Investigation of Multifocal Visual Evoked Potential in Anisometropic and Esotropic Amblyopes

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PURPOSE. To investigate the variation of visual evoked potential (VEP) function at different eccentricities of the visual field in esotropic amblyopes and anisometropic amblyopes.

METHODS. Data from 5 esotropic amblyopic eyes, 6 anisometropic amblyopic eyes, and 45 control eyes were analyzed. A VERIS system was used to generate a stimulus matrix containing 61 hexagons on a computer monitor. Each hexagon of the display contained a number of small black and white hexagonal patches that reversed in polarity during stimulation according to a pseudorandom binary m-sequence. The VERIS system extracted the local responses by cross-correlating the input and output signals. The latencies and amplitudes of the responses from the central 8.6 degrees of arc in the visual field were analyzed.

RESULTS. In esotropic amblyopia, the multifocal VEP latency is prolonged, and the amplitude is reduced in the central region of the visual field. The mean amplitude is significantly smaller, and the mean latency is significantly longer in the temporal visual field than in the nasal visual field. In anisometropic amblyopia, latencies are markedly prolonged, and the amplitudes of multifocal VEP are attenuated in the central region of the visual field, and these effects are lessened in the periphery.

CONCLUSIONS. The results are in agreement with psychophysical studies reporting a greater foveal deficit in amblyopia and a greater visual loss in the temporal field than in the nasal field in esotropic amblyopia. (Invest Ophthalmol Vis Sci. 1998;39:2033–2040)

Amblyopia is thought to primarily affect the function of the lateral geniculate nucleus and visual cortex, and not surprisingly, it has been found that the conventional pattern visual evoked potential (VEP) in amblyopia is abnormal.1,2 A number of studies have shown that visual acuity impairment in amblyopes is greater at the fovea than in the periphery of the visual field.3,4

The contrast sensitivity for a fixed spatial frequency across the visual field of amblyopes also shows greater depression foveally than peripherally. Sireteanu and Fronius reported that the grating acuity deficits of strabismic amblyopes are frequently asymmetrical, with one hemifield being spared relative to the other, whereas those of anisometric amblyopes are symmetrical.5 Thomas observed that contrast sensitivity loss in esotropic amblyopes was more serious in the central visual field than in the periphery, with the greatest peripheral loss of sensitivity in the temporal visual field (i.e., in the nasal retina).6

Thomas predicted a specific pattern of asymmetry in strabismus—a greater loss in the temporal field of esotropes and in the nasal field of exotropes. In the present experiment, the multifocal VEP was measured across the visual field in esotropic amblyopes and anisometric amblyopes to provide objective evidence of the distribution of the depression of visual function in these types of amblyopia.

METHODS

Subjects

All subjects were volunteers who had been diagnosed as normals or amblyopes in the Optometry Clinic at The Hong Kong Polytechnic University. The research followed the tenets of the Declaration of Helsinki, and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. One right and four left esotropic amblyopic eyes (Table 1) and one right and five left anisometropic amblyopic eyes (Table 2) were tested. The amblyopes, whose ages ranged from 13 to 24 years, had corrected distance visual acuity (VA) from 6/9 to 6/24 in the amblyopic eye. All the amblyopic eyes tested had foveal fixation and no observable fundus pathology; fixation was evaluated using the cup-disc ratio graticate of an ophthalmoscope, to a precision of approximately 0.5 degree of arc. None of the strabismic eyes had been operated on before the test. Refractive errors were corrected for the working distance used during the VEP test.

The data for normal eyes analyzed in this study are from 22 right and 23 left eyes (from 26 subjects). Eighteen of these subjects had both eyes tested; four had right eyes tested, and
five had left eyes tested. The normal (healthy) subjects' ages ranged from 13 to 40 years (mean 17.5 years). Subjects had corrected distance VA of 6/6 or better and near VA of at least N6 with each tested eye. They had less than 3 diopters of spherical refractive error and less than 2 diopters of astigmatism; refractive errors were corrected for the working distance during the VEP test.

Stimulus

A VERIS I multifocal ERG/VEP system (EDI Associates, San Francisco, CA) was used to record the multifocal VEP. This system is similar to that used by Sutter and Tran for multifocal ERG measurements and used by Baseler and Sutter for multifocal VEP measurement. The stimulus arrangement was the same as in our previous studies. The stimulus matrix, containing 61 hexagons (see Fig. 1), was generated on a standard Apple color high resolution RGB monitor, which was controlled by a video card in a Macintosh computer. A stimulus matrix has been designed by the manufacturer in which the hexagons are scaled with eccentricity, to achieve higher spatial resolution near the center of the visual field and to improve the signal-to-noise ratio in the periphery. The hexagons were approximately 0.6 degree of arc across at the foveal hexagon and approximately 2.3 degrees of arc across in the hexagon of the outermost stimulus ring. The diameter of the entire stimulating field was approximately 13.6 degrees of arc. The viewing distance was 80 cm, and the diameter of the foveal patch was approximately 0.6 degree of arc. The diameter of the first stimulus ring was from 0.6 to 3.3 degrees of arc, the second stimulus ring from 3.3 to 5.0 degrees of arc, and the third stimulus ring from 5.0 to 8.6 degrees of arc. The frame rate of the monitor was 67 Hz. The maximum luminance of the pattern was 111 candela (cd)/m², and the minimum luminance was 5 cd/m². Thus, the contrast was 91%.

Every hexagon of the display contained a number of small black and white hexagonal patches with a center-surround configuration that reversed during stimulation. The diameters of the small patches increased with eccentricity from approximately 15 minutes of arc in the foveal hexagon to approximately 30 minutes of arc in the hexagons of the outermost stimulus ring. The optimal check size for producing the VEP response is smaller for the central retina than for the periphery, with the optimal size in the foveal area being 10 to 20 minutes of arc. On each displayed frame of the monitor, approximately 50% of the hexagons were changed compared with the previous display. These hexagons were simultaneously but independently modulated in time by the controlling computer program, which simultaneously recorded the raw VEP signal. The changed elements were selected on each frame according to a computer-generated binary pseudorandom time series (m-sequence), which allows rapid computation of the cross-correlation of input and output signals. The response contributions from each of the individual stimulus elements were extracted from the cross-correlation function.

Recording

Silver-silver chloride cup electrodes were placed on the scalp according to the International Ten-Twenty System. The active electrode was placed at OZ, the reference electrode was placed at FZ, and the ground electrode at A2 according to the 10-20 system. The impedances of electrodes were less than 5 kΩ. Amplification was provided by a Grass P511 amplifier (Grass Instrument Co., Quincy, MA) with a gain of 200,000 and bandwidth of 3 to 100 Hz.

The subject sat in a darkened and quiet room; natural pupils were used. When one eye was being tested, the fellow eye was occluded. The central hexagon of the display can be easily recognized while the stimulating pattern is reversing, so that maintaining fixation is not difficult. Subjects were asked to relax and maintain fixation at the center of the stimulus pattern, and to refrain from blinking during the stimulus se-
The length of each nonrepetitive sequence was $2^{15} - 1 = 32,767$ frames, and at the frame rate of 67 Hz, the whole stimulus sequence took 8.15 minutes. Each of these sequences was divided into 256 segments. The segment overlapped by 1 second on either end, thus each segment lasted 3.91 seconds. This very short stimulus segment reduces the possibility of eye movements and blinks contaminating the record. Occasionally, segments were obviously contaminated; these segments were rejected and re-recorded immediately.

The researcher controlled the procedure of the experiment, but the subject used a computer mouse to control when each segment of the test was started. This helped subjects to concentrate and to better maintain fixation on the center of the test pattern.

**Analysis**

The first slice of the second order kernel was calculated using the VERIS I system. The response traces were numbered in a spiral from center to periphery for convenience of analysis (Fig. 1). The traces from the right eye were numbered counterclockwise, and the traces of the left eye were numbered clockwise, so that the corresponding trace numbers in the right and left eyes were in mirror symmetry. The variation of the VEP waveform in amblyopia is too large to measure trace by trace, and so the VEPs from different eccentricities were divided, ring by ring from center to periphery, into five groups. Because the intersubject and the intrasubject variances of the waveforms in the traces of the outermost ring (traces 38 to 61) were very large, only the parameters of traces 1 to 37 (i.e.,...
Variation of VEP Latencies with Eccentricity

Latencies are approximately 20 msec longer in the fovea, and 7 to 10 msec longer in the first ring for both types of amblyopes than for the control subjects (Fig. 5). The mean latencies at the fovea are significantly different \((F = 38, df = 2,53, P < 0.0001)\), and the groups that are different are the normals and the esotropes \((P < 0.001)\), and the normals and the anisometropes \((P < 0.001)\). The mean latencies measured from the first ring were also significantly different \((F = 7.856, df = 2,53, P = 0.001)\), and again the groups that are different are the normals and the esotropes \((P < 0.01)\); the normals and the anisometropes show differences only at the \(P < 0.05\) level. No two groups were significantly different in the second or third ring.

Statistical Treatment of Data

Because the data obtained for different eccentricities are not independent, differences between the normal, esotropic, and anisometropic groups cannot be tested with a conventional two-way ANOVA (vision group X eccentricity group). We used a series of four one-way ANOVAs, and, to correct for the increased likelihood of making a type I error, we rejected the null hypothesis if the probability value was less than 0.0127 instead of 0.05.\(^{17}\) If we rejected the null hypothesis we then used a conventional post-hoc test (Student-Newman-Keuls) to determine which pairs of groups were different. This test allows for multiple comparisons and we considered \(P = 0.05\) to be statistically significant.

RESULTS

Figure 2 shows a typical normal monocular pattern reversal multifocal VEP recorded in the experiment. The inset shows a typical single response trace from the foveal response. Figure 3 shows a monocular pattern VEP in an esotropic amblyope. Amplitude attenuation can be seen in the central and temporal field responses. Figure 4 shows a typical monocular pattern multifocal VEP recorded in an anisometropic amblyope, in which the amplitude in the center stimulus hexagon is attenuated compared to the trace of the normal control eye (Fig. 2) and to those traces adjacent to it. For the convenience of the presentation of the data as a function of eccentricity, the eccentricities corresponding to fovea and rings 1, 2, and 3 are expressed as the mean eccentricity of each ring (i.e., 0, 1.0, 2.1, and 3.4, respectively, degrees of arc).
Variations of VEP Amplitudes with Eccentricity

On average, all three groups show a larger response amplitude foveally than in the periphery (Fig. 6). The differences in amplitudes between the normal eyes and the two amblyopic groups are greater at the fovea than in the periphery, especially for the P1-N2 amplitude (Fig. 6B). The mean N1-P1 amplitude measured at the fovea in the esotropic amblyopes is only 45% of the amplitude in the normal subjects. In the third ring, it is approximately 70% of that in normal subjects.

The N1-P1 amplitudes are significantly different between the three groups only at the fovea ($F = 4.912$, $df = 2.53$, $P = 0.011$). The groups that differ are the normals and the esotropes ($P < 0.05$). There is a trend for the attenuation of the N1-P1 amplitude for the anisometropic amblyopes to be less than that of the esotropic subjects at all tested eccentricities. However, the differences are not statistically significant.

In the foveal response, the mean P1-N2 amplitude for the esotropic amblyopes is only 31% of that in normal subjects, whereas in the third ring the mean P1-N2 amplitude is 76% of that in the normal subjects. In anisometropic amblyopes, the P1-N2 amplitude measured at the fovea is about half of that in the normal subjects, and in the third ring is similar to that of normal subjects. The mean amplitudes measured in the three groups are significantly different only in the fovea ($F = 7.586$, $df = 2.53$, $P = 0.0013$). The normals and the esotropes ($P < 0.01$) and the normals and anisometropes ($P < 0.05$) are different.

The mean P1-N2 amplitudes decrease more than do the mean N1-P1 amplitudes in both types of amblyopes. To show the variation of the waveform with eccentricity more clearly, the ratios of the N1-P1 to P1-N2 amplitudes have been calculated for all subjects, and the mean ratios for the three groups are shown in Figure 7A. In general, the P1-N2 amplitude is greater than the N1-P1 amplitude and the ratios tend to be higher in the fovea than in the periphery in all three groups.

The mean ratios of N1-P1 to P1-N2 amplitudes are significantly different at the fovea ($F = 13.353$, $df = 2.53$, $P < 0.0001$). Both the normals and the esotropes ($P < 0.01$) and the normals and the anisometropes ($P < 0.001$) are different. The mean ratios are different in the first ring ($F = 9.570$, $df = 2.53$, $P = 0.0003$). In this case the mean ratios for normals and anisometropes ($P < 0.01$) and for esotropes and anisometropes ($P < 0.05$) are significantly different. The mean ratios are also different in the second ring ($F = 5.192$, $df = 2.53$, $P = 0.0087$), the normals and the esotropes ($P < 0.05$) and the normals and the anisometropes ($P < 0.05$) being significantly different. There was no statistically significant difference between the ratios in the third ring.

The ratios of the mean VEP amplitudes in esotropic amblyopia and anisometropic amblyopia to those of the normals are shown in Figures 7B (N1-P1) and 7C (P1-N2). In each type of amblyopia the ratios are lower in the fovea than in the outer rings, with a steady increase as eccentricity increases. For each eccentricity, the ratios are lower for the P1-N2 component than for the N1-P1 component.
TABLE 3. Mean PI Latencies of Nasal and Temporal Fields in Normal Subjects and Esotropic and Anisometropic Amblyopes

<table>
<thead>
<tr>
<th>Latency</th>
<th>Normal</th>
<th>Amblyopes</th>
<th>Paired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal Field</td>
<td>Temporal Field</td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>100.1(5.2)</td>
<td>101.2(5.2)</td>
<td>t = -0.92, df = 44, P = 0.362</td>
</tr>
<tr>
<td>Amblyopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esotropic</td>
<td>100.6(6.7)</td>
<td>111.8(6.3)</td>
<td>t = 3.20, df = 4, P = 0.033</td>
</tr>
<tr>
<td>Anisometropic</td>
<td>96.5 (9.4)</td>
<td>100.5 (10.0)</td>
<td>t = 2.07, df = 5, P = 0.092</td>
</tr>
</tbody>
</table>

Latency values are mean (SD) msec. Bold t denotes statistically significant.

Differences of VEP between Nasal Field and Temporal Field

In esotropic amblyopes, the mean PI latency in the temporal field is approximately 10 msec longer than that in the nasal field, and this difference is statistically significant (see Table 3 for data and statistical test values). The mean PI latency in the temporal field of the esotropic amblyopic eyes was also approximately 10 msec longer than that from the temporal field of the normal control eyes, and the difference is statistically significant. In normal subjects and anisometropic amblyopes, the differences in PI latency between the nasal field and temporal field are not statistically significant (Table 3).

Esotropic amblyopes show statistically significant differences in N1-P1 amplitude and in P1-N2 amplitude between nasal field and temporal field; the amplitudes in the nasal field are about three times larger than those in the temporal field (see Table 4 for statistical test and data values). Anisometropic amblyopes show no statistically significant amplitude differences between nasal field and temporal field, although their absolute values are much reduced compared with the normal values. In healthy subjects, there are no statistically significant differences in N1-P1 amplitude or in P1-N2 amplitude between nasal and temporal fields (Table 4).

DISCUSSION

Variations of VEP with Eccentricity in Esotropic Amblyopia and Anisometropic Amblyopia

The VEP results in this experiment are consistent with data showing losses of vision in amblyopia. VEP latencies are prolonged and amplitudes attenuated in the central area of the field. These differences from normal diminish with increased eccentricity and are asymmetrical in esotropic amblyopia. Many studies have suggested that VA is closely related to the amplitude \(^{18-24}\) and the latency of the VEP. \(^{25-29}\) Acuity losses in amblyopia are reduced in peripheral vision, \(^{3,4}\) grating acuity deficits in strabismic amblyopes are frequently asymmetrical between nasal field and temporal field, \(^{5}\) and the contrast sensitivities of amblyopes show similar trends. \(^{6}\)

The latency and amplitude of the pattern VEP obtained using large stimulating fields have been reported to be abnormal in amblyopia by many researchers. \(^{30-33}\) However, these data do not show how latency prolongation and amplitude attenuation vary with eccentricity in amblyopia. The present study showed that the latency is increased and the P1-N2 amplitude is reduced and that these effects are marked at the fovea and diminish with increased eccentricity in the two types of amblyopes. This is consistent with results from the study of Levi et al., \(^{4}\) who showed that VA was impaired more severely in the foveal area than in the periphery in amblyopic eyes. A

TABLE 4. Mean Amplitudes of Nasal and Temporal Fields in Normal Subjects and Esotropic and Anisometropic Amblyopes

<table>
<thead>
<tr>
<th>Amplitude</th>
<th>Normal</th>
<th>Amblyopes</th>
<th>Paired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal Field</td>
<td>Temporal Field</td>
<td></td>
</tr>
<tr>
<td>N1-P1 Amplitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>53.8 (30.7)</td>
<td>50.2 (32.4)</td>
<td>t = 1.19, df = 44, P = 0.239</td>
</tr>
<tr>
<td>Amblyopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esotropic</td>
<td>58.2 (19.5)</td>
<td>18.2 (7.9)</td>
<td>t = 4.32, df = 4, P = 0.012</td>
</tr>
<tr>
<td>Anisometropic</td>
<td>38.5 (11.6)</td>
<td>34.3 (13.0)</td>
<td>t = 0.98, df = 5, P = 0.374</td>
</tr>
<tr>
<td>P1-N2 Amplitude</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>100.4 (46.8)</td>
<td>93.07 (48.0)</td>
<td>t = 1.43, df = 44, P = 0.160</td>
</tr>
<tr>
<td>Amblyopes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esotropic</td>
<td>87.2 (19.7)</td>
<td>31.2 (20.5)</td>
<td>t = 5.92, df = 4, P = 0.004</td>
</tr>
<tr>
<td>Anisometropic</td>
<td>74.8 (36.6)</td>
<td>54.2 (19.8)</td>
<td>t = 1.47, df = 5, P = 0.203</td>
</tr>
</tbody>
</table>

Amplitude values are mean (SD) nanovolts. Bold type denotes statistical significance.
possible explanation for the development of this phenomenon is that in the normal eye the center of the visual field has keen acuity, the development of which demands an accurately focused image, whereas the periphery of the visual field has poorer acuity requiring less accurate focus of the image. Sengpiel et al. suggested that the binocular facilitation for matched stimuli, thought to underlie fusion and stereopsis, is superimposed on a nonselective inhibitory interaction between the two eyes in normals. Sengpiel and Blakemore believe that binocular facilitation occurs only for matched orientations, and that this facilitation is specifically lost in strabismus. This phenomenon is more significant in the central and nasal hemiretina of an esotropic eye.

Harrad et al. postulated that amblyopia might develop if the neurons in the ocular dominance columns with input from the deviating eye are constantly inhibited by the ocular dominance columns of the other eye; their inactivity would be likely to cause constant and long-term reduction of excitatory synaptic efficacy. According to Rauschecker, the primary cause of the cortical latency increase to the visual stimulation most probably lies in a reduction of excitatory synaptic efficacy of the cortical cell. For a cell to reach threshold and fire action potentials, considerable summation of excitatory postsynaptic potentials (EPSPs) has to occur in a short time. If smaller EPSPs are generated with less temporal precision, more EPSPs need to be integrated for the cell to reach threshold, and this takes longer.

The P1-N2 amplitude is twice as large as the N1-P1 amplitude in each VEP waveform in normals, whereas the central P1-N2 amplitude is only slightly larger than the N1-P1 amplitude in each VEP waveform in the two types of amblyopes. Figure 7A shows that the increase of this ratio in amblyopes is caused by a decrease in P1-N2 amplitude. This may reflect the different origins of N1 and N2 components. Lesèvre and Joseph considered that the N1 peak of the pattern reversal VEP reflected activity of part of area 17, whereas P1 was more likely to originate in area 19, and N2 probably originated in area 18. Schroeder et al. reported that the N1 component of the VEP corresponded to sources in input layer 4C of the primary visual cortex (area 17), and the P1 component of the VEP localized to layer 3 of area 17. Activity later than the human P1-equivalent has been attributed to the sum of complex activity throughout the striate layers, particularly those above layer 5, and to extrastriate sources.

Although the studies noted above do not agree on the origins of the VEP components, all these authors consider that the N1 and N2 components have different sources. Yamazaki observed that the development of the N2 latency in children was different from those of the N1 and P1 components. This also implies that the source of the N2 component is different from those of the N1 and P1 components.

Differences in VEPs between the Nasal and Temporal Fields in Esotropic Amblyopia

In esotropic amblyopia, the mean latency was approximately 10 msec longer, and the mean amplitude was 70% smaller in the temporal field than in the nasal field, whereas there were no significant differences in the mean latencies or mean amplitudes between nasal field and temporal field in normals or anisometropic amblyopes.

These findings are consistent with those from studies that have compared visual acuity between nasal and temporal hemifields in esotropia and in anisometropia. Grating acuity deficits of strabismic amblyopes are frequently asymmetrical, with the nasal hemifield being relatively spared in the esotropic eye, whereas visual acuity measurements of anisometropic amblyopes are symmetrical. Thomas observed that esotropic amblyopes showed the greatest loss of contrast sensitivity in the temporal visual field. The three exotropes and one esotrope investigated by Hess and Pointer showed marked asymmetries in contrast sensitivity, as did the contrast sensitivity measures for esotropes reported by Katz et al. and the vernier acuity data for esotropes reported by Bradley and Freeman.

The current experiment shows objective indexes of the variation of visual function of specific types of amblyopia across the visual field and provides reference values for the application of the multifocal VEP technique in monitoring visual function during therapy for amblyopia.

Acknowledgments

The authors thank Carly Lam, PhD, Lucia San, and Fan Chi Shing, PhD, for their assistance with the study.

References


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