Degradation of Swept-Parameter VEP Responses by Neutral Density Filters in Amblyopic and Normal Subjects

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Purpose. To determine whether objective visual function, measured by swept-parameter visual evoked potential (sVEP), is preferentially degraded by neutral density filtration (NDF) in normal control and fellow eyes compared to amblyopic eyes, and to determine whether the response to NDF is a function of stimulus type, using grating and vernier stimuli.

Methods. Monocular Snellen acuity and both grating and vernier sVEP responses were measured in each eye of 23 children or adolescents with amblyopia and 21 visually and neurologically normal children or adolescents. Acuity and sVEP responses were measured with and without a 2.0 log unit neutral density filter before the viewing eye.

Results. Suprathreshold sVEP grating responses were more sensitive than vernier to degradation by amblyopia in the unfiltered state and to NDF-induced preferential degradation of responses from fellow and normal control eyes. For threshold measurements, on the other hand, vernier responses were more sensitive to degradation by amblyopia in the unfiltered state and to NDF-induced preferential depression. Threshold vernier responses of amblyopic eyes were paradoxically enhanced by NDF.

Conclusions. Neutral density filtration causes preferential degradation of both threshold and suprathreshold sVEP responses in normal control eyes and fellow eyes of amblyopes, compared to amblyopic eyes. The degradation is stimulus specific and dependent upon whether threshold or suprathreshold responses are measured. Grating responses are more likely to identify suprathreshold abnormalities, while vernier stimuli are more likely to detect threshold abnormalities. These findings may be used to optimize the stimulus parameters and design of future studies utilizing evoked potential techniques in amblyopic subjects.

Keywords: amblyopia, strabismus, vernier acuity, visual evoked potential

Amblyopia is a major public health problem. It affects 2% to 5% of the population in developed countries where it is the most common cause of monocular visual impairment in children as well as young and middle-aged adults.[1–5] Both clinical[6–10] and experimental[11–14] studies have demonstrated that treatment for amblyopia is most successful during a "window of opportunity" in early childhood, when neural circuits for visual processing may be more easily created or modified. This window of opportunity includes the early preverbal period. However, clinical testing for amblyopia in preverbal and developmentally delayed children presently relies primarily on behavioral techniques (fixation preference testing). This procedure may have limited sensitivity in amblyopes with small or no ocular misalignment and an unacceptably high false-positive rate in those with large deviations.[15,16]

Swept-parameter visual evoked potential (sVEP) is a rapid and objective test with promising capabilities for overcoming the limitations of subjective testing. Previous studies using both steady-state[17–24] and transient[25–29] VEP have shown significant differences between amblyopic and fellow or normal control eyes with respect to threshold, amplitude, and/or latency. However the optimal stimulus paradigm for differentiating amblyopic from normal eyes has not yet been determined. The major purpose of this study was to test the hypothesis that amblyopic and fellow eyes might respond differently to neutral density filtration (NDF), as is the case with subjective acuity, and to determine whether this phenomenon might offer an additional useful parameter for the objective diagnosis of amblyopia by sVEP. This hypothesis was motivated by earlier studies by von Noorden and Burian,[30] followed later by Leonards and Sireteanu,[31] demonstrating that subjective optotype acuity is preferentially degraded by NDF in fellow eyes of amblyopic subjects. In addition, this study compares the responses to NDF in normal and amblyopic eyes using both vernier and grating stimuli. This comparison was driven by recent psychophysical and electrophysiological studies suggesting that responses to vernier stimuli may be intrinsically more sensitive to visual degradation by amblyopia[32–34] as well as other disorders of visual cortical development.[35,36]

Although the eventual goal of this line of research is to develop an objective test for amblyopia in preverbal and nonverbal children, the methodology must first be tested in older children in whom the diagnosis of amblyopia has been validated by the "gold standard" of optotype acuity. If the sensitivity and specificity of an optimized sVEP protocol are

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satisfactory in this group, further studies may be performed on preverbal and developmentally delayed children.

Methods

Subjects

The research protocol followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board (IRB) of the Penn State University Hershey Medical Center. Written informed consent was obtained from the parents of all participants after explanation of the nature and possible consequences of this study. Verbal assent was obtained from all children or adolescents.

Twenty-three children and adolescents with unilateral amblyopia, ages 8 to 17 years at the time of sVEP testing (mean = 11.0, SD = 2.4, 10 males), served as experimental subjects. Twenty-one visually and neurologically normal children and adolescents, ages 8 to 17 years (mean 12.4, SD 3.0, 9 males), served as control subjects (age difference $P = 0.14$, Student's *t*-test). All amblyopic subjects were recruited from the Pediatric Ophthalmology Clinic of the Penn State M.S. Hershey Medical Center. Normal control subjects were recruited from the same clinic or from the community at large by word of mouth or in response to IRB-approved recruitment posters.

Amblyopic and normal control subjects had no neurological or developmental disorders, and no systemic disorders or drug therapy that might affect vision or VEP responses. All amblyopic subjects had a complete ophthalmologic examination including visual acuity, ocular motility exam, stereoacuity, pupillary testing, confrontation visual fields, slit-lamp exam, dilated fundus exam, and cycloplegic refraction. Visual acuity was tested with Snellen letters at 15 feet using a computer-based system (M&S Technologies Smart System; M&S Technologies, Niles, IL, USA). Acuity was at least 20/20 in the normal eye. Unilateral amblyopia was defined as an interocular acuity difference greater than or equal to two Snellen lines in conjunction with amblyogenic factors of anisometropia and/or strabismus and not accounted for by other organic abnormalities of the visual system. Patients with deprivation amblyopia, for example, due to cataracts, were not admitted to the study. Patients were classified based on clinical criteria into anisometropic, strabismic, or mixed mechanism amblyopia. Fourteen subjects were diagnosed with strabismic amblyopia, four with anisometropic amblyopia, and five with mixed mechanism amblyopia.

All normal control subjects also had a complete ophthalmologic exam as described for amblyopic subjects, except that an undilated fundus examination was performed. All components of their exam were normal, including best-corrected Snellen visual acuity of 20/20 or better in each eye, with an interocular difference less than one Snellen line. Normal subjects with greater than 5.00 diopters of myopia, 4.00 diopters of hyperopia, 2.00 diopters of astigmatism, or 1.50 diopters of anisometropia in any meridian were not admitted to the study.

Data Acquisition

The testing procedure for amblyopic and normal control subjects was identical. All testing was done with spectacles if they were prescribed. On the day of sVEP testing, monocular Snellen visual acuity was obtained from each eye, both with and without a 2.0 log unit neutral density filter (NDF) in front of the viewing eye. Snellen acuity was converted to logMAR units for analysis. Student's correlated *t*-test was used for comparisons between the two eyes of the same subject (e.g., amblyopic versus normal fellow eye) or between two stimulus conditions (grating/vernier, unfiltered/filtered) for the same eye. Student's uncorrelated *t*-tests were used for comparison of acuities between diagnostic groups. All *P* values for acuity include Bonferroni correction. Following acuity testing, subjects were seated comfortably on a chair 150 cm from a high-resolution monochrome cathode ray tube monitor (Philips MGD403; Royal Philips Electronics N.V., Eindhoven, The Netherlands), which displayed either grating or vernier stimuli (see below). The stimulus display subtended an angle of 12° by 9° at a viewing distance of 150 cm and was controlled by PowerDiva v.2.90 software (A.M. Norcia; Smith Kettlewell Eye Institute, San Francisco, CA, USA). Gold cup electrodes (10-mm; Grass Technologies, West Warwick, RI, USA) were placed on the scalp at three active electrode sites, O1, O2, and Oz, following the International 10–20 system. A reference electrode was placed at the vertex (Cz), and a ground electrode was placed over the midforehead (Fz). Electrode sites were prepped with a mild abrasive and were attached using water-soluble conductive paste. Electrodes were secured with a soft elastic headband. An impedance of <5 to 10 K was maintained. Visual evoked potential responses were acquired by Grass amplifiers (model 12, Neurodata Acquisition System; Grass Technologies) for each channel, amplified at either 20 K or 50 K depending upon signal strength, and processed by a low-pass filter of 100 Hz and a high-pass filter of 1 Hz to eliminate noise. Electroencephalographic (EEG) signal processing and threshold estimation were performed according to the procedure outlined by Norcia and associates.[24,37,38] Signals were acquired at a data acquisition rate of 601.08 Hz and partitioned into 10 sequential epochs, designated "bins," corresponding to sequential stimulus presentations at different spatial frequencies or offset sizes. A recursive, least-squares algorithm generated the amplitude and phase of the response at each spatial frequency or offset size as a complex-valued Fourier component of the scalp EEG signal. The Fourier coefficients were averaged across trials to obtain an averaged amplitude and phase corresponding to each stimulus condition (i.e., each spatial frequency or offset size/NDF or no NDF). The averaged response for each stimulus condition was compared for statistical significance to baseline noise using the T^2-circ statistic.[39,40] Amplitude and phase of noise for each condition were estimated by averaging Fourier components of the response in frequency bins immediately above and below the stimulus fundamental frequency. Response thresholds for each condition were estimated by linear regression of amplitudes and extrapolation to zero response and amplitude. The following criteria were used to select bins for regression: (1) Response probability was better than $P \leq 0.16$; (2) phase responses in consecutive bins were stable or declining, with the difference in phase between consecutive bins between 80° and −100°; (3) responses in at least one of any pair of consecutive bins was significant at $P \leq 0.077$; and (4) for any bin included in the regression range, the amplitude of adjacent bins could not be less than 30% of the amplitude of the included bin.

Swept-source VEP was recorded monocularly for each eye using four testing conditions: grating and vernier stimuli with and without a 2.0 log unit NDF in front of the viewing eye. Fixation was carefully monitored by a video camera during the recording procedure. Recordings were stopped and the data were deleted if fixation was inconsistent. The grating stimulus consisted of a vertical square-wave grating, Michelson contrast of 80%, mean luminance 72.50 cd/m^2, alternating at a rate of 7.50 Hz with a matched space-average field of equal uniform luminance (Fig. 1). Gratings increased in spatial frequency from 2.00 to 25.00 cycles per degree (cyc/deg) (Snellen

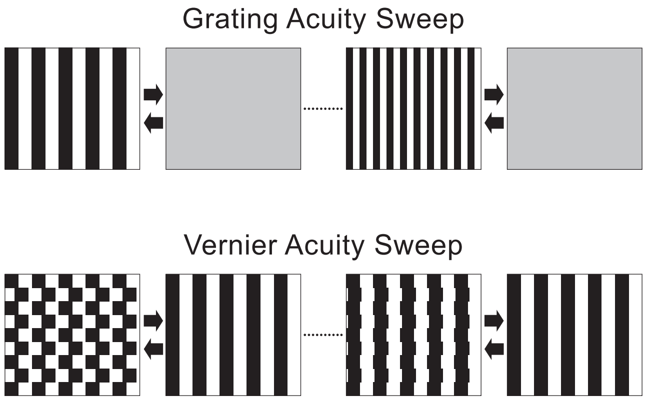

FIGURE 1. Schematic diagrams of visual stimuli.

equivalent 20/350–20/24) in 10 linear steps. Each of the 10 spatial frequencies was displayed for 1000 ms. Spatial frequencies were swept from lowest to highest over a 10-second interval, designated as a "sweep." A range of 6 to 12 sweeps was performed for each condition, until a stable threshold was achieved for three successive trials or a maximum of 12 trials. The vernier stimulus consisted of vertical square-wave bars of spatial frequency 2.00 cyc/deg with Michelson contrast of 80% and mean luminance 72.50 cd/m^2 (Fig. 1). The display alternated at 6.00 Hz for 1000 ms between a fully collinear grating and a grating containing horizontal vernier offsets. Offset size ranged from 4.00 to 0.25 min in 10 logarithmic steps. Six to 12 sweeps were performed for each condition. No further trials were performed after a stable threshold was obtained for three successive trials or after the 12th trial. Each of the 10 offset sizes was displayed for 1000 ms. Offset sizes were swept from highest to lowest over a 10-second interval.

The order of the eye to be tested (right/left) was first randomized using a random number generator, followed by randomization of the stimulus (grating/vernier), followed by randomization of the filter/no-filter condition. Prolonged dark adaptation for each eye/filter condition could not be performed because of the number of conditions tested. Approximately 60 seconds between conditions was allowed for changing the patches and filters and changing the computer stimulus parameters.

Suprathreshold sVEP Data Analysis

For each spatial frequency or offset size, the probability of a suprathreshold interocular difference (IOD) at identical testing conditions (i.e., identical stimulus and filter conditions) was calculated for each subject using the two-sample T^2-circ statistic.[39,40] This statistic considers both amplitude and phase of the response at each spatial frequency or offset size. Amplitude and phase for each condition were plotted as a polar vector with 95% confidence intervals derived from the 6 to 12 trials (Fig. 2). The set of right and left eye vectors for each subject at a given testing condition was compared at each of the 10 spatial frequencies or offsets (interocular comparisons). A set of all 10 suprathreshold IOD comparisons was considered significant if (1) the T^2-circ statistic indicated a value of $P <$ 0.05 at four out of any five consecutive spatial frequencies (grating) or offsets (vernier),[23] with a larger amplitude in the same eye for all four responses, and (2) signal-to-noise ratio (SNR) was >1.0 for all four responses. The proportion of subjects demonstrating an IOD for each stimulus condition (grating or vernier/NDF or no NDF) was compared across diagnostic groups (amblyopic/normal) using Fisher's exact test,

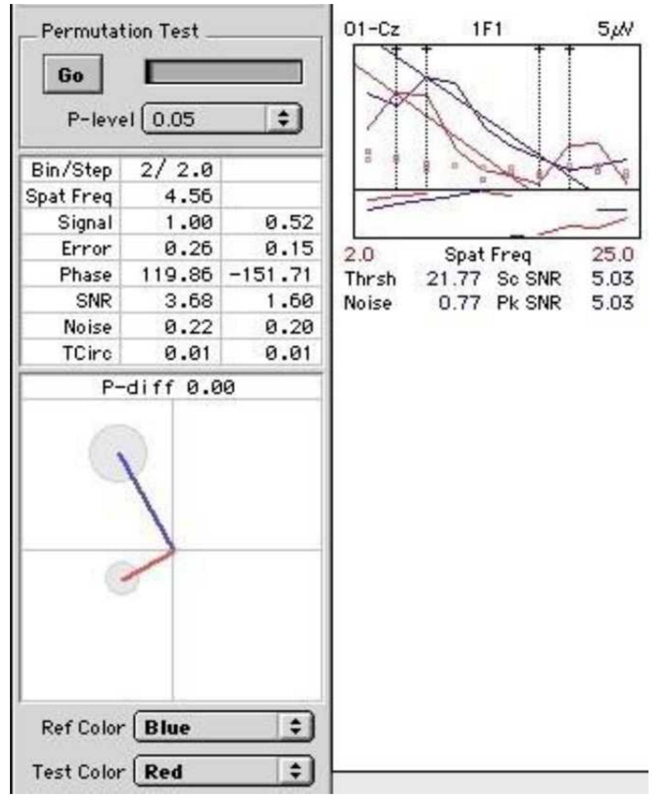

FIGURE 2. Polar vector comparing suprathreshold grating responses of amblyopic to normal eye at indicated 4.56 cyc/deg, showing a significant IOD between the two eyes (T^2-circ statistic to two significant decimal places). Spat Freq, spatial frequency; Bin, a repository of data for the displayed stimulus condition. Number of bin represents sequential order of presentation of this stimulus; for example, bin 2 contains data from the second stimulus presented, a grating stimulus of 4.56 cyc/deg. Step, same as bin for this study. Error, standard error of signal amplitude for displayed data. Phase, phase component of Fourier signal for displayed data. Noise, Fourier amplitude component of raw EEG averaged at adjacent temporal frequencies for this bin. TCirc, statistical comparison of the two conditions displayed in the polar graphs using the T^2-circ statistic. P-diff, the T^2-circ statistic for the two polar vectors to two significant decimal places (actual value $P < 0.005$). P-level is user determined parameter for acceptable level of significance for T^2-circ statistic. Ref Color/Test Color, not shown. Program displays polar factors in red and blue.

or within groups but across conditions using McNemar's test for correlated proportions. The Bonferroni correction was used to adjust for testing multiple proportions.

In order to compare the responses of normal control eyes to amblyopic and fellow eyes, intraocular comparisons were performed in a fashion analogous to that for interocular comparisons. An amblyopic eye, normal fellow eye, or a randomly selected right or left eye from each control subject was compared to itself with versus without NDF using the T^2-circ test. A result of $P < 0.05$ at four out of any five consecutive spatial frequencies or offset sizes, with SNR > 1.0, was considered a significant result. Eyes that met thiscondition were labeled as having "NDF-induced suprathreshold depression" for a given stimulus (grating or vernier). The proportion of eyes with NDF-induced suprathreshold depression was compared across ocular categories (amblyopic eye/normal fellow eye/normal control eye) and across stimuli (grating, vernier). Comparisons were performed using either Fisher's exact test for uncorrelated proportions or McNemar's test for

Table 1. Interocular Comparisons: Frequency of IOD With and Without NDF in Amblyopic and Normal Control Subjects as a Function of Stimulus

	Grating	Vernier	Comparisons, Across Row
Without NDF			
Amblyopes	70%	39%	$P = 0.086$*
Normal controls	14%	0%	$P = 1.0$*
Amblyopes vs. controls	$P = 0.003$†	0.017†	
With NDF			
Amblyopes	30%	9%	$P = 0.77$*
Normal controls	0%	0%	$P = 1.00$*
Amblyopes vs. controls	$P = 0.094$†	$P = 1.00$†	
Comparisons: with vs. without NDF			
Amblyopes	$P = 0.12$*	$P = 0.086$*	
Normal controls	$P = 1.00$*	$P = 1.00$*	

* McNemar test with Bonferroni correction.
† Fisher's exact test with Bonferroni correction.

correlated proportions. Bonferroni correction was used to correct for multiple comparisons.

Threshold Data Analysis

In each amblyopic subject, and for each testing condition (grating/vernier, unfiltered/filtered), the threshold at the electrode site with the best SNR was chosen for analysis. Threshold data were fit to a linear mixed-effects model. Fixed effects were eye (amblyopic eye/normal fellow eye), stimulus (grating/vernier), and filter condition (with NDF/without NDF). Random effect was subject. Main effects and two- and three-way interactions were calculated. Significant interactions were further explored with paired comparisons using Tukey's honestly significant difference (HSD) test, which accounts for the effect of multiple comparisons. All statistical analyses were performed using Systat 13 (Systat Software, Inc., Chicago, IL, USA).

Results

Snellen Visual Acuity

Student's correlated *t*-test was used for comparisons between the two eyes of the same subject (e.g., amblyopic versus normal fellow eye) or between two stimulus conditions (grating/vernier, unfiltered/filtered) for the same eye. Student's uncorrelated *t*-tests were used for comparison of acuities between diagnostic groups. All *P* values include Bonferroni correction.

Neutral density filtration reduced logMAR Snellen visual acuity in amblyopic eyes from a mean and 95% confidence interval (CI) of 0.48 ± 0.070 to 0.55 ± 0.070 ($P = 0.00088$, Student's correlated *t*-test with Bonferroni correction, $t = 4.69$, $df = 22$). In normal fellow eyes, acuity was reduced by NDF from 0.034 ± 0.050 to 0.23 ± 0.057 ($P = 0.4.27e^{-10}$, Student's correlated *t*-test with Bonferroni correction, $t = 11.82$, $df = 22$). The mean reduction by NDF was significantly greater in fellow eyes (0.20 ± 0.03) than in amblyopic eyes (0.075 ± 0.03) ($P = 5.96e^{-5}$, Student's correlated *t*-test with Bonferroni correction, $t = 5.82$, $df = 22$). The mean acuity before and after NDF for randomly selected right or left eyes from each of the 21 normal control subjects was 0.02 ± 0.02 and 0.20 ± 0.035, respectively ($P = 3.29e^{-9}$, Student's correlated *t*-test with

Table 2. Intraocular Comparisons: Frequency of NDF-Induced Suprathreshold Depression in Amblyopic and Normal Control Eyes as a Function of Stimulus

	Grating	Vernier	Comparisons, Across Row
Amblyopic eyes	43%	39%	$P = 1.0$*
Normal control eyes	90%	24%	$P = 0.00067$*
Normal fellow eye	70%	39%	$P = 0.042$*
Comparisons			
Fellow vs. control	$P = 0.137$†	$P = 1.0$†	
Amblyopic vs. fellow	$P = 0.039$*	$P = 1.0$*	
Amblyopic vs. control	$P = 0.006$†	$P = 1.0$†	

* McNemar test with Bonferroni correction.
† Fisher's exact test with Bonferroni correction.

Bonferroni correction, $t = 10.91$, $df = 20$). The mean reduction of these normal control eyes by NDF (0.20 ± 0.04) was also significantly more than the reduction for amblyopic eyes (0.075 + 0.030) ($P = 3.14e^{-5}$, Student's uncorrelated *t*-test with Bonferroni correction, $t = 6.27$, $df = 42$), and was not significantly different from the reduction for fellow eyes of amblyopes (0.20 + 0.035) ($P = 0.45$, Student's uncorrelated *t*-test with Bonferroni correction, $t = 0.28$, $df = 42$).

Suprathreshold sVEP Results

The results and statistical analysis of suprathreshold data are summarized in Table 1 for interocular comparisons and Table 2 for intraocular comparisons. In the unfiltered state, grating stimuli detected significant suprathreshold IODs in 70% of amblyopic subjects and 14% of normal control subjects; that is, sensitivity = 70% and specificity = 14% (Table 1). Vernier stimuli detected significant suprathreshold IODs in 39% of amblyopes and 0% of normal control subjects ($P = 0.017$), for a sensitivity and specificity of 39% and 100%, respectively. The difference between grating and vernier did not reach statistical significance for either sensitivity ($P = 0.34$) or specificity ($P = 1.0$).

The proportion of amblyopic subjects with significant suprathreshold IODs (by T^2-circ test, per Methods) with grating stimuli dropped from 70% without filter to 30% when both eyes were filtered. A similar phenomenon occurred using suprathreshold vernier stimuli, with a reduction in amblyopia detection rate from 39% unfiltered to 9% when both eyes were filtered. However, the frequency of NDF-induced suprathreshold depression did not reach statistical significance for either grating ($P = 0.12$) or vernier stimuli ($P = 0.086$). There was also no difference in the frequency of NDF-induced suprathreshold depression when comparing grating to vernier stimuli ($P = 1.0$).

The possibility of NDF-induced depression of suprathreshold sVEP responses was further investigated by comparing responses of each eye directly to itself, with versus without NDF, using the T^2-circ test (intraocular comparisons, Table 2). Neutral density filtration–induced suprathreshold depression was defined for intraocular comparisons as a significantly larger response of any given eye without versus with NDF (by T^2-circ test), under identical stimulus conditions (grating/vernier). Using grating stimuli, 43% of amblyopic eyes demonstrated NDF-induced suprathreshold depression, compared to 90% of randomly selected right or left normal control eyes ($P = 0.006$) and 74% of normal fellow eyes ($P = 0.039$). For vernier stimuli, NDF-induced suprathreshold depression occurred in 39% of amblyopic eyes, 39% of normal fellow eyes, and 24% of normal control eyes ($P = 1.0$ all comparisons). The proportion of eyes

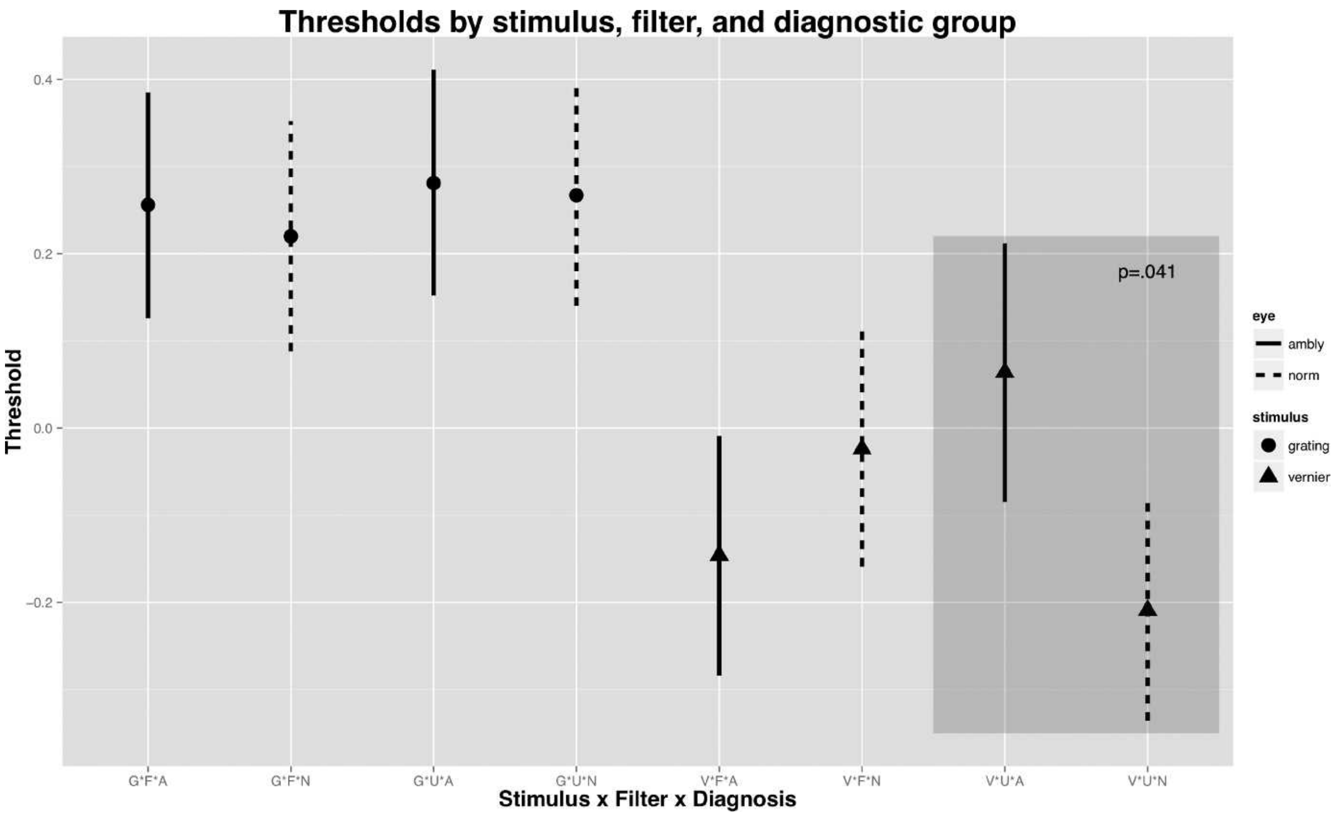

FIGURE 3. Note lower threshold for vernier stimuli for both amblyopic and normal fellow eyes, with or without NDF. A three-way interaction ($P =$ 0.015) is demonstrated by relative lack of effect of both amblyopia and NDF on grating thresholds (GFA, GFN, GUA, GUN) but elevation of vernier thresholds by amblyopia (VUA, VUN) and paradoxical decrease in threshold of amblyopic eye with NDF. In the unfiltered state, vernier thresholds were significantly different between amblyopic and fellow eyes ($P = 0.41$, Tukey's HSD test). A, amblyopic eye; N, normal fellow eye; G, grating; V, vernier; F, filtered; U, unfiltered.

experiencing NDF-induced suprathreshold depression was not significantly different for fellow versus control eyes ($P =$ 0.137). The proportion of eyes experiencing NDF-induced depression was significantly greater for grating as opposed to vernier stimuli in both normal control eyes ($P = 0.000122$) and fellow eyes ($P = 0.042$), but not in amblyopic eyes ($P = 1.0$). Also of note, no amblyopic eyes demonstrated significantly enhanced responses when compared with versus without NDF, strongly suggesting that the reduced proportion of amblyopic subjects showing IOD in the filtered state (see interocular comparisons noted above) was due to preferential depression by NDF of suprathreshold responses in the fellow eye, rather than paradoxical enhancement of responses in the amblyopic eye.

Threshold sVEP Results

A linear mixed model comparing thresholds of amblyopic and normal fellow eyes, across filtered and unfiltered states and across grating and vernier stimuli, revealed a significant three-way interactions between diagnosis × stimulus × filter ($P =$ 0.015), the latter indicating that the effect of NDF for a given diagnosis depended strongly upon which stimulus was chosen (Fig. 3). In particular, there was very little effect of either NDF or amblyopia, or both combined, using grating stimuli (Fig. 3). However, using NDF with vernier stimuli increased the threshold in fellow eyes, as expected, but paradoxically improved the threshold in amblyopic eyes, again demonstrating differential responses to NDF in amblyopic versus normal fellow eyes. Paired comparisons using Tukey's HSD test, which accounts for the effect of multiple comparisons, revealed a significant difference between unfiltered thresholds in amblyopic versus normal fellow eyes when using vernier but not grating stimuli (vernier, $P = 0.041$, grating, $P = 0.99$).

DISCUSSION

Snellen Acuity

As in prior reports,[30,31] Snellen acuity showed significantly less degradation by NDF in amblyopic eyes (0.075 logMAR) than either normal fellow eyes (0.20 logMAR) or randomly selected right or left normal control eyes (0.20 logMAR).

sVEP

The purpose of this study was to determine the effect of NDF upon both threshold and suprathreshold sVEP responses, and to determine whether these effects were stimulus dependent (grating versus vernier). As with subjective Snellen acuity, NDF also caused differential depression of both threshold and suprathreshold sVEP in both fellow eyes and normal control eyes, compared to amblyopic eyes. For suprathreshold measurements, interocular comparisons (Table 1) demonstrated a trend toward a smaller IOD between the amblyopic and fellow eyes when tested with versus without NDF for both grating (70% vs. 30%) and vernier (39% vs. 9%) stimuli, but this trend did not reach statistical significance. These findings are consistent with, but do not prove, the existence of preferential depression by NDF of suprathreshold responses in the fellow eye. This interpretation is further supported by the absence on intraocular testing of any amblyopic subjects with NDF-induced enhancement of the amblyopic eye response, suggesting that the decrease in IOD with NDF is due to a decreased response in the fellow eye rather than an increased response of the amblyopic eye.

The phenomenon of NDF-induced suprathreshold depression was most convincingly demonstrated by intraocular comparison of each eye to itself, with versus without NDF (Table 2). These comparisons demonstrated a significantly higher frequency of NDF-induced suprathreshold depression for grating, but not vernier, stimuli in fellow eyes (70%) and in normal control eyes (90%) compared to amblyopic eyes (43%). The frequency of depression was not significantly different between fellow and control eyes. These comparisons also demonstrated that the frequency of NDF-induced suprathreshold depression was significantly greater for grating than vernier stimuli for both control eyes (90% vs. 24%) and fellow eyes (70% vs. 39%). For amblyopic eyes, on the other hand, NDF-induced suprathreshold depression occurred equally frequently with grating (43%) and vernier (39%) stimuli.

Although suprathreshold depression of fellow and control eyes occurred significantly more frequently with grating than with vernier stimuli, threshold responses were more severely depressed by NDF for vernier stimuli. Threshold responses to grating stimuli were comparatively unaffected by amblyopia, NDF, or both (Fig. 3). Vernier responses, on the other hand, demonstrated the expected increased threshold with NDF in fellow eyes, but an unexpected and paradoxical improvement in threshold with NDF in amblyopic eyes. This was evidenced by a three-way interaction of diagnosis × stimulus × filter (Fig. 3). The presence of a significant three-way interaction indicates that the decrease in the filtered vernier threshold for amblyopic eyes and the increase for fellow eyes, with very little change due to either amblyopia or NDF for grating stimuli, is in fact a real phenomenon. Specifically, this finding indicates that the differential effect of NDF on amblyopic versus normal fellow eyes is highly dependent upon which stimulus is used (grating or vernier). This is true even though paired comparisons between filtered and unfiltered vernier conditions do not meet statistical significance (amblyopic eyes $P = 0.30$, fellow eyes $P = 0.32$). As expected from the nature of the vernier response as a hyperacuity phenomenon, vernier thresholds were lower than grating thresholds for both amblyopic and fellow eyes in both the filtered and unfiltered conditions (Fig. 3).

For unfiltered recordings, responses to vernier stimuli also demonstrated significantly higher thresholds in amblyopic compared to normal fellow eyes (Fig. 3, VUA versus VUN), while threshold responses to grating stimuli did not differ between amblyopic and fellow eyes (GUA versus GUN). These results are consistent with psychophysical studies that show greater sensitivity of subjective threshold vernier responses, as opposed to grating, for detection of amblyopia.[32–34] Taken together, these results suggest that grating responses are more likely to identify suprathreshold abnormalities, including both increased threshold in the amblyopic and NDF-induced depression of the fellow eye, while vernier stimuli are more likely to detect corresponding threshold abnormalities, along with paradoxically decreased threshold in the amblyopic eye.

The effects of amblyopia and NDF seem to parallel each other; that is, they both cause greater degradation of grating responses for suprathreshold measurements and greater degradation of vernier responses for threshold measurements. For grating and vernier stimuli, these findings presumably reflect fundamental differences in central processing. Vernier acuity, often described as a form of "hyperacuity,"[41] is primarily a measure of spatial localization and is significantly finer than grating acuity, with a normal adult ratio of vernier to grating resolution of approximately 1:4–5.[33,34] While normal grating resolution is limited by photoreceptor separation (i.e., normal grating resolution is approximately equal to the intercone distance), vernier resolution may reach 5 to 10 times this level. These observations suggest that development of normal vernier resolution may be limited by higher-order processing, presumably in the primary visual cortex and perhaps also involving visual association areas, though hyperacuity performance may be obtained at the retinal level.[42] Both psychophysical and electrophysiologic studies have also shown that vernier acuity matures considerably later than grating acuity. While grating acuity is fully mature by age 6, vernier acuity may not reach adult levels until late childhood or early adolescence.[32,43,44] Vernier acuity also correlates more closely with optotype acuity than grating in normal eyes, and is more severely depressed than grating by amblyopia.[32–34] The disparity between grating and vernier acuity is greatest in subjects with strabismic amblyopia or other forms of amblyopia accompanied by severe loss of binocular vision.[32,34] Indeed, vernier acuity is preferentially disrupted not only in amblyopia but also in children with cerebral visual impairment (CVI) acquired at an early age.[35,36] Both late maturation and more extensive higher-level cortical processing may account, at least in part, for the greater susceptibility of both psychophysical and sVEP vernier thresholds to disruption in infancy and early childhood. However, it is not clear why suprathreshold vernier responses are less susceptible to degradation by amblyopia than suprathreshold grating responses.

The responses of amblyopic eyes to NDF are similar to sVEP responses under low luminosity conditions in patients with CVI, as described by Good and Hou.[45] These authors described paradoxical enhancement of sVEP threshold and suprathreshold responses to grating stimuli under reduced luminosity conditions in 17 patients with CVI. Though responses of CVI patients were diminished compared to normal controls under both normal and low luminosity conditions, responses paradoxically improved with reduced luminosity in CVI patients and were unchanged or diminished in normal controls. Both CVI and amblyopia cause aberrant cortical processing, and enhanced responses to low luminance stimuli seem to be a common feature of both. Amblyopia is common in patients with CVI, and it is unclear whether this may have been a contributory factor. The authors did not specify the prevalence of amblyopia in their patients.

Our results are also consistent with a model of binocular vision proposed by McKee et al.[33] They found that, although there is a great deal of overlap between clinical categories, nonbinocular amblyopes, especially strabismic amblyopes, tend to have poorer optotype acuity and paradoxically better, frequently supranormal, contrast sensitivity, while binocular amblyopes tend to have better optotype acuity and poorer contrast sensitivity. McKee et al.[33] explain the relatively normal or even supranormal levels of contrast sensitivity in nonbinocular amblyopes by "re-organization of primary visual cortex after the binocular units disappear." They further suggest that "many or all of the binocular connections that are destroyed by ocular misalignment rearrange to drive the remaining monocular cells." Their model predicts an increase in contrast sensitivity of approximately 1.22 in nonbinocular subjects compared to fully binocular subjects (see their Appendix A and Fig. A1). These findings and their model may help to explain our NDF findings, including the paradoxical decrease in sVEP vernier threshold in amblyopic eyes. Nineteen of our 23 patients had either strabismic amblyopia ($n = 14$) or mixed mechanism amblyopia ($n = 5$) and would be expected, on the basis of this model, to have better, sometimes supranormal contrast sensitivity and poorer optotype acuity. In our primarily strabismic population of amblyopes, the NDF-induced preferential depression of threshold vernier responses (Fig. 3) and of suprathreshold grating responses (Table 2), as well as the paradoxical improvement of threshold vernier responses in the amblyopic eye (Fig. 3), may be a manifestation of this or a similar type of cortical reorganization following loss of binocular neurons. It is not clear, however, why this phenomenon was not also detected for suprathreshold vernier responses.

There are several reasons why VEP techniques have not gained widespread clinical acceptance for the detection of amblyopia in preverbal and developmentally delayed children. These include the need for technical expertise to perform and interpret recordings, untestability of some subjects,[26] lack of universally accepted stimulus parameters and recording protocols (grating, vernier, checkerboard, and contrast targets),[46–50] and disparate criteria for calculating threshold.[46–48] It is therefore unclear at this point which technique is the most sensitive and specific. Simon et al.[23] reported sensitivity for suprathreshold sVEP of 97% and specificity of 81%, but this series included patients with a broad range of pathology including amblyopia, optic nerve hypoplasia, glaucoma, and other causes of organic visual loss. In addition, the diagnosis of decreased vision in preverbal children was based upon fixation preference testing, which, as noted earlier, may have significant limitations[15,16] and may have affected the calculated sensitivity and specificity.

The major purpose of this study was to determine whether NDF induced the same phenomenon of differential depression of fellow eyes that is seen with subjective optotype acuity. A secondary goal was to determine whether this phenomenon was stimulus specific. Ideally, answers to these questions would provide information complementary to clinical findings and would aid in the diagnosis of amblyopia in preverbal and developmentally delayed children. Although we answered these questions, clinical application of these findings is limited at this point by several unresolved issues. First, our protocol was necessarily lengthy, requiring up to 45 minutes to complete, because we obtained recordings to both grating and vernier stimuli and performed each of these with and without NDF. This was done in an attempt to identify the optimal stimulus protocol. Infants and developmentally delayed children would be unlikely to cooperate for this length of time. Second, an optimal stimulus protocol did not emerge from our study. Unfortunately (or perhaps fortunately), useful diagnostic information was gleaned from both grating and vernier responses and from both filtered and unfiltered conditions. Although these results may not be directly applicable to clinical testing at this time, they do have important implications for the design of future VEP protocols for the detection of amblyopia. For rapid suprathreshold testing, grating stimuli without NDF offers the best sensitivity (70%) and reasonable specificity (86%). Adding a filter criterion, failure to show NDF-induced suprathreshold depression (by intraocular comparison) increased sensitivity in our study to 83% but decreased specificity to 70%. This protocol would require testing with grating stimuli with and without NDF and would increase testing time further. For threshold testing, vernier stimuli with and without NDF seems to offer greater sensitivity for amblyopia and would be the logical candidate for a threshold protocol. At this point in time, however, testing with NDF, employing the parameters used in this study, does not improve sensitivity or specificity and is therefore not yet applicable to clinical practice. Further research will be needed to determine the optimum stimulus and recording parameters. Parameters that may be altered in an effort to obtain optimal sensitivity and specificity include NDF density, temporal frequency of the steady-state stimulus, and stimulus contrast.

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