Validation Study of VEP Vernier Acuity in Normal-Vision and Amblyopic Adults

Chuan Hou, William V. Good, and Anthony M. Norcia

Purpose. Vernier displacement thresholds can be measured with swept-parameter visual evoked potentials (sVEPs) and may therefore be useful in pre- or nonverbal subjects. This study was conducted to test whether sVEP vernier thresholds are valid measures of the visibility of vernier offsets in two different settings.

Methods. Vernier acuity thresholds were measured psychophysically and electrophysiologically using square-wave gratings containing vernier displacements modulated at 3.76 Hz. The detectability of the vernier alignment cue was degraded by introducing either gaps or standing offsets in the stimulus. These manipulations were performed in normal-vision observers. In a second experiment, psychophysical and sVEP vernier acuity were measured in amblyopic observers.

Results. sVEP thresholds and overall amplitudes in normal observers were strongly affected by the introduction of gaps or standing offsets, as were psychophysical thresholds. Psychophysical and sVEP vernier offset thresholds were significantly correlated in the amblyopic eyes, as were sVEP and optotype interocular threshold differences. sVEP amplitudes of patients with strabismus were lower than those of patients with anisometropic amblyopia, even though optotype acuities were the same in the two groups.

Conclusions. Vernier acuity thresholds derived from the sVEP tap mechanisms that are specific for the relative position of stimulus elements, and they correlate with perceptual visibility in normal and amblyopic observers. Because of this correlation and because sVEP thresholds can be measured without the need for instruction or behavioral responses, they may be useful in assessing visual function in pre- and nonverbal patients. (*Invest Ophthalmol Vis Sci.* 2007;48:4070–4078) DOI:10.1167/iovs.06-1368

Vernier acuity, the ability to discriminate the relative positions of features, is one of the visual hyperacuities and can be measured psychophysically[1–9] and with visual evoked potentials (VEPs).[10–20] Several psychophysical studies have reported that vernier acuity is more strongly affected in amblyopia than is grating acuity,[1–3,21–24] and it has also been reported that vernier acuity correlates more precisely with optotype acuity than does grating acuity.[1,24] Vernier acuity assessment is thus an alternative method for detecting and quantifying amblyopia.

VEPs have the desirable property that they can be recorded in pre- and nonverbal subjects. However, a necessary prerequisite for any nonverbal test of visual function, including vernier acuity, is that its results correlate with visibility in verbal subjects who can reliably report their perceptual experience. Several studies have reported VEP techniques for measuring vernier acuity. Levi et al.,[10] using transient VEPs, reported that VEP vernier thresholds are similar to psychophysical thresholds measured with the same stimulus. This initial work was later confirmed and expanded.[11,12] Steinman et al.[11] showed that introducing gaps and interfering lines reduces vernier VEP responses in a fashion similar to the effects that these manipulations have on psychophysical thresholds. A similar effect of interfering lines was also found in another study.[12] These early VEP studies of vernier acuity used time-domain methods, in which it is difficult to separate the vernier onset–offset responses from responses to simultaneously presented motion cues. To avoid this problem, Norcia et al.[14] used a combination of the frequency-domain analysis method of Zemon and Ratliff[25,26] and a swept parameter presentation (sVEP). Norcia et al. showed that sVEP vernier acuity correlate well with psychophysical vernier acuity from the fovea to the near periphery and VEP amplitude decreases with increasing separation of features.[14] However, psychophysical data was not presented for this comparison. Using a similar sVEP paradigm, Chen et al.[20] reported significant vernier acuity differences between the amblyopic and fellow eyes of a group of children whose thresholds were measured shortly after refractive error correction but before the initiation of occlusion therapy. Amblyopia was diagnosed in most of their patients based on optotype acuity (Lea chart), but vernier acuity was not assessed psychophysically.

To assess further the validity of sVEP vernier thresholds as neural correlates of perceptual thresholds, we measured the covariation of sVEP and psychophysical thresholds over a range of vernier acuities in normal-vision and amblyopic adults. A range of thresholds was obtained in normal subjects by degrading vernier sensitivity either by increasing the separation between features or by introducing standing offsets in the position of the elements composing the targets. Both these manipulations produced systematic decreases in sVEP and psychophysical vernier acuity. We also compared psychophysical and electrophysiological estimates of vernier acuity in a group of patients with amblyopia who had a large range of optotype acuity. We found that sVEP and psychophysical thresholds were similarly affected in patients with amblyopia.

Methods and Materials

Observers

The research protocol was approved by the Institutional Review Board of the California Pacific Medical Center and conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from the observers after the VEP recording and psychophysical procedures were explained.

Eight normal-vision observers between 24 and 52 years of age (mean, 42.5 ± 8.5) and 36 patients with amblyopia between 18 and 68 years of age (mean, 41 ± 13.8) participated. All participants underwent

From The Smith-Kettlewell Eye Research Institute, San Francisco, California.

Supported by National Eye Institute Grants K23EY000384 (WVG), EY0115228 (WVG), and EY06579 (AMN).

Submitted for publication November 13, 2006; revised February 28 and April 16, 2007; accepted June 13, 2007.

Disclosure: **C. Hou**, None; **W.V. Good**, None; **A.M. Norcia**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Chuan Hou, The Smith-Kettlewell Eye Research Institute, 2318 Fillmore Street, San Francisco, 94115; chuanhou@ski.org.

Investigative Ophthalmology & Visual Science, September 2007, Vol. 48, No. 9

refraction refracted under noncycloplegic conditions by a pediatric ophthalmologist (WVG or CH) before the experiments. Visual acuity was evaluated with a constant crowding logMAR (logarithm of the minimum angle of resolution) chart (Bailey-Lovie) and was measured with best optical correction. Stereo acuity was measured with Randot stereotests (Stereo Optical Co., Inc., Chicago, IL). All normal-vision observers had 0.0 logMAR (20/20) or better optotype acuity in each eye and stereo acuity of at least 30 arc sec. They also had no prior history of strabismus, amblyopia, or any other eye diseases. The mean optotype acuity in normal-vision observers was −0.05 ± 0.03 logMAR in the dominant eye and −0.025 ± 0.02 logMAR in the nondominant eye.

In the observers with amblyopia, horizontal, and vertical angles of deviation were quantified with a prism cover test at 0.3 and 6 m, with and without optical correction. During unilateral and alternating cover tests, each eye was covered for at least 5 seconds. The presence of eccentric fixation in the amblyopic eye was determined with a visuoscope while the fellow eye was occluded. Monocular fixation of more than 0.5° from the center of the fovea was classified as eccentric fixation. Stability of fixation was also judged during visuoscopy. Inclusion criteria for observers with amblyopia included: (1) 0.1 logMAR (20/25) or worse acuity in one eye, with the other eye being 0 logMAR or better; (2) no history of visual deprivation (e.g., cataract, ptosis); and (3) no other eye disease (e.g., cataract, glaucoma, lens implant). We recruited patients with a large range of optotype acuity from 0.1 (20/25) to 1.2 logMAR (20/300). Mean acuity was 0.54 ± 0.05 (SEM) logMAR in the amblyopic eyes and −0.06 ± 0.01 (SEM) logMAR in the fellow eyes. The observers with amblyopia were classified, based on clinical criteria, into anisometropic or strabismic groups. Patients with anisometropia and strabismus were grouped with those with only strabismus for analysis. Amblyopic observers with unequal refractive error between the two eyes of at least 1 D in any meridian and with no constant ocular deviation or history of strabismus surgery were classified as having anisometropic amblyopia ($n = 20$). Amblyopic observers with a constant ocular deviation or a history of prior strabismus surgery, with or without anisometropia were defined as having strabismic amblyopia ($n = 16$). The mean optotype acuity in the anisometropic group was −0.07 ± 0.02 (SEM) logMAR in the fellow eye and 0.56 ± 0.07 logMAR in the amblyopic eye. The mean optotype acuity in the strabismic group was −0.06 ± 0.02 logMAR in the fellow eye and 0.51 ± 0.06 logMAR in the amblyopic eye. There were no significant differences in optotype acuity between anisometropic and strabismic groups ($P = 0.72$ in the fellow eye and $P = 0.58$ in the amblyopic eye). Refractive errors were fully corrected for the testing distance (150 cm) in all observers during the experiments. Details regarding the patients are shown in Table 1 along with their logMAR acuity.

Table 1. Optotype Acuity of Observers with Amblyopia

Subject*	Fellow Eye	Amblyopic Eye
1	−0.1	0.1
2	−0.1	0.5
3	0	0.5
4	0	0.2
5	0	0.1
6	−0.1	0.3
7	−0.1	0.4
8	0	1.2†
9	−0.2	0.8
10	0	0.8
11	0	0.8
12	−0.1	0.4
13	−0.1	0.5
14	−0.2	0.7
15	−0.1	0.2
16	0	1
17	0	0.5
18	−0.2	0.8
19	0	0.4
20	0	1.1
21	0	0.5‡
22	0	0.7‡
23	−0.2	0.8‡
24	0	0.3
25	0	0.3
26	0	0.3
27	−0.1	0.8
28	−0.2	0.7†‡
29	0	0.6‡
30	−0.2	0.7‡
31	0	0.4
32	0	0.4
33	−0.1	0.1
34	−0.1	0.6
35	0	0.8†‡
36	0	0.2

* 1–20, observers with anisometropic amblyopia; 21–36, observers with strabismic amblyopia or mixed strabismic and anisometropic amblyopia.

† Unsteady fixation.

‡ Eccentric fixation.

Stimuli

Stimulus generation and signal analysis were performed by in-house software running on separate computers (both Power Macintosh G3; Apple Computer, Cupertino, CA). Horizontal square-wave luminance gratings containing vertical vernier displacements were generated on a multisync video monitor (1600 × 1200 pixels; 60 Hz vertical refresh, video bandwidth, 150 MHz; MRHB2000; Richardson Electronics, Inc., LaFox, IL) at a space average luminance of 110 cd/m^2 and a Michelson contrast of 80%. The vernier onset–offset stimuli (Figs. 1a, 2) alternated at 3.76 Hz between two states: a 2-cyc/deg collinear square-wave grating (first state) and the same grating containing a set of vernier displacements (second state). The distance between vernier breaks was 0.5°. Viewing distance was 150 cm, which generated a display size of 12° × 9° yielding approximately 400 offsets. A small fixation point in the center of the stimuli was given during the experiments.

A control stimulus with the same amount of motion as the vernier onset–offset test condition consisted of symmetric vernier displacements of the moving elements relative to the static elements (Fig. 1b). The difference between these two displays was only that the alternation occurred between the symmetrical upward- and downward-offset gratings in the local transient control stimulus (Fig. 1b) instead of the collinear and offset gratings in the vernier onset–offset stimulus (Fig. 1a). The configuration shown in Figure 1a served as the baseline condition for the gap and standing-offset experiments and was the primary measure in the amblyopes. The baseline condition shown in Figure 1a and local transient control condition in Figure 1b in the present study were similar to stimuli used in previous studies.[14,20]

To obtain sVEP voltage versus displacement functions, vernier displacement was systematically increased from 0.25 to 4 arc min in 10 logarithmic steps over a period of 10 seconds in both test and control conditions in the normal-vision observers and the fellow eyes of observers with amblyopia. The sweep range was 0.5 to 8 arc min in the amblyopic eyes of most observers ($n = 29$). Larger displacement ranges were used for the amblyopic eyes of observers with deep amblyopia ($n = 7$).

Gap Stimulus. In the gap experiment, a series of vertical mean luminance gaps was introduced at the location of the vernier breaks (Fig. 2a). Gap sizes of 0, 1, 2.5, 3.5, 5, 10, and 20 arc min were used in sVEP recording but only gap sizes of 1, 2.5, and 3.5 arc min were used in the psychophysics, because the gaps beyond 5 arc min reduced the sVEP amplitudes to levels at which thresholds could not be estimated reliably. The 0.25- to 4-arc min sweep range was used.

Standing-Offset Stimulus. In the standing-offset experiment, the relative position of the static and jittered panels was progressively

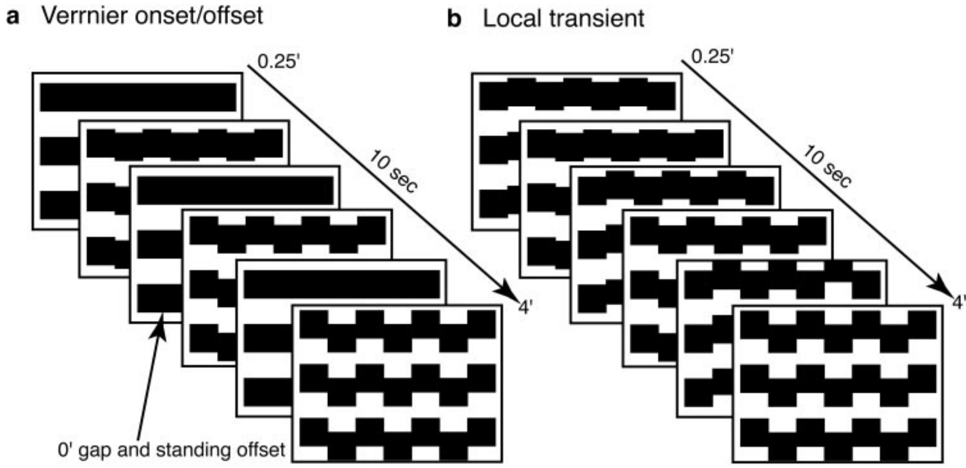

FIGURE 1. sVEP stimuli without gaps or standing offsets for the vernier onset–offset (**a**) and local transient (**b**) conditions. Square-wave gratings contained vernier displacements of every other column of the grating, which alternated between two states (also, see Fig. 2) at 3.76 Hz. Over a period of 10 seconds, the size of the displacement was increased from 0.25 to 4 arc min in 10 equal logarithmic steps for normal-vision observers and the fellow eyes of observers with amblyopia. Larger displacement ranges were used for the amblyopic eyes of the patients. There were approximately 400 vernier offsets over the 12° × 9° display area.

shifted away from perfect alignment, and the display thus alternated between two different values of misalignment at each step in the sweep (Fig. 2b). Standing offset sizes of 0, 0.2, 0.4, 0.8, 1.25, 2, and 3.75 arc min were used. A 3.75-arc min direction shift is the maximum unambiguous one for a 2-cyc/deg grating. The 0.25- to 4-arc min sweep range was used.

sVEP Recording

Gold-cup surface electrodes (F-E5GH; Grass Telefactor, West Warwick, RI) and a model 12 A5 amplifier (Grass Telefactor) were used to record the EEG at a gain of 50,000. An amplitude band-pass filter setting of 0.3 to 100 Hz was used. Three electrodes were placed over the occipital pole at O_1, O_Z, and O_2 of the 10-10 electrode placement system.[27] Reference and ground electrodes were placed at C_Z and P_Z, respectively. Differential voltages were measured between the reference (C_Z) and the electrodes placed at O_1, O_Z, and O_2. Bipolar differences were also measured between O_1 and O_Z and between O_2 and O_Z. The derivations (hereafter termed "channels") were thus O_1–C_Z, O_z–C_Z, O_2–C_Z, O_1–O_Z, and O_2–O_Z. Impedance was measured and maintained below 10 KΩ.

sVEP Signal Analysis

sVEP signal analysis was similar to the procedure described previously.[20] In brief, a recursive least square (RLS) adaptive filter[28] was used to determine sVEP amplitude and phase for the first several harmonics of the 3.76-Hz stimulus frequency. All stimulus conditions were swept-parameter conditions. Vernier offset displacements were swept in logarithmic steps over a 10.6-second recording period (hereafter termed the trial) divided into 10 sequential epochs of 1.06-second duration (hereafter termed bins). We recorded eight 10.6-second trials for each stimulus condition. Voltage-versus-displacement functions were obtained by coherently averaging the spectral coefficients for each bin across trials for each observer, channel, harmonic, and stimulus condition. These functions were used to estimate thresholds for each observer's individual conditions and were also averaged coherently across observers, to obtain group response functions.

VEP Threshold Estimation

For each swept stimulus condition of each observer, response thresholds were estimated by regression of amplitudes from the trial-average bins in which the response increased linearly to the point of stimulus visibility. The range of bins eligible for regression depended on the statistical significance and phase consistency of the response according to a modification of a previously published algorithm.[29] The regression range was limited to those bins in which the criteria described in previous study[20] were met. Once the regression range was established, the threshold was determined by extrapolating the regression line to 0 response amplitude. When applied to spectral data from background EEG, these criteria yield a 5% false-alarm rate over a full set of harmonics and recording channels (data not shown).

The sVEP group thresholds (Fig. 7) were determined in two ways. First, we applied the regression procedure to the sweep response function of each individual observer. For those individuals whose response functions passed the regression criteria for a given stimulus condition, we calculated the mean and SE of the resultant thresholds. This quantity is hereafter termed the sVEP average-individual threshold. The method for calculating sVEP average-individual threshold was the same as the method used in the study of Chen et al.[20] Because we were not able to estimate a threshold for all observers in all conditions from their individual thresholds, we also calculated thresholds by first averaging the sweep response functions of the individual observers, followed by the regression. This second quantity is termed the sVEP

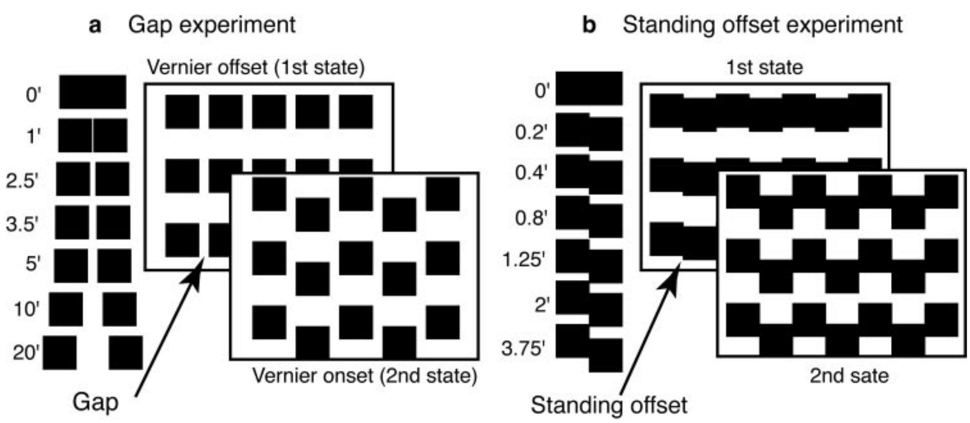

FIGURE 2. Gaps (**a**) and standing offsets (**b**) were introduced at the location of the vernier displacements. The offsets used are indicated to the *left* of each panel. When gaps were introduced at the location of the vernier displacements, the square-wave gratings still contained vernier displacements that alternated between aligned and misaligned at 3.76 Hz. When standing offsets were introduced, the relative position of the static and displaced panels was progressively shifted away from perfect alignment, and the display thus alternated between two different values of misalignment at each step in the sweep.

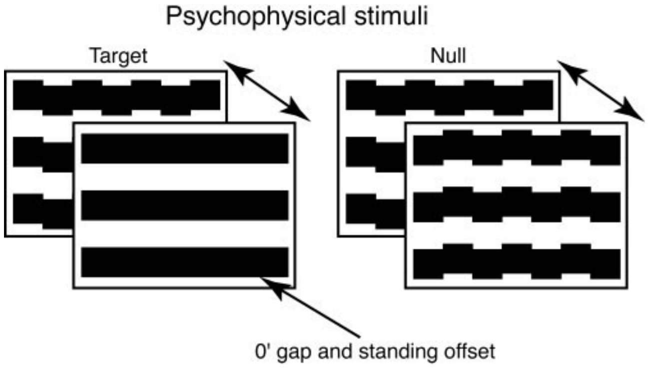

FIGURE 3. Psychophysical stimuli for 2AFC procedure. The baseline condition (no gap or standing offset) is illustrated. Vernier offsets that appeared and disappeared defined the *target* interval. Symmetrically jittered offsets defined the *null* interval. A 2AFC staircase was used to estimate the 82% correct level of the psychometric function.

group-average threshold. In this analysis, each observer contributed equally to all conditions. Error bars in the figures depicting sweep responses are vector standard errors of the vector mean. A jackknife procedure[30] was used to estimate the threshold standard errors and the slopes of the regression line. The average amplitude at frequencies 0.94 Hz above and below each response harmonic frequency was used to estimate the background EEG noise level.

Psychophysical Procedure

The same spatial and temporal parameters from the sVEP recordings were used in the psychophysical measurements. A two-alternative, forced-choice (2AFC) staircase was used to estimate the 82% correct level of the psychometric function. The vernier onset–offset configuration defined the *target* interval and the symmetrically jittered offsets defined the *null* interval (Fig. 3). The size of the vernier offset in both *target* and *null* intervals was the same, and both were changed by the same amounts during the staircase procedure. This comparison ensured that the threshold discrimination was based on the relative position of the static and dynamic elements of the display. The moving regions of the display were temporally modulated at 3.76 Hz and the *target* and *null* intervals were each presented for 1 second with tones indicating the beginning and end of each interval. The observer's task was to indicate which interval contained the *target*. For normal-vision observers, the staircase values ranged from 1 to 0.05 arc min in the baseline vernier acuity condition and also when gaps (1, 2.5, and 3.5 arc min) and standing offsets (0.2, 0.4, 0.8, 1.25, 2, and 3.75 arc min) were introduced at the location of the vernier breaks (Fig. 2). We tested only the smallest three gaps because the larger gaps had reduced the sVEP amplitudes to levels where thresholds could not be estimated reliably. For the observers with amblyopia, the staircase values ranged from 1 to 0.05 arc min in the fellow eyes and 2 to 0.1 arc min in the amblyopic eyes of most observers with amblyopia ($n = 29$). Higher starting values were used in the deeply amblyopic eyes ($n = 7$).

In the first experiment, both sVEP and psychophysical procedures were performed binocularly in the normal-vision observers, to determine whether the two vernier thresholds were similarly affected by degrading the detectability of the vernier alignment cue, either by introducing gaps or standing offsets in the stimulus. All eight normal-vision observers had sVEP recordings, and four of them finished the psychophysical measures. In the second experiment, we observed the covariation of monocular sVEP and psychophysical vernier thresholds in all 36 patients with amblyopia. The nonviewing eye was occluded with black eye patch during the second experiment.

RESULTS

sVEP Signature of Position Sensitivity

Because the sVEP vernier stimulus contains local motion, contrast, and luminance cues, as well as vernier offset cues, it is important to establish which components of the response are specific to the position of the stimulus features. To do this, we compared responses from the vernier onset–offset baseline condition (Fig. 1a) to those obtained in the control condition (local transient control) which had the same range of stimulus displacements but that never resulted in an aligned state of the pattern (Fig. 1b). In this control stimulus, the display differed from the vernier onset–offset test condition in that the alternation occurred between symmetrical upward- and downward-offset gratings instead of between collinear and offset gratings. Figure 4 plots vector averaged sVEP amplitude as a function of displacement for the vernier onset–offset and local transient stimuli across eight normal-vision observers at the first harmonic (Fig. 4a) and at the second harmonic (Fig. 4b). Because there are motion and dynamic contrast cues present in both the local transient control and vernier onset–offset test stimuli, both stimuli produced second-harmonic responses. However, only the vernier onset–offset stimulus produced a reliable first-harmonic response. This result indicates that the first-harmonic component arises from mechanisms that encode spatial aspects of the stimulus. The second-harmonic component is presumably generated by a mixture of motion and contrast responses. Of interest, the amplitude of the second-harmonic responses in the vernier onset–offset condition was larger than in the local transient condition, although the same amount of motion and contrast change occurred in both stimuli. This finding indicates that the second-harmonic response to the motion cue is also sensitive to the spatial configuration

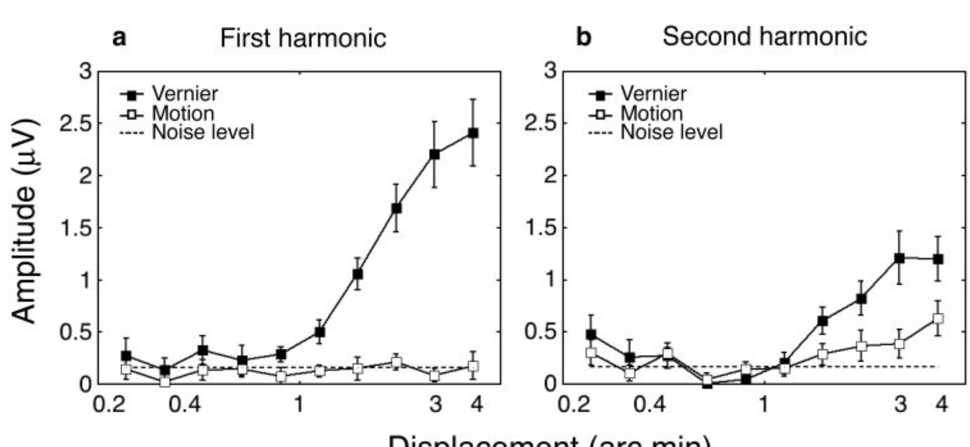

FIGURE 4. Vector averaged sVEP amplitudes as a function of displacement for vernier onset–offset stimuli and local transient stimuli across eight normal-vision observers. Error bars plot SEMs. *Dashed lines*: EEG background. (**a**) Only vernier onset–offset stimuli produced a reliable first-harmonic response. (**b**) Both vernier onset–offset stimuli and local transient stimuli produced second-harmonic responses, but the amplitude of second-harmonic response in the vernier onset–offset condition was larger than in the local transient condition, even though the same amount of motion was present in both stimuli.

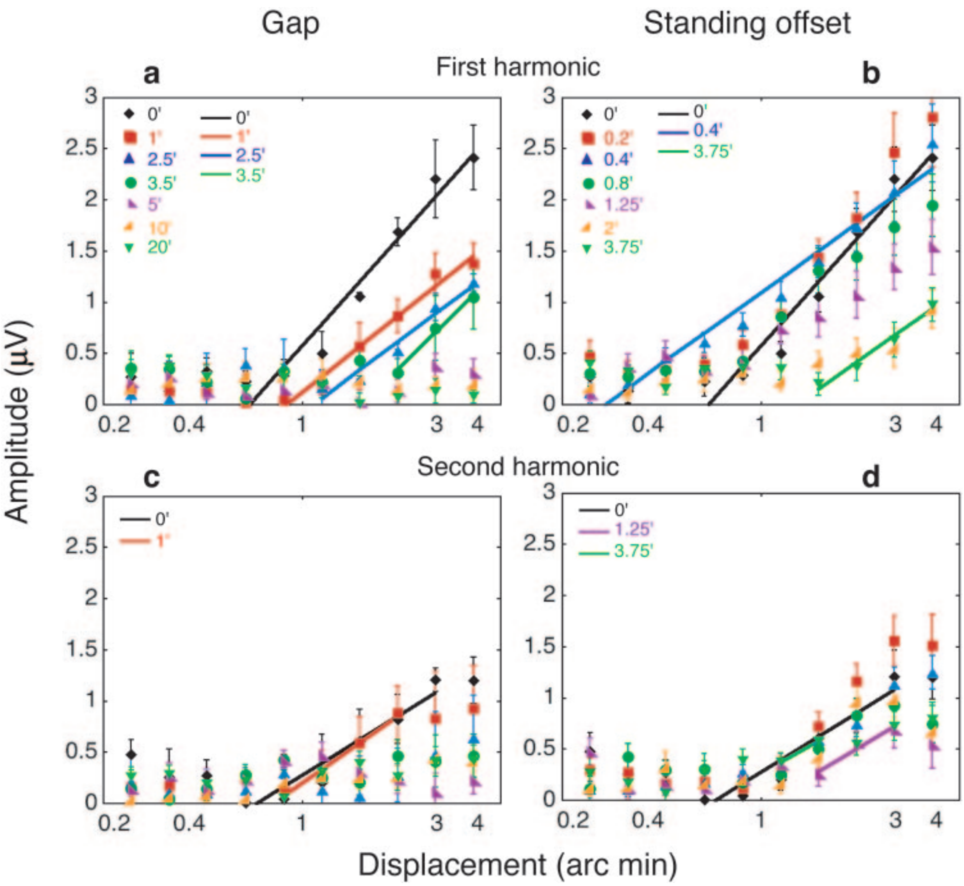

FIGURE 5. Vector-averaged sVEP amplitudes as a function of displacement for vernier onset–offset for (**a**, **c**) gap and (**b**, **d**) standing-offset experiments at the first (**a**, **b**) and second harmonics (**c**, **d**) across eight normal-vision observers. Error bars, SEM. *Solid lines*: the regression lines for color-coded stimulus condition. sVEP amplitudes decreased with increasing gaps and standing offsets for both first- and second-harmonic responses. The first harmonic was more strongly affected than the second harmonic.

of the target. However, in contrast to the first harmonic, the second harmonic is not uniquely or exclusively dependent on the relative position of the stimulus elements. We also examined the third-, fourth-, and fifth-harmonic responses. These higher harmonic responses showed low signal-to-noise ratios and less reliable responses to the stimuli. Because of the specificity of the first harmonic to relative position and its higher signal-to-noise-ratio, it was used in the remainder of the analysis.

sVEP Gap and Standing-Offset Effects

The introduction of either gaps or standing offsets caused substantial reductions in the amplitude of the sVEP. Figure 5 is a plot of the vector-averaged sVEP amplitude as a function of vernier displacement across eight normal-vision observers at the first (Figs. 5a, 5b) and second harmonics (Figs. 5c, 5d). The error bars indicate the SEM. The solid lines are the regression lines used to estimate threshold for a subset of the conditions. The different conditions are color-coded according to the legend provided in the figure. Figures 5a and 5c show the effect of gaps, and Figures 5b and 5d show the effects of standing offsets. Overall, sVEP amplitudes at both the first and second harmonic were affected by the introduction gaps or standing offsets, with the first harmonic being more strongly affected than the second.

For the first harmonic in the gap experiment (Fig. 5a), the sVEP amplitude with abutted targets showed the highest response and lowest threshold (0.649 ± 0.044 arc min). sVEP amplitude systematically decreased, and group thresholds systematically increased at gap sizes of 1, 2.5, and 3.5 arc min (0.873 ± 0.072, 1.085 ± 0.094, and 1.635 ± 0.241 arc min, respectively). Threshold differences between the average responses to each pair of successive gap sizes were all significant (P = 0.039, 0.005, and 0.012, respectively). The sVEP amplitude dropped to the noise level beyond the 5-arc-min gap and did not pass the criteria for regression analysis.

In the standing-offset experiment, both first (Fig. 5b) and second (Fig. 5d) harmonic sVEP amplitudes did not decrease until 0.8 arc min of offset. At larger standing offsets, sVEP amplitudes systematically decreased with increasing standing offsets from 1.25 to 3.75 arc min. The first harmonic thresholds of the average response functions depended on the magnitude of the standing offset of the vernier stimulus. For the smallest standing offset, the thresholds decreased from the baseline (no offset) condition to a minimum of 0.281 ± 0.024 at the 0.4-arc min standing-offset condition. Thresholds then rose as larger standing offsets (0.8, 1.25, 2, and 3.75 arc min) were introduced, with the highest threshold, 1.316 ± 0.262, occurring at the largest offset (3.75 arc min). As a crude estimate of the tuning of the threshold versus standing-offset function, we note that the minimum threshold at 0.4-arc min offset was significantly smaller than the thresholds at 0-arc min offset ($P < 0.001$) and at 0.8-arc min offset ($P < 0.005$).

For a direct comparison of the effects of gaps and standing offsets, we replotted the last bin amplitudes (the amplitudes measured for the largest vernier offset in the sweep) from Figure 5 on the same scale (Fig. 6). We did not measure the effect of gaps smaller than 1 arc min because of display limitations and did not measure standing offsets beyond 3.75 arc min because the direction of offsets larger than this is ambiguous. For both first- (Fig. 6a) and second- (Fig. 6b) harmonic responses, the amplitudes decreased with increasing gap and standing-offset sizes. For the overlapping part of the gap and standing-offset scales (between 1 and 3.75 arc min), the first- and second-harmonic responses showed similar reductions for both gaps and standing offsets, indicating that the introduction

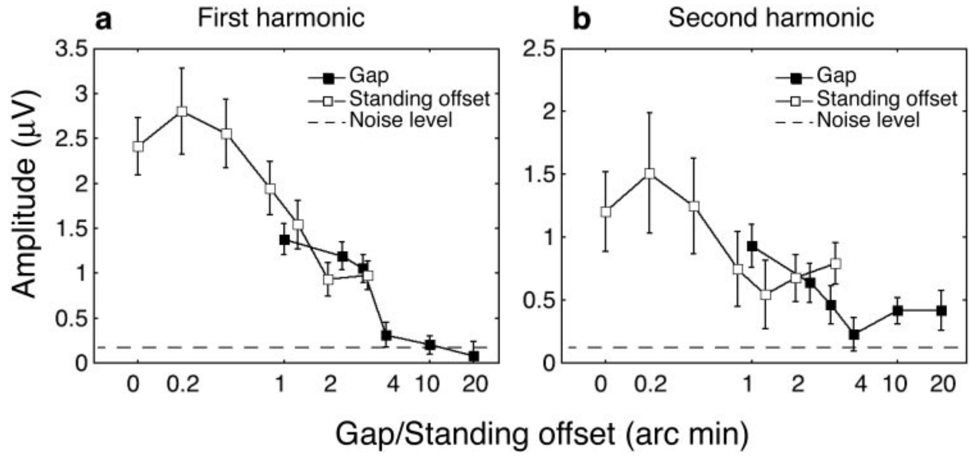

FIGURE 6. Replot of the last bin amplitudes (the amplitudes measured at 4-arc min vernier displacement) from Figure 5 for the first (**a**) and second (**b**) harmonics on the same scale for both gap and standing-offset experiments. Error bars, SEM. *Dashed lines*: EEG background. Both first- and second-harmonic response amplitudes decreased with increasing gap/standing-offset size. The first- and second-harmonic responses showed similar reductions for the overlapping part of gap and standing-offset scales (between 1 and 3.75 arc min).

of comparable coaxial gaps or standing offsets produced similar reductions in response amplitude.

Comparison of sVEP and Psychophysical Vernier Thresholds

Separating the static and moving elements of the display by introducing gaps produced similar elevations in both psychophysical and VEP vernier thresholds. Figure 7 is a plot of sVEP average individual-thresholds, group-average thresholds at the O_z–C_z derivation, and psychophysical thresholds from the four observers who had both sVEP and psychophysical measures. The data for the gap experiment are shown in Figure 7a. For the sVEP average individual-thresholds in Figure 7a, all eight observers had measurable sVEP thresholds for the baseline condition and for the 0.1- and 2.5-arc min gaps and six of eight had measurable sVEP thresholds for the 3.5-arc min gap. The rate of increase of the sVEP group average thresholds, to which each observer contributed equally, and the psychophysical thresholds did not differ significantly ($t = 2.2$; $P = 0.09$). The rate of increase was determined by the slope of the regression lines (dashed lines for sVEP group average thresholds and solid lines for psychophysical thresholds) for the gap and offset experiments (Figs. 7a, 7b, respectively).

Similar to the effects of gaps, the addition of standing offsets between the static and moving elements also produced elevations in both psychophysical and VEP thresholds. The data for the standing-offset experiment are shown in Figure 7b. For the sVEP average individual-thresholds, all eight observers had measurable sVEP thresholds at 0 (the baseline condition), 0.2, and 0.4 arc min. sVEP thresholds were obtained in seven of eight observers at 0.8- and 1.25-arc min shifts, in six of eight at 2 arc min and in four of eight at 3.75 arc min. The rate of increase in threshold with increasing offset was the same for psychophysical and sVEP group-average thresholds ($t = 0.40$, $P = 0.71$).

sVEP and Psychophysical Vernier Acuity in Amblyopia

Monocular sVEP and psychophysical vernier acuity thresholds were measured in the baseline condition (no gaps or standing offsets) for both amblyopic and fellow eyes of patients with amblyopia. Figure 8 shows vector-averaged first-harmonic sVEP amplitudes as a function of displacement for the fellow (Fig. 8a) and amblyopic (Fig. 8b) eyes for the 15 observers with anisometropic amblyopia, and the 14 observers with strabismic amblyopia who provided data on the standard vernier displacement sweep ranges (0.5–8 arc min in the amblyopic eyes and 0.25–4 arc min in the fellow eyes). The response function was of lower amplitude in the amblyopic eyes (Fig. 8b) compared with the fellow eyes (Fig. 8a) in both the anisometropic and strabismic groups, with the amplitude difference increasing as vernier displacement increased. The amplitudes of the strabismic group were lower than those of the anisometropic group in both fellow and amblyopic eyes, even though there were no significant differences between the two groups on optotype

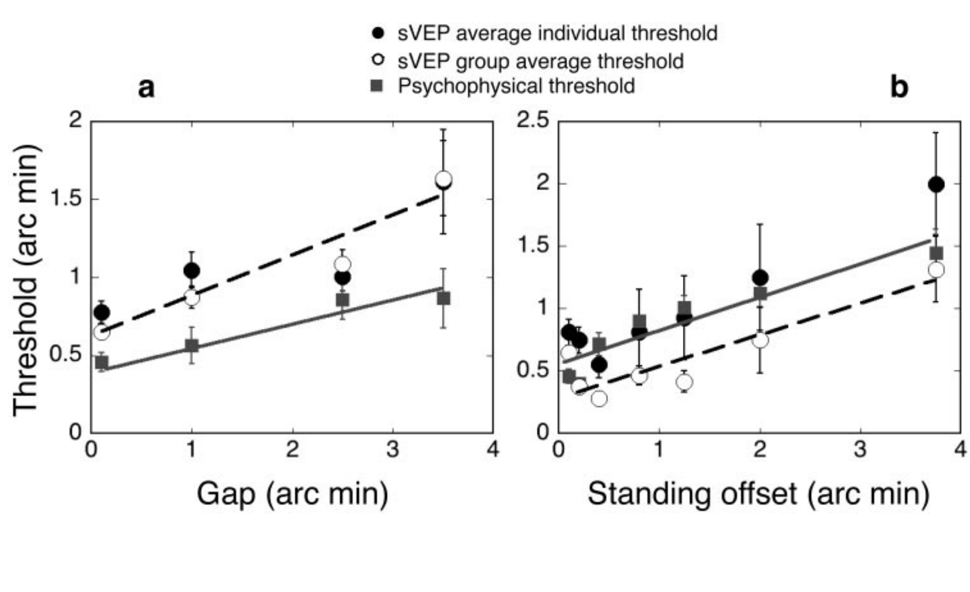

FIGURE 7. Comparison of sVEP and psychophysical thresholds for the gap (**a**) and standing offset (**b**) experiments. Error bars, SEM. *Dashed lines*: are the regression lines for sVEP group average thresholds; *solid lines*: the regression lines for psychophysical thresholds. sVEP data are from the O_z–C_z derivation. In the gap experiment (**a**), sVEP group average thresholds and psychophysical thresholds increased at a similar rate with increasing gap size. In the standing-offset experiment (**b**), sVEP group average thresholds and psychophysical thresholds increased by similar amounts over the range of standing offsets tested.

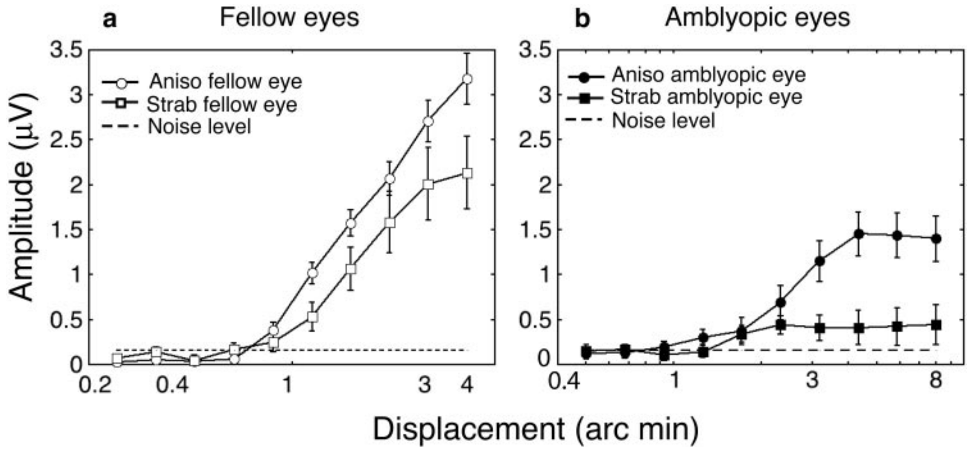

FIGURE 8. Vector-averaged, first-harmonic sVEP amplitudes as a function of displacement for fellow eyes (**a**) and amblyopic eyes (**b**) in 15 observers with anisometropic amblyopia and 14 observers with strabismic amblyopia. Error bars, SEM. *Dashed lines*: EEG background level. The fellow eyes (**a**) showed larger amplitudes in response to vernier onset–offset stimuli than the amblyopic eyes (**b**) for both anisometropic and strabismic groups, with the amplitude difference increasing as vernier displacement increased. The amplitudes of strabismic group were lower than those of the anisometropic group in both the fellow and amblyopic eyes.

acuity, psychophysical vernier acuity, or sVEP vernier acuity (Table 2).

The sVEP and psychophysical vernier acuity thresholds of all 36 fellow and amblyopic eyes are shown in Figure 9a (anisometropic group, $n = 20$; strabismic group, $n = 16$). Interocular threshold differences for the two measures are compared in Figure 9b. Figure 9c plots sVEP and psychophysical interocular threshold differences for each observer as a function of their interocular differences in optotype acuity. In Figure 9a, sVEP thresholds correlated significantly with psychophysical thresholds in the 36 amblyopic eyes ($r = 0.65$, $P < 0.0001$) and within the anisometropic ($r = 0.78$, $P < 0.0001$) and strabismic subgroups ($r = 0.51$, $P < 0.05$). The 36 fellow eyes showed no correlation between sVEP thresholds and psychophysical thresholds ($r = 0.26$, $P > 0.05$), which is not surprising, given the limited range of acuities in the fellow eyes. In two of the patients, the disagreement between sVEP and psychophysical thresholds was a full log unit. The optotype acuities in these patients were 20/40 and 20/50, in line with the VEP result, suggesting that the psychophysical thresholds were limited by task-performance factors beyond the visibility of the targets. The correlations just provided included these two patients.

In Figure 9b, sVEP interocular threshold differences also correlated with psychophysical vernier interocular differences in the full group of patients ($r = 0.76$, $P < 0.0001$) as well as in the anisometropic ($r = 0.81$, $P < 0.0001$) and the strabismic ($r = 0.71$, $P < 0.001$) groups.

We also compared sVEP and psychophysical vernier acuity interocular differences to the optotype interocular acuity differences (Fig. 9c) and found similar correlations between the two vernier acuity measures and the optotype measure. The correlation coefficient relating sVEP and optotype interocular differences was 0.67 ($P < 0.0001$) and that relating psychophysical vernier and optotype interocular acuity differences was 0.72 ($P < 0.0001$).

DISCUSSION

Vernier Onset–Offset versus Control Responses

This study has shown that the first harmonic of the sVEP vernier acuity paradigm is dependent on the relative position of the stimulus features. As in previous studies,[14,15,20] we have found that symmetric displacement of the moving elements of an offset grating relative to the stationary elements eliminates the first harmonic of the response. The only difference between the asymmetric alignment-misalignment pattern of the vernier acuity stimulus and the symmetric misalignment–misalignment pattern of the control stimulus is the relative position of the dynamic and static parts of the display. Similar to previous studies, we showed that the relative position of the static and moving elements of the display has been encoded all the way down to threshold. The absolute thresholds we observed were well under 1 arc min and are thus in the range of the other hyperacuities.[9]

The present study, unlike a previous study[14] has shown that the magnitude of the second harmonic is also sensitive to the relative position of the moving and stationary elements of the display (Figs. 4b). Although both the first and second harmonics are sensitive to relative position, the first harmonic is more specifically interpretable as a position signal, because it is eliminated in the local transient control condition, whereas the second harmonic is not. The second-harmonic response of the vernier onset–offset stimulus depends less on retinal eccentricity than does the first harmonic, and it may derive from mechanisms responsible for the detection of relative motion.[14]

Changing the position of the static elements of the display relative to the dynamic elements leads to differences in several possible cues, such as collinearity versus noncollinearity when the stimuli are described in the space domain and changes in the spatial frequency and orientation of spectral components when the stimuli are described in the frequency domain. Our experiments do not directly determine which cues underlie

TABLE 2. Comparison of Anisometropic and Strabismic Amblyopes Who Provided Data in Figure 8

	sVEP Threshold		Psychophysical Threshold		Optotype Acuity	
	Fellow Eye	Amblyopic Eye	Fellow Eye	Amblyopic Eye	Fellow Eye	Amblyopic Eye
Aniso	−0.47 ± 0.04	−0.22 ± 0.07	−0.3 ± 0.07	0.16 ± 0.06	−0.07 ± 0.02	0.43 ± 0.06
Strab	−0.41 ± 0.03	−0.12 ± 0.07	−0.42 ± 0.08	0.09 ± 0.09	−0.04 ± 0.02	0.48 ± 0.06
P	0.23	0.28	0.26	0.48	0.14	0.54

Data are the mean logMAR ± SEM.

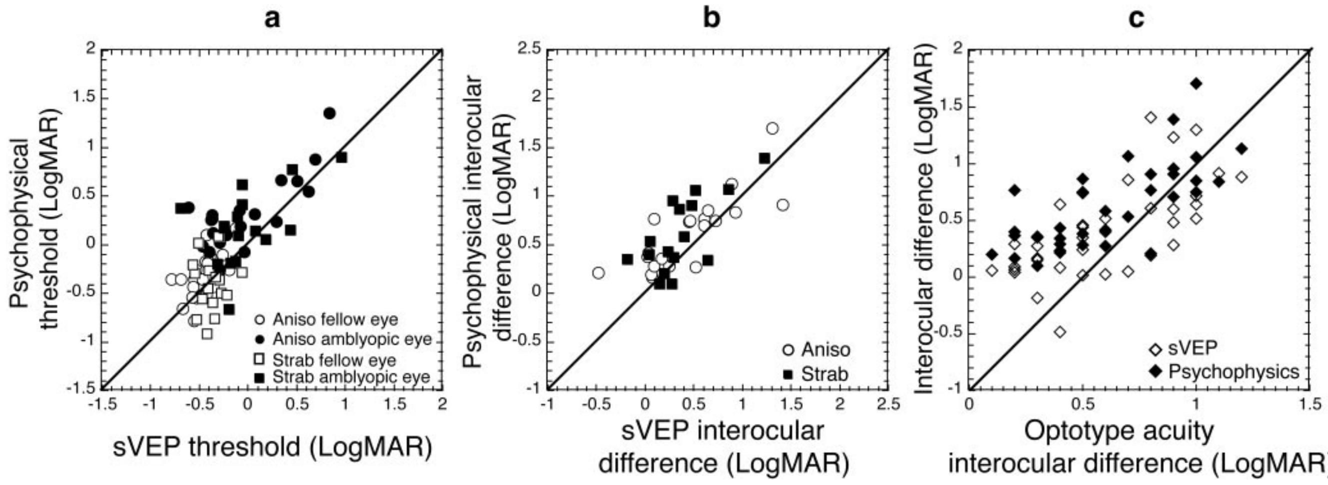

FIGURE 9. Comparison of sVEP thresholds and psychophysical thresholds for vernier onset–offset stimuli without gaps and standing offsets across each eye of the 36 patients with amblyopia. *Solid lines*: 1:1 ratio. (**a**) sVEP thresholds correlated significantly with psychophysical thresholds in all 36 amblyopic eyes and in each subgroup. (**b**) sVEP threshold interocular differences were also significantly correlated with psychophysical threshold interocular differences in the full group and in the sub-groups. (**c**) A similar correlation was found between the two vernier acuity measures (sVEP and psychophysical thresholds) and the optotype measure.

the VEP response or the psychophysical discrimination, but they do show that the two co-vary over a range of thresholds in both normal and amblyopic observers.

Covariation of VEP Vernier Responses and Perception

Our study and previous ones have used covariation of psychophysical performance and VEP responses in situations where manipulations of the stimulus conditions lead to changes in visibility. The logic here is that if the two co-vary, they must be sharing common mechanisms. It has been shown that the amplitude of the VEP response to suprathreshold vernier offsets can be reduced by the introduction of gaps[14] as can psychophysical thresholds.[7] In the present study, gaps had comparable effects on threshold performance within the same observers viewing the same stimuli, further establishing a connection between the sVEP vernier threshold and psychophysical visibility. Steinman et al.[11] found a similar effect of gaps with the transient vernier-VEP using a 7.5-arc min gap and line targets, as did Zemon and Ratliff[26] with the windmill–dartboard target. In our gap experiments, the second harmonic degraded with increasing gaps, too, but this harmonic was not measured in previous sVEP recordings.[14] The sVEP vernier paradigm also shows specificity for the standing-offset position of stimulus features. Here again, sVEP and psychophysical thresholds increased at comparable rates, especially for large standing offsets.

In a similar vein, thresholds measured from the first harmonic of the vernier VEP (using methods very similar to those of the present study) show another hallmark characteristic of the family of positional hyperacuities: VEP thresholds measured at the first harmonic are well below the limit set by the photoreceptor lattice in the fovea (15–20 arc sec vs. 1 arc min), and they fall off rapidly with retinal eccentricity, as do psychophysical left–right position judgments on the same stimuli.[14] The second harmonic showed a shallower eccentricity function and that study thus concluded that the first and second harmonics were generated by different mechanisms.

The first harmonic of the vernier VEP is also sensitive to the relative position of stimuli placed in the other eye (retinal disparity).[31] A static, offset-grating placed in one eye greatly reduces the amplitude of the vernier VEP first harmonic measured from a vernier onset–offset stimulus in the other eye. This effect is not due to contrast masking (a low level effect), but rather is dependent on interocular disparity (a type of relative position). The dichoptic experiment is also relevant to the question of whether spatial position has been encoded by the vernier VEP generator. The addition of a shifted grating in one eye to the vernier alignment–misalignment stimulus in the other eye produces a perceptual shift in the apparent visual direction of the stimulus elements: A physically aligned stimulus on the retina appears to be misaligned, as well as in depth. The observation of a reduction of the first harmonic to a stimulus that alternates between two states that are *perceptually* misaligned is similar to that observed when the two states are *physically* misaligned as in the standing-offset experiment of the present study. Psychophysical thresholds are also elevated for a similar dichoptic stimulus arrangement.[32]The results of Norcia et al.[31] indicate that the Vernier VEP arises after the site at which visual direction (e.g., *perceived* position) is computed.

sVEP Vernier Offset Responses in Amblyopia

Establishing that the sVEP tracks the visibility of degraded stimuli in normal observers does not guarantee that it will also track the visibility of full-cue stimuli in patients with degraded visual systems. We have shown that the sVEP threshold is correlated with the visibility of vernier offsets in adults with amblyopia. sVEP thresholds were correlated with psychophysical thresholds in both subgroups of amblyopic eyes. Moreover, sVEP interocular differences were also correlated with optotype acuity interocular differences in amblyopic patients, indicating that the sVEP vernier thresholds can predict optotype acuity, at least in patients with amblyopia.

Beyond its use in threshold measurement, the sVEP also provides a response function that describes visual activity at suprathreshold levels. We found that both fellow and amblyopic eyes of the patients with strabismus showed lower amplitudes than those of the patients with anisometropic amblyopia, even though the optotype acuity and vernier acuities of the two groups were the same. This finding indicates that suprathreshold VEP amplitude provides information that is independent of the threshold values. Differences between groups in the response to suprathreshold vernier offset adds to psychophysical results that have suggested that the two types of amblyopia involve different mechanisms.[1,24,33,34] Combining threshold measurements with a measure of suprathreshold amplitude may provide additional diagnostic value and also provide clues to the developmental processes underlying amblyopia with different etiologies.

Conclusion

The results of the present study further validate the use of the sVEP vernier paradigm as a direct physiological measure of the visibility of vernier offsets. Beyond threshold, the sVEP detected differences between fellow and amblyopic eyes of patients with different types of amblyopia that could not be accounted for by differences in optotype acuity. Because sVEP vernier acuity can be measured without the need for instruction or behavioral responses, it may be useful in assessing visual function in pre- and nonverbal patient populations and for studying the early phases of the development of amblyopia and its response to treatment.

Acknowledgments

The authors thank Mark W. Pettet for help with the data analyses and Vanitha Sampath for assistance in recruiting the observers with amblyopia.

References

1. Levi DM, Klein S. Hyperacuity and amblyopia. *Nature*. 1982;298:268–270.
2. Levi DM, Klein S. Differences in vernier discrimination for grating between strabismic and anisometropic amblyopes. *Invest Ophthalmol Vis Sci*. 1982;23:398–407.
3. Levi DM, Klein SA. Vernier acuity, crowding and amblyopia. *Vision Res*. 1985;25:979–991.
4. Levi DM, Klein SA, Wang H. Discrimination of position and contrast in amblyopic and peripheral vision. *Vision Res*. 1994;34:3293–3313.
5. Levi DM, Klein SA, Wang H. Amblyopic and peripheral vernier acuity: a test-pedestal approach. *Vision Res*. 1994;34:3265–3292.
6. Westheimer G, Hauske G. Temporal and spatial interference with vernier acuity. *Vision Res*. 1975;15:1137–1141.
7. Westheimer G, McKee SP. Spatial configurations for visual hyperacuity. *Vision Res*. 1977;17:941–947.
8. Westheimer G, McKee SP. Integration regions for visual hyperacuity. *Vision Res*. 1977;17:89–93.
9. McKee SP, Levi DM. Dichoptic hyperacuity: the precision of nonius alignment. *J Opt Soc Am A*. 1987;4:1104–1108.
10. Levi DM, Manny RE, Klein SA, Steinman SB. Electrophysiological correlates of hyperacuity in the human visual cortex. *Nature*. 1983;306:468–470.
11. Steinman SB, Levi DM, Klein SA, Manny RE. Selectivity of the evoked potential for vernier offset. *Vision Res*. 1985;25:951–961.
12. Zak R, Berkley MA. Evoked potentials elicited by brief vernier offsets: estimating vernier thresholds and properties of the neural substrate. *Vision Res*. 1986;26:439–451.
13. Victor JD, Conte MM. Two-frequency analysis of interactions elicited by Vernier stimuli. *Vis Neurosci*. 2000;17:959–973.
14. Norcia AM, Wesemann W, Manny RE. Electrophysiological correlates of vernier and relative motion mechanisms in human visual cortex. *Vis Neurosci*. 1999;16:1123–1131.
15. Skoczenski AM, Norcia AM. Development of VEP Vernier acuity and grating acuity in human infants. *Invest Ophthalmol Vis Sci*. 1999;40:2411–2417.
16. Wesemann W, Norcia AM, Manny RE. Measuring vernier acuity and motion perception with the parameter sweep VEP (in German). *Klin Monatsbl Augenheilkd*. 1996;208:11–17.
17. Manny RE. The visually evoked potential in response to vernier offsets in infants. *Hum Neurobiol*. 1988;6:273–279.
18. Mirabella G, Kjaer PK, Norcia AM, Good WV, Madan A. Visual development in very low birth weight infants. *Pediatr Res*. 2006;60:435–439.
19. Good WV, Hou C. Normal vernier acuity in infants with delayed visual maturation. *Am J Ophthalmol*. 2004;138:140–142.
20. Chen SI, Norcia AM, Pettet MW, Chandna A. Measurement of position acuity in strabismus and amblyopia: specificity of the vernier VEP paradigm. *Invest Ophthalmol Vis Sci*. 2005;46:4563–4570.
21. Levi DM, Klein SA, Yap YL. Positional uncertainty in peripheral and amblyopic vision. *Vision Res*. 1987;27:581–597.
22. Bradley A, Freeman RD. Is reduced vernier acuity in amblyopia due to position, contrast or fixation deficits? *Vision Res*. 1985;25:55–66.
23. Birch EE, Swanson WH. Hyperacuity deficits in anisometropic and strabismic amblyopes with known ages of onset. *Vision Res*. 2000;40:1035–1040.
24. McKee SP, Levi DM, Movshon JA. The pattern of visual deficits in amblyopia. *J Vis*. 2003;3:380–405.
25. Zemon V, Ratliff F. Intermodulation components of the visual evoked potential: responses to lateral and superimposed stimuli. *Biol Cybern*. 1984;50:401–408.
26. Zemon V, Ratliff F. Visual evoked potentials: evidence for lateral interactions. *Proc Natl Acad Sci USA*. 1982;79:5723–5726.
27. Nuwer MR, Comi G, Emerson R, et al. IFCN standards for digital recording of clinical EEG. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl*. 1999;52:11–14.
28. Tang Y, Norcia AM. An adaptive filter for steady-state evoked responses. *Electroencephalogr Clin Neurophysiol*. 1995;96:268–277.
29. Norcia AM, Tyler CW, Hamer RD, Wesemann W. Measurement of spatial contrast sensitivity with the swept contrast VEP. *Vision Res*. 1989;29:627–637.
30. Sprent P. *Applied Nonparametric Statistical Methods*. 2nd ed. Boca Raton, FL: Chapman & Hall/CRC; 1989.
31. Norcia AM, McKee SP, Bonneh Y, Pettet MW. Suppression of monocular visual direction under fused binocular stimulation: evoked potential measurements. *J Vis*. 2005;5:34–44.
32. McKee SP, Harrad RA. Fusional suppression in normal and stereoanomalous observers. *Vision Res*. 1993;33:1645–1658.
33. Hess RF, Bradley A. Contrast perception above threshold is only minimally impaired in human amblyopia. *Nature*. 1980;287:463–464.
34. Hess RF, Campbell FW, Zimmern R. Differences in the neural basis of human amblyopias: the effect of mean luminance. *Vision Res*. 1980;20:295–305.