Effect of Contrast, Stimulus Density, and Viewing Distance on Multifocal Steady-State Visual Evoked Potentials (MSVs)

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PURPOSE. We investigated the effects of image contrast, stimulus density, and viewing distance upon a multifocal steady-state visual evoked potential (MSV) method.

METHODS. Fourteen adults with normal vision (mean age = 27.0 ± 6.6 years; 6 males) participated in the study. Each of the stimulus regions of the multifocal ensembles presented a contrast modulated grating, displaying spatial and temporal frequencies that evoke the spatial frequency doubling illusion. All subjects were tested at five contrasts: 0.06, 0.11, 0.22, 0.45, and 0.89; viewed at 16, 32, and 128 cm. A multivariate linear model estimated factors for each stimulus region, recording channel, number of stimuli (9 or 17 regions), and sex; and covariates for contrast, and octaves of viewing distance, and age.

RESULTS. The responses per unit area for the 17-region display were significantly larger than for the 9-region display (P < 10⁻¹²). The contrast-response function could be described by a power law with exponent 0.068 (P < 0.008). The effect of viewing distance was small but significant: response amplitude dropped by −0.17 ± 0.03 dB per octave of viewing distance (P < 10⁻⁶), or 10% over 8 octaves.

CONCLUSIONS. The response per unit area indicated that cortical folding diminishes responses to larger stimuli. Viewing distance did not greatly affect response amplitude. This suggested that we can use similar, but scaled, stimuli to study central and peripheral disease. The rapidly saturating contrast responses imply that there would be nothing lost from testing stimuli in an attempt to isolate responses from every part of the V1 cortex and the sulcus mean that cortical folding, particularly by the calcarine sulcus. The geometry of Brodmann area 17 (V1) and the sulcus mean that stimuli in an attempt to isolate responses from every part of the occipital cortical sheet, but this requires long recording durations. Here, we will examine the effect on response amplitudes of dicing the area of our stimulus regions by a factor of 2; thus, producing stimuli that sample the VF at two densities.

Another factor that influences VEP responses is cortical magnification, which is largely determined by the retinal ganglion cell (RGC) density of the retina, and the fact that each millimeter squared of V1 receives approximately the same number of afferent fibers; thus, the part of the V1 representing the fovea is larger than more peripheral areas. Under that mapping, if a person is fixating on the center of an object that looms toward them, such that its VF area increases by x, the cortical representation of the object maintains its size and shape and translates along the log (VF radius) axis of the cortex by an amount proportional to log(x). Thus, one might expect little change in VEP response amplitude for radial scaling of peripherally presented stimuli that correspond to the effects of looming. Accordingly, we also examined the effect on the MSVs of the viewing distances: 16, 32, and 128 cm.

Finally, we have previously reported strongly saturating CRFs for the MSV method, and this could affect the results obtained here. Therefore, the three viewing distances and two stimulus densities were assessed at five contrast levels in each of 14 subjects.

MATERIALS AND METHODS

Subjects
The study included 6 males and 8 females (mean ± SD, 27.0 ± 6.6 years). The subjects had vision corrected to 6/6 or better (Bailey-Lovie

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The multifocal visual evoked potential (mfVEP) was first introduced by Baseler and Sutter, and permits localized visual field (VF) deficits to be quantified. Here we examine multifocal steady-state visual evoked potentials (MSV). The basis for the method has been reported in two previous studies, and is derived from a multifocal electroretinogram (mERG) method that has been shown to have predictive value in diagnosing glaucoma. One reason for using a steady-state method here is to permit multifocal stimuli that all display the spatial frequency doubling (FD) illusion, like the stimuli used in the suprathreshold test of the Frequency Doubling Technology (FDT) perimeter (Carl Zeiss, Meditec, Dublin, CA). Visual evoked potential (VEP) responses are affected by cortical folding, particularly by the calcareous sulcus. The geometry of Brodmann area 17 (V1) and the sulcus mean that when stimulating two parts of the VF they may produce largely equal but opposite electric field vectors on either side of the sulcus, thus, producing no net recorded response at some points on the scalp. Less severe interference between response generators located in smaller cortical folds can also reduce VEP amplitudes. To get around these cortical folding effects researchers have attempted to use 200 or more very small multifocal stimuli in an attempt to isolate responses from every part of the occipital cortical sheet, but this requires long recording durations. Here, we will examine the effect on response amplitudes of dicing the area of our stimulus regions by a factor of 2; thus, producing stimuli that sample the VF at two densities.
chart), and refractive errors less than ± 6 dioptres of spherical and 1.5 diopters of cylindrical correction. All subjects certified that they were not medicated for any systemic or ocular disorders. Their undilated right eyes were tested, and their left eyes were occluded. All procedures were fully explained to the subjects and informed written consent was obtained. Subjects were treated in accordance with the Declaration of Helsinki and the experiments were regulated by the Human Research Ethics Committee of the University of New South Wales.

The MSV Stimuli

The MSV stimuli were generated by a graphics card (Vista; Truevision, Shadeland Station, IN) and were displayed on a HP1230 CRT monitor (Hewlett-Packard, Palo Alto, CA) with a resolution of 512 by 424 pixels. The mean luminance of the screen was 54.5 cd/m². The stimuli were sinusoidal achromatic gratings having bars oriented horizontally (Fig. 1). Grating contrasts were corrected for the room lighting (3.80 cd/m²). The two densities of stimulus ensembles displayed either 9 or 17 regions per VF (Fig. 1). There were 2-mm gaps between each of the stimulus regions, which were designed to remove short range interactions. A piece of white cardboard with a 290-mm diameter hole was mounted on the monitor to define an outer circular border resulting in three concentric rings of stimuli as illustrated in Figures 2A and 2B. At the viewing distance of 16 cm, each ring had a fixed spatial frequency (SF), central 0.25 cycles per degree (cpd), middle 0.125 cpd, and outermost at 0.0625 cpd (Fig. 1). The octave intervals of SF scaling with eccentricity accords with RGC densities. A red fixation spot, 2 pixels square, was presented at the center of the screen.

Stimulus Frequencies and Data Acquisition

The display rate of the monitor was 101.50 frames/s. The VEP signals were sampled at four times the video frame rate synchronous with the onset of each frame. The stimulus length was 2048 frames for the 9-region stimulus, and 4096 frames for the 17-region stimulus. The durations of the two types of stimulus ensemble were, therefore, 20.18 s (i.e., 2048/101.50) and 40.35 s, producing frequency resolutions (df) of 0.0496 or 0.0248 Hz.

The incommensurate contrast modulation frequencies of the 9 and 17 regions occurred in the Fourier spectrum at the zero based indices $jfreq = 444, 448, 453, 459, 466, 476, 490, 502, 510$, and $888, 892, 897, 903, 910, 920, 934, 946, 954, 975, 994, 1025, 1055, 1088, 1121, 1176, 1192$, respectively. The actual temporal frequencies, therefore, were in the range 22.02 to 25.28 Hz for the 9-region stimuli (Fig. 2A), and 22.02 to 29.56 Hz for the 17-region stimuli (Fig. 2B), and were, thus, within bands 3.27 Hz (25.28 – 22.01) and 7.53 Hz (29.54 – 22.01) wide for the 9- and 17-region stimuli, respectively. The resulting response frequencies were, therefore, in a band double the width of the stimulus frequencies. Within the response band for the 9-region stimuli there were 45 unique second order response frequencies and 88 noise (i.e., nonsignal = noise) frequencies that were used to estimate the signal to noise ratios (SNR) within the narrow band of response frequencies, and significance of the responses was based on an F-statistic for each response as previously described. For the 17-region stimuli there were 605 frequencies, 452 of which were noise frequencies. The 17-region design is similar to that used for a dichoptic MSV that had 16-stimulus regions in total. The gratings modulated at the temporal and spatial frequencies described here exhibit the FD illusion for VF locations like those used here. Importantly, the selected frequencies in the stimulus band mean that any frequencies generated by second order interactions between regions are separate from the second harmonic and noise frequencies.

Testing Conditions

Stimuli were presented at three different viewing distances, 16, 32, and 128 cm (Figs. 2, 5). Measurements for each viewing distance and stimulus density were repeated at five contrast steps: 0.06, 0.11, 0.22, 0.45, and 0.89. The three distances, five contrasts, and four densities of display (9 or 17 regions) made for 30 stimulus conditions per subject. Each subject viewed the 30 conditions in a random presentation order. Two repeats of each of the 30 conditions were taken and the complex valued response coefficients were averaged on each of the three recording channels, ensuring that signals with consistent phase were enhanced. A companion study showed that two repeats is adequate for estimating adequate effect sizes when measuring contrast-response functions (CRFs).

In order to understand the effects of viewing distance on recorded VEPs it is necessary to understand that for the outer two rings of the stimulus the cortical representation in V1 is well approximated by a log-polar transformation (i.e., where the coordinates are the logarithm of the distance from fixation and the polar angle around fixation). A log-polar transform has advantages for the recognition of looming objects that are centered on the fovea. As mentioned previously, when objects loom (expand) by a factor $x$ in the retinal image, their cortical...

Figure 1. Illustration of the 9 (left) and 17 region (right) FDT-like stimuli used in the experiments. The contrasts of the gratings are as if the stimulus sequence had been stopped partway through the experiment. At the standard viewing distance of 16 cm, the spatial frequency was fixed within each ring, having a central value of 0.25 cpd, 0.125 cpd for the middle ring, and 0.0625 cpd for the outer ring. The numbers labeling each region indicate the increasing order of the temporal frequencies of the stimuli (see Fig. 2). A circular cardboard mask defined the outer border of the outer ring. The angles subtended at the retina reduced as viewing distance was altered from nearer 16 cm (84.4°), and 32 cm (48.8°) to the farthest 128 cm (12.9°).
representation remains the same size and just translates along the log-radius axis by an amount proportional to \(\log(x)\). This representational invariance probably aids the visual system to recognize looming objects as they approach.

Figure 3 illustrates this effect for the outer two rings of the stimulus ensemble over the 8-fold change of viewing distance used here (Figs. 3A, 3C, 3E). The calculated log-polar representations on the cortical sheet of an area like V1 are shown in Figures 3B, 3D, and 3F. The transformation calculations were done with Matlab (The Mathworks, Natick, MA). Notice also that the cortical spatial frequency of the grating stimuli, in terms of cycles per millimeter of cortex, also remains fixed as the viewing distance changes. The number of afferent inputs per millimeter squared of the cortical sheet is fairly constant, so this scaling also means that each cycle of the stimulus is always spanned by an equal number of cortical afferent receptive fields. Thus, given that the cortical excitation by the stimuli remains relatively unchanged for the different view distances, the expectation is that VEP amplitudes should be little affected by changes in viewing distance. If correct this could be a useful property for assessing different areas of the VF by stimuli of various scales.

**Electrode Positioning and Recording**

VEPs were amplified 100,000 times using a four channel amplifier (built at Australian National University by Mark Snowball) and were bandpass filtered between 3 and 100 Hz. The amplified data were acquired using a Labmaster (Scientific Solutions Inc., Solon, OH) board. A three channel recording configuration was employed (Fig. 4). The inion was set as reference for the first two channels, and electrodes (C and D) were placed lateral to the inion. Electrode placement lateral to the inion has been reported to enhance the signals from the center of the field as well as those just below the horizontal VF meridian. Channel 3, C-D, was a differential record with D being the reference for C, an arrangement that has been reported to be sensitive to movement and \(z\) the exponent. The effects of the viewing distance (Figs. 5A, 5B), recording channels (Figs. 5C, 5D), and regions (Figs. 5E, 5F) appear to be largely multiplicative rather than additive. These factors and the heteroskedastic variance suggested that both contrast and response required log-transformation before fitting with the linear model. Both contrast and the responses were converted to decibels (10 log 10).

For the 9-region stimuli the mean response amplitude appears to be slightly larger at the closest viewing distance, 16 cm, reducing as the distance increases; however, the differences are small and fall well short of scaling linearly with the 8-fold change in distance (Fig. 5A). There was no apparent effect of viewing distance in the 17-region data (Fig. 5B). As for the electrode placements (Figs. 5C, 5D), both stimulus ensembles produced maximum response amplitudes on channel 3 (C-D), and minima on Channel 2 (B-Inion). The signal Fz (position A, Fig. 4) referenced to the inion (Channel 1, Fig. 4) was also large (Fig. 5C) and roughly corresponds to Fz referenced to Oz.

**RESULTS**

The major effects of the number of regions, contrast, channels, and viewing distance were explored by plotting the means of the response amplitudes across various stimulus parameters. Being averaged responses, several confounding factors may contribute to the shapes of the plotted curves. For this reason a multiple regression linear model was also used to quantify the independent effects of the various stimulus parameters (the Table). Several factors are evident from these plots. The mean CRFs in Figures 5A through 5D, are about log-linear and so would be well approximated by the power function

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R = kC^z
\]

where \(R\) is the response, \(C\) the test contrast, \(k\) a scaling factor, and \(z\) the exponent. The effects of the viewing distance (Figs. 5A, 5B), recording channels (Figs. 5C, 5D), and regions (Figs. 5E, 5F) appear to be largely multiplicative rather than additive. These factors and the heteroskedastic variance suggested that both contrast and response required log-transformation before fitting with the linear model. Both contrast and the responses were converted to decibels (10 log 10).

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The area of each region of the 9-region ensemble corresponds to the sum of two regions of the 17-region stimuli.
Overall the 9-region stimuli produced larger mean response amplitudes (Fig. 5E) than the 17-region stimuli (Fig. 5F), but not twice as large. We, therefore, decided to incorporate the response per unit area in each part of the field in our linear model. The linear model incorporated factors for regions, sex, channel, stimulus regions (see below), and covariates for decibel contrast, viewing distance, and age. There was also a reference term, which was the grand mean across nonfitted conditions (i.e., region 1 of the 9-region protocol; and responses of males, recorded on channel 1). The factors were fitted as contrasts against the reference condition, which meant the $t$-statistics and $P$ values for the fitted items indicate the significance of the difference of the fitted values from the reference. That is the significance of female response relative to males, the other electrodes relative to electrode one, and so on. The Table shows the results of the model.

The reference value was 90.36 nV ($t$-statistic 58.4). There was a small but significant increase in responses with age (0.02 dB/yr, $P = 0.01$). Females had significantly larger responses than males by 2.63 dB (1.83 times), and relative to Channel 1 (reference) channels 2 and 3 had smaller responses (0.72 and 0.90 times), all $P$ was less than 10$^{-12}$. As in previous reports, the CRFs were well represented by a power-law contrast-response function, $\text{Response} = k \times \text{Contrast}^w$, with an exponent less than 1, 0.10 ± 0.01.

The Table shows that relative to region 1 only regions 8 and 9 gave significantly different responses (the Table, rows “9 Region, r2” to “r9”). In terms of comparing the 9- and 17-
region stimuli then, as mentioned above, a reasonable thing to do would be to estimate the response per unit area. In order to accomplish this, the amplitudes of the responses to pairs of the 17-region stimuli were summed to make nine measures that sampled the equivalent parts of the VF as in the 9-region stimulus. These summed equivalents are termed r1s to r9s in the Table. By examining Figure 1, we can see that r1s is the sum of the responses to regions 1 and 5 of the 17-region stimuli, r2s is the sum regions 2 and 6 of the 17-region stimuli, and so on. If the responses of the summed equivalents were significantly larger than their 9-region counterparts, then the 17-region stimulus would be more efficient in generating larger responses per unit area of stimulus. The Table shows that these equivalent stimulus regions typically gave responses that were approximately 1.5 times their 9-region counterparts. Interestingly, the central stimulus of the 17-region array gave a smaller response than the same region in the 9-stimulus array (0.54 times), perhaps due to the large difference in stimulus frequency (Fig. 2).

The other aim of the study was to investigate the effect of changing viewing distances on the response amplitude. Viewing distance was fitted as the log2 values for 16, 32, and 128 cm: 4, 5, and 7. On average responses dropped by −0.17 ± 0.03 dB per octave of viewing distance (P < 1.3 × 10−7). The fitted values correspond to −0.17 dB; 4, 5, 7 = −0.68, −0.85, −1.19 dB, respectively; or 1, 0.96 and 0.89 times smaller relative to the results at 16 cm. Thus, the 8-fold (i.e., 128/16) change in viewing distance made approximately a 10% difference in mean response amplitude (1 to 0.89).

The Table and Figure 5 indicated that the 17-region stimulus had small responses making a rapid test of visual function unlikely. For comparison with a companion study, which also used a 9-region MSV method, we, therefore, examined the distributions of the number of significant regions obtained for the current 9-region tests (Fig. 6). Note that the method for computing significance in the complex plane (Materials and Methods section) explicitly takes into account variability in the phase of the signals. Distributions are shown for significance at P less than 0.05 and P less than 0.001.

D I S C U SS I O N

As reported in a previous study even, the CRFs were roughly log-linear, being well described by a power function with an
exponent around 0.1, that is straight lines with shallow slopes when plotted on a log-contrast abscissa (Figs. 5A–5D). These CRFs are also similar to those reported in other mfVEP studies using either pseudorandom pattern-reversal stimuli, \(^26,27\) pseudorandom flash stimuli, \(^28\) or conventional steady-state VEPs. \(^29\) This behavior may originate from the saturating responses of M-cells. \(^30\) Our result of larger responses for females concurs with findings from other pattern mfVEP studies, \(^31\) which is thought to be related mainly to skull thickness. \(^32\)

As expected for responses arising from a log-polar representation of the visual world (Fig. 3), viewing distance had little effect on response size, changing by just 10% over an 8-fold change in viewing distance, \(-0.17 \pm 0.05\) dB per octave (the Table). The results are similar to those reported by Hood et al. \(^33\) who used three concentric rings of 18-multifocal stimuli with similar angular subtenses and viewing distances to those used here. Results were presented for three subjects, but provided no explanation of the lack of response change with

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**FIGURE 5.** Mean responses plotted for the 9- (left column) and 17-regions (right column) stimuli. Mean response amplitude computed across. (A, B) Subjects \((n = 14)\), regions, and the three electrode placements, showing the effect of viewing distance. (C, D) Means across subjects \((n = 14)\), regions, and the three viewing distances, showing the mean response at the three electrodes. (E, F) Means across subjects, viewing distance, and electrode positions, resulting in one curve for each of the five contrast steps. Standard errors of the mean (SEM) bars were not plotted above to avoid clutter; however, for (A, C, E), their mean values were 1.60, 2.28, and 1.62 nV; for (B, D, F), these were 0.62, 1.30, and 0.87 nV.
In terms of response per unit area, the pairs of 17-region stimuli produced responses that were approximately 50% larger than the 9-region stimuli of the same area. In fact, one might have expected the responses of the pairs of 17-region stimuli to be less than their 9-region counterparts due to the different temporal frequencies used for each pair. This raises the issue of how large the frequency effects were. In two previous studies, we showed small but significant frequency dependence in the distribution used here, of approximately 1 uV/Hz. For the two stimulus arrays used here the responses for different regions were quite similar at all contrasts (Figs. 5E, 5F), indicating the scaling of the stimuli was reasonable. Notable exceptions were regions 9 and 8 of the 9-region array, the central region, and one peripheral region (Fig. 1). Such fluctuations are evident even when the experimental design allows separate frequency and regional effects to be estimated, but seem to be reduced when higher spatial frequencies are used in the stimulus regions. Much of the remaining variation in Figures 5E and 5F are due to overall, smaller responses in the superior field, although most are not significant (the Table). In any case, it would appear that the frequency effects are small and so we interpret the improvement in the response/stimulus array as being mainly due to cortical fold effects. Interestingly, the present results mirror a companion study, in which the summed responses of the same 9-regions stimulus used here were larger than the response to a single large stimulus that had the same area as the 9-region ensemble.

That companion study indicated that two repeats of the 20 second 9-region stimulus was adequate to estimate models like those used here, but that for VF testing perhaps 8 to 10 repeats would be needed. The study examined the number of significant responses and also the sum of those responses to look at signal quality. Here, we examined the distribution significant results (Fig. 6) and have come to similar conclusions. The 17-region test, with its smaller responses, would perhaps take too long for a clinically useful test. Across the two repeats responses were averaged in the complex plane. We, therefore, used F-static based significance testing to take into account that the real and imaginary axes represent two independent random variables. Thus, the significance testing took into account phase variability, random signals having random phase do not add constructively across averages.

The method has shown very consistent phase repeatability in our other studies. One of those studies was very similar to the one presented here, and contained a great number of repeats allowing phase stability to be quantified. The stimuli were at 95% contrast and had no central region, but the spatial frequencies and layout of the remaining eight regions were like those in this study for a viewing distance of 25.6 cm. Three monocular conditions were repeated at stimulus frequencies in the range 15.45 to 16.67 Hz, and three in the range 16.84 to 21.51 Hz. Four repeats were done on each of those six tests, and that was repeated on 16 subjects. The whole process was repeated again on a second visit, and the recording electronics were identical to the present study. To examine phase stability here the SD for the four repeats per test was calculated. Figure 7 shows box plots for resulting 384 SDs (16 x 3 x 8) for each visit and frequency band. The median SD was approximately 12° (1/30 of a cycle), and the 90th percentiles were in the range 38.5° to 51.2° (1/9.4 to 1/7.0 cycle).

We have not described the outcomes for frequencies that were designed to capture nonlinear interactions between the stimulus regions. Two earlier MSV studies have shown that these are very small and better recorded by electrodes placed to be biased toward extrastriate cortical responses. The important message for this study is that these small interactions did not overlap with the main second harmonics responses. The stimuli used in this study resemble those of the FDT perimeter, in producing the spatial FD illusion, and so might have some utility in measuring the effects of glaucoma.

**Figure 6.** Box plots indicating the number of tests that contained significant responses for two repeats of the 20 second 9-region stimuli. The value at the notch is the median, the upper and lower box edges are the 25th and 75th percentiles, and the whiskers mark the 5th and 95th percentiles. (A) At the $P$ less than 0.05 level there was little difference in the distribution of significant responses between contrasts 0.24 and 0.96. (B) A surprising number of responses were significant even at $P$ less than 0.01. Significance levels were based on F-statistics that took into account consistency of response phase (Materials and Methods section).
Certainly the ERG version showed some promise in that area.\textsuperscript{4,5} The FD illusion occurs when stimuli are presented at spatial frequencies of less than 1 cpd that are rapidly contrast modulated at greater than 10 Hz. The effect was initially attributed to a nonlinear M\textsubscript{s} subgroup of the M-system,\textsuperscript{21,35} but more recently, others have attributed it to other nonlinearities.\textsuperscript{36–39} That being said, an anatomic substrate for primate retina that by virtue of their low coverage factor, may mean cell stimuli appear to preferentially stimulate large ganglion cells, My-cells has recently been reported.\textsuperscript{40,41} In any case, the responses to low contrasts gave responses approximately as large and reliable as responses to high contrasts. When assessing glaucomatous VF damage contrasts might be prudent. Although the response per unit area was larger for the summed 17-region stimulus ensemble, the absolute response sizes were smaller than the 9-region area.\textsuperscript{4,5} The FD illusion occurs when stimuli are presented at spatial frequencies of less than 1 cpd that are rapidly contrast modulated at greater than 10 Hz. The effect was initially attributed to a nonlinear M\textsubscript{s} subgroup of the M-system,\textsuperscript{21,35} but more recently, others have attributed it to other nonlinearities.\textsuperscript{36–39} That being said, an anatomic substrate for primate retina that by virtue of their low coverage factor, may mean cell death is more easily measured.\textsuperscript{35}

\textbf{CONCLUSIONS}

Responses to low contrasts gave responses approximately as large and reliable as responses to high contrasts. When assessing glaucomatous VF damage contrasts that result in saturation could mask some VF defects, so using less saturating contrasts might be prudent. Although the response per unit area was larger for the summed 17-region stimulus ensemble, the absolute response sizes were smaller than the 9-region ensemble. As expected, varying the viewing distance did not greatly affect the amplitude of the recorded responses. This should allow peripheral and (relatively) central disease to be tested with mfVEPs that exploited zoomed versions of the stimulus array.

\textbf{References}