J. Physiol. (1975), **247**, *pp.* 711–724
With 8 *text-figures*
Printed in Great Britain

VISUAL EVOKED RESPONSES IN HUMANS WITH ABNORMAL VISUAL EXPERIENCE

BY R. D. FREEMAN AND L. N. THIBOS
From the School of Optometry, University of California, Berkeley, California 94720, U.S.A.

(*Received 12 August 1974*)

SUMMARY

1. The visual evoked response to a grating target of varying spatial frequency was examined for normal subjects and for subjects with meridional amblyopia. This condition, reduced visual resolution for specific target orientations, is associated with, and thought to result from, marked ocular astigmatism.

2. For normal subjects, the general relation between spatial frequency and the evoked response is similar to psychophysical contrast sensitivity data. Evoked response amplitudes to oblique gratings are typically reduced and this is analogous to the lower acuity for oblique compared to horizontal and vertical detail.

3. In addition to the oblique effect, the magnitude of the evoked response for meridional amblyopes depends upon grating orientation over most of the spatial frequency range tested (0·5–16 cycles/deg). The lowest evoked amplitude is found when stimulus grating orientation matches that for which acuity is reduced.

4. The evoked potential spatial frequency response functions are qualitatively similar to contrast sensitivity functions determined with the same abnormal subjects.

5. From these results, it may be concluded that the physiological locus of meridional amblyopia is confined primarily to structures at or prior to the site of evoked potential generation.

INTRODUCTION

Most of what is known about vision has been determined using psychophysical measurements. The relatively recent contributions of electrophysiology have been very important but they have been partly limited because related psychophysical data are predominantly from humans. By using the visual evoked response (VER), this gap may be bridged. Unfortunately, although many VER studies have been conducted (see Riggs

& Wooten, 1972, for a review) there remains a lack of knowledge about the precise physiological genesis of the evoked response. Therefore, it appears at present that productive evoked potential experiments with humans may be limited to two primary issues. The first is whether psychophysical measurements and evoked responses for a given parameter are parallel. If this is the case, then evoked response tests can be very valuable in situations where psychophysical measurements are inconvenient or impossible as, e.g., with young children. Secondly, if a particular phenomenon can be demonstrated using evoked potentials, its origin can be assigned to a region at or prior to neurones responsible for the evoked response.

With these applications in mind, we carried out a preliminary study using evoked potential measurements to demonstrate a direct neurophysiological correlate to a psychophysical finding (Freeman & Thibos, 1973). The psychophysical tests had shown visual resolution deficiencies for certain target orientations in subjects with pronounced ocular astigmatism, even when full ophthalmic corrections were used. Since optical explanations of the effect had been ruled out (Freeman, Mitchell & Millodot, 1972), our evoked potential results, which showed analogous deficits with the same subjects, limit the defect to regions which are peripheral to or which generate evoked potentials, presumably visual cortex.

The study has been extended in the experiments described in the present paper. Our original evoked potential data was obtained using stimulus gratings of one spatial frequency. We now report details of preliminary findings of differences in the evoked potential specific to grating orientation over a broad range of spatial frequencies (Freeman & Thibos 1974). These results are compared directly with psychophysical data from the same subjects of the preceeding paper (Freeman & Thibos, 1975).

METHODS

Stimulus

In order to compare directly the electrophysiological results of this study with the psychophysical findings of the preceding paper, the same visual stimulus equipment was used to generate sinusoidal grating targets. However, two modifications were necessary for the VER experiments. First, preliminary tests indicated that a large stimulus field gave a stronger evoked response, so we chose a field size of 7°, corresponding to a 57 cm viewing distance. Secondly, the grating contrast was sinusoidally modulated at a temporal frequency of 9 or 12 Hz. With this procedure, total space-average luminance is kept constant at 22 cd m^{-2} while the luminance of alternate bars is modulated in antiphase. A maximum contrast of about 0·64 was used to avoid saturation effects of either the oscilloscope grating or the evoked potential mechanisms (Spekreijse, van der Tweel & Zuidema, 1973).

Procedure

All subjects used ophthalmic lens corrections based upon careful clinical examination and further checks were made while the subjects were seated before the stimulus grating. Standard bipolar recording electrodes were placed on mid line at the inion and 6–9 cm above the inion, with the ear as ground.

We arranged a quasi-random sequence of test targets before each experimental run. Subjects were then seated and monocularly viewed the target only upon command in order to minimize fatigue or adaptation effects that might occur with prolonged viewing. Test periods were typically 30–60 sec, with about 15 sec breaks between runs. During the test period the e.e.g. was recorded with an FM magnetic tape recorder and simultaneously it was analysed for the VER content. Each experiment ran continuously until the predetermined sequence had been finished in order to control for the variability effects between experimental sessions due to differences in electrode preparation.

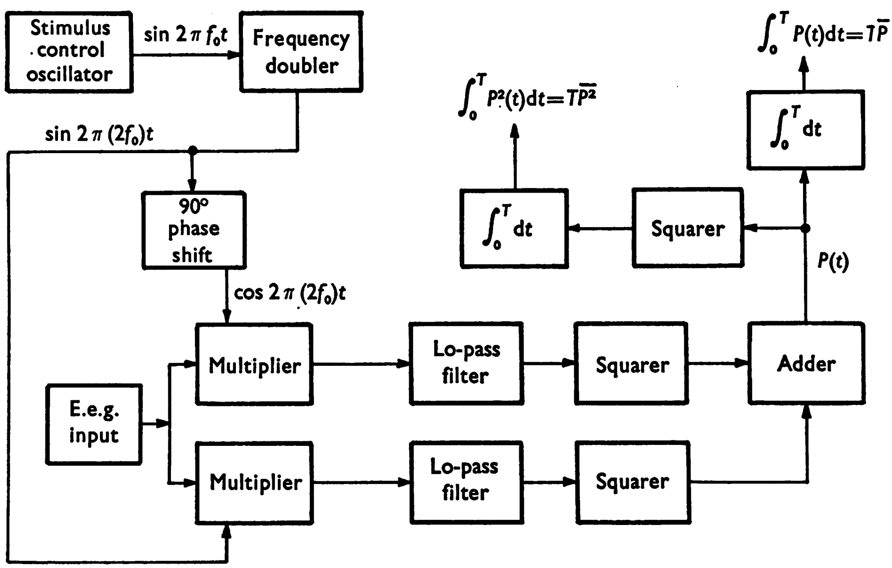

Fig. 1. Block diagram of a synchronous filter used to compute visual evoked response power. The evoked response to an alternating bar pattern is nearly sinusoidal with frequency twice the alternation frequency, f_0. This component of the raw e.e.g. is extracted by the circuit using a simple demodulation process. $P(t)$ is real-time VER power; $\bar{P}$ is the average signal power obtained by averaging $P(t)$ for T sec; $\bar{P}^2$ is the second moment of $P(t)$, used in calculating the variance of $P(t)$.

Data analysis

We have found, in accord with previous results, that the primary change in the e.e.g. resulting from a grating pattern stimulus is the generation of a nearly sinusoidal signal with frequency twice the stimulating frequency (Campbell & Maffei, 1970; Cobb, Morton & Ettlinger, 1967; Perry, Childers & Falgout, 1972). To determine the magnitude of the steady-state evoked response at this second harmonic frequency, we measured signal power using two techniques. For the first method, a digital computer is used to sample the e.e.g., compute a discrete Fourier transform from the samples, and calculate a power spectral density function from the transform. The spectral function is graphed by an $X-Y$ recorder to show how the signal power of the e.e.g. is distributed in the frequency domain, and the size of the VER component is readily obtained from the graph. Because this method is not convenient for routine on-line measurements, we used it primarily to confirm results obtained by the second method, synchronized filtering.

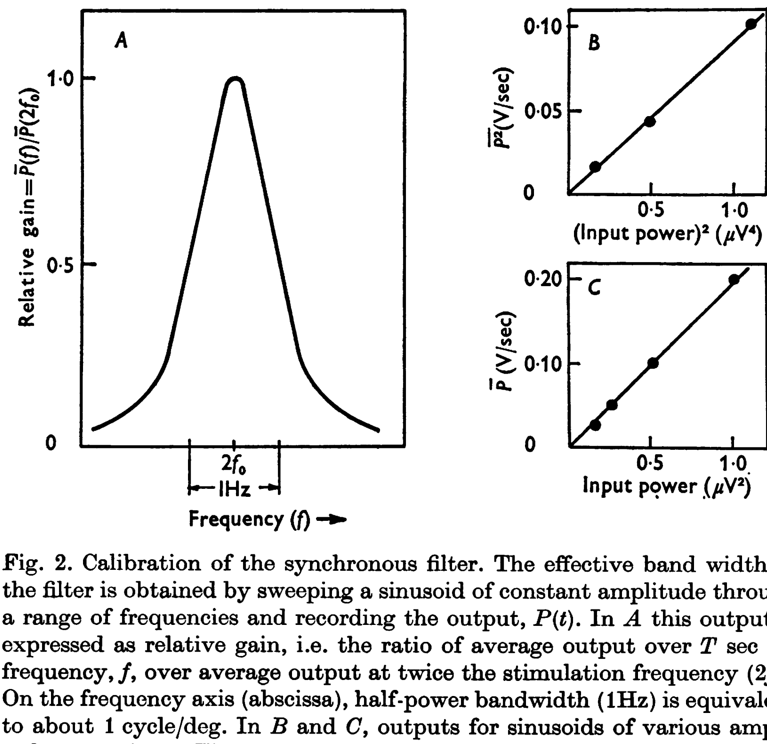

Fig. 2. Calibration of the synchronous filter. The effective band width of the filter is obtained by sweeping a sinusoid of constant amplitude through a range of frequencies and recording the output, $P(t)$. In A this output is expressed as relative gain, i.e. the ratio of average output over T sec for frequency, f, over average output at twice the stimulation frequency ($2f_0$). On the frequency axis (abscissa), half-power bandwidth (1Hz) is equivalent to about 1 cycle/deg. In B and C, outputs for sinusoids of various amplitudes are given. These data show that the system accurately measures first (C) and second (B) moments of real-time evoked response.

For the second technique, the synchronous (phase-locked) filter shown in Fig. 1 was designed to measure VER power in a standard way (Regan, 1966). With this system, the e.e.g. signal is multiplied by a sine wave and cosine wave with frequencies of twice the stimulus modulation frequency. The average values of the products are found by low-pass filtering and they are then squared and added to give a real-time measure of VER power, $P(t)$. This signal is integrated over time T (typically 30 to 60 sec) to obtain $\bar{P}$, the measure of average VER power used for the results we present below. To facilitate comparison with other results, we have computed for some data values of $\sqrt{\bar{P}}$, i.e. the r.m.s. amplitude. The synchronous filter was calibrated by applying sinusoids of known power to the apparatus. Bandwidth was measured by sweeping a sinusoid of constant amplitude through a small range of frequencies and the calibration results are shown in Fig. 2.

With this filtering procedure, we can estimate confidence bounds on the measurement of average VER power in two ways. First, the second statistical moment of $P(t)$, $\bar{P}^2$ is computed. This gives a direct estimate since Var $(P) = \bar{P}^2 - (\bar{P})^2$. The second estimate assumes that the evoked response for our stimulus configuration is a narrow-band Gaussian random process. In this case, $\bar{P}$ has a χ-squared distribution. Both methods indicate S.E. of the order of 15 per cent.

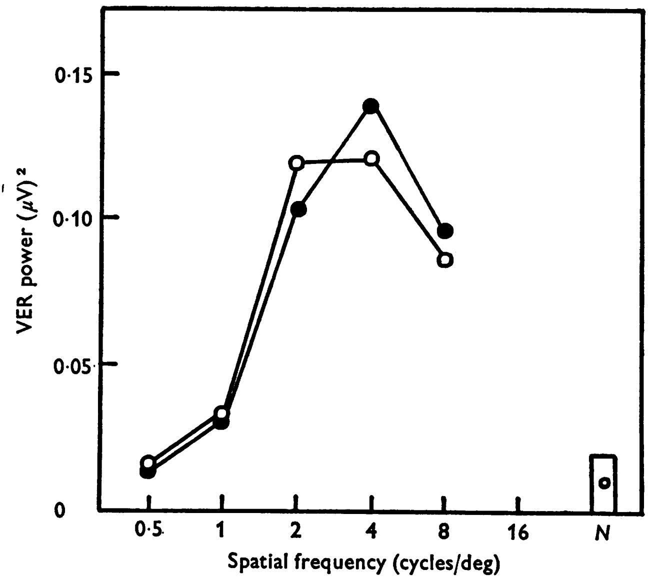

Fig. 3. The evoked responses for a normal subject, L.T., who viewed a grating target of fixed contrast 0·65 and variable spatial frequency. Evoked potential power is obtained by spectral analysis using a digital computer (filled circles) and by synchronous filtering (open circles) using the circuit shown in Fig. 1. Noise level for the synchronous filtering procedure was measured while the subject viewed a blank screen and it is indicated by the rectangle, N.

RESULTS

Normal spatial frequency response functions

Fig. 3 shows VER power for a peak grating contrast of 0·65 as a function of spatial frequency for a typical non-astigmatic subject. The two sets of data represent measurements by spectral analysis and synchronized filtering. In this graph and the ones that follow, the rectangle denoted N represents an estimate of the noise power of the e.e.g. in the range of the filter's passband. The height of the rectangle is the output of the synchronized filter for a control experiment in which the subject viewed a blank screen, i.e. a grating with zero contrast. As the graph shows, results for the two measurement techniques are in accord. It is also clear that the size of the evoked response to a grating varies with spatial frequency and in this case there is a peak at 4 cycles/deg.

In previous work, it has been found that the relation between log contrast and evoked potential amplitude for a given spatial frequency is approximately linear (Campbell & Maffei, 1970). Using this linear relation, Campbell & Maffei extrapolated suprathreshold VER values to find contrast for zero amplitude VER. Close agreement was found with corresponding psychophysically determined contrast threshold data. But apparently, there also would have been close agreement between psychophysical data and suprathreshold VER amplitude for a given target contrast. This follows from the Campbell & Maffei finding that regression lines for evoked response amplitude as a function of log contrast have a nearly constant slope for several spatial frequencies and target orientations. So the intercepts on the contrast axis differ only by a scaling factor, the common slope of the regression lines, from VER amplitude at a given value of contrast. This accounts for the congruence of the shapes of threshold and suprathreshold VER functions of spatial frequency. We thus have a basis, if psychophysically determined contrast thresholds coincide with extrapolated VER 'thresholds', to compare suprathreshold VER data with threshold psychophysical results.

In Fig. 4, an example of this comparison is shown for the subject of Fig. 3. VER amplitude and suprathreshold grating contrast (0·65) (*A*) and psychophysical contrast sensitivities (reciprocals of thresholds) (*B*) are given as functions of spatial frequency. Both sets of data show low and high frequency attentuation and overall shapes are similar. This is a typical result, but for some subjects little or no attenuation is found in the low frequency region. Inter-subject variability in evoked potentials for low spatial frequencies extending to zero cycles/deg (uniform luminance field) has been found in other experiments (Padmos, Haayman & Spekreijse, 1973). At present, we cannot explain this finding and it certainly warrants further investigation.

A final observation about the data in Fig. 4 concerns orientation. It has been shown that the psychophysical oblique effect, i.e. reduced resolution for obliquely oriented detail compared to horizontal and vertical orientations, has a counterpart in the VER. Evoked potential amplitude for an oblique grating stimulus is lower than that for a vertical or horizontal grating (Maffei & Campbell, 1970; Freeman & Thibos, 1973). We have extended this result in the present study by investigating orientation differences over a range of spatial frequencies. The results shown in Fig. 4*A*, which have been confirmed in other normal subjects, indicate that the oblique reduction in VER amplitude is more prominent at the higher frequencies tested. A similar finding is apparent for the psychophysical data of Fig. 4*B*.

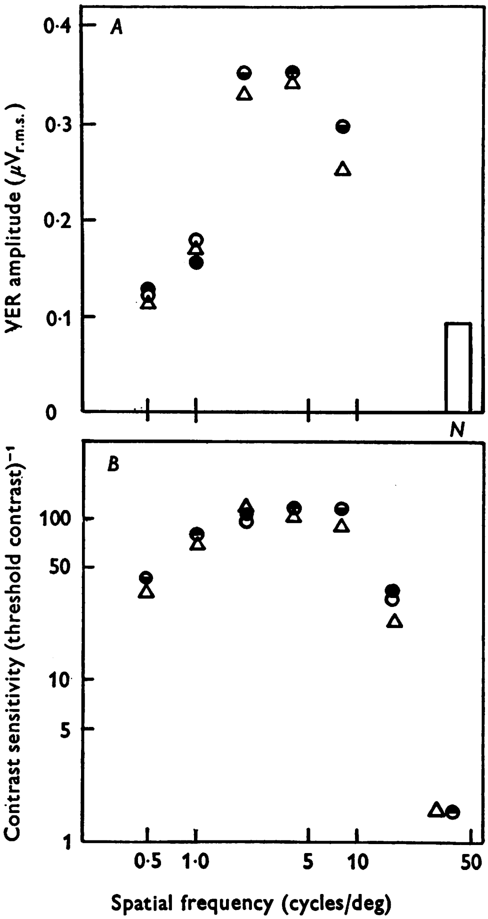

Fig. 4. Evoked response as a function of spatial frequency (*A*) is shown along with contrast sensitivity data determined psychophysically (*B*) for the same subject of Fig. 3. Three target orientations were used: vertical (open circles), horizontal (filled circles) and oblique, 45° from horizontal (open triangles). Evoked amplitudes are given (square roots of average power) to facilitate comparison with the psychophysical data.

The influence of defocus

We are interested in the effect of target orientation on the visual evoked potential for subjects who show a meridional resolution deficit (meridional amblyopes). Since relatively small amounts of defocus can depress the theoretical modulation transfer function significantly (Freeman & Thibos, 1975), it is important to see how defocus influences the evoked potential. We will then have an idea of magnitude for comparison with the data for meridional amblyopes.

Previous experiments have shown that evoked response is a function of the eye's refractive state (Harter & White, 1968; Millodot & Riggs, 1970). The effect is demonstrated for the subject of Fig. 3 and the result is shown in Fig. 5*A*. Gratings of several spatial frequencies were observed through spherical lenses of 0, + 1, and + 3 D over the proper correction. The + 1 and + 3 D lenses reduce the evoked response, and as expected the reduction is greater for the higher lens power. Also, higher frequencies are more affected.

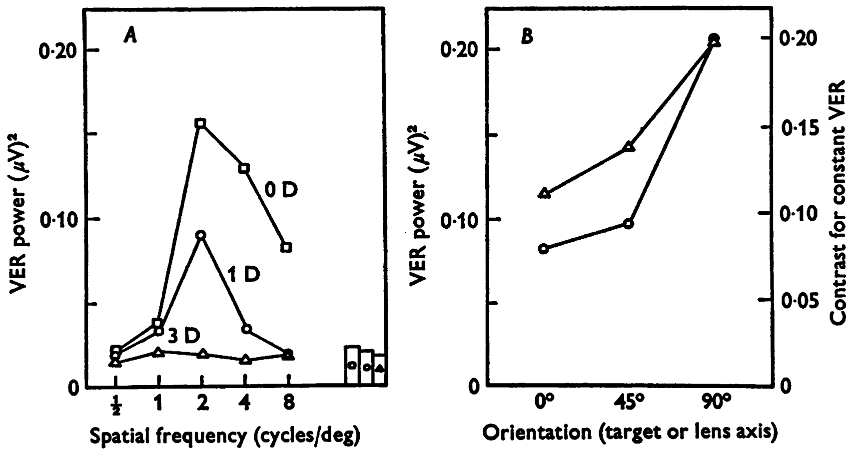

Fig. 5. *A*, the effect of spherical defocus on the evoked response is shown for the subject of Fig. 3. The top curve (open squares) represents optimal correction and the other curves (open circles and triangles) show the defocus effects at various spatial frequencies for additional lenses of + 1 and + 3 D, respectively. The noise levels for each set of data are represented by the rectangles in the lower right corner. In *B*, meridional defocus effects are shown for two normal subjects, D.R. (open circles) and P.K. (open triangles). D.R. viewed an 8 cycles/deg grating oriented horizontally, obliquely, or vertically through a cylindrical lens which blurred the horizontal grating, and this is expressed in the corresponding evoked response power (left ordinate). P.K. viewed a vertical 8 cycles/deg target through a cylindrical lens with axis horizontal, vertical or oblique. For each axis orientation, target contrast was adjusted to cause a near-threshold level evoked response and the highest contrast required corresponds to the axis of maximum target defocus.

Now, we wish to see the influence of meridional defocus on the VER for different orientations of the stimulus grating. Astigmatism was simulated in an emmetropic subject who viewed an 8 cycles/deg grating through a cylindrical lens which caused maximal defocus of the grating when it was oriented horizontally. The resulting evoked responses for horizontal, oblique (45°) and vertical orientations of the stimulus are shown in Fig. 5*B* and it is clear that VER power (left ordinate) varies directly with the amount that each orientation is defocused. This effect is shown in Fig. 5*B* for another subject who viewed a fixed vertical target through the cylindrical lens. For this subject the axis of the lens was rotated and target contrast was adjusted to obtained near-threshold constant level evoked potential for lens axis orientations of horizontal, oblique and vertical. Target blur is greatest when the lens axis is vertical and correspondingly, the highest target contrast is required for constant VER. These results show that spherical and meridional defocus affects both threshold and suprathreshold evoked potentials in a predictable way.

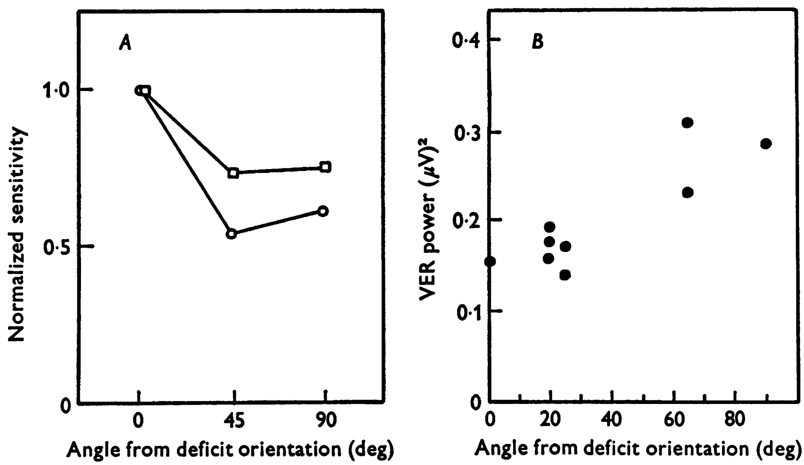

Fig. 6. *A*, evoked response (open circles) and psychophysically determined cut-off frequency (open squares) is shown for a meridional amblyope, A.D. The evoked data is for a 6 cycles/deg target and normalized ordinate values are used to facilitate comparison. *B*, evoked response is shown for another meridional amblyope, P.G., for an 8 cycles/deg grating of several orientations. The abscissa shows the angle between the test target and the deficit orientation. The correlation coefficient is 0·88.

Spatial frequency response functions for meridional amblyopes

Orientation differences. As mentioned above, evoked potentials for horizontal and vertical orientations of a given target are about equal and oblique orientations yield lower responses. However, for the meridional amblyope, this oblique reduction is accompanied by a lowered VER for the target orientation which yields poor acuity (Freeman & Thibos, 1973). This effect is only found in astigmats and the reduced sensitivity always corresponds to the meridian which would be most defocused if corrective lenses were not used. Fig. 6*A* shows orientation differences in evoked responses to a 6 cycles/deg stimulus grating for a typical meridional amblyope. Without correction, horizontal lines are blurred for this subject. Tests with optimal lens correction show that, in addition to the oblique effect, responses to the horizontal grating are substantially reduced. Sensitivity is on a normalized scale to facilitate comparison with the other set of data, psychophysically determined cut-off frequencies for the orientations shown. Similar orientation differences are evident for both types of measurement.

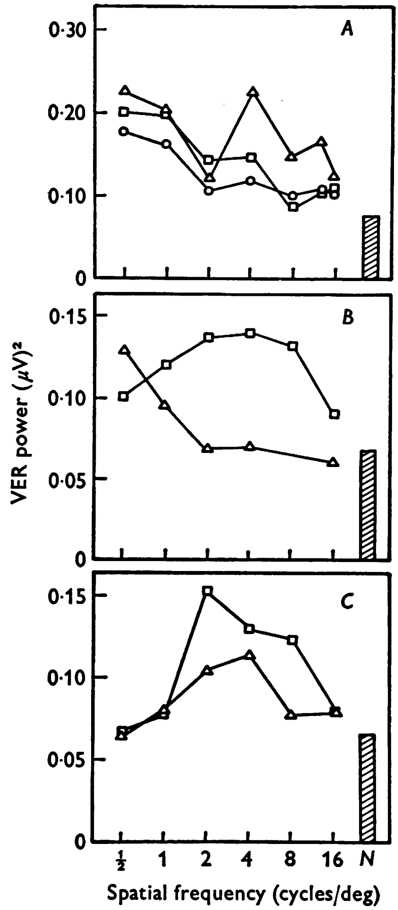

Fig. 7. Evoked potential-spatial frequency functions are shown for three meridional amblyopes, C.M. (*A*), R.B. (*B*) and C.L. (*C*). Target contrast is 0·65 and results for orientations of horizontal (open triangles) oblique (open circles) and vertical (open squares) are indicated. Noise levels are indicated by rectangles in the lower right corners.

Evoked responses to several target orientations are shown for another meridional amblyope in Fig. 6*B*. Once again, VER power is higher with increasing angular distance of the target from the deficit orientation.

Although as Fig. 6*A* shows, this relation is not linear, a linear approximation can be used and it shows that the correlation between evoked potential amplitude and angular distance from the reduced meridian is very high ($r = 0{\cdot}88$; significant at $P = 0{\cdot}01$).

Spatial frequency differences. We have demonstrated that orientation differences in evoked responses of meridional amblyopes parallel psychophysical results. Next, it is important to see if the relation holds across a range of spatial frequencies in order to establish if the complete psychophysical finding is accounted for by evoked potential measurements. Results for three meridional amblyopes are given in Fig. 7 where VER power is shown for various stimulus grating spatial frequencies of 0·65 contrast.

The subject shown in Fig. 7*A* has reduced visual acuity for vertical gratings and for nearly all the spatial frequencies tested there is a corresponding reduction in evoked potential amplitude. The deficit is prominent in the low frequency range including the lowest frequency tested, 0·5 cycles/deg. In Fig. 7*B*, the most severe deficit is shown. This subject has poor resolution for horizontal gratings and, except for the lowest frequency tested, there is also a considerable reduction in the VER at various spatial frequencies for a horizontal grating stimulus. As with the other meridional amblyopes, the subject of Fig. 7*C* has an orientational acuity deficit, in this case for horizontal gratings. And once again, for most spatial frequencies, the evoked responses are correspondingly reduced for horizontal gratings.

These evoked potential results are parallel with psychophysical tests on the same subjects (Freeman & Thibos, 1975). With few exceptions, psychophysical contrast sensitivity differences at given spatial frequencies are qualitatively expressed in evoked potential measurements. This relation is shown in more detail for one other meridional amblyope in Fig. 8. The subject here is of particular interest because her principal astigmatic axes are oblique and acuity tests show maximum and minimum values for grating orientations which match the astigmatic meridians. This difference applies also to contrast sensitivity over nearly the entire range of spatial frequencies tested, as shown in Fig. 8*B*. Evoked response data are shown for the same subject in Fig. 8*A*. Following the suggestion that VER amplitude is directly proportional to log contrast (Campbell & Maffei, 1970), we plot the square root of average VER power in the Figure. The close similarity in spatial frequency functions between psychophysical contrast sensitivity data and evoked responses is quite striking for this subject. An important implication is that the spatial weighting functions computed from psychophysical data in the preceding paper will approximate similar functions calculated from evoked response data. As a consequence, neurones having the asymmetrical receptive fields predicted by these weighting functions must reside on the input side of evoked potential generators. Although there is inter-subject variability in the degree of concordance between psychophysical and evoked potential spatial frequency response functions, we conclude that the primary features of meridional amblyopia are evident in the visual evoked potential.

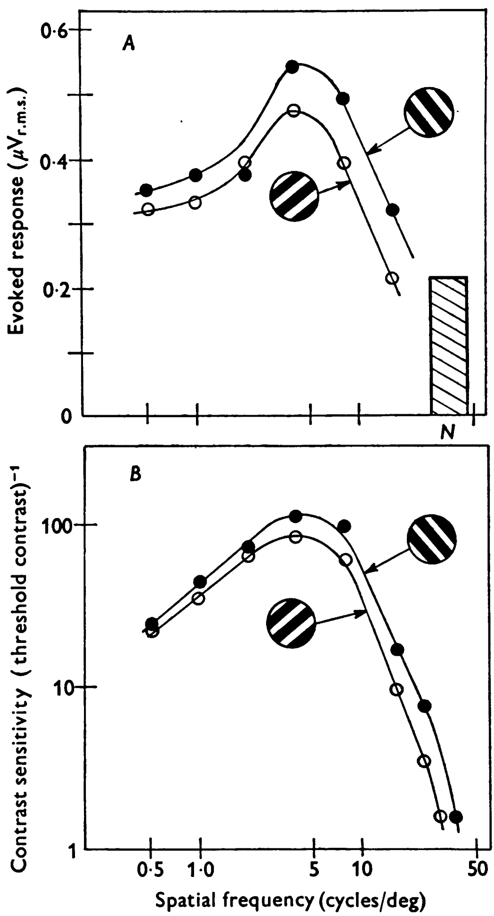

Fig. 8. Spatial frequency response functions are shown for a meridional amblyope who has oblique astigmatism. Evoked responses are given in *A* and psychophysical contrast sensitivity data are shown in *B*. Filled circles represent the normal meridian and open circles are the results for the orthogonal orientation.

DISCUSSION

We confirm here that the visual evoked response to a continuously modulated bar grating target in subjects with meridional amblyopia is strongly dependent on orientation. The results extend related studies (Freeman & Thibos, 1973; Fiorentini & Maffei, 1973) to include a range of spatial frequencies. In general, the weakest evoked response occurs when the target is oriented in the meridian which would be most defocused in the absence of ophthalmic lenses. Evoked responses are largest for orthogonally oriented targets. Meridional differences are usually more prominent at the higher spatial frequencies but some subjects show pronounced orientation effects for a 0·5 cycles/deg target. These features of evoked responses in meridional amblyopes agree well with psychophysically determined contrast sensitivity data for the same subjects. Since contrast sensitivity values for the deficit meridian are definitely below normal, we would expect analogous evoked amplitudes to be also lower than normal. However, absolute values for evoked responses depend on so many variables that we can only refer with certainty to relative differences. There are indications, though, that the deficit target orientation results in very low evoked amplitudes which sometimes approach the noise level (Fig. 7*B*, for example).

The implications of these results depend on what is known about visual evoked potentials. Evoked responses monitor activity at a point intermediate to light transduction and perception. The primary VER generator for patterned light stimulation appears to be visual cortex although there remain some areas of dispute (Halliday & Michael, 1970; Jeffreys, 1971; Rush & Driscoll, 1969). From experimental stimulation of selected areas of visual field and known retinotopic projections to visual cortex, it has been suggested that the major portion of the occipital signals is due to fovea (Spekreijse, 1966; Rietveld, Tordior & Hagenouw, Lubbers & Spoor 1967; Regan & Heron, 1969). Since orientation differences for meridional amblyopes are evident in evoked potential data, we may imply that the responsible physiological mechanisms reside in early visual pathways.

This work was supported by a grant from the National Eye Institute, U.S. Public Health Service, N.I.H. (EY 01175) and by N.I.H. Training Grant 1 TO1 EY00076-010A.

REFERENCES

CAMPBELL, F. W. & MAFFEI, L. (1970). Electrophysiological evidence for the existence of orientation and size detectors in the human visual system. *J. Physiol.* **207**, 635–652.

COBB, W. A., MORTON, H. B. & ETTLINGER, G. (1967). Cerebral potentials evoked by pattern reversal and their suppression in visual rivalry. *Nature, Lond.* **216**, 1123–1125.

FIORENTINI, A. & MAFFEI, L. (1973). Evoked potentials in astigmatic subjects. *Vision Res.* **13**, 1781–1783.

FREEMAN, R. D., MITCHELL, D. E. & MILLODOT, M. (1972). A neural effect of partial visual deprivation in humans. *Science, N.Y.* **175**, 1384–1386.

FREEMAN, R. D. & THIBOS, L. N. (1973). Electrophysiological evidence that abnormal early visual experience can modify the human brain. *Science, N.Y.* **180**, 876–878.

FREEMAN, R. D. & THIBOS, L. N. (1974). Psychophysical and electrophysiological determinations of contrast sensitivities in meridional amblyopes. *Association for Research in Vision and Ophthalmology*, Sarasota, Fla.

FREEMAN, R. D. & THIBOS, L. N. (1975). Contrast sensitivity in humans with abnormal visual experience. *J. Physiol.* **247**, 687–710.

HALLIDAY, A. M. & MICHAEL, W. F. (1970). Changes in pattern-evoked responses in man associated with the vertical and horizontal meridians of the visual field. *J. Physiol.* **208**, 499–513.

HARTER, M. & WHITE, C. T. (1968). Effects of contour sharpness and check-size on visually evoked cortical potentials. *Vision Res.* **8**, 701–711.

JEFFREYS, D. A. (1971). Cortical source locations of pattern related visual evoked potentials recorded from human scalp. *Nature, Lond.* **229**, 502–504.

MAFFEI, L. & CAMPBELL, F. W. (1970). Neurophysiological localization of the vertical and horizontal coordinates in man. *Science, N.Y.* **167**, 386–387.

MILLODOT, M. & RIGGS, L. A. (1970). Refraction determined electrophysiologically. *Archs Ophthal. Paris* **84**, 272–277.

PADMOS, P., HAAYMAN, J. & SPEKREIJSE, H. (1973). Visually evoked cortical potentials to patterned stimuli in monkey and man. *Electroenceph. clin. Neurophysiol.* **35**, 153–163.

PERRY, N. W., JR., CHILDERS, D. G. & FALGOUT, J. C. (1972). Chromatic specificity of the visual evoked response. *Science, N.Y.* **177**, 813–815.

REGAN, D. (1966). Some characteristics of average steady-state and transient responses evoked by modulated light. *Electroenceph. clin. Neurophysiol.* **20**, 238–248.

REGAN, D. & HERON, J. R. (1969). Clinical investigations of lesions of the visual pathway: a new objective technique. *J. Neurol. Neurosurg. Psychiat.* **32**, 479–483.

RIETVELD, W. J., TORDIOR, W. E. M., HAGENOUW, J. R. B., LUBBERS, J. A. & SPOOR, TH. A. C. (1967). Visual evoked responses to blank and checkerboard patterned flashes. *Acta physiol. pharmac. neerl.* **14**, 259–285.

RIGGS, L. A. & WOOTEN, B. R. (1972). Electrical measures and psychophysical data on human vision. *Handbook of Sensory Physiology*, vol. VII/4, ed. JAMESON, D. & HURVICH, L. Berlin, Heidelberg, New York: Springer-Verlag.

RUSH, S. & DRISCOLL, D. A. (1969). EEG electrode sensitivity – an application of reciprocity. *IEEE Trans. bio-med. Engng BME*-**16**, 15–22.

SPEKREIJSE, H. (1966). Analysis of EEG responses in man evoked by sine wave modulated light. Ph.D. Thesis. The Hague: W. Junk.

SPEKREIJSE, H., VAN DER TWEEL, L. H. & ZUIDEMA, TH. (1973). Contrast evoked responses in man. *Vision Res.* **13**, 577–1601.