certainty, ascribe the uveitogenic activity of S-antigen to the ~50,000 dalton polypeptide. The ability to readily purify this species to homogeneity should facilitate both the absolute identification of the polypeptide and elucidation of the molecular mechanism of its uveitogenic activity.

Key words: retinal S-antigen, purification to homogeneity, high performance liquid chromatography (HPLC), experimental autoimmune uveitis

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References

VEPs in Humans Reveal High and Low Spatial Contrast Mechanisms

P. Bobak,* I. Bodis-Wollner,† C. Hornois,‡ and J. Thornton†

The effect of contrast on visual evoked potential (VEP) amplitude was examined in nine observers. A 6.0 cycles/deg (cpd) grating was modulated in an “on-off” mode at 7.5 Hz. The VEP response contains significant first and second harmonic components: their growth with contrast is parallel, each function consisting of two limbs. The data are consistent with the hypothesis that the pattern VEP obtained with “on-off” presentation may reflect the contributions of “low” and “high” contrast neuronal populations demonstrated in physiological studies of the primate. Invest Ophthalmol Vis Sci 25:980–983, 1984

Both psychophysical and visual evoked potential (VEP) methods have been used to investigate the spatial contrast properties of the human visual system. There is a good correlation between the VEP and psychophysical contrast sensitivity when VEP threshold is relating VEP amplitude to contrast have been described in humans below 3.0 cycles/deg (cpd) and over a range of spatial frequencies in the monkey and in the rat. A nonmonotonic growth of the human contrast response is consistent with the psychophysical contrast modulation results of Bodis-Wollner and Hendley. In this study, we examined both the function relating VEP amplitude to grating contrast and the relationship between psychophysical and VEP thresholds. A 6.0 cpd sinusoidal grating pattern was presented in an “on-off” mode at 7.5 Hz. The VEP response to this type of presentation contains both first and second harmonic components as has been reported previously. The contrast response for each harmonic was analyzed separately to assess agreement or lack of it between evoked potential amplitude and psychophysical flicker and pattern detection thresholds. A 6.0 cpd grating was chosen, as criteria for pattern and flicker detection are well separated at 8 Hz at this spatial frequency. Materials and Methods. A Joyce Electronics oscilloscope was used to present a 6.0 cpd sinusoidal grating that was sinusoidally modulated in an “on-off” mode at the rate of 7.5 Hz. The display field subtended 9 by 12 deg at a viewing distance of 144 cm. The mean...
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VEP (6.0c/d)

Fig. 1A, Mean VEP amplitude data of nine observers as a function of grating contrast (note log scale) for each harmonic component. "On-off" pattern presentation at 7.5 Hz was used with a 6.0 cpd sinusoidal grating. The solid lines are regression lines used to estimate the VEP threshold. Our VEP noise estimate was obtained by viewing a "blank screen" (lower left of figure) as explained in the text. The psychophysical threshold for pattern perception is represented by a filled arrow on the X-axis and for flicker perception, by an open arrow. B, Mean VEP phase data of four observers as a function of grating contrast (note log scale) for each harmonic component.

luminance of the screen was 171 cd/m² and was equal during the pattern “on” and “off” periods.

VEP recording: Monocular VEPs were recorded with the active electrode positioned at Z₁, the reference electrode at Z₂, and the forehead was grounded (“Z” refers to the midline and the subscript, to the percentage inion-to-nasion distance). The signals were amplified with a Grass P511 preamplifier (band-pass half amplitude settings at 3 Hz (low) and 100 Hz (high) and then fed into a PDP-11/23 microcomputer that averaged the response. The amplitude and phase of the first and second harmonic components of the VEP responses were measured by Fourier analysis, where the bandwidth of each spectral component was 0.03 Hz. The uncorrelated background activity at the response frequencies, that is the average noise level, was estimated by recording the response to a nonpatterned unmodulated field of the same mean luminance (“blank” screen). The responses were of small amplitudes with random phases. The mean contrast of the grating, defined as one-half the grating contrast during the “on” period, was varied on 0.15 log unit steps over a contrast range of 1.7% to 27.5%. Contrast was defined as the mean of the duty cycle in accordance with the definition of an “on-off” pattern (using sinusoidal temporal modulation) as the endpoint in contrast modulation.⁶ Contrast presentation was randomized.

Psychophysics: The method of constant stimuli was used to determine flicker and pattern detection thresholds. The test stimulus was presented at least 12 times within a bracketed range differing by 0.10 log units. For each trial, the subject stated if both flicker and pattern, either, or none were seen. Threshold was defined as that contrast for 50% seeing, derived from a psychometric template.⁶

Observers: Nine observers participated in all nine VEP contrast conditions. Each observer was tested twice. Psychophysical thresholds for both flicker and pattern detection were obtained from seven of these observers.

Results. The VEP responses to “on-off” presentation contain both first and second harmonic components. The amplitude and phase of each of these components will be addressed first, followed by a comparison between VEP and psychophysical thresholds.

VEP contrast response functions: We found a double-limb function relating VEP amplitude to contrast, the slope of the two limbs being similar. In Figure 1A it can be seen that the shape of the response function is similar for the fundamental and second harmonic components: there are two ascending limbs, the second limb beginning above 13.8% contrast. Each observer was given two testing sessions. Since no statistically significant session effects were found using a repeated measures analysis of variance, the data obtained from the two testing sessions were averaged for each observer. These data were analyzed using a repeated measures analysis of variance. The results of the analysis are given in Table 1. The absence of any significant harmonic by contrast interaction indicates that these data are consistent with the hypothesis that the difference in mean amplitude between

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Table 1. Analysis of variance table—amplitude

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<th>Df</th>
<th>MS</th>
<th>F</th>
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<tbody>
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<td>7.29</td>
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<tr>
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<td>1</td>
<td>12.39</td>
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<tr>
<td>Error</td>
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<td>8</td>
<td>2.59</td>
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<tr>
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<td>8</td>
<td>4.08</td>
<td>14.57†</td>
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<tr>
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<td>7</td>
<td>3.03</td>
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<tr>
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<td>64</td>
<td>0.28</td>
<td>—</td>
</tr>
<tr>
<td>Harmonic × contrast</td>
<td>0.1870</td>
<td>8</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>Error</td>
<td>11.1494</td>
<td>64</td>
<td>0.17</td>
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</tr>
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</table>

* P ≤ 0.06
† P ≤ 0.001

Table 2. Analysis of variance table—phase

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<tr>
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<td>5,677</td>
<td>67.58</td>
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<tr>
<td>Error</td>
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<td>12</td>
<td>84</td>
<td>—</td>
</tr>
<tr>
<td>Harmonic × contrast</td>
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<td>6</td>
<td>2,616</td>
<td>11.13*</td>
</tr>
<tr>
<td>Error</td>
<td>2,817</td>
<td>12</td>
<td>235</td>
<td>—</td>
</tr>
</tbody>
</table>

* P ≤ 0.001

VEP and psychophysical thresholds: The VEP contrast threshold for each harmonic component was defined as that contrast where the VEP was extrapolated to zero amplitude. The extrapolated value was determined by fitting a linear regression line to the mean amplitude of all observers for each of the five lowest contrast levels (Figure 1A, solid lines). The equations for the fundamental and second harmonics are 0.38 + 1.56 (log[C]), (r² = .99) and -0.17 + 1.55 (log[C]), (r² = .96), respectively. The regression lines provide a good fit to the obtained mean amplitude within this contrast range. The VEP threshold of the first harmonic is 0.35 log units lower than that of the second harmonic.

Psychophysical thresholds for pattern and flicker detection are indicated by arrows on the X-axis. The pattern threshold of each observer was on the average 0.30 log units lower than that for flicker. This difference in thresholds was significant as indicated by the results of a paired t-test (t(6) = 13.59, P ≤ 0.001, two-tailed t-test). In Figure 1A, it can be seen that there is a good correspondence between the threshold for pattern detection and that of the first harmonic of the VEP and between the threshold for flicker detection and that of the second harmonic.

This apparent correspondence between VEP and psychophysical thresholds was examined further by evaluating the threshold results of individual observers. For each of seven observers, a linear regression line was fitted to the VEP amplitude obtained at each of the five lowest log contrast levels. The antilog of the X-intercept defined the VEP contrast threshold, which was determined separately for each harmonic component. The difference in thresholds between psychophysical pattern detection and the first harmonic of the VEP was insignificant as indicated by the results of a paired t-test (t(6) = 0.13). The mean threshold of the first harmonic was at a contrast of 0.64% and that of psychophysical pattern detection, at a contrast of 0.61%. The standard error of the mean difference was 0.18. The difference in thresholds between psychophysical flicker detection and the second harmonic of the VEP was also insignificant (t(6) = 0.34). The mean threshold of the second harmonic was at a contrast of 1.24% and that of psychophysical flicker detection, at a contrast of 1.12%. The standard error of the mean difference was 0.35.

Discussion. Our major finding is that the VEP amplitude function at 6.0 cpd using "on-off" presentation can not be simply accepted as representing a unitary process of contrast response in the human visual system. First of all, there are two response functions, one based on the fundamental, and another, on the second harmonic. Secondly, neither shows a simple linear relationship between amplitude and contrast: the functions consist of both a high and a low contrast limb.
with similar slopes. The similarity of slopes is contrary to previous findings: steeper slopes have been reported for the high than the low contrast range in man,1 monkey,4 and rat.5 However, all these studies used counterphase modulation, while we used “on-off” presentation. Double-limb amplitude functions have been reported for the human VEP using counterphase modulation only below 3 cpd. It was suggested that the nonmonotonic function was due to a parafoveal contribution at low spatial frequencies.1 Our results at 6 cpd are not explained easily by simply assuming parafoveal contribution. They are consistent, however, with later findings in both the rat5 and monkey4 in which the presence of a two-limb function was independent of spatial frequency.

The existence of two limbs in the VEP contrast response function may be consistent with the existence of at least two populations of neurons, those responding at low and those at high contrast. This segregation is suggested by recent electrophysiologic evidence in the monkey at both the LGN and cortical levels: the contrast sensitivity of cells in the magnocellular layer of the LGN was found to be higher than that of cells in the parvocellular layer.10,11 Both X- and Y-cells were found in the magnocellular layer with equally high contrast sensitivities. Consistent with these LGN findings is the report that contrast sensitivity is higher for cortical cells in layer 4-C alpha which receive projections from the magnocellular LGN than for cells in layer 4-C beta, which receive projections from the parvocellular LGN.12

The noteworthy finding of our studies is that one needs to separately analyze the VEP for separate harmonic components. Under our stimulation conditions, we found similar amplitude contrast response functions for both harmonics but different phase functions: although the amplitude of both components increased with contrast, there was a steeper change in the phase of the second harmonic relative to that of the first harmonic. Finally, the displaced amplitude functions resulted in different VEP thresholds for each harmonic component that appear to correspond with the difference obtained psychophysically between pattern and flicker detection thresholds. In a recent study, Cannon7 also studied the relationship between the VEP threshold to “on-off” pattern presentation and psychophysical pattern and flicker thresholds. His analysis, however, neglected to include the VEP thresholds for both harmonic components that, based on our findings, differentially correspond to these perceptual attributes.

Key words: parallel pathways, visual evoked potentials, psychophysics, contrast sensitivity, suprathreshold contrast, spectral analysis

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