Low-Luminance Contrast Stimulation Is Optimal for Early Detection of Glaucoma Using Multifocal Visual Evoked Potentials

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PURPOSE. The blue-on-yellow multifocal visual evoked potential (BonY mfVEP) stimulus is more sensitive than the conventional black-and-white pattern-reversal stimulus in identifying early glaucoma. BonY employs pattern-onset stimulation and lower luminance contrast (40%) in addition to color. This study was conducted to elucidate the mechanism responsible for the enhanced performance of the BonY stimulus.

METHODS. Multifocal pattern-onset VEPs were recorded in response to BonY, high-luminance contrast achromatic (HLA) and low-luminance contrast achromatic (LLA) stimulations in 30 normal subjects (to construct normative databases) and 23 patients with early glaucoma (mean deviation [MD] < 6 dB). In addition, the specificity of BonY and LLA stimulation was examined in a subset of 25 normal subjects.

RESULTS. In normal subjects, LLA mfVEPs had significantly lower amplitudes than did BonY and HLA mfVEPs (P < 0.001), which were not significantly different from each other. In glaucomatous eyes, all three stimuli demonstrated significantly reduced amplitudes in comparison with those of normal eyes. Although the sensitivities of both BonY and LLA in identifying subjective visual field defects were similarly high (93% and 89.7%, respectively), HLA showed only a 79.3% detection rate. BonY and LLA demonstrated significantly higher detect sever- ity scores than did HLA (P < 0.05 for both). Specificities for BonY and LLA were similar (96%).

CONCLUSIONS. BonY and LLA mfVEPs performed comparably, and both were significantly better than the HLA mfVEP in identifying early glaucoma. Enhanced performance of BonY stimulation is most likely due to its low-luminance contrast component rather than the pattern-onset mode of presentation or its chromatic properties. (Invest Ophthalmol Vis Sci. 2011; 52:3744–3750) DOI:10.1167/iovs.10-6057

Multifocal visual evoked potentials provide an objective method to identify functional losses in glaucoma. Since the technique was first described in 1994,1 several advances have made it more accurate and repeatable, with improved intersubject and intrasubject repeatability. Bipolar recording2,3 and scaling of signals based on background EEG4 have improved the consistency of recording, and asymmetry analysis5,6 has enhanced the ability to identify early losses. In earlier studies7–9 a black-and-white pattern-reversal checkerboard stimulus was used, and it performed very well in identifying moderate and severe losses, but showed lower sensitivity in early glaucoma.

In a recent study, we described a blue-on-yellow (BonY) stimulus.10 mfVEPs recorded with this stimulation technique demonstrated significantly better sensitivity in identifying glaucomatous visual field loss than did the pattern-reversal achromatic high-luminance contrast stimulation. Further, the BonY stimulus identified visual field defects in nearly 50% of cases of preperimetric glaucoma, which corresponded extremely well with the structural changes identified on optic disc photographs.11

BonY is a pattern-onset stimulus, however, and is spatially and temporally sparse compared with the pattern-reversal type of stimulation (James AC, et al. IOVS 2005;46:ARVO E-Abstract 3602).12,13 It is slower (four presentations per second) and produces larger signal-to-noise ratios of mfVEP amplitude. This alone may increase the sensitivity of the stimulation technique. In addition, the BonY stimulus displays a 40% luminance contrast between the yellow background and blue checks. This level of contrast may be well suited to stimulate magnocellular neurons,13 which have been reported to show early functional deficits in glaucoma.15,16

To elucidate the mechanisms by which the BonY stimulus performs better than black-and-white pattern-reversal stimulation, we compared the performance of the BonY stimulus to achromatic high- and low (40%)-contrast stimulation, using sparse stimuli for all three tests. Identifying the particular mechanisms that enhance performance of the stimulus may help refine and improve stimulation techniques for identifying early glaucomatous loss.

METHODS

The study was conducted in three parts: part A, construction of normative databases for all three stimulus conditions; part B, testing patients with early proven glaucomatous defects; and part C, specificity testing. Approval was obtained from the institutional review board, written informed consent was obtained from all participants, and the study was conducted in accordance with the tenets of the Declaration of Helsinki.17

In part A, normal subjects (n = 30) were recruited to construct normative databases for all three stimulus conditions, as described below. All subjects classified as normal had best corrected visual acuity ≥6/6 in both eyes and entirely normal results in an ophthalmic examination, including IOP < 21 mm Hg and normal discs and maculae. In part B, early-glaucoma patients (n = 23) were recruited from a glaucoma practice. The diagnosis of glaucoma was made on the basis

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Supported by the Sydney Medical Foundation (AK), in part by the Adolf Besser Foundation (HA), and in part by a grant from the National Health and Medical Research Council.

Submitted for publication June 16, 2010; revised September 4, October 19, and November 17, 2010; accepted November 27, 2010.

Disclosures: H. Arvind, None; A. Klistorner, P; J. Grigg, None; S.L. Graham, P.

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of glaucomatous cupping of the optic disc, as judged by stereoscopic ophthalmoscopy with a corresponding visual field defect on Humphrey visual field analysis (HFA 24-2 SITA Standard; Carl Zeiss Meditec, North Ryde, NSW, Australia). All patients fulfilled the following inclusion criteria: best corrected visual acuity ≥6/12 in the worse eye; reliable and repeatable early (mean deviation [MD] ≤ 6 dB) visual field defects on SITA Standard 24-2 perimetry, which corresponded to disc excavation; and absence of other ocular disease. A minimum scotoma on HFA examination was defined as a set of three contiguous abnormal points in the same hemifield at P < 5%, with at least two points at P < 2% on the pattern standard deviation (PSD) plot. Points immediately above and below the blind spot did not qualify as part of the scotoma. Peripheral rim points qualified as part of the overall scotoma, but at least two of the points qualifying as the scotoma nucleus had to be nonrims. Patients with significant cataract or other media opacities and/or inconsistent or nonrepeatable visual field defects were excluded.

The qualifying patients underwent mfVEP tests with all three stimulus conditions: BonY, low-luminance achromatic stimulation (LLA), and high-luminance achromatic stimulation (HLA), as described in detail later.

After parts A and B were completed based on the results of the mfVEP, in part C, specificities of BonY and LLA stimulus conditions were examined in a separate group of 25 normal subjects. All subjects included for specificity testing had best corrected visual acuity ≥6/6 in both eyes and entirely normal results in an ophthalmic examination.

Tests
Normal subjects (for the normative database) and glaucoma patients were tested under the three stimulus conditions mentioned earlier: BonY, HLA, and LLA. Normal subjects invited to examine the specificity of BonY and LLA stimulus conditions underwent only these two tests. The order of the tests was randomized for all three parts of the study. The visual stimulus was generated on a 19-inch, high-resolution LCD display (response time, 2 ms; model L1954; LG Electronics, Slough, UK) with a refresh rate of 60 Hz. All subjects were refracted optimally for near and seated 30 cm from the display. All recordings were conducted monocularly, right eye first.

BonY Stimulus. A BonY mfVEP stimulus paradigm based on sparse stimulus presentation\(^{10}\) was used (Fig. 1a). The stimulus consisted of a cortically scaled dartboard pattern of 58 segments; 56 segments were arranged in five concentric rings (eccentricities 1–2.5°, 2.5–5°, 5–10°, 10–16°, and 16–24°), and 2 segments straddled the horizontal nasally (24°–33°). A fixation target occupied the central 1°. Each segment contained a 4 × 4 grid of blue checks scaled proportional to segment size (luminance, 20 cd/m\(^2\); Commission Internationale d’Eclairage (CIE) coordinates, 0.15, 0.06) that appeared briefly on a bright yellow background (luminance, 125 cd/m\(^2\); CIE coordinates, 0.46, 0.49) according to a pseudorandom binary sequence. The temporal characteristics of the stimulus have been detailed elsewhere.\(^{10}\) Briefly, the sequence had a total length of 440 elements and consisted of two types of elements (elements 0 and 1) distributed pseudorandomly. Each element of the sequence lasted nine frames of the monitor (16.67 × 9 = 150 ms). Element 1 of the binary sequence was represented by two consecutive states: a blue pattern-on (checker-board blue-and-yellow pattern) state, which lasted two frames, and a pattern-off (diffuse, bright yellow illumination of the entire segment) state, which lasted seven frames. For the element 0 of stimulating sequence, the pattern-off state (diffuse yellow illumination) was active for all nine frames of the element.

HLA Stimulus. Sparse stimulus presentation was also used for HLA stimulation (Fig. 1b). The dartboard stimulus was similar to that used for BonY, except that black-and-white checks (Michelson contrast, 99%), were presented on a gray background that was the mean luminance of black and white.

LLA Stimulus. This stimulus was identical with the BonY one, but was its equivalent in gray scale (Fig. 1c). The yellow background was replaced by light gray of the same luminance as the yellow, and the blue color of the checkerboard was replaced by a darker gray of the same luminance as the blue.

Recording. Four gold-cup electrodes (Grass-Telefactor, Warwick, RI), placed in a custom-built electrode holder, were used for bipolar recording: two electrodes, positioned 4 cm on either side of the inion; one electrode in the midline 2.5 cm above the inion; and one electrode 4 to 5 cm below the inion.\(^{5}\) Electrical signals were recorded along four channels, as the difference between superior and inferior, left and right, and obliquely between left and inferior and between

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**FIGURE 1.** (a) BonY, (b) HLA, (c) and LLA stimuli. The examples show a representative frame of the stimulus sequences. During the course of the sequence, different regions are active.
right and inferior electrodes. The ground electrode was placed on an ear lobe. The visual evoked responses were amplified 100,000 times (sampling rate, 512 Hz) and band-pass filtered (1–20 Hz). Recording of each eye lasted 5 to 7 minutes, depending on the number of runs needed to maximize the signal-to-noise ratio. All recordings were performed monocularly. The custom-designed software correlated the electrical responses with the stimulus appearance and assigned signals to the corresponding segments. The software also scaled the responses to the background electroencephalogram to reduce the interindividual variability, as described elsewhere.4

Analysis
For every segment, the largest peak-to-trough amplitude for each wave within the interval of 60 to 200 ms was determined for each channel. The wave of maximum amplitude from each segment in the field from the four channels was selected automatically, and the software created a combined topographic map. The difference in intereye amplitudes for each segment (except the nasal step region) was also calculated and compared with the intereye difference in the normal database for each segment. The two segments constituting the nasal step did not have corresponding segments in the contralateral eye and were therefore not included for asymmetry analysis. Areas of amplitude asymmetry were documented by constructing an asymmetry probability plot. A visual defect for mfVEPs was defined as a cluster of three or more abnormal segments on the amplitude deviation or intereye asymmetry deviation plots with \( P < 0.02 \), with at least one of them with \( P < 0.01 \), or two or more zones with \( P < 0.005 \) on the asymmetry deviation plot. The ASI (abnormality severity index) is used to assign scores to individual abnormal segments and clusters of segments, with a weighting for location and presence on the asymmetry plot. The ASI provides an overall index of whether the mfVEP amplitude results are within normal limits (score, 0–11), borderline (score, 11–19), or outside the normal range (score, >20). Mean amplitudes among normal and glaucomatous subjects and ASIs were compared between the three stimuli.

Results
Normative Database
The mean (±SD) age of normal subjects who constituted the normative database was 65.1 ± 10.1 years (range, 49–76). There were 15 men and 15 women. Mean amplitudes (±SD) of responses to BonY, HLA, and LLA stimulation were 775 ± 95, 780 ± 113, and 666 ± 85 nV, respectively (significantly different from each other; one-way ANOVA; \( P < 0.001 \)). Post hoc tests revealed that LLA had significantly lower amplitudes than the other two methods of stimulation (\( P < 0.001 \) for both), which were not significantly different from each other (\( P > 0.05 \)). Figure 2 displays average amplitudes for each ring of eccentricity recorded with the different methods of stimulation.

Glucoma Patients
Twenty-three subjects with early perimetric defects (MD ≤ 6 dB) in the worse eye were enrolled. The average age of the subjects was 66.8 ± 7.06 years. There was no significant difference between the ages of normal and glaucomatous subjects (\( P = 0.5 \), t-test). Six participants were men, and the rest were women. HFA defects were bilateral in 6 subjects and unilateral in 18; 29 eyes of 23 patients were analyzed. The average MD of eyes with HFA defects was −1.75 ± 1.8 dB, with average PSD of 3.9 ± 2.12.

The mean amplitude (±SD) of eyes with HFA defects detected by BonY, HLA, and LLA stimulation were 655 ± 142, 713 ± 120, and 569 ± 127 nV, respectively (Fig. 3). These represented reductions of 14.6% for LLA, 15.5% for BonY, and 8.6% for HLA compared to average amplitudes of the normative database. With all three stimuli, amplitudes of eyes with glaucoma were significantly lower than those of the normative database (\( P < 0.0001 \), \( P = 0.0007 \), and \( P = 0.04 \) for BonY, LLA, and HLA, respectively).

Multivariate analysis using multiple linear regression was performed on the combined (normal and glaucomatous subjects) dataset, to analyze the effects of glaucoma, age, and sex on the amplitudes of each stimulus condition. For all three stimuli, amplitudes were significantly affected by glaucoma (\( P < 0.0001 \), \( P = 0.004 \), and \( P = 0.036 \) for BonY, LLA, and HLA, respectively), but not by age (\( P = 0.8, 0.4 \), and 0.5 for BonY, LLA, and HLA, respectively) or sex (\( P = 0.3, 0.5 \), and 0.5 for BonY, LLA, and HLAZ, respectively).

Table 1 shows the performance of each of the tested mfVEP stimuli, in comparison to defects identified by HFA. Repeated-measures ANOVA revealed significant differences in ASI scores between the three groups (\( P = 0.006 \)). A Tukey post hoc multiple-comparison test revealed significant differences between HLA and LLA (\( P < 0.05 \)) and between HLA and BonY (\( P < 0.05 \)), but no significant differences between LLA and BonY (\( P > 0.05 \)).
Table 2 compares results of BonY versus LLA stimulation for hemifields that were abnormal on subjective visual field testing. Figures 4 and 5 are case examples. Figure 4 shows consistent defects identified on all methods of stimulation, while in Figure 5, the defect was not detected by HLA stimulation.

Specificity

Part C of the study was the final phase. In view of excellent and comparable sensitivities of BonY and LLA, specificities of these two stimulus conditions were examined in a separate group of 25 normal subjects. HLA was not included in the specificity study in view of its poor sensitivity, which would limit clinical use. The mean age of the subjects was 48.9 ± 14.9 years. There were 16 women and 9 men. BonY and LLA identified defects sufficiently severe to be classified as scotomas in one subject each (96% specificity). The two stimuli identified different subjects.

DISCUSSION

The advantages of the BonY stimulus over the conventional black-and-white pattern-reversal checkerboard in identifying early perimetric losses has been reported. Subsequently, its ability to identify preperimetric losses in nearly 50% of patients with disc changes but normal white-on-white visual fields was also demonstrated. The technique also had excellent specificity (>90%). The purpose of the present study was to identify the component of the BonY mVEP stimulus responsible for superior performance of the technique in detecting early glaucoma. There are several factors that differentiate the BonY stimulus from the conventional black-and-white stimulus. The BonY stimulus was initially designed to stimulate the koniocellular pathway. The bright yellow background was intended to adapt red and green cones, while the blue checks were intended to selectively stimulate blue cones. Therefore, the pattern-onset mode of stimulation was mandatory (as opposed to pattern reversal). In addition, the stimulus also had 40% luminance contrast, which was necessary, considering the differences in luminance of the blue versus the red and green colors of the monitor. The saturating nature of the contrast response function of the VEP has been reported before, and the use of low contrasts may have rendered the stimulus more sensitive to early losses. Although using a tritanopic stimulus (isoluminant blue-yellow) would have been the ideal way to stimulate the blue-yellow pathway, its clinical applicability was limited due to the extremely small magnitude of the responses, as established in a pilot study (Klistorner A, unpublished data, 2009).

The BonY stimulus therefore combined the blue-yellow color component, low-luminance contrast characteristics, and sparse (both temporally and spatially) mode of presentation. In an attempt to separately examine those components, we used high-contrast, pattern-onset achromatic stimulation to test the performance of the sparse mode of stimulus presentation and low-luminance contrast stimulation to address the possible role of achromatic low-luminance contrast and compared them both with the original BonY stimulation in a group of patients with early, repeatable perimetric defects. Subjects with early glaucoma were recruited because differences between the stimulation techniques are most likely to be well demonstrated at this stage of the disease, due to the dynamic range of the mVEP signals. Also perimetric defects with corresponding structural disc changes ascertained that we were looking at areas of true glaucomatous field loss.

We demonstrated that high-contrast stimulation, while generating mVEPs similar in magnitude to that evoked by BonY stimulation (among normals), identified field defects in only 27% of eyes compared to 93% for BonY. The BonY and LLA stimulation techniques were compared in a separate group of 25 normal subjects. HLA was not included in the specificity study due to its poor sensitivity. The mean age of the subjects was 48.9 ± 14.9 years, with 16 women and 9 men. BonY and LLA identified defects sufficiently severe to be classified as scotomas in one subject each (96% specificity). The two stimuli identified different subjects.

Table 1. Comparison of BonY, LLA, and HLA Stimulation Techniques in Eyes with Visual Field Defects

<table>
<thead>
<tr>
<th></th>
<th>HFA</th>
<th>HLA</th>
<th>LLA</th>
<th>BonY</th>
</tr>
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<tbody>
<tr>
<td>Abnormal eyes</td>
<td>29</td>
<td>23</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Abnormal hemifields</td>
<td>37</td>
<td>24</td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>ASI*</td>
<td>41.5 ± 30.3</td>
<td>55.6 ± 23.6</td>
<td>55.2 ± 27.7</td>
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Data are the number of eyes with defects (percentage of eyes detected by HFA).

* A score calculated for each eye based on the number and severity of abnormal segments.

Table 2. Comparison of the Number of Hemifields Classified as Abnormal by BonY and LLA Stimulation Techniques in Eyes with Visual Field Defects

<table>
<thead>
<tr>
<th></th>
<th>BonY</th>
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<tbody>
<tr>
<td>Abnormal</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

FIGURE 3. Average amplitudes of glaucomatous subjects at each ring of eccentricity for each of the stimulus conditions used. The rings are as described in Figure 2. In glaucoma patients, BonY and LLA reduced significantly, whereas HLA did not.
approximately 80% of the glaucomatous eyes and showed significantly lower averaged ASI value. This result was even lower than our previously published sensitivity (95%)8 of high-contrast pattern-reversal stimulation in early glaucoma. This finding indicates that sparse stimulation by itself is very unlikely to play a significant role in the enhanced performance of BonY mfVEPs in glaucoma.

Achromatic LLA, on the other hand, demonstrated similar sensitivity in identifying early glaucomatous defects, compared with BonY stimulation. Comparable performance of BonY and LLA stimuli indicates that there was no additional benefit in introducing the color component or, in other words, the enhanced performance of blue-yellow stimulation was most likely due to its luminance properties rather than chromatic properties.

It is a well-known fact that visual information is processed along three major retino-geniculo-cortical pathways: parvocellular, magnocellular, and koniocellular.20 The parvocellular pathway, which comprises approximately 80% of retinal ganglion cells is responsible for processing of high-contrast and low-temporal, high-spatial-frequency information, whereas the magnocellular pathway conveys information about low-contrast, low-spatial-frequency achromatic images and has higher temporal resolution. The third, the koniocellular pathway, conveys blue-on, yellow-off color signals to the brain. For subjective functional tests, using stimuli that preferentially target either the magnocellular or koniocellular pathways has been shown to enable earlier identification of scotomas,21 because of the lower functional redundancy in cells subserving these pathways,22,23 since each of these pathways constitutes, on average, only approximately 10% of the ganglion cell population.24,25

Using high achromatic contrast may therefore preferentially stimulate parvocellular neurons, which, by having a high degree of redundancy would probably not be very sensitive to small losses of neurons. The lower sensitivity of high-luminance contrast, sparse stimulation compared with pattern-reversal stimulation may be explained by the much slower rate of stimulation, which again may preferentially stimulate parvocellular neurons. mfVEP responses to achromatic stimulation saturate at approximately 40% to 50% contrast,14,19 which is similar to what we used in this study for LLA and BonY. Response at this level of luminance contrast has been described to have predominant magnocellular contributions, which may be the reason for excellent performance of LLA as well as BonY stimuli.14,26

**Figure 4.** Patient 4 showed consistent defects on visual field analysis and mfVEP tests with BonY, LLA, and HLA stimulation. Traces are shown on the left with amplitude asymmetry (middle) and amplitude (right) deviation plots for each stimulus.
This study was limited by our inability to separate the blue–yellow component completely from the luminance characteristics. This difficulty, as mentioned previously, was due to the extremely small amplitudes obtained with isoluminant blue–yellow stimulation, rendering it clinically inapplicable. We therefore could study this component only indirectly, by comparing achromatic and chromatic stimuli with the same level of luminance contrast. Second, although LLA stimulation worked very well and although its luminance characteristics show that its performance may be due to stimulation of magnocellular neurons, the temporal characteristics of this stimulus were much slower than what is optimal for magnocellular neurons.

However, this study established that the low-luminance contrast, rather than the chromatic, characteristics of the BonY stimulus contributed to its excellent performance in the early detection of glaucoma. Further work will be directed toward optimizing the stimulation technique further for magnocellular neurons (i.e., increasing temporal frequency, while maintaining the same level of luminance contrast). Also, the reproducibility and robustness of the signal in the presence of early cataract should be examined and will be additional factors that determine the clinical usefulness of the technique.

References


