AOS THESIS

Inter-Ocular Fixation Instability of Amblyopia: Relationship to Visual Acuity, Strabismus, Nystagmus, Stereopsis, Vergence, and Age

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• PURPOSE: Amblyopia damages visual sensory and ocular motor functions. One manifestation of the damage is abnormal fixational eye movements. Tiny fixation movements are normal; however, when these exceed a normal range, the behavior is labeled "fixation instability" (FI). Here we compare FI between normal and amblyopic subjects, and evaluate the relationship between FI and severity of amblyopia, strabismus angle, nystagmus, stereopsis, vergence, and subject age.

• METHODS: Fixation eye movements were recorded using infrared video-oculography from 47 controls (15.3 ± 12.2 years of age) and 104 amblyopic subjects (13.3 ± 11.2 years of age) during binocular and monocular viewing. FI and vergence instability were quantified as the bivariate contour ellipse area (BCEA). We also calculated the ratio of FI between the 2 eyes: right eye/left eye for controls, amblyopic eye/fellow eye for amblyopes. Multiple regression analysis evaluated how FI related to a range of visuo-motor measures.

• RESULTS: During binocular viewing, the FI of fellow and amblyopic eye, vergence instability, and inter-ocular FI ratios were least in anisometropic and most in mixed amblyopia ($P < .05$). Each correlated positively with the strabismus angle ($P < .01$). During monocular viewing, subjects with deeper amblyopia ($P < .01$) and larger strabismus angles ($P < .05$) had higher inter-ocular FI ratios. In all, 27% of anisometropic and >65% of strabismic/mixed amblyopes had nystagmus. Younger age and nystagmus increased FI and vergence instability ($P < .05$) but did not affect the inter-ocular FI ratios ($P > .05$).

• CONCLUSIONS: Quantitative recording of perturbed eye movements in children reveal a major functional deficit linked to amblyopia. Imprecise fixation, measured as inter-ocular FI ratios, may be used as a robust marker for amblyopia and strabismus severity. NOTE: Publication of this article is sponsored by the American Ophthalmological Society. (Am J Ophthalmol 2024;267: 230–248. © 2024 Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.)

AMBLYOPIA IS THE MOST COMMON CAUSE OF VISUAL impairment in children, affecting 2% to 4%.[1] Amblyopia arises as a result of discordant binocular visual inputs during the critical period of development (from birth to 7 years of age).[2-6] It is caused most commonly by anisometropia or strabismus. Amblyopia is characterized by reduced grating, vernier, and optotype visual acuity,[7,8] as well as subnormal contrast sensitivity.[9,10] Amblyopic subjects also have reduced stereopsis and increased inter-ocular suppression.[11-14]

Video-oculography–based eye tracking is useful for quantifying strabismus[15-18] and holds promise as an automated marker for the depth of amblyopia. Pathologic fixation instability is a feature of vision loss.[19,20] Amblyopia also damages oculomotor functions, evident as increased fixation instability (FI).[21-25] FI can be quantified as an oval of fixation scatter or a bivariate contour ellipse area (BCEA).[23,26] [21,24] FI in amblyopes correlates with both visual acuity and contrast sensitivity deficits.[23,27-29] Vergence instability in amblyopes, a form of FI, also correlates with stereo-acuity deficits[21,23,28,30-32] and interocular suppression.[30,33]

Normal human beings exhibit tiny, involuntary physiologic fixational eye movements (FMs). These comprise micro-saccades (<1°): binocular, conjugate movements occurring at a frequency of 1 to 2 Hz, with interspersed slow drifts and tremors.[34,35] Fixation disparity, which is a normal minor difference between right and left eye alignment on a visual target, arises from constant micro-disconjugacies of binocular eye movement. Physiological FMs create controlled image motion, keeping it within the foveola, promoting the achievement of the highest visual acuity.[36-38] Similarly, normal FMs facilitate stereopsis without causing diplopia.[39] The specific fast and slow FMs components contributing to the observed FI in amblyopia have been described.[22-25,40] The alterations comprise larger fixational

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saccades and higher drift velocities. FI may be aggravated further by the presence of fusion maldevelopment nystagmus.[25,28,41] Nystagmus other than fusion maldevelopment nystagmus may also contribute to FI in amblyopes.[42] Furthermore, amblyopic individuals have more disconjugate FMs. The disconjugacies are pronounced in individuals who lack binocularity, those with large angle strabismus, and stereo blindness.[15-17]

Recent studies have shown that FM abnormalities are influenced by viewing conditions. For instance, FI, when measured as the amplitude of fast FMs, is most evident when the amblyopic eye is viewing and the fellow eye is occluded.[23,42] Previous research has focused primarily on individual fellow eye or amblyopic eye FI or vergence instability in binocular viewing.

The current study quantifies FI and vergence instability under binocular and monocular viewing, and provides a detailed analysis of fast and slow FM components to identify nystagmus waveform (if any). It also aims to assess potential factors contributing to FI and vergence instability, including the depth of amblyopia, strabismus angle, stereopsis, nystagmus waveforms, and affected individuals' age. Furthermore, the current study is a new effort to quantify relative changes in FI of the amblyopic eye compared to the fellow eye under different viewing conditions by computing inter-ocular FI ratios. The inter-ocular FI ratio measurements in our study are akin to the clinical diagnosis of amblyopia based on inter-ocular visual acuity difference. This approach minimizes the influence of age and potential noise artifacts in fixation eye movement recordings in children.[43-48] It will enhance our understanding of the relationship among visual feedback, fixation movement alterations, and binocular interactions in children with amblyopia. In addition, it contributes to establishing a comprehensive database of FM parameters for future clinical assessment of amblyopic children.

METHODS

The Cleveland Clinic Institutional Review Board approved the protocol. Written informed consent was obtained from each participant or parent/legal guardian, as mandated by the Declaration of Helsinki.

• STUDY COHORT, INCLUSION/EXCLUSION CRITERIA, AND CLINICAL PARAMETERS: We recruited 47 controls and 104 amblyopic subjects. Inclusion criteria were the presence of amblyopia attributable to anisometropia, strabismus, or a combination of anisometropia and strabismus (hereinafter labeled "mixed mechanism" amblyopia). Amblyopia was defined as subnormal corrected distance visual acuity in the absence of any structural optic nerve, retinal, or visual pathway abnormalities. Exclusion criteria included any coexisting ocular or systemic disease, congenital infections/malformations, or developmental delay. Subjects with idiopathic infantile (sensory anomaly or congenital motor) nystagmus were also excluded. Idiopathic infantile nystagmus is characterized by an increasing-velocity, slow-phase component of the nystagmus, or, alternatively, pendular nystagmus (no fast phase), which is a confound in measurement of amblyopia. Participants' ages ranged from 3 to 69 years.

Control subjects in the study were chosen based on the absence of any ocular or systemic abnormalities affecting visual acuity, except for refractive errors. Subjects' demographic data including age, sex, and race were collected based on self-report. A total of 104 subjects with amblyopia (mean [SD] age, 13.3[11.2] years; 58 female [55.1%]; 46 male [44.9%]), along with 47 controls (mean [SD] age, 15.3[12.2] years; 28 female [59.5%]; 18 male [40.4%]) were included in the study. There was no difference in age (t test, $P = .20$) and sex (χ^2, $P = .47$) between controls and amblyopic subjects. Similarly, there was no difference in the distribution of race between controls (28 Caucasian [59.5%]; 7 African American [14.8%]; 3 Hispanic [6.3%]; 5 Asian [10.6%]; 4 Multi-cultural [8.5%]) and amblyopia subjects (68 Caucasian [65.3]; 16 African American [15.3%]; 10 Hispanic [9.6%]; 4 Asian [3.9%]; 6 Multi-cultural [6.7%]) (χ^2, $P = .18$).

Clinical characteristics of each subject were extracted from a retrospective chart review. The corrected distance visual acuity, stereo-acuity, cycloplegic refraction, and strabismus angle at distance and near were tabulated at the time of eye movement recordings. Corrected distance visual acuity was measured monocularly, starting from the right eye, using the participant's optimal spectacle correction with Snellen linear optotypes. For subjects <6 years of age, alternatively, crowding-bar HOTV optotypes or picture optotypes (Allen optotypes with crowding bars presented with commercially available computer-based system Accomodata Stimuli) were used. Corrected distance visual acuity was measured at 20 feet of distance, and was recorded as the line at which all letters (or symbols) were read. Stereoacuity was measured with the Titmus Stereo Test at 40 cm. Subjects with no detectable (nil) stereoacuity were assigned a value of 7000″. Corrected distance visual acuity scores were converted to logMAR, and stereo-acuity scores in seconds of arc were converted to log arcsec.

The clinical categorization of amblyopia subtype (anisometropic, strabismic, or mixed) and severity (treated, mild, moderate, or severe) at the time of diagnosis was based on Pediatric Eye Disease Investigator Group criteria.[49] The type of amblyopia was assessed as described below.

Anisometropic amblyopia

For anisometropic amblyopia, at least 1 of the following criteria were required to be met: ≥0.50 diopters (D) difference between eyes in spherical equivalent or ≥1.50 D difference between eyes in astigmatism in any meridian.

Strabismic amblyopia

For strabismic amblyopia, at least 1 of the following criteria were required to be met, and criteria not met for mixed amblyopia: heterotropia at distance and/or near fixation on examination (with or without spectacles); history of strabismus surgery; and previous history of strabismus that had resolved with glasses and/or surgery

Mixed mechanism amblyopia

For mixed mechanism amblyopia, both of the following criteria were required to be met: criteria for strabismus met (see above); and ≥1.00 D difference between eyes in spherical equivalent or ≥1.50 D difference between eyes in astigmatism in any meridian. The severity of amblyopia was assessed as follows: (1) treated: if worse eye corrected distance visual acuity was ≤0.09 logMAR; (2) mild: if >0.09 but <0.30 logMAR; (3) moderate if ≥0.30 and <0.70 logMAR; and (4) severe if ≥0.70 logMAR. Amblyopic subjects were grouped by severity of disease as treated (n = 24), mild (n= 18), moderate (n= 44), and severe (n = 18), and by *etiology* as strabismic (n = 35), anisometropic (n = 33), and mixed mechanism (n = 36).[49]

• **EYE MOVEMENT RECORDINGS:** Remote infrared video-oculography (EyeLink 1000 plus, SR Research) was used to measure horizontal and vertical eye positions as detailed in previous studies[28,50-53] This video tracking method tracks the pupil with the corneal reflection. This system has a spatial resolution of 0.01° and a temporal resolution of 500 Hz. It captures horizontal and vertical eye positions of both eyes that are important for quantifying each eye's amplitude, direction, and position under different viewing conditions. Experiments were conducted in a semi-darkened room. Children with appropriate refractive correction were seated in an examination chair, with their heads placed on a chin rest 84 cm away from the liquid crystal display (LCD) screen. A small target sticker was placed on the child's forehead to allow measurements of head movements and distance of the subject in relation to the camera while capturing binocular eye position data.[54-56]

Protocol

Monocular calibration and validation were done using a cruciform 5-point constellation of horizontal–vertical target positions. At the initiation of each trial, a large color animal cartoon image appeared on an LCD monitor. When the recordings were about to commence, the animal's sound was broadcast, and its image shrank in 3 rapid steps. A similar technique has been used to capture a child's attention while recording visual evoked potentials.[57]

Target stimuli

After calibration, FMs were recorded as the subject fixated a white circular target (0.5° visual angle) projected against a black background on the LCD monitor (30-inch diameter, resolution 2560 × 1600 pixels at 60 Hz, brightness 350 cd/m^2). The animal sound was re-broadcast to recapture the child's attention if they deflected their gaze beyond a 4 × 4° fixation window for >500 milliseconds.

Trials

Three 45-second trials were recorded under binocular viewing and under right vs left eye monocular viewing (1 eye blocked) conditions in a randomized order. The monocular viewing trials were categorized as monocular fellow eye viewing and monocular amblyopic eye viewing conditions. An infrared permissive filter was used to block visible light while allowing the non-viewing eye to be tracked.

• **DATA ANALYSIS:** Horizontal and vertical eye positions of right and left eye were analyzed using Matlab TM (MathWorks). Blinks and partial blinks were identified and removed. Blinks were defined as portions of raw data where the pupil information was missing, and partial blinks were defined as portions of data where there was a sudden change in pupil size >50 units per sample. In addition, 100 units (200 milliseconds) of data before and after each blink and partial blink were removed to account for periods when the pupil may have been partially occluded by the eyelid. Eye position signals were differentiated and smoothed with a Savitzkey–Golay filter to measure eye velocity. An interface that incorporated an automated Engbert and Kleigl algorithm was used to identify fixational saccades and quick phases of nystagmus.[58-60] Both types of fast FMs are generated by the same neural circuitry,[61-66] and both follow the main-sequence relationship of saccades to target steps.[67,68] To recognize and exclude noisy data points, we evaluated the main-sequence of fast FMs (quick phases and fixational saccades were pooled together).[27,42] Drifts and slow phases were defined as epochs between fixational saccades and quick phases in patients without or with nystagmus.[27,42]

Subjects were categorized based on the presence of absence of nystagmus, defined as repetitive cycles of a fast-phase saccade and a slower, prolonged, slow-phase linear or decreasing-velocity drift of eye position during attempted steady fixation (subjects with idiopathic infantile nystagmus were excluded, as mentioned above).[69] Fusion–maldevelopment nystagmus is common in individuals with early-onset strabismus.[70-73] Fusion maldevelopment nystagmus was identified by the following: a nasalward slow-phase drift with respect to the fixating eye; and an instantaneous reversal of the direction of the fast and slow phases when fixation changed from 1 eye to the other. Subjects who did not fit the criteria for fusion maldevelopment nystagmus (and did not have idiopathic infantile nystagmus, an exclusion criterion) were defined as having "other nystagmus." Thus, all included subjects fell into 1 of 3 nystagmus categories: no nystagmus; fusion maldevelopment nystagmus; or other nystagmus.

Fixation and vergence stability (bivariate contour ellipse) metric
The fixation stability of the amblyopic eye was quantified by calculating the bivariate contour ellipse (BCEA) encompassing 68% of fixation points, using the following formula[21,22,74]:

$$\text{BCEA} = \pi \text{ X } 2\,\sigma \ \times \ \sigma\text{y}\sqrt{1 - p^2}$$

where 2.291 is the χ^2 value (2 degrees of freedom) corresponding to a probability of 0.68; σ_x and σ_y are the standard deviations of horizontal (x) and vertical (y) eye position of the amblyopic eye, respectively; and p is the product–moment correlation of 2-position components (ie, the horizontal (x) and vertical (y) position components). BCEA were used to quantify the fixation stability of the amblyopic eye obtained under binocular viewing, monocular fellow eye viewing, and monocular amblyopic eye viewing conditions. Similarly, BCEA was used to quantify the fixation stability of the fellow eye obtained under binocular viewing, monocular fellow eye viewing, and monocular amblyopic eye viewing conditions.

To calculate vergence instability, we computed the difference between the horizontal and vertical eye positions of the amblyopic eye and fellow eye at a given time. Vergence instability was quantified using the same BCEA formula as above. Instead of using the actual horizontal (x) and vertical (y) eye positions of the amblyopic eye or fellow eye, we computed the standard deviations and product–moment correlation on the difference in horizontal and vertical eye positions, respectively, between the amblyopic eye and fellow eye at a given time. We quantified vergence instability for each subject under binocular viewing, monocular fellow eye viewing, and monocular amblyopic eye viewing conditions. A log10 transformation was used to normalize the resulting BCEAs. The higher the BCEA values, the greater the FI and vergence instability.

Inter-ocular FI ratios
We computed 2 inter-ocular FI ratios as follows. (1) The binocular viewing inter-ocular FI ratio was computed by taking the ratio of the fixation stability of the amblyopic eye and fixation instability of the fellow eye obtained under binocular viewing, that is, the FI of the amblyopic eye under binocular viewing divided by FI of the fellow eye under binocular viewing. (2) The monocular viewing inter-ocular FI ratio was computed by the taking the ratio of the FI of the viewing obtained under the monocular amblyopic eye viewing and monocular fellow eye viewing conditions, that is, the FI of the amblyopic eye under monocular amblyopic eye viewing divided by the FI of the fellow eye under monocular fellow eye viewing.

The inter-ocular FI ratio allowed us to evaluate the relative increase in FI of the amblyopic eye compared to the fellow eye under binocular and monocular viewing conditions. Inter-ocular ratios of >1 are indicative of greater FI of the amblyopic eye compared to the FI of the fellow eye.

• **STATISTICAL ANALYSIS:** Statistical analysis was performed using SPSS (Version 25). The age between controls and amblyopic subjects was compared using the unpaired *t* test, whereas the sex and race distribution between the 2 cohorts was compared using χ^2 analysis. Also, the frequency of different FM waveforms by clinical type and severity of amblyopia was compared using χ^2 analysis. Post hoc comparisons were made for statistically significant results. A hierarchical multiple regression was run to evaluate the factors affecting fellow eye and amblyopic eye fixation instability, vergence instability, and binocular viewing and monocular viewing inter-ocular FI ratios. In multiple regression model 1, age, corrected distance visual acuity, stereo acuity, strabismus angle, extent of anisometropia, and multi-categorical waveform characteristics (no nystagmus, FMN, other nystagmus) were included. In model 2, multi-categorical waveform information was removed. For all multiple regression models, linearity was assessed by partial regression plots and a plot of studentized residuals against the predicted values. The independence of residuals was assessed by a Durbin–Watson statistic, which was <2.4 for all models. There was homoscedasticity, as assessed by visual inspection of a plot of studentized residuals vs nonstandardized predicted values. There was no evidence of multicollinearity, as assessed by tolerance values of >0.1 with VIF values of <3. There were no studentized deleted residuals greater than ±3 standard deviations. All statistical tests had a critical alpha (significance) value of 0.05. To obtain summative statistical measures of the FM variables diagnostic discrimination abilities in distinguishing different types of amblyopia from controls, the individual eye fixation instability (ie, fellow eye and amblyopic eye FI) and vergence instability obtained under binocular viewing, monocular _fellow eye viewing and monocular _amblyopic eye viewing and the binocular viewing and monocular viewing inter-ocular fixation instability ratios were subjected to receiver operating characteristic (ROC) curve analyses. The specificity and sensitivity at a predetermined cut off values were computed from the ROC curve analyses of each FM variable. The cut-off points were defined using the upper bound of 95% confidence intervals of the respective FM variables of control subjects (higher values of FM variables are indicative of greater FI). The area under the curve (AUC) and 95% confidence intervals of the AUC and statistical significance were computed.

RESULTS

• **FIXATION INSTABILITY IN ANISOMETROPIC, STRABISMIC, AND MIXED-MECHANISM AMBLYOPIA:** Fixation stability in a normal subject and FI in 2 different types of amblyopes are displayed in the graphs in Figure 1. The top row 1A is a normal subject. Within each box, on the right are the ovals (BCEA plots) of each eye. The 3 boxes of the

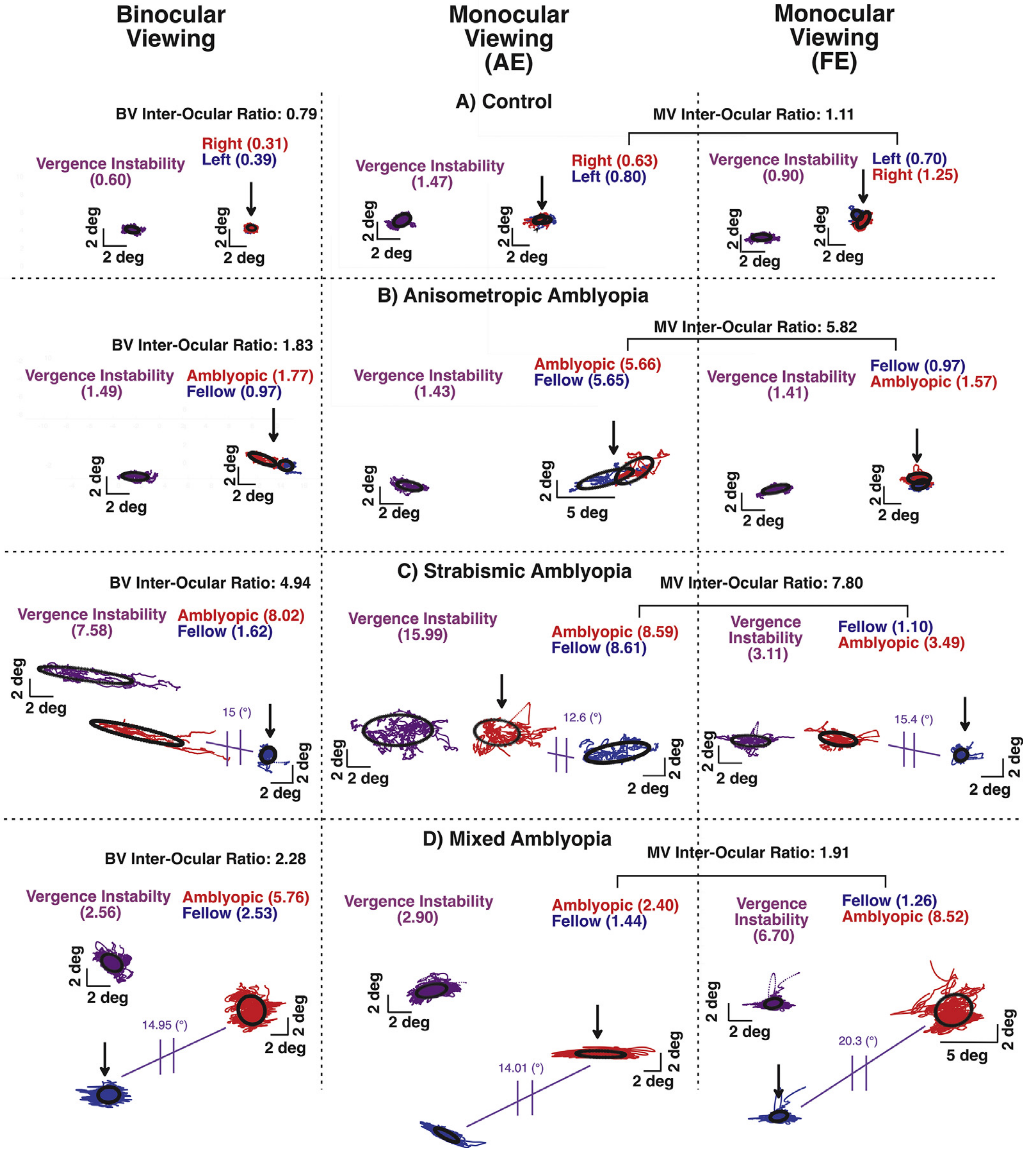

FIGURE 1. Horizontal and vertical eye positions of right and left eye of control subject (A), fellow eye and amblyopic eye of anisometropic amblyopia subject (B), strabismic subject with amblyopia (C), and mixed amblyopia subject (D) obtained during a 45-second visual fixation trial in primary position during binocular viewing (BV), monocular fellow eye viewing (MV_FE), and monocular amblyopic eye viewing (MV_AE). The 68% BCEA values of fixation stability for each eye are included next to the eye position plots as a quantitative measure of fixation scatter: the greater the BCEA values, the more unstable is the fixation (blue = fellow eye or left eye in control, red = amblyopic eye or right eye in control). Black arrows indicate the location of the target. The vergence stability for each subject under different viewing conditions is depicted in purple. Binocular and monocular viewing inter-ocular fixation instability (FI) ratios are computed for each subject.

row depict recordings during binocular viewing, monocular amblyopic eye viewing, and monocular fellow eye viewing respectively. To the left of each BCEA plot is the plot of vergence stability (purple). The BCEA plots superimpose the horizontal (x-axis) and vertical (y-axis) eye positions of the right or amblyopic (red) vs left or fellow (blue) eye. The greater the size of the BCEA oval, the greater the FI. Similarly, the greater the size of the vergence instability oval, the greater the vergence instability. The numbers within each box represent the calculated BCEA for each eye, the inter-ocular FI ratio, and the calculated vergence instability. Lower numbers equate to more stability; higher numbers equate to more instability.

All of the amblyopic subjects in Figure 1 had larger BCEA than the control subject, indicating greater FI, and all of the amblyopes had greater vergence instability. The FI and vergence instability abnormalities were apparent during binocular viewing (left column) in each type of ambly-

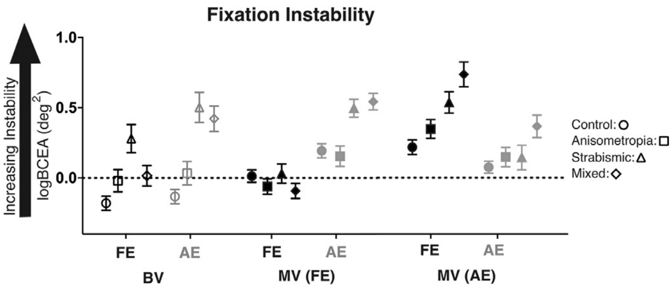

FIGURE 2. Mean and standard error of the mean of fixation instability (FI) of the fellow eye (black) and amblyopic eye (gray) in binocular viewing (BV), monocular fellow eye viewing (MV_FE), and monocular amblyopic eye viewing (MV_AE) in controls and in subjects with anisometropic, strabismic, and mixed amblyopia.

opia. The anisometropic amblyope had twice the binocular viewing inter-ocular FI ratio of the control (1.83 vs 0.79). The strabismic amblyope had a 6-fold greater binocular viewing inter-ocular FI ratio compared to the control (4.94 vs 0.79), and the mixed amblyope had a 2.5 greater binocular viewing inter-ocular FI ratio compared to the control (2.28 vs 0.79). Likewise, vergence instability was least in the control (0.60), more in the anisometropic amblyope (1.49), greatest in the strabismic amblyope (7.58), and intermediate (2.56) in the mixed-mechanism amblyope.

Under conditions of monocular viewing, the FI in all 3 types of amblyopes was worse than under conditions of binocular viewing. The monocular viewing inter-ocular FI ratio increased in the anisometropic amblyope when compared to the binocular viewing inter-ocular FI ratio. Vergence instability also increased when viewing changed from BV to monocular viewing in the strabismic and mixed-mechanism amblyopes. In the strabismic amblyope, the greatest vergence instability occurred during the monocular amblyopic eye viewing condition. In the mixed amblyope, the greatest vergence instability occurred during the monocular fellow eye viewing condition. Thus, monocular viewing made FI worse in all 3 types of amblyopia, and, in subjects with a history of strabismus, monocular viewing made vergence instability worse.

• **FIXATION INSTABILITY AND INTER-OCULAR FI RATIOS AVERAGE VALUES FOR THE ENTIRE CONTROL AND AMBLYOPIA COHORT:** Individual eye plots of FI of fellow eye and amblyopic eye are displayed in Figure 1. In Figure 2, average FI values of the fellow eye and amblyopic eye are shown for all controls and for all amblyopes, separated according to amblyopia subtype. The severity of FI is plotted along the y-axis as the size of the $\log_{10}$ BCEA. The viewing conditions on the x-axis are assigned as binocular viewing, monocular fellow eye viewing, and monocular amblyopic eye viewing. For binocular viewing, controls had mean FI of <0, that is, highly stable fixation. The FI of controls did increase in monocular viewing, particularly in the eye that was occluded, which was attributable to minor instability caused by lack of binocular summation and elimination of binocular-disparity eye position feedback. During binocular viewing, the mean FI in all 3 amblyopia groups was worse than controls, and was worse in the amblyopic eye compared to fellow eye. During monocular viewing, the mean FI of fellow eye and amblyopic eye in all 3 amblyopia groups increased, with a greater increase observed in the non-viewing (blocked) eye. The highest mean FI values occurred in subjects with strabismic and mixed-mechanism amblyopia under binocular viewing and in the non-viewing eye under monocular viewing, indicating that strabismus and diminshed visual feedback aggravate FI. Increase FI in the viewing eye under monocular viewing was seen in anisometropic and strabismic amblyopia groups, indicating that FI of viewing eye under monocular viewing is reflective of the visual acuity deficit of the amblyopic eye.

The inter-ocular FI ratios of the individual subjects depicted in Figure 1 are representative of the results found by averaging measurements for all subjects within a given subgroup (Figure 3). The inter-ocular FI ratio was least for controls and greatest for strabismic and mixed mechanism amblyopes under binocular viewing. Inter-ocular FI ratio increased in all groups, except the control group, when viewing changed from binocular to monocular viewing.

• **EFFECT OF AMBLYOPIA SUBTYPE ON VERGENCE INSTABILITY:** To assess how subtype of amblyopia could influence the stability of vergence, mean vergence instability was calculated and is plotted in Figure 4. Following previous convention, the x-axis notes the viewing condition: binocular viewing, monocular fellow eye viewing, or monocular amblyopic eye viewing. Control subjects had the lowest vergence instability, <0 under conditons of binocular viewing. The next most stable subgroup was the one with anisometropic amblyopia. The largest vergence instability was recorded in those with strabismus: the strabismic and mixed mechanism amblyopia groups. In all groups, including the control, changing from binocular to monocular viewing increased vergence instability, and changing from monocular fellow eye viewing to monocular amblyopic eye viewing increased vergence instability further.

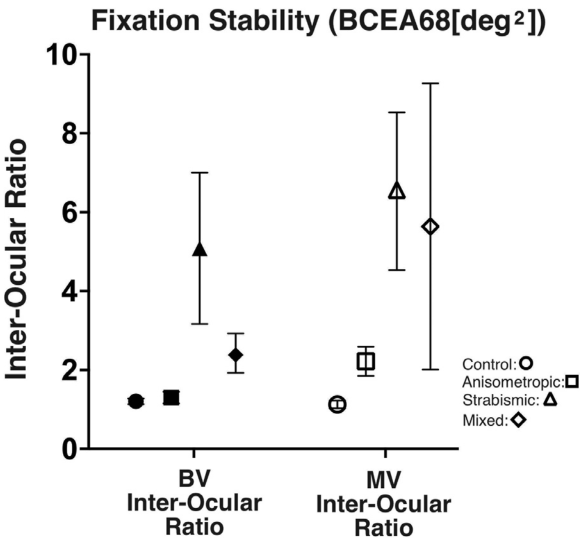

FIGURE 3. Mean and standard error of the mean of inter-ocular fixation instability ratio of amblyopic eye vs fellow eye (BCEA 68) obtained under binocular viewing (BV ratio) and per the viewing eye fixation stability obtained under monocular viewing, that is, amblyopic eye obtained under monocular amblyopic eye viewing divided by fellow eye obtained under monocular fellow eye viewing (MV ratio) in controls and in subjects with anisometropic amblyopia, strabismic amblyopia, and mixed amblyopia. To compute the inter-ocular fixation instability (FI) ratios in controls, we divided the data of the left eye by the right eye.

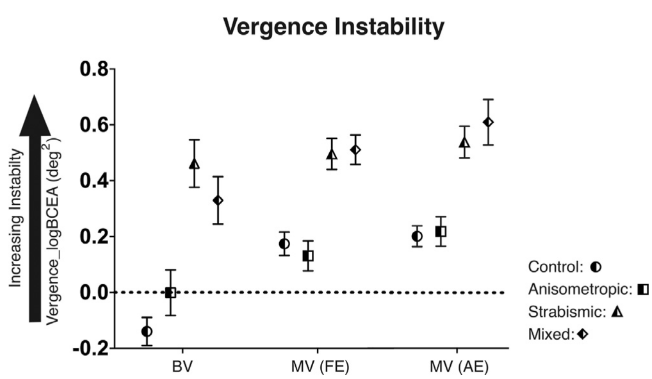

FIGURE 4. Mean and standard error of the mean of vergence instability in binocular viewing (BV), monocular fellow eye viewing (MV_FE), and monocular amblyopic eye viewing (MV_AE) in controls and in subjects with anisometropic, strabismic, and mixed amblyopia.

• **NYSTAGMUS AS AN ADDITIONAL ELEMENT OF AMBLYOPIC FIXATION INSTABILITY:** FI and vergence instability, as measured using BCEA, exceeded control values in all the amblyopes of the present study. Both FI and vergence instability were aggravated when subjects were deprived of the fixation cues provided by binocular viewing; that is, FI and vergence instability worsened in monocular viewing. However, as valuable as FI and vergence instability measures are, BCEA measurement does not capture the dynamic features of FM in some amblyopes, evident as fixational nystagmus.[28] Furthermore, clinical examination may not accurately detect nystagmus.[69,73,75-78] Accurate nystagmus detection entails careful analysis of eye movement recordings with particular attention to fast and slow-phase oscillations. To detect nystagmus, eye position traces were analyzed during binocular and monocular viewing epochs (Figure 5). Subjects were categorized for the presence or absence of nystagmus, as well as type: no nystagmus, other nystagmus and fusion maldevelopment nystagmus.

Figure 5 shows fast and slow components of FMs in a control subject, and in amblyopes who displayed no nystagmus, other nystagmus and fusion maldevelopment nystagmus. Eye movement traces were analyzed for the direction of repetitive fast and slow phases.[67] The control subject (Figure 5, A) and amblyopia subject without nystagmus (Figure 5, B) tracings show some fixational saccades interspersed with inter-saccadic drifts, but lack repetitive oscillations. They were categorized as no nystagmus. The subject in Figure 5, C showed evidence of a minor albeit inconsistent nystagmus in binocular viewing, interspersed with micro-square-wave jerks. When viewing converted to monocular fellow eye viewing, the nystagmus in both eyes converted to a repetitive cycle of conspicuous, conjugate slow and fast phases. When viewing changed to monocular amblyopic eye viewing, the repetitive nystagmus cycles disappeared and were supplanted by a series of conjugate macro-square-wave jerks on a baseline of slower drift. The nystagmus in this subject was labeled as having other nystagmus.

In Figure 5, D, binocular viewing showed a conjugate horizontal jerk nystagmus with a slow phase velocity of ~3 degrees per second rightward and a frequency of ~2.5 Hz. Monocular fellow eye viewing showed equivalent, rightward slow-phase nystagmus. Monocular amblyopic eye viewing produced an inversion of the nystagmus; slow phases were now directed leftward in both eyes at a higher velocity (~15 degrees per second). The nystagmus was categorized as fusion maldevelopment nystagmus, typified by a conjugate nasalward slow phase with respect to the viewing eye, rightward when viewing with the left fellow eye and leftward when viewing with the right amblyopic eye.

• **FIXATION NYSTAGMUS IN DIFFERENT SUBTYPES AND SEVERITIES OF AMBLYOPIA:** FM tracing waveforms were analyzed to assess the relationship to subtype and severity of amblyopia. In amblyopic subjects as a whole, 46.1% (n = 48) had no nystagmus; 40.5% (n = 42) had other nystagmus; and 13.5% (n = 14) had fusion maldevelopment nystagmus. Nystagmus type was plotted for each of the 3 subtypes of amblyopia (Figure 6, A). The majority of anisometropic subjects had no nystagmus (P < .001), whereas the majority of strabismic and mixed amblyopia

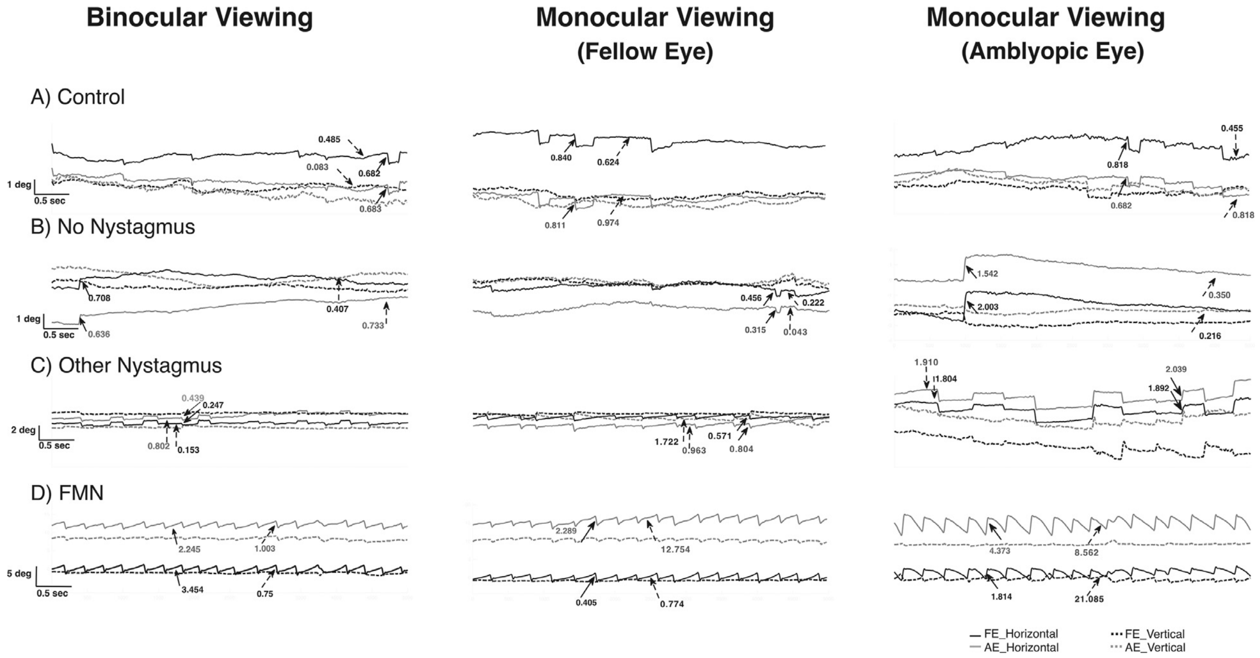

FIGURE 5. Examples of fixation eye movement (FMs) obtained during a 5-second epoch under conditions of binocular viewing (on the left), monocular viewing (fellow eye) (in the center), and monocular viewing (amblyopic eye) (on the right) from a control subject (A) and amblyopic subjects, including a subject without nystagmus (B), a subject with fusion maldevelopment nystagmus (C), and a subject with other nystagmus that did not meet the criteria for fusion maldevelopment nystagmus or idiopathic infantile nystagmus (D). The x-axis represents time, and the y-axis represents horizontal (solid line, black: fellow eye, and gray: amblyopic eye) and vertical (dotted line, black: fellow eye, and gray: amblyopic eye) positions. Solid black arrows represent the fast FMs, whereas dotted black arrows represent slow FMs. Numbers in black and gray represent fast FM amplitude and eye velocity of slow FMs of the fellow eye and amblyopic eye, respectively. Note that the amplitude of fast FMs and eye position variance of slow FMs are increased in the amblyopic eye during monocular amblyopic eye viewing (MV_AE) (gray numbers) than in the fellow eye during monocular fellow eye viewing (MV_FE) (black numbers) in amblyopic subjects without and with nystagmus.

subjects had other nystagmus ($P < .001$). Fusion maldevelopment nystagmus was absent in anisometropic amblyopes and was present in a minority of subjects with strabismic or mixed amblyopia ($P < .001$). Nystagmus waveform was also assessed for any relationship to severity of amblyopia (Figure 6, B). There was no correlation observed (chi square, $P = .40$).

• **MULTIPLE FACTORS THAT COULD INFLUENCE FIXATION INSTABILITY AND VERGENCE INSTABILITY:** FI could be influenced potentially by a number of factors, in addition to those noted in the previous results.

To examine this possibility, multiple regression analyses were conducted to weigh the influence of the following: subject age, amblyopic eye corrected distance visual acuity, fellow eye corrected distance visual acuity, refractive error differences, stereo-acuity, strabismus angle, and FM waveforms. The results of the analyses are listed in Table 1. Model 1 includes FM waveform, whereas model 2 excludes waveform data. The cells at the top row of the table display the viewing condition, the eye measured, and the effect on the inter-ocular FI ratio. The analysis was run to also take into account any confounding effect caused by FM waveforms.

Age analysis showed that younger children had a greater FI ($P < .05$). FI was also likely to be greater in eyes with the following: poorer corrected distance visual acuity, whether amblyopic eye ($P < .001$) or fellow eye ($P < .05$); larger strabismus angle ($P < .05$); and either no nystagmus or other nystagmus ($P < .05$). Greater monocular viewing inter-ocular FI ratio was linked to poorer amblyopic eye corrected distance visual acuity ($P < .001$) and larger strabismus angle ($P < .05$). Greater binocular viewing inter-ocular FI ratio was linked to larger strabismus angle ($P < .001$). The removal of waveform information does not significantly affect the ability of the model to predict the FI of fellow eye and amblyopic eye (no statistically significant change in Δ R2, Δ F) under monocular fellow eye viewing as well as monocular amblyopic eye viewing. However, the removal of waveform information had a statistically significant effect on the model's performance (Δ R2, Δ F were reduced, $P < .05$) in predicting FI of fellow eye and amblyopic eye under binocular viewing. In other words, characterizing FM waveforms is important while evaluating fixation instability under binocular viewing. Interestingly, age and FM wave-

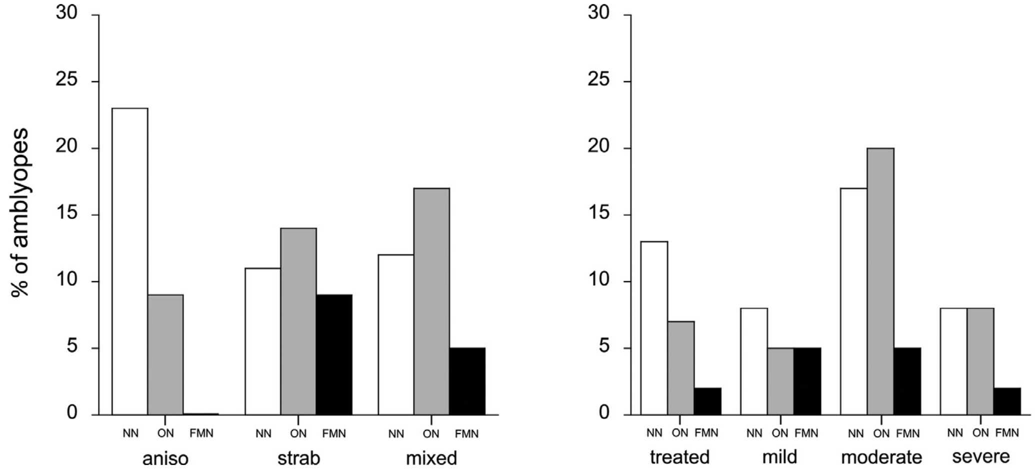

FIGURE 6. The distribution of fixation eye movement (FM) waveforms per the clinical type (A) and severity (B) of amblyopia (n = 104).

forms did not have an impact on the binocular viewing and monocular viewing inter-ocular FI ratios.

Mulitple regression analysis was also performed to determine the effect of other factors on vergence instability (Table 2). As in Table 1, the analysis was run with or without the addition of waveform information. During monocular viewing, vergence instability tended to be worse in subjects with larger strabismus angles ($P < .001$) and those with fusion maldevelopment nystagmus ($P < .05$). During binocular viewing, vergence instability tended to be worse in younger children ($P < .05$), in those with larger strabismus angles ($P < .001$), and in subjects with either no nystagmus or fusion maldevelopment nystagmus ($P < .05$). The removal of waveform information had a statistically significant effect on the model's performance (Δ R2, Δ F were reduced, $P < .05$) in predicting vergence instability under binocular viewing and monocular fellow eye viewing conditions. In other words, characterizing FM waveforms is important while evaluating vergence instability.

• **ROC ANALYSIS FOR FM VARIABLES IN DETECTION OF AMBLYOPIA:** Figure 7 plots the results of the ROC analysis for the FM variables in detecting different types of amblyopia. The parameters included separate analysis of FI of the fellow eye and amblyopic eye obtained under different viewing conditions (top panel), vergence instability under different viewing conditions (middle panel), and binocular and monocular viewing inter-ocular FI ratios (bottom panel). In our study, we established the cut-off values for each FM variable by setting them at 95% of the upper bound of the confidence intervals for the median value observed in the control group. Although this approach may reduce sensitivity by raising the threshold for positive identification, it was chosen to enhance specificity and to minimize the likelihood of false-positive results in diagnostic assessments. For strabismic and mixed amblyopia subjects, separate fellow eye and amblyopic eye FI instability obtained under binocular viewing and of the non-viewing eye under monocular viewing had very good capabilities in differentiating them from controls. Similarly, vergence instability under all 3 viewing conditions and the inter-ocular binocular viewing ratio had good discriminative capablities in differentiating strabismic and mixed amblyopia subjects from controls (Table 3). On the other hand, the inter-ocular monocular viewing ratio was the only FM parameter with a fair discriminative capability (AUC = 0.68) in differentiating anisometropic amblyopia subjects from controls. These results underscore that various FM variables can reflect the different clinical types of amblyopia. Specifically, the comparison of inter-ocular fixation instability ratio obtained under monocular and binocular viewing can be particularly informative, as it is not affected by age and the presence of nystagmus, unlike the individual fellow eye and amblyopic eye FI and vergence instability parameters.

DISCUSSION

The chief goal of this study was to examine fixation stability in subjects with different subtypes of amblyopia. A related goal was to introduce a new metric—namely, the inter-ocular FI ratio—to aid the understanding of the ocular motor deficits incurred by amblyopia. Our analysis of precise eye movement recording revealed that FI was more pronounced in amblyopic subjects with strabismus under binocular viewing, whereas the increased FI under monocular viewing was related to the depth of amblyopia (Figures 1-4). The FI was evident as larger BCEA plots for each eye, larger inter-ocular BCEA differences (ratios) between eyes, and larger magnitudes of vergence instability. Our results also show a systematic increase in FI and inter-ocular FI

TABLE 1. Multiple Regression Model Evaluating Separately (ie, Not Pooled) Fellow Eye and Amblyopic Eye Fixation Stability, and Inter-Ocular Fixation Stability in Controls and Amblyopia Under Different Viewing Conditions

Separate Fellow Eye and Amblyopia Eye Fixation Instability			Inter-Ocular Fixation Instability Ratios	
	FI of FE in BV [FI of AE in BV]	FI of FE in MV_FE [FI of AE in MV_AE]	BV Inter-Ocular FI Ratio = FI of AE in BV/ FI of FE in BV	MV Inter-Ocular FI Ratio = FI of AE in MV_AE/ FI of FE in MV_FE
Variables		MODEL_1 WITH FM WAVEFORM		
Age	−0.31***	−0.25*	0.09	0.03
	[−0.24]*	[−0.17]*		
AE visual acuity	0.17	−0.05	0.04	0.53***
	[0.21]	[0.65]***		
FE visual acuity	0.12	0.24*	0.05	−0.01
	[0.08]	[0.09]		
Refractive error difference	−0.20	−0.10	0.06	−0.19
	[−0.15]	[−0.09]		
Stereo-acuity	0.06	−0.00	−0.02	−0.01
	[0.04]	[−0.04]		
Strabismus angle	0.16	0.05	0.35***	0.22*
	[0.27]*	[0.14]		
No nystagmus	0.25*	0.08	−0.07	−0.19
	[0.17]	[−0.18]		
Fusion maldevelopment nystagmus	0.18	−0.07	0.00	−0.13
	[0.32]	[−0.03]		
Other nystagmus	0.06	−0.04	0.05	−0.11
	[0.13]	[−0.26]*		
R^2, F	0.30, 5.68***	0.17, 2.72*	0.18, 2.88*	0.25, 4.38*
	[0.37,7.89]***	[0.35, 7.20]***		
Variables		MODEL_2 WITHOUT FM WAVEFORM		
Age	−0.34***	−0.27	0.10	0.04
	[−0.23]*	[−0.14]		
AE visual acuity	0.19	−0.02	0.04	0.51***
	[0.21]	[0.59]***		
FE visual acuity	0.16*	0.23	0.04	−0.04
	[0.15]	[0.13]		
Refractive error difference	−0.15	−0.09	0.05	−0.18
	[−0.13]	[−0.11]		
Stereo-acuity	0.14	−0.06	0.00	−0.05
	[0.21]*	[−0.06]		
Strabismus angle	0.17	0.034	0.35*	0.21*
	[0.29]***	[0.10]		
R^2, F	0.25, 6.80***	0.16, 3.83*	0.17, 4.12***	0.23, 6.13***
	[0.32, 3.10]***	[0.31, 9.50]***		
(ΔR^2, Δ F)	(−0.05, 2.84)*	(−0.01, 0.59)	(−0.01, 50)	(−0.02, 0.90)
(after removing FM waveforms)	[(−0.05, 3.09)]*	[(−0.03, 2.07)]		

AE = amblyopic eye; BV = binocular viewing; FE = fellow eye; FI = fixation instability; MV = monocular viewing; MV_AE = monocular amblyopic eye viewing; MV_FE = monocular fellow eye viewing.

The top row in each cell in the individual eye position FI section represents data of the FE, whereas the data in square brackets [] represent values of the AE.

*$P < .05$

***$P < .001$.

when the ocular motor system is deprived of binocular feedback; FI under conditions of monocular viewing exceeded FI under conditions of binocular viewing.

An additional component of the study was waveform analysis, with the goal of revealing the prevalence and subtype of nystagmus in subjects with amblyopia (Figures 5 and 6). Nystagmus was recorded in >65% of subjects with strabismic or mixed mechanism amblyopia and in 27% of subjects with anisometropic amblyopia. Multiple regression analysis (Table 1) was used to examine the relationship be-

TABLE 2. Multiple Regression Model Evaluating Vergence Stability in Controls and Amblyopia Under Different Viewing Conditions

	Vergence Instability in BV	Vergence Instability in MV_FE	Vergence Instability in MV_AE
Variables		MODEL_1 WITH FM WAVEFORM	
Age	–0.19*	–0.13	0.00
AE visual acuity	0.19	0.14	0.15
FE visual acuity	0.12	0.02	0.1
Refractive error difference	0.16	–0.10	–0.03
Stereo-acuity	–0.03	–0.02	0.14
Strabismus angle	0.35***	0.30***	0.31***
None	0.22*	0.06	0.09
FMN	0.31*	0.35*	0.11*
Other nystagmus	0.14	0.16	–0.07
R^2, F	0.39, 8.55***	0.26, 4.83***	0.25, 4.41***
Variables		MODEL_2 WITHOUT FM WAVEFORM	
Age	–0.19*	–0.08	0.02
Visual acuity of AE	0.19	0.10	0.13
Visual acuity of FE	0.18*	0.09	0.14
Refractive error difference	–0.13	–0.09	–0.01
Stereo-acuity	0.14	0.17	0.16
Strabismus angle	0.38***	0.33***	0.29***
R^2, F	0.34, 10.52***	0.21, 5.39***	0.22, 5.82***
(Δ R^2, Δ F) [after removing FM waveforms]	(–0.05, 3.40)*	(–0.06, 3.15)*	(–0.03, 1.46)

AE = amblyopic eye; BV = binocular viewing; FE = fellow eye; FI = fixation instability; MV = monocular viewing; MV_AE = monocular amblyopic eye viewing; MV_FE = monocular fellow eye viewing.

*$P < .05$.

***$P < .001$.

tween FI, inter-ocular FI ratio, and a variety of contributing factors: age, corrected distant visual acuity, stereo-acuity, magnitude of anisometropia, angle of strabismus, and subtype of nystagmus. Multiple regression analysis (Table 2) was also used to evaluate the impact of these same factors on vergence instability. ROC analysis was used to determine the discriminative abilities of fixation eye movement variables in detecting various types of amblyopia (Table 3).

• **VALUE OF QUANTITATIVE EYE MOVEMENT RECORDING ADVANCES FOR STUDIES OF CHILDREN:** Advancements in eye-tracking technology now allow precise recording of fixational eye movements (FMs) in children, including those with developmental delay and autism.[79-81] These innovations facilitate objective assessments of eye movement in infants, toddlers, and pre-school children. Over the last 2 decades, the FM measures have allowed the construction of normative databases.[43,44] The primary objective of these studies was to establish benchmarks for evaluating FM abnormalities in children with amblyopia and strabismus. An additional application is to describe FMs in other pediatric visual brain disorders, such as cerebral visual impairment. Collectively, these studies have demonstrated the feasibility of obtaining FM recordings in children. Pediatric FM recording has also aided the detection and diagnosis of nystagmus.[81-85]

The current study leverages advances in pediatric eye tracking technology, using remote infrared video-oculography, which offers several advantages.[50-53] First, it is child-friendly, with quick calibration.[17,52,82,86,87] Second, it precisely captures binocular eye position data. Third, infrared video-oculography captures head movements and subject–camera distance variations to eliminate analysis confounds.[54-56] The high sampling frequency and spatial resolution are akin to the gold standard of scleral search coil recordings, but avoid the discomfort and eye safety concerns associated with scleral contact.[88,89] Infrared video-oculography ensures simultaneous capture of horizontal and vertical eye positions for both eyes under different viewing conditions. That capability is instrumental for quantifying amplitude, direction, position, alignment stability, and disconjugacy (ie, difference in eye position between the 2 eyes). Infrared video-oculography is further enhanced by automated (Engbert algorithm) FM analysis.[27,59,66,90] The analysis software enhances data quality, which includes plotting the main-sequence relationship of fast FM (fixational saccades and quick phases) to facilitate the recognition and exclusion of noisy data points.

• **FIXATION ABNORMALITIES AND THE NEURAL INTEGRATOR:** Fixation in normal subjects is more stable when viewing a well-lit target; increased instability occurs in

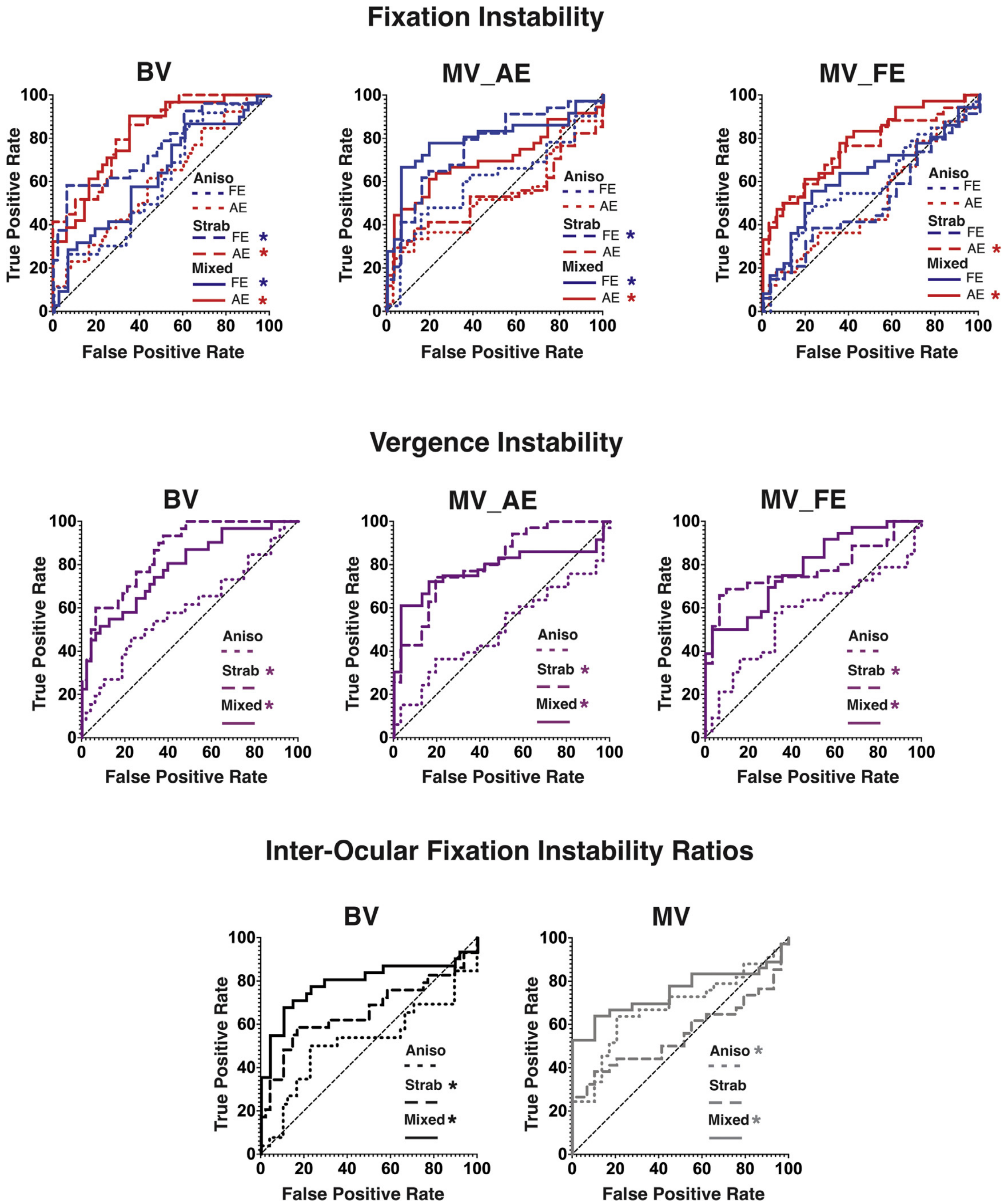

FIGURE 7. Receiver operating characteristic curves of various fixation eye movement (FM) variables in predicting anisometropic, strabismic, and mixed amblyopia. The top panel plots fellow eye and amblyopic eye fixation instability, whereas the middle panel plots vergence instability in binocular viewing (BV), monocular fellow eye viewing (MV_FE), and monocular amblyopic eye viewing (MV_AE). The bottom panel plots binocular and monocular viewing inter-ocular fixation instability (FI) ratios.

darkness.[52,91,92] Likewise, fixation is more stable when viewing with both eyes as opposed to 1 eye. The improvement is attributed to binocular summation.[22,28,30] Fixation instability (FI) is more pronounced in eyes with reduced vision (Figures 1-4). Key to FI is the role of the brainstem neural integrator, which integrates premotor velocity into eye position signals.[93] Neural integrators specify the position of each eye by receiving the individual eye's visual information. A mechanism for FI is degraded visual input into the neural integrator.

TABLE 3. ROC Analysis of Fixation Eye Movement Variables in Different Types of Amblyopia

Fixation Eye Movement Variables	AUC	95% CI	Cut-Off values	Sensitivity	Specificity
Fellow Eye and Amblyopic Eye Fixation Instability					
Fellow eye (BV)	(0.61)	(0.47-0.74)	(> −0.03)	(46.5%)	(65.9%)
	[0.76*]	**[0.65-0.88]**	**[> −0.03]**	**[65.5%]**	**[65.9%]**
	{0.64*}	**{0.51-0.76}**	**{> −0.03}**	**{58.1%}**	**{65.9%}**
Amblyopic eye (BV)	(0.59)	(0.46-0.73)	(> −0.01)	(46.5%)	(65.9%)
	[0.82*]	**[0.73-0.91]**	**[> −0.01]**	**[86.2%]**	**[65.9%]**
	{0.81*}	**{0.72-0.91}**	**{> −0.01}**	**{90.3%}**	**{65.9%}**
Fellow eye (MV_FE)	(0.57)	(0.43-0.72)	(< −0.09)	(51.5%)	(71%)
	[0.50]	[0.36-0.64]	[< −0.09]	[38.2%]	[71%]
	{0.63}	{0.49-0.76}	{< −0.09}	{58.3%}	{71%}
Amblyopic eye (MV_FE)	(0.50)	(0.36-0.65)	(> 0.32)	(33.3%)	(70%)
	[0.75*]	**[0.64-0.87]**	**[> 0.32]**	**[67.5%]**	**[70%]**
	{0.79*}	**{0.68-0.89}**	**{> 0.32}**	**{63.89%}**	**{70%}**
Fellow eye (MV_AE)	(0.62)	(0.48-0.76)	(> 0.32)	(48.8%)	(70%)
	[0.78*]	**[0.66-0.89]**	**[> 0.32]**	**[67.6%]**	**[70%]**
	{0.81*}	**{0.70-0.92}**	**{> 0.32}**	**{77.8%}**	**{70%}**
Amblyopic eye (MV_AE)	(0.54)	(0.39-0.68)	(> 0.07)	(48.4%)	(63.3%)
	[0.54]	[0.39-0.68]	[> 0.07]	[52.9%]	[63.3%]
	{0.71*}	**{0.59-0.84}**	**{> 0.07}**	**{66.6%}**	**{63.3%}**
Vergence Instability					
BV	(0.59)	(0.45-0.74)	(> −0.03)	(53.8%)	(64.5%)
	[0.86*]	**[0.78-0.94]**	**[> −0.03]**	**[90.0%]**	**[64.5%]**
	{0.78*}	**{0.68-0.89}**	**{> −0.03}**	**{74.1%}**	**{64.5%}**
MV_FE	(0.55)	(0.40-0.69)	(< 0.22)	(63.6%)	(43.3%)
	[0.79*]	**[0.68-0.90]**	**[> 0.25]**	**[74.29%]**	**[66.7%]**
	{0.79*}	**{0.69-0.90}**	**{> 0.25}**	**{75.0%}**	**{66.7%}**
MV_AE	(0.50)	(0.36-0.65)	(> 0.25)	(39.3%)	(66.6%)
	[0.81*]	**[0.71-0.91]**	**[> 0.25]**	**[77.1%]**	**[66.6%]**
	{0.78*}	**{0.66-0.90}**	**{> 0.25}**	**{75%}**	**{66.6%}**
Inter-Ocular Fixation Instability Ratio					
Inter-Ocular BV ratio	(0.52)	(0.36-0.67)	(> 1.2)	(53.8%)	(64.5%)
	[0.66*]	**[0.52-0.80]**	**[> 1.2]**	**[62%]**	**[64.5%]**
	{0.79*}	**{0.67-0.91}**	**{> 1.2}**	**{80%}**	**{64.5%}**
Inter-Ocular MV ratio	**(0.68*)**	**(0.54-0.81)**	**(> 1.2)**	**(63.6%)**	**(72.4%)**
	[0.55]	[0.41-0.69]	[>1.2]	[44.1%]	[72.4%]
	{0.75*}	**{0.63-0.87}**	**{> 1.2}**	**{69.4%}**	**{72.4%}**

AUC = area under the curve; BV = binocular viewing; MV= monocular viewing; MV_AE = monocular amblyopic eye viewing; MV_FE = monocular fellow eye viewing; ROC = receiver operating characteristic.

The inter-ocular MV ratio compares the fixation instability of the amblyopic eye obtained under monocular amblyopic eye viewing condition/fellow eye obtained under monocular fellow eye viewing condition, whereas the inter-ocular BV ratio compares the fixation instability of amblyopic eye/fellow eye obtained under binocular viewing. All values for anisometropic amblyopes are indicated by parentheses (); for strabismic amblyopes, square brackets []; and for mixed amblyopia, wavy brackets {}. Statistical significance is denoted by asterisks.

$^{*}P < .05$.

• **FI AND MALDEVELOPMENT OF THE VISUAL CORTEX:** Studies of non-human primate models of amblyopia and strabismus lend important insights into degraded visual input and ocular motor control.[4,6,16,70,71,94-105] Non-corresponding binocular inputs during the critical period lead to anatomical and functional abnormalities in striate cortical area.[96,99,101,106] The disruption reduces binocular neurons in the striate cortex and causes loss of excitatory connections, persistence of inhibitory connections, and imprecise spatial signaling. The binocular maldevelopment is promulgated to downstream visual areas, including middle temporal and medial superior temporal, responsible for conjugate gaze holding.[70] Medial superior temporal drive remains unbalanced, manifested as a nasalward gaze drift with respect to the viewing eye. The imbalance is implicated as the cause of fusion maldevelopment nystagmus.[6,70,71,99-101,103,107,108] Subcortical areas driving eye movements that receive inputs from the visual cortex areas are also damaged by amblyopia. Damage to those neurons is associated with FI.[4,39,96,108,109] Strabismus also impairs fixation stability. Strabismus is characterized by eye position variability, in both the deviating and fixating eye.[15,17,25] The instability is an abnormality of the vergence pathways.[15,17,21,110,111] Our results agree with these findings. FI in both the viewing and non-viewing eyes was more prominent in subjects with strabismus and stereo blindness (Table 1).

• **BCEA AND NYSTAGMUS WAVEFORM ANALYSIS AS TOOLS FOR MEASUREMENT OF FI:** Fixation stability measured using the bivariate contour ellipse area (BCEA) serves as a crucial metric for quantifying FI in individuals with amblyopia and strabismus.[21-24,46,112,113] BCEA values are larger in amblyopic eyes of both children and adults compared to fellow eyes.[21-24,26,28,30,31] Although measurement of BCEA is a valuable tool for quantifying the dispersion of eye positions, it does not accurately detect the presence of nystagmus.[28] In previous work, we have described nystagmus patterns in amblyopes, categorized as no nystagmus, other nystagmus, and fusion maldevelopment nystagmus.[28] An examination of FM waveforms revealed that subjects with strabismic or mixed amblyopia were more likely to have nystagmus (Figure 6). The most common form of nystagmus in anisometropes was the form designated as other nystagmus. Amblyopic subjects without nystagmus can also exhibit other abnormalities in FMs. The abnormal FMs can be present in both the amblyopic and fellow eye.[21-23,42] These observations underscore the importance of evaluating eye position traces obtained under binocular and monocular viewing conditions to accurately characterize waveforms.

• **VERGENCE INSTABILITY IN AMBLYOPIA WITH AND WITHOUT STRABISMUS:** Vergence instability, or FI in depth, is computed as the difference in right and left eye position over time. Vergence instability increases in the presence of strabismus or amblyopia.[15,16,28,30] Our results show that vergence instability is greater in strabismic and mixed mechanisms amblyopes and is less in anisometropic amblyopes.[16,30] The current results identified strabismus, rather than amblyopia, as the primary determinant of vergence instability (Figure 4). Vergence instability was greater in controls and anisometropic amblyopes during monocular viewing. However, vergence instability in subjects with strabismus was large in both binocular and monocular viewing conditions. Loss of binocular disparity cues is implicated, therefore, as a major driver of vergence instability. Vergence instability was also more pronounced in subjects with fusion maldevelopment nystagmus, reinforcing the notion that lack of binocular fusion is a major cause of vergence instability. Parallel findings of maldeveloped disparity vergence in strabismic non-human primates complement our observations.[114]

• **INTER-OCULAR FI IN AMBLYOPIA WITH AND WITHOUT STRABISMUS:** A few studies in the literature have evaluated fixation stability, noting greater instability in younger children.[43,44,115] The capacity to maintain a steady fixation is not well developed at birth, and is characterized by increased instability and variability during infancy.[116] However, over the course of development, there is a discernible reduction in FI, a decrease in the frequency of intrusive saccades and larger amplitude saccades during fixation, persisting through mid-adolescence in typically developing children.[43,44] Investigation into the developmental trajectory of eye movements has revealed the same observations for other classes of eye movements, including smooth pursuit, saccades, and binocular eye alignment. The proficiency of smoothly tracking a moving target, termed ocular pursuit, is immature at birth, but it improves in the first year of life.[117-121] The neural infrastructure to generate saccades is in place at infancy, but saccadic accuracy improves throughout the childhood. Ocular misalignment observed in newborns during the first 2 months is indicative of a normally developing vergence system.[122] The incidence of such misalignment progressively diminishes in infancy, coinciding with the introduction of retinal disparity cues to the visual stimulus.[123]

To address the inherent challenges posed by inter-subject variabilities, specifically considering the influences of age, we did a meticulous assessment of inter-ocular FI. In agreement with previous studies, we found an increase in both individual eye FI and vergence instability in younger children; however, the inter-ocular FI was not impacted by age. Our results emphasize the importance of inter-ocular FI measurement. For binocular viewing, the strabismus angle emerged as the most robust predictor of inter ocular FI (Table 1). For monocular viewing, the corrected distance visual acuity of the amblyopic eye emerged as the best predictor, followed by the strabismus angle. We have previously reported increased FI (BCEA) values for the amblyopic eye compared to the fellow eye, regardless of nystagmus.[28] That finding was replicated in the current study.

• **UTILITY OF EYE MOVEMENT RECORDINGS AND FUTURE MACHINE-LEARNING TOOLS FOR AMBLYOPIA MANAGEMENT:** The shortage of pediatric ophthalmologists in the United States and globally has prompted a quest for innovative solutions to optimize patient care, to reduce costs, and to overcome barriers to access.[124 125] Artificial intelligence, or machine learning, offers promise for detection of amblyopia and strabismus. Eye movement analysis is amenable to machine learning. Commercially available remote video eye trackers are now portable, accurate, and possess high sampling rates. Coupled with machine learning and the kind of normal and abnormal FI data generated in the current study, the technology could advance considerably the future diagnosis and treatment of binocular vision disorders.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Fatema Ghasia: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lawrence Tychsen:** Writing – review & editing.

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