The mean reduction in moderate to severe glaucoma pupil responses using blue stimuli was larger but more variable than that of the shorter yellow stimuli (blue: −1.32 dB [t(40) = −2.29; P = 0.027]; yellow: −0.93 dB [t(40) = −3.13; P = 0.003]). Color discrimination decreased significantly with age and glaucoma, with type III blue-yellow anomalies dominating. ROC analysis revealed similar diagnostic accuracies (AUC for eyes classified as moderate to severe; blue: 81.7%, yellow: 83.7%). Slightly higher sensitivity and specificity were obtained using blue stimuli in mild disease (AUCs blue: 71.1, cf. yellow: 67.7), although this difference was not significant.

Conclusions. In moderate to severe glaucoma, diagnostic accuracy of yellow and blue was similar, but blue stimuli showed limited ability to resolve scotomas. Blue mfPOP stimuli, however, may have advantages over yellow in detecting early glaucoma.

Keywords: glaucoma, melanopsin, pupil, visual field

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component and enable topographic assessment of the intrinsic ipRGC response.

The mfPOP test protocols currently under development use yellow stimuli, which minimizes any confounding effects of lens brunescence or variations in macular pigment density. These transient (33-ms) yellow stimuli favor the excitatory extrinsic input of M- and L-cones to ipRGCs. M- and L-cones also contribute to pupillary responses via cortical projections to the pretectum originating in midget and parasol ganglion cells. The targeting of ipRGCs through their intrinsic melanopsin response and minimization of midget and parasol ganglion cell involvement, however, may confer benefits in glaucoma assessment due to the sparse distribution and limited spatial redundancy of these melanopsin-containing cells. This study therefore compares the response characteristics and diagnostic utility for glaucoma of a yellow stimulus protocol using transient stimuli, with a protocol using blue stimuli of long duration designed to target the intrinsic component of the ipRGC response.

METHODS

This study consisted of two parts. The first part was a preliminary experiment to assess the characteristics of pupil responses to 750-ms red and blue mfPOP stimuli at four luminance levels, to determine whether the results for blue stimuli matched expectations from published studies for melanopsin-driven responses. The main experiment compared responses to 750-ms blue mfPOP stimuli with responses to our standard 33-ms yellow in glaucomatous and normal eyes.

Subjects

Four normal subjects participated in the preliminary experiment (Table 1). Their visual acuity was checked and visual fields assessed using Humphrey FDT C-20 full threshold perimetry (Carl Zeiss Meditec, Inc., Dublin, CA, USA). Exclusion criteria are outlined below.

In the main experiment, 43 subjects were tested with blue and yellow variants of mfPOP. Diagnostic status was confirmed using Humphrey (HFA II) achromatic SITA-fast perimetry, Matrix 24-2 perimetry, and Stratus OCT (all from Carl Zeiss Meditec, Inc.), fundus photography, slit-lamp biomicroscopy, and applanation tonometry. Color vision was assessed using the Farnsworth 100-hue test (F100; Luneau Ophthalmologie, Chartres, France). One putatively normal subject’s data were excluded from analysis due to a F100 result exceeding the 95th percentile of published norms. Final study group characteristics are shown in Table 1 and Supplementary Table S1. Both eyes of each subject were tested concurrently (n = 86 visual fields). Informed written consent was given by all participants after the nature and possible consequences of the study were explained, under ANU Human Experimentation Ethics Committee approval 238/04. All research adhered to the tenets of the Declaration of Helsinki.

Glaucoma subjects were recruited from the Canberra Eye Hospital and were required to have a diagnosis of open-angle glaucoma with evidence of glaucomatous scotomas in at least one eye (four subjects had normotensive glaucoma). Eyes of glaucoma subjects were classified based on HFA mean deviation (MD) as follows: moderate to severe glaucoma was defined as MD less than −6 dB (13 eyes); mild glaucoma as MD equal to or greater than −6 dB (22 eyes); and ND denoted no apparent field defects (3 fellow eyes). Normal subjects, recruited from local optometric practices or by word of mouth, were required to have no detectable glaucomatous abnormalities, open angles, discs within normal limits, and intraocular pressure of <21 mm Hg. Clinical characteristics of subjects are provided in Supplementary Table S1.

Exclusion criteria for all subjects in both experiments included evidence of other ocular pathology or previous ocular surgery (argon or selective laser trabeculoplasty excepted in patients), refractive errors greater than ≥6 diopters or more than 2 diopters of cylinder, or systemic disease or medication that might impair vision or pupillary responses. Subjects were requested to not consume caffeine or alcohol for 1 hour before testing.

Multifocal Infrared Pupillography

Presentation of mfPOP stimuli and monitoring of pupil diameter were carried out using a prototype of the US Food and Drug Administration–approved nuCoria Field Analyzer (nuCoria Pty. Ltd., Acton, Australia). This tabletop device uses concurrent, dichoptic presentation of temporally and spatially sparse multifocal stimuli at 60 frames/s. Infrared light was used to illuminate subjects’ pupils and their responses were monitored by separate video cameras at 30 frames/s/eye. During testing, subjects fixated on a small cross in the center of the viewing field. Stimuli were presented at optical infinity to minimize accommodative responses. Binocular fusion of the two images was aided by large crosshairs and the low-contrast (less than ±0.1) radial sinusoidal variation of the stimulus background (Fig. 1B). Gaze was monitored online, and data from

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**TABLE 1. Subject Group Characteristics, Plus Temporal and Luminance Characteristics of the Stimulus Protocols for the Main and Preliminary Experiments**

<table>
<thead>
<tr>
<th>Stimulus Characteristics</th>
<th>Preliminary Experiment</th>
<th>Main Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n, mean ± SD age, y</td>
<td>Normal: 4 (2 males), 48.3 ± 10.4</td>
<td>Glaucoma: 19 (10 males), 64.1 ± 9.8</td>
</tr>
<tr>
<td>Color</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Luminance, cd/m²</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Mean interval between presentations/region, s</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Average number of stimuli shown in each test-region</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Recording duration, min</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Experimental protocols differed in color, stimulus luminance and duration, presentation rate, and recording duration. Stimuli were presented on a photopic background the same color as the stimuli but with a mean luminance of 10 cd/m² (Fig. 1B). For details of the stimulus spectra, CIE coordinates and relative cone activations refer to Figures 1D, 1E, 1F.
blinks and fixation losses were deleted. The mfPOP method can tolerate up to 15% data loss before a given 30-second segment must be repeated. Corrective lenses compensated for refractive errors to within 1.5 diopters; the stimuli contained no spatial frequencies above 2 cyc/deg, making them tolerant of this degree of misrefraction.41

**Stimuli**

The stimulus layout used in both experiments extended ±30° from fixation and consisted of 44 visual field test-regions arranged in an overlapping dartboard configuration (Fig. 1A).11 The two experimental conditions (stimulus protocols) of the main experiment used either transient 33-ms stimuli consisting
mainly of medium- and long-wavelength light: the M- and L-cone-biased “yellow” protocol, or longer duration 750-ms stimuli consisting mainly of shorter wavelength light: the melanopsin-biased "blue" protocol (see details below). The integration time of melanopsin is long: blue stimuli much shorter than this may exclude the intrinsic response. 25,26 Stimuli in the preliminary experiment had temporal characteristics identical to those of the blue protocol (duration: 750 ms) but used red and blue stimuli presented at four different luminance levels (Table 1).

Each stimulus sequence was separated into segments lasting 30 seconds; the yellow protocol contained 8 of these segments, giving a total recording duration of 4 minutes (Table 1). The blue and red protocols, due to their much longer stimuli and lower presentation rate, consisted of 16 segments with a total recording duration of 8 minutes. Stimuli in all protocols were presented on a photopic background (mean luminance of 10 cd/m²), the same color as the stimuli. The presence of some spectral components over 500 nm in the blue background should serve to maintain levels of the 11-cis melanopsin isomer and enable continuous ipRGC firing. 42 The CIE x-y coordinates for yellow protocol test-regions were 0.377 and 0.464, respectively, and 0.408 and 0.515, respectively, for the background (Fig. 1D). This protocol used “partially luminance-balanced” stimuli to minimize the effects of response saturation12; therefore, the luminance of test-regions ranged between 67 and 150 cd/m², depending on their location in the visual field10,14 (Fig. 1C). Balancing results in reduced topographic variation in response amplitudes and improves diagnostic power for glaucoma (Maddess T, et al. IOVS 2009;50:ARVO E-Abstract 5281). The luminance of all test-regions in the blue protocol was 75 cd/m². CIE x-y coordinates of these regions were 0.145 and 0.113, respectively, and 0.137 and 0.120, respectively, for the background (Fig. 1E). Luminance-balanced stimuli were not used in this protocol due to finding in the preliminary experiment that response saturation was not present at this level of blue illumination (Fig. 2). Proportional cone activations for the color channels used in both experiments are shown in Figure 3. These were estimated using human cone sensitivity functions43 and measured spectra at luminances of 75 cd/m² for the nuCoria field analyzer red and blue channels and 108 cd/m² for yellow.

**Response Estimation**

Signal processing was carried out using custom-designed software developed using Matlab (release R2010b; MathWorks Inc., Natick, MA, USA). Response waveforms for each test-region were extracted from raw pupillary responses using multiple linear regression as previously described. 6,7,10 This method provided a set of 176 response estimates (waveforms) for each subject and protocol: both direct and consensual responses for left and right eyes for each of the 44 test-regions. Thus, for each region, these response estimates are effectively the mean of the responses to either 60 (yellow protocol) or 15

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** Mean responses from a preliminary experiment comparing red and blue stimulus protocols. (A) Average response waveforms for blue and red protocols. (B) Mean amplitudes of pupillary responses to blue and red mfPOP protocols were plotted against stimulus luminances (45, 55, 65, or 75 cd/m²) of each set of protocols. The red stimuli produced functions (dashed lines) that saturated as luminance increased. In contrast, the blue response waveform (solid line) demonstrated increasing gain with stimulus luminance and no evidence of saturation. (C) Mean waveforms are scaled as proportions of peak constriction amplitudes. White headed arrows indicate the PIPR for each blue protocol: black- and open-headed arrows show the positive components for red and blue protocols, respectively.
(blue protocol) individual stimulus presentations to that region. Pupil diameter measurements were normalized to a mean pupil diameter of 3500 μm as in previous studies, providing constriction amplitudes that are relative to that standard diameter.9,11,12,19

Analysis
Sources of variation in the F100 total error scores (TESs) of glaucoma and normal subjects were quantified using multiple linear regression. The effects of relevant study variables on pupillary constriction amplitudes were calculated using a mixed-effects model incorporating restricted maximum likelihood estimation44 in R software (R Core Team 2013, Vienna, Austria). The distribution of variance in response amplitudes was stabilized initially using a generalized logarithmic transform as described previously10,18; model outputs are therefore reported in dB. Random effects were fitted for the nested factors eye within subject, and the remaining variances fitted as fixed effects.

Receiver operating characteristic (ROC) analysis, which offsets the true positive rate of a method against its false positive rate, was used to assess diagnostic performance.11,19 ROC curves and their area under the curve (AUC) values were calculated for each protocol and severity classification. This involved selecting responses for each test-region from the mean of varying-sized subsets of the most deviating test-regions of each eye, from the single worst-performing region (n-worst = 1), the mean of the 2 worst-performing regions (n-worst = 2), to the mean of all 44 test-region deviations of each field (n-worst = 44).

Results
Preliminary Experiment
Mean response waveforms and constriction amplitudes across test-regions, eyes, and subjects are presented in Figure 2. Mean amplitudes of red protocol responses were slightly larger than those of blue (Figs. 2A, 2B). This is most likely due to the larger baseline pupil diameters, and therefore higher retinal illumination, during testing with red protocols (Fig. 1D; Supplementary Table S2). Constriction amplitudes increased with illumination in both blue and red protocols (Figs. 2A, 2B). Red protocol amplitudes, however, plateaued at 65 cd/m² and above; in contrast, blue amplitudes accelerated at equivalent luminances. In blue protocols, this marked increase in amplitude was replicated in the PIPR and also in the decrease of the positive dilation component. There was little evidence of a PIPR in red protocols; the amplitude of the small deflection that was present did not change. In contrast to blue, higher luminance also increased the strength of the positive component. The scaled responses plotted in Figure 2C show that these patterns hold when taken as a proportion of the overall constriction amplitude (Fig. 2C, white-headed arrows indicate the increase in blue PIPR, and black- and open-headed arrows highlight the opposing effects of increased red and blue luminance on the positive response component). In contrast to the responses to red stimuli, the response waveforms and stimulus response function for blue stimuli were consistent with substantial melanopsin involvement.20,46

Main Experiment
F100 Color Discrimination. A possible confounding factor for these experiments is color vision deficits from various sources, so color vision was tested in all subjects. F100 TESs ranged from 4 to 169 in normal subjects and from 43 to 169 in patients. Multiple linear regression revealed significant independent effects of both diagnosis and age on color discrimination. The mean TES for normal subjects was 46.5, increasing on average by 16.4 per 10-year increment in age \[t(40) = 2.6; \ P = 0.013\]. Independent of this age effect, patients’ scores averaged 35.2 higher than normal subjects \[t(40) = 5.1, \ P = 0.004\]. Mean and median test results for each group are shown in Figure 3, which indicates that the majority of variation in these subjects is due to a tendency toward type III tritan-like anomalies, this being more pronounced in patients.

Pupillary Response Characteristics. Of the 1032 thirty-second protocol segments presented to the subjects in this study’s main experiment, only 4 segments needed to be repeated due to the criterion lack of more than 15% of that segment’s data. The mean pupillary constriction waveforms for the two protocols differed slightly; the time course of constrictions elicited by the blue protocol was substantially longer, with evidence of a PIPR sustained component following the peak of the response (Fig. 4A).

Pupillary response characteristics were assessed by stimulus protocol, diagnostic status, age, sex, color discrimination, and location of the test-region in the visual field. The yellow protocol produced mean constriction amplitudes that were significantly smaller than those of the blue \(16.26 - 11.64 = 4.62 \ \text{dB;} \ \langle t(7481) = -104.3; \ P < 0.0001\rangle\) (Table 2). Mean baseline pupil diameters differed in a manner similar to those in the preliminary experiment, with the longer wavelength yellow protocol producing a larger diameter of 3.47 mm than that of blue at 2.70 mm. The smaller responses obtained using this yellow protocol are therefore most likely due to the short
duration of the yellow stimuli. In both yellow and blue protocols, the largest regional constriction amplitudes were obtained to stimuli in the temporal field (Fig. 4B). Mean constriction amplitudes of eyes with moderate to severe glaucoma were significantly smaller than those of normal subjects in both protocols: blue by −1.32 dB (t(49) = −2.29; P ≤ 0.027), yellow by a smaller but more significant margin of −0.95 dB (t(40) = −3.13; P = 0.003) (Table 2). No significant difference was observed between mild glaucoma and normal subjects or for any other of the fitted variates.

**Diagnostic Accuracy.** ROC plots for the n-worst test-regions that produced the highest AUC for each severity are shown in Figure 5A. The diagnostic performance of the two protocols was quite similar (Table 3). On inclusion of all subject eyes (i.e., all severities) in the analysis, an overall AUC value of 70.9% (±5.6% SE, n-worst = 41) was obtained using the blue protocol, and 71.3% (±5.9% SE, n-worst = 1) using the yellow protocol. Both of the protocols were able to detect eyes classified as mild glaucoma and normal subjects or for any other of the fitted variates.

**DISCUSSION**

The potential of mfPOP as an emerging diagnostic tool for glaucoma is considerable, with its diagnostic accuracy comparable to commonly used forms of perimetry.9,19 This study

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**Table 2.** Fixed Effects of Study Variables From Linear Mixed-Effects Models for Blue and Yellow mfPOP Protocols

<table>
<thead>
<tr>
<th></th>
<th>Blue Protocol</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b, dB</td>
<td>df</td>
<td>t</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>16.259</td>
<td>3698</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>DecadeRel60</td>
<td>0.157</td>
<td>39</td>
<td>0.55</td>
<td>0.5823</td>
<td></td>
</tr>
<tr>
<td>Right eye</td>
<td>−0.059</td>
<td>40</td>
<td>−0.41</td>
<td>0.6871</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>−0.164</td>
<td>39</td>
<td>−0.37</td>
<td>0.7153</td>
<td></td>
</tr>
<tr>
<td>Glaucoma, mild/ND*</td>
<td>0.005</td>
<td>40</td>
<td>0.01</td>
<td>0.9919</td>
<td></td>
</tr>
<tr>
<td>Glaucoma, moderate/severe</td>
<td>−1.318</td>
<td>40</td>
<td>−2.29</td>
<td>0.0272</td>
<td></td>
</tr>
<tr>
<td>F100</td>
<td>−0.021</td>
<td>39</td>
<td>−0.31</td>
<td>0.7590</td>
<td></td>
</tr>
</tbody>
</table>

Additive effects (b) are relative to the y intercept or Constant, which describes the mean of constriction amplitudes across the 44 test-regions of left eyes of male subjects aged 60 with normal vision and average color sensitivity. DecadeRel60 refers to an additive slope in dB/decade relative to 60 years. For the variable F100, 120 (the normative mean TES for a 60-year-old subject) was subtracted from subjects’ total error scores in order to maintain parity with DecadeRel60. The result was then divided by 10 to provide a more practical measure; therefore, the fitted values refer to the additive effect of F100 increments of 10, relative to a TES of 120 on constriction amplitudes.

* Putatively normal eyes of glaucoma patients.

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The two protocols differed, however, in the pattern of which n-worst regions produced the highest AUCs. In moderate to severe eyes, the accuracy of the yellow protocol was highest when only each subject’s single most deviating test-regions were included in the analysis. The blue protocol, however, demonstrated increasing accuracy on inclusion of larger numbers of these worst performing regions (Fig. 5B). Thus, this protocol appeared to have lesser capacity than yellow to detect localized damage in advanced disease, relying instead on measures akin to the mean defect to derive diagnostic power. In contrast, in eyes classified as mild, both of the protocols were most accurate using just the single worst-performing region. The blue protocol produced a slightly higher AUC, but this value was not significantly different from that produced by the yellow protocol.

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**FIGURE 4.** (A) Averaged pupillary response waveforms showing the general form taken by responses to the two different stimulus protocols of the main experiment (means for glaucoma and normal subjects computed across eyes, pupils, and visual field regions). The blue response waveforms show a PIPR component in the redilation phase. (B) Constriction amplitude means by region from a mixed-effects model incorporating effects for sex, eye, age, F100 TES, and diagnosis of subjects. Thus, these regional means represent the responses of left eyes of male subjects aged 60 with normal vision and average color sensitivity, and are subject to modification by the effects reported in Table 2. All results are mapped as left-eye–equivalent visual fields with the temporal field shown here on the left. Constriction amplitudes were largest in the temporal field in both blue and yellow protocols.
aimed to determine whether improvements could be made to methods currently being developed by targeting the intrinsic component of the ipRGC response. Pupil perimetry using single blue stimuli have been undertaken\(^47,48\); however, these studies used dark-adapted subjects, so responses will have also involved rod photoreceptors. The use of a photopic blue adapting background in this experiment aimed to minimize rod involvement and facilitate the observation of melanopsin responses.

**Preliminary Experiment and Melanopsin Involvement**

As well as being largely responsible for the PIPR, some results suggest that melanopsin has a substantial influence on early response components at luminance levels and durations similar to those used in our main experiment (Fig. 1).\(^{20}\) Additionally, at pupillary diameters equivalent to those achieved using blue mfPOP (Supplementary Table S2), the spectral sensitivity of these early response components appears to be dominated by melanopsin.\(^{26}\) Therefore, the potential to obtain melanopsin-influenced mfPOP responses exists; several characteristics of the responses obtained to blue stimulation in these experiments appear to confirm this.

The accelerating stimulus-response function shown in Figure 2B and the differences between red and blue later response components (Figs. 2A, 2C) are consistent with observations of the progressive domination of pupil responses by melanopsin at higher luminances.\(^{26}\) This contrasts with the saturating stimulus-response functions we obtained previously using yellow stimuli\(^12\) and which were also observed using red stimuli in the preliminary experiment (Fig. 2B). The decrease in the positive component of blue responses is consistent with the greatly reduced \(b\)-wave seen in isolated melanopsin responses to flash electroretinography in macaques\(^{20}\) and lends strength to the assertion that melanopsin is involved in these responses to 750-ms blue mfPOP stimuli. The congruence between the changes in peak amplitude of constrictions and both the PIPR and positive dilation component is highly suggestive of a substantial melanopsin contribution to early (i.e., response amplitudes), as well as later components. It seems reasonable therefore to conclude that, although cone photoreceptors have undoubtedly participated in responses to the blue protocol of the main experiment as sources of both excitatory and inhibitory input (Fig. 3, cone activations), pupil constriction amplitudes in the blue protocol were also substantially influenced by melanopsin.

**Short-Wavelength Discrimination**

Normal aging results in increases in absorption of shorter wavelength light by the crystalline lens, observable as

![Figure 5](https://example.com/figure5.png)

**Table 3.** Percent ROC Area Under the Curve (AUC) and AUC Standard Error (SE) Values

<table>
<thead>
<tr>
<th>Severity (n eyes)</th>
<th>Blue Protocol</th>
<th>Yellow Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC, %</td>
<td>SE, %</td>
</tr>
<tr>
<td>Moderate to severe (13)</td>
<td>81.7</td>
<td>6.1</td>
</tr>
<tr>
<td>Mild (22)</td>
<td>71.1</td>
<td>8.0</td>
</tr>
<tr>
<td>ND* (3)</td>
<td>47.2</td>
<td>13.4</td>
</tr>
</tbody>
</table>

ROC estimates were obtained using response amplitudes and the optimal number of \(n\)-worst deviations from normal from any position in the visual field. Diagnostic accuracy using the yellow protocol was high when only a limited number of the worst performing regions were included in the analysis (Fig. 5B). The blue protocol, however, displayed a tendency for accuracy to increase with the inclusion of more regions. This was particularly apparent for eyes with moderate to severe defects.

* Putatively normal eyes of glaucoma patients.
Blowing or brunescence, as well as changes in the function of S-cone pathways. Evidence of this is seen in the increase in F100 TEs with age and the reduced blue-yellow discrimination illustrated in Figure 3. The antagonistic effect of S-cone stimulation on the intrinsic and M- + L-cone-derived ipRGC response is postulated to arise in DB6 bipolar cells, the major source of synaptic input to ipRGCs. This is proposed to be due to S-cone ON inactivation of ionotropic glutamatergic ion channels, preferentially expressed at S-cone synapses, leading to hyperpolarization of the DB6 cell. This would counteract the depolarization caused by the M- + L-cone inactivation of metabotropic ion channels in these same cells and reduce excitatory input to ipRGCs. The potential effect of brunescence on ipRGC responses is therefore twofold: (1) an overall reduction in the intrinsic melanopsin response; and (2) an increased bipolar cell synaptic input due to a reduction in antagonistic signal arising in S-cones. No significant independent effect of age or color sensitivity was observed in responses to the blue protocol (Table 2). A possible explanation for this is that any effects of brunescence may have counteracted each other (i.e., reduced melanopsin responses balanced by reduced S-cone antagonism). Similarly, age was not seen to significantly affect responses to the yellow protocol (Table 2), perhaps spared by the longer wavelengths of the stimuli. The alternative explanation, that brunescence did not affect retinal function, is unlikely because age was shown to have a significant effect on F100 error scores.

Abnormalities in blue-yellow discrimination also occur in glaucoma, independent of the effects of aging or media opacities, and probably account for the greater type III opacities. Evidence of this is seen in the increase in F100 TEs with age and the reduced blue-yellow discrimination illustrated in Figure 3. The antagonistic effect of S-cone stimulation on the intrinsic and M- + L-cone-derived ipRGC response is postulated to arise in DB6 bipolar cells, the major source of synaptic input to ipRGCs. This is proposed to be due to S-cone ON inactivation of ionotropic glutamatergic ion channels, preferentially expressed at S-cone synapses, leading to hyperpolarization of the DB6 cell. This would counteract the depolarization caused by the M- + L-cone inactivation of metabotropic ion channels in these same cells and reduce excitatory input to ipRGCs. No significant independent effect of age or color sensitivity was observed in responses to the blue protocol (Table 2). A possible explanation for this is that any effects of brunescence may have counteracted each other (i.e., reduced melanopsin responses balanced by reduced S-cone antagonism). Similarly, age was not seen to significantly affect responses to the yellow protocol (Table 2), perhaps spared by the longer wavelengths of the stimuli. The alternative explanation, that brunescence did not affect retinal function, is unlikely because age was shown to have a significant effect on F100 error scores.

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Abnormalities in blue-yellow discrimination also occur in glaucoma, independent of the effects of aging or media opacities, and probably account for the greater type III opacities. Evidence of this is seen in the increase in F100 TEs with age and the reduced blue-yellow discrimination illustrated in Figure 3. The antagonistic effect of S-cone stimulation on the intrinsic and M- + L-cone-derived ipRGC response is postulated to arise in DB6 bipolar cells, the major source of synaptic input to ipRGCs. This is proposed to be due to S-cone ON inactivation of ionotropic glutamatergic ion channels, preferentially expressed at S-cone synapses, leading to hyperpolarization of the DB6 cell. This would counteract the depolarization caused by the M- + L-cone inactivation of metabotropic ion channels in these same cells and reduce excitatory input to ipRGCs. No significant independent effect of age or color sensitivity was observed in responses to the blue protocol (Table 2). A possible explanation for this is that any effects of brunescence may have counteracted each other (i.e., reduced melanopsin responses balanced by reduced S-cone antagonism). Similarly, age was not seen to significantly affect responses to the yellow protocol (Table 2), perhaps spared by the longer wavelengths of the stimuli. The alternative explanation, that brunescence did not affect retinal function, is unlikely because age was shown to have a significant effect on F100 error scores.

Identification of Localized Dysfunction

The restriction of comparisons between normal and glaucoma subjects to different sized subsets of n-worst regions can be used as a measure of a test’s ability to identify localized areas of dysfunction. Although much damage to retinal ganglion cells in glaucoma is diffuse, localized scotomas are an obvious feature. In severe eyes, reliance of the blue protocol on mean deviations to achieve best diagnostic accuracy contrasts with the yellow protocol, where the best results were obtained using only the few worst performing test-regions, highlighting its ability to detect highly diagnostic localized depressions in sensitivity (Fig. 5B). Considering only the mean defect (i.e., n-worst = 44), diagnostic performance was quite different for yellow and blue stimuli. If these protocols’ diagnostic accuracies were comparable, they would be expected to produce similar mean defect-related ROC values. This is clearly not the case. Taken together, the larger mean reduction in regional amplitudes for yellow and the very different response patterns of the two protocols in eyes with established scotomas (Fig. 5B) suggest that different physiology and possibly even different neural pathways are involved.

Postreceptor retinal factors, such as differently sized RGC receptive fields, are most likely not responsible; cone-mediated receptive fields of ipRGCs are colocalized with their dendritic arborization, and blue-yellow RGC receptive fields in primates, although slightly larger than those of red-green sensitive RGCs, are not different enough to account for this. Forward scatter of light transmitted by the aging crystalline lens, although more prevalent at shorter wavelengths, is not likely to result in sufficient blurring of stimuli to render scotomas invisible. Cortical input to the pupillary pathway could potentially play a role, although using a similar global mechanism to the accommodative response to optical defocus, although exactly how this would result in these differences between blue and yellow is not clear.

Conclusions

This study’s aim was to determine the viability of mfPOP stimuli that target the intrinsic melanopsin response of ipRGCs. Little difference was observed between the overall diagnostic accuracies of the blue and yellow stimuli, the slightly higher ROC AUC of the blue protocol not being significantly different from yellow. The blue protocol produced much larger constriction amplitudes and signal-to-noise ratios than the yellow protocol, without evidence of response saturation. Yellow stimuli produced less variable reductions in patients’ response amplitudes and had better sensitivity to localized defects in established disease. In advanced disease, the diagnostic value of the blue protocol appeared somewhat prone to confounding factors related to aging and the disease process; similar issues have previously been found to hamper short-wavelength automated perimetry. Although this melanopsin-targeted protocol did not appear to lend any advantage in identification of later-stage glaucoma, it may have potential in the detection and management of early disease or possibly in other diseases in which sensitivity to short-wavelength light is affected. The replication of this study using a larger cohort would likely clarify the potential benefits of blue mfPOP stimuli relative to those of yellow in early glaucoma.

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References

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