Simultaneous electroretinograms and visually evoked potentials from adult amblyopes in response to a pattern stimulus

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Electroretinograms (ERGs) and visually evoked potentials (VEPs) were recorded simultaneously from each eye of three adult amblyopes. A spatially alternating checkerboard pattern stimulus of constant mean luminance was used to eliminate the effect of stray light on the ERG. The VEP was affected in the amblyopic eye of all subjects. In two subjects the VEP amplitude was reduced; in the third subject the amplitude was not attenuated, but the waveform of the VEP was markedly altered. Photopic ERGs recorded from the normal and amblyopic eye of each subject with an unpatterned flashing light were equal in amplitude. However, ERGs elicited by a patterned stimulus were affected in the amblyopic eye of all three subjects; the after-potential showed larger reductions in amplitude than the b-wave. These results suggest there may be some retinal involvement in human amblyopia.

Key words: human electroretinogram, visually evoked potential, pattern stimulus, amblyopia

The visually evoked potential (VEP) is abnormal in amblyopia, particularly when patterned stimuli are used. However, investigations of the electroretinogram (ERG) in human adult amblyopes have utilized unpatterned flashing lights to elicit electrical signals from the retina. Some authors report that they find no differences in the amplitude of the ERG of the normal and amblyopic eyes, whereas others report reduced or increased amplitudes. The wide variance in the ERG data obtained thus far should not be surprising. First, the stray light produced by flashing stimuli elicits signals from retinal regions outside the area directly stimulated. Second, it has been shown that flash-elicited ERGs can be normal in patients with macular lesions, therefore there is no reason to necessarily expect the ERG from an amblyopic eye to be affected by the use of a flashing unpatterned stimulus.

Riggs and co-workers used a stimulus comprising spatially alternating dark and light bars of constant mean luminance to record ERGs; since the stray light produced by this stimulus remains constant, it does not contribute to the ERG. Their technique also eliminates the contribution from rods to the...
ERG, they confirmed this by demonstrating that the electroretinographic spectral sensitivity curve derived from the responses to the bar pattern stimulus agreed with the photopic spectral sensitivity curve. Also, the Stiles-Crawford effect, a property exhibited by cones but not rods, has been demonstrated electrophysiologically with a spatially alternating pattern stimulus. Finally, ERGs elicited by gratings and checkerboards are more sensitive in detecting retinal pathology involving the macula than are ERGs obtained in the same patients with unpatterned flashing lights.

The purpose of the present study was to determine whether a patterned stimulus would, as in the case of macular disease, provide a more sensitive index of retinal function in human amblyopia. In addition, monocular ERGs and VEPs were simultaneously recorded to compare the electrical activity at the retina and occipital cortex.

Methods

Subjects were three females who, after receiving a full explanation of the reasons for the experiment, agreed to be tested. Subject 1, 32 years old, was an anisometropic amblyope, with a refractive error of +5.25 +1.75 x 180° in the amblyopic eye and +0.25 in the normal eye. Her acuities were 20/300 and 20/15, respectively. Subject 2, age 26, was a strabismic amblyope with a refractive error of +5.25 +1.75 x 180° in the amblyopic eye; her best corrected acuities were 20/25 and 20/200, respectively. Subject 3, 27 years old, had a right esotropia of 10 prism diopters. Corrected acuity in her amblyopic eye was 20/100 with a refractive error of -2.00 -0.75 x 170°.

A checkerboard-pattern reversal stimulus was generated by a TV monitor. The field size was 13° by 16°; two check sizes were used subtending 24 and 48 min arc and alternated at a rate of 1.88 reversals/sec. The mean luminance of the TV screen was 22 ft-lamberts and the contrast of the checks was 0.84. Signals from the retina and cortex were led into two differential amplifiers with frequency bandpass settings of 1 and 35 Hz. Signals were then led from the amplifiers and processed by a minicomputer programmed to average the ERG and VEP. In addition to the averaging function, the computer also had an artifact-rejection buffer which removed any artifacts created by eye blinks or large eye movement.

The ERG was recorded in each of these subjects with a piggyback hard-soft lens combination; the details of this lens are described elsewhere. A soft lens was placed on the subject’s cornea; then a hard lens with a silver wire attached to it was placed on top of the soft lens. All subjects comfortably tolerated this lens combination after a few minutes of wear. The cheekbone was used as reference, and the ear served as ground. VEPs were recorded from an electrode placed 1 cm above the inion along the midline and referenced to the ear.

There are at least two factors which may contribute to a reduced ERG: (1) blurring of the retinal image and (2) eye movements caused by unsteady or wandering eccentric fixation. The first factor was controlled by recording the VEP from the normal and amblyopic eye of each subject with and without the contact lenses. Since it is known that the amplitude of the VEP is sensitive to optical blur, it would be reasonable to assume that if the lenses were blurring the retinal image, the VEP amplitude would be attenuated for small checks or increased with large checks. The second factor, eye movements caused by unsteady eccentric fixation, was measured by taking a series of fundus photographs of each eye of the three subjects with the use of a fixation target to quantify their fixation and to determine the steadiness of the amblyopic eye. The photographs showed that Subject 1 had a steady eccentric fixation that was 2.5° superior-nasal whereas Subjects 2 and 3 had central fixation.

The sequence used for monocular and simultaneous recording of the ERG and VEP from each subject was as follows. The normal eye was tested first. The soft lens was placed on the cornea, and electrodes were then positioned on the scalp, cheekbone, and ears. The hard lens was then placed on the soft lens. If necessary, trial lenses were placed in a frame located in front of the hard lens, and the subject was refracted to the same acuity level measured without the piggyback arrangement. The eye without the contact lens was patched, and 128 accumulations with 24 min checks were recorded followed by 128 accumulations with 48 min checks. This pairing was replicated three times in one session; the same procedure was used to test the amblyopic eye. The data for each block of 128 accumulations were stored on a floppy diskette; ERGs and VEPs were recorded.
Fig. 1A. Flash-elicited ERGs recorded from the normal (upper record) and amblyopic (lower record) eyes of Subject 1 after 5 min of light adaptation. Ganzfeld stimulus was used in the presence of an 8 ft-lambert background light. Vertical line, 50 μV; horizontal line, 25 msec.

from each subject on 3 separate days. This resulted in nine pairs of data from the retina and cortex of each subject for 24 min and 48 min checks.

Since the normal fellow eye of each amblyope can be used as a control, the data from each subject can be treated as matched pairs, and the difference between pairs can be analyzed. Accordingly, the b-wave amplitude of the ERGs from the normal and amblyopic eye of each subject for nine replications of each stimulus condition was analyzed by a two-tailed sign test. The results for three subjects were significant (p < 0.01) and therefore favored the hypothesis that the amplitude of the pattern-elicited ERG is larger in the normal eye of an amblyope. On this basis, the ERG and VEP data from each subject were retrieved from diskette and summed by the computer, giving a total of 1152 (9 x 128) accumulations for each stimulus condition.

Photopic ERGs were also recorded from each subject with an unpatterned flashing light. The stimulus was a ganzfeld comprising a photosimulator mounted on top of a 30-inch diameter globe containing a background adaptation light of 8 ft-lamberts. Each subject’s pupils were dilated with 1% tropicamide, and the corneas were anesthetized with proparacine hydrochloride. Since a ganzfeld was used and fixation was not critical, ERGs were simultaneously recorded from both eyes with Burian-Allen lenses after 5 min of light adaptation.

**Results**

Fig. 1A shows the photopic flash ERGs recorded from the normal and amblyopic eyes of Subject 1. There was no appreciable difference between the normal and amblyopic eye in the b-wave amplitude or implicit time. Similar results were also found in Subjects 2 and 3.

Figs. 1B, 2, and 3 show the pattern ERGs and VEPs from the normal and amblyopic eyes of the three subjects. ERGs are shown in the left column and VEPs in the right. The VEPs recorded from the normal eye of the three subjects were similar. There was an initial negative component between 70 and 80 msec followed by a large positive deflection between 100 and 110 msec. A second negative deflection occurred between 130 and 170 msec. The VEP recorded from Subject 1 showed a prominent second positive component at approximately 180 msec. Subjects 2 and 3 showed a similar component, but it was smaller and less prominent.

VEPs recorded from the amblyopic eyes of Subject 1 (Fig. 1B) and Subject 2 (Fig. 2) were similar: a reduction in amplitude for both check sizes and no noticeable change in implicit time. The VEP of Subject 3 had a markedly different waveform in her amblyopic eye. The first negative component showed no reduction in amplitude for either 24 or 48 min checks, and the implicit times were equal to those in the normal eye. The first positive component of the VEP obtained from the amblyopic eye with 24 min checks had an implicit time which was nearly 50 msec longer than the implicit time of the normal eye and showed no reduction in amplitude. The record obtained with 48 min checks exhibited a shoulder around the same time as the P1 component of the normal eye, but there was a more prominent positive wave at approximately 155 msec.

The ERGs obtained from the normal eyes of three subjects were similar. The waveform and implicit time of these ERGs were in
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ERG

24

VEP

A

48

N

A

Fig. 1B. Monocular pattern ERGs and VEPs from the normal (N) and amblyopic (A) eyes of Subject 1. Check size is shown above the records. Note the third VEP in response to 24 min checks which was recorded without the contact lens electrodes but with spectacle correction in place. Vertical line, 5 μV for VEP, 2.5 μV for ERG; horizontal line, 50 msec.

agreement with those recorded by Armington et al., who used checkerboard stimuli of similar size and alternation rate. The a-waves were either very small or absent; the peak of the b-wave occurred between 40 and 45 msec and a negative after-potential occurred between 85 and 90 msec.

Both the b-wave and after-potential of the ERGs recorded from the amblyopic eye of Subject 1 were reduced in amplitude for 24 and 48 min checks. There was no significant change in implicit time of b-wave or after-potential. The ERG of Subject 2 showed a slight reduction in the amplitude of the b-wave for 24 min checks and a larger reduction of the after-potential. For 48 min checks the retinal signal from Subject 2 showed reduced amplitudes for both the b-wave and after-potential. The amplitudes of the b-wave from the normal and amblyopic eye of Subject 3 in response to 24 min were similar. However, with 24 min checks there was a reduction in the amplitude of the after-potential from the amblyopic eye. Both the b-wave and after-potential of Subject 3 were reduced with 48 min checks.

The results also showed that Subject 1, who had the largest acuity deficit (20/300), had the greatest attenuation in amplitude of the b-wave of the ERG for 24 min checks and that in the case of Subject 3, with an acuity of 20/100, the effect on the b-wave was minimal. The after-potential was affected in all subjects regardless of their level of acuity. The results obtained with 48 min checks showed that the b-wave and after-potential were affected regardless of acuity.

It is noteworthy that Subject 1 had eccentric fixation, and although the stimulus field was large enough to cover the fovea, reduction in her ERG could be due in part to the directional sensitivity of the cones. If the angle of the light striking the retinal cones is oblique to the receptor, the sensitivity of the receptor mosaic is reduced, and the ERG amplitude will fall. This was not the case in
Subjects 2 and 3, however, since they had central fixation.

The ERG data have been summarized more quantitatively in Table I. The ratios of the amplitudes of the normal to the amblyopic eye of each subject were calculated for the b-wave of the flash ERG (Fig. 1) and the b-wave and after-potential of the patterned ERG. The amplitude, in microvolts, of the b-wave elicited by the unpatterned and patterned stimuli was measured from the trough of the a-wave, if one was present, to the largest positive peak following the stimulus onset. If there was no a-wave, b-wave amplitude was measured from the baseline. The amplitude of the after-potential was measured from the trough of the a-wave, or the baseline, to the first negative deflection that followed the b-wave. If the signals elicited from the two eyes are equal, the ratio is 1.00.

The ratios obtained from the flash-elicited b-waves were very close to 1.00 for all subjects. On the other hand, the ratios obtained for the pattern ERGs were larger than 1.00, with the after-potential affected more than the b-wave. The after-potential ratios ranges from 1.47 to 3.00, whereas the b-wave ratios ranged from 1.13 to 2.43. Subject 3 had a ratio of 1.13 for 24 min checks, a value which we do not consider significant. The highest ratio we have found in normal subjects with normal acuity in both eyes is 1.25 for the b-wave and after-potential.13

Discussion

The VEP results of two of the subjects confirms other studies14-16 which have shown that the amplitude of the pattern evoked potential is attenuated in amblyopia. However, the VEP of Subject 3 is unique, since she...
elicited an electrical signal with an altered waveform. This finding demonstrates that some amblyopes may have large-amplitude pattern-elicited VEPs but with a waveform made up of components which differ from those obtained with stimulation of the normal eye. Since the VEP from the normal eye of Subject 3 is similar to the VEPs from the normal eyes of Subjects 1 and 2, the alteration of her VEP waveform is most likely the result of amblyopia and not of differences in VEP waveforms between subjects. Also, given this type of change in the waveform of the VEP recorded from the amblyopic eye, we do not feel that one can meaningfully describe the waveform in terms of increased implicit time as done in the case of de-myelinating disease of the optic nerve.22

To our knowledge, these findings are the first to show that pattern ERGs are more affected in amblyopia than ERGs recorded with flash-elicited stimuli. Tuttle23 used an alternating bar pattern grating of constant mean luminance to record ERGs and VEPs from four amblyopic subjects. He reported no difference in the amplitude of the b-wave of the ERGs recorded from the amblyopic and normal eyes. Also, he found no differences between the amplitudes of the VEPs recorded from the normal and the amblyopic eye, although he did report an increase in the implicit time of the VEP from the amblyopic eye. It is unusual that no differences in amplitude were found, since most investigators find significant alterations of the VEP amplitude in amblyopic eyes, particularly when small checks are used.14-6 One notable fact is that three of the four amblyopes tested by Tuttle had acuities in their amblyopic eye of 20/40, 20/40, and 20/60, respectively,
whereas the fourth had acuity of 20/100. Two of our subjects had acuities which were much worse: 20/200 to 20/300. Subject 3 with an acuity of 20/100 had a normal amplitude b-wave for 24 min checks. Consequently, Tuttle's finding of equal ERG and VEP amplitudes may be due in part to the fact that his subjects had only mild to moderate acuity loss.

The major question then is: why are the pattern-elicited ERGs more affected in ambylophia than flash-elicited ERGs? There are a number of possible explanations. First, if the retinal image of the patterned stimulus were blurred, it would have the effect of reducing the contrast level between checks and therefore reduce the amplitude of the signal. This is unlikely, since the subjects were refracted to the acuity levels that they had before the contact lenses were put in place and we found no differences in the amplitude or implicit time of the VEPs recorded with and without the contact lenses.

A second possibility is that eye movements may have contributed to the reduction of amplitude of the ERG components. If the ambylopic eye is unsteady in fixation, eye movements which occur during recording might affect the amplitude of the ERG. Eye movements probably had little effect on the results of our subjects, since our fundus photographs show steady fixation in all subjects. In addition, if eye movements were involved, one would expect all components to be affected equally. However, the b-wave ratios obtained with 24 min checks in Subjects 2 and 3 were borderline-normal, but the after-potential ratio was abnormal in all subjects.

A third possibility is that eye movements have contributed to the reduction of amplitude of the ERG components. If the ambylopic eye is unsteady in fixation, eye movements which occur during recording might affect the amplitude of the ERG. Eye movements probably had little effect on the results of our subjects, since our fundus photographs show steady fixation in all subjects. In addition, if eye movements were involved, one would expect all components to be affected equally. However, the b-wave ratios obtained with 24 min checks in Subjects 2 and 3 were borderline-normal, but the after-potential ratio was abnormal in all subjects.

A third possibility is that the pattern-elicited ERG, particularly the after-potential, reflects a disorder in the photopic mechanisms of an ambylopic eye. It is believed that processing of contrast information in the human visual system occurs beyond the retinal elements which are responsible for generation of the ERG. Nevertheless, it has been well established that a pattern stimulus of constant mean luminance elicits ERGs which are not contaminated by the intrusion of stray light and are photopic in nature. Therefore, it appears that a patterned stimulus of constant mean luminance provides a more sensitive means of testing retinal function in ambylophia than does an unpatterned flash stimulus.

A significant finding of this study is that the after-potential of the pattern-elicited ERG is more affected than the b-wave in an ambylopic eye. The activity of the after-potential is believed to be retinal in origin. Marg recorded ERGs with unpatterned flashing stimuli and found that the amplitude changes of the after-potential paralleled changes in the amplitude of the a-wave but not the b-wave. Using a number of controls, he excluded the possibility that the after-potential was due to nonretinal contributions such as electrode artifacts, accommodation, or pupil size. Arming et al. found that the amplitude of the after-potential elicited by a checkerboard pattern stimulus increased monotonically with increases in retinal illumination. Although the a-wave amplitudes were too small to be reliably measured, they did note that the amplitude of the after-potential changed more rapidly than the b-wave. This difference in the time course of the after-potential and b-wave implies different retinal sources. Arming et al. present further evidence that the after-potential is primarily photopic and is elicited by the central retina. They report that the amplitude of the after-potential is large when the central 17° of the retina is stimulated with a bar pattern stimulus. However, when the stimulus is placed 7.5° temporally or nasally from the fovea, the after-potential is no longer present. The present findings that the after-potential is the most affected of the ERG components in ambylophia, a visual defect of the photopic mechanisms, suggests that this component should be studied more closely in other ocular disorders which involve central retinal function.

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