Effect of Viewing Conditions on Fixation Eye Movements and Eye Alignment in Amblyopia

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Purpose. Patients with amblyopia are known to have fixation instability, which arises from alteration of physiologic fixation eye movements (FEMs) and nystagmus. We assessed the effects of monocular, binocular, and dichoptic viewing on FEMs and eye alignment in patients with and without fusion maldevelopment nystagmus (FMN).

Methods. Thirty-four patients with amblyopia and seven healthy controls were recruited for this study. Eye movements were recorded using infrared video-oculography during (1) fellow eye viewing (FEV), (2) amblyopic eye viewing (AEV), (3) both eye viewing (BEV), and (4) dichoptic viewing (DcV) at varying fellow eye (FE) contrasts. The patients were classified per the clinical type of amblyopia and FEM waveforms into those without nystagmus, those with nystagmus with and without FMN. Fixational saccades and intersaccadic drifts, quick and slow phases of nystagmus, and bivariate contour ellipse area were analyzed in the FE and amblyopic eye (AE).

Results. We found that FEMs are differentially affected with increased amplitude of quick phases of FMN observed during AEV than BEV and during DcV at lower FE contrasts. Increased fixation instability was seen in anisometropic patients at lower FE contrasts. Incomitance of eye misalignment was seen with the greatest increase during FEV. Strabismic/mixed amblyopia patients without FMN were more likely to demonstrate a fixation switch where the AE attends to the target during DcV than patients with FMN.

Conclusions. Our findings suggest that FEM abnormalities modulate with different viewing conditions as used in various amblyopia therapies. Increased FEM abnormalities could affect the visual function deficits and may have treatment implications.

Keywords: amblyopia, fixation, dichoptic, eye alignment, latent nystagmus

Amblyopia is a neurodevelopmental disorder that arises from binocularly discordant visual experience.[1] Current amblyopia therapies comprise of part-time patching that forces the amblyopic eye (AE) to fixate at near and far distances under monocular viewing, whereas atropine penalization forces the AE to fixate at a near distance while the fellow eye (FE) vision is blurred under binocular viewing. Recent research suggests that interocular suppression is the key factor resulting in diminished binocularity and visual acuity in amblyopia.[2,3] Newer therapies that aim to reverse the suppression by rebalancing the contrast presented to each eye (low contrast to the FE and high contrast to the AE under dichoptic viewing conditions) are being extensively investigated.[4–7] Thus, amblyopia therapies aid recovery of vision through distinct mechanisms and require different viewing conditions.

Fixation instability of the AE and, to some extent, the FE has been reported during monocular and binocular viewing.[8–12] Normally, during attempted visual fixation, our eyes are not entirely still but instead show small involuntary physiologic "fixational eye movements" (FEMs). Physiologic FEMs produce motion that causes variability in eye position confined mainly within the foveola, thereby achieving the highest visual acuity, which is referred to as stable fixation.[13] The FEMs comprise of microsaccades (fixational saccades), which are binocular conjugate movements with amplitude (<1 degree) that occur at 1 to 2 hertz (Hz) separated by intersaccadic drifts and tremors. Microsaccades, the fastest of all FEMs, have shown (1) to improve visual acuity by precisely relocating the fovea[14,15]; (2) to aid in contrast sensitivity estimates,[16] and (3) are utilized to scan small and informative visual regions.[17,18] Microsaccades and saccades share the same properties and are generated by the same neural circuitry.[19–24] Non-human primate (NHP) studies have shown that the microsaccades are generated within the rostral superior colliculus, whereas the larger visually guided saccades are generated within the caudal superior colliculus.[22–24] NHP studies have also shown that the micro-saccade

production is associated with an increase in neural activity in cortical area V1.[25] It helps explain the role of microsaccades in the maintenance of perception during steady visual fixation by thwarting neural adaptation and visual fading.[25–28]

Animal models of amblyopia have shown that the earliest functional and anatomic changes occur in area V1.[29–35] There is a shift in neuronal "acuity" to lower spatial frequencies, which results in impaired acuity and spatial resolution[36–38] with loss of V1 horizontal axonal connections between ocular dominance columns of the opposite eye, which are key components of fusion development. The effects of discordant input from the 2 eyes on the V1 neurons are greatest if they occur in the first 3 to 5 months of postnatal life at the time of emergence of stereopsis.[39–41] It causes an unbalanced middle temporal/medial superior temporal drive, where one hemisphere becomes more active,[42–44] resulting in persistence of nasal-ward bias that is the basis for developing fusion maldevelopment nystagmus (FMN).[33,39,40,45–48] Beyond infancy, discordant binocular input alters the architecture of V1 cortex with involvement of downstream subcortical eye movement sensitive areas resulting in unstable fixation.[41,49–51] We have characterized the fixation instability in patients with amblyopia and have found that these patients can have nystagmus with and without FMN.[52] Patients with amblyopia without nystagmus have alterations of physiologic FEMs.[53,54] These include increased amplitude of fast FEMs with a corresponding decrease in physiologic microsaccades and increased eye velocities of slow FEMs in AE than the FE, which correlate with the severity of visual acuity and stereo-acuity deficits.[52–54] The FEM abnormalities have shown to limit visual acuity in amblyopia.[55] A prior study from our laboratory has shown reduced microsaccade and saccade frequencies with greater difficulties performing a visual search task, which correlated with increasing amblyopia severity.[56]

Evidence suggests that FEM abnormalities can vary per the viewing conditions (e.g. the intensity of latent nystagmus now referred to as FMN) increases under monocular viewing.[57,58] Forty percent of patients treated with part-time patching and atropine penalization of the FE have recurrent/residual amblyopia.[59,60] The results from large randomized-control dichoptic treatment studies in children have been mixed.[61–63] There is a paucity of data on how the monocular, binocular, and dichoptic viewing as used in various amblyopia therapies affect FEMs and eye alignment. These metrics can have implications for treatment response[64–68] and can affect the ability to attend to the presented stimuli during therapies effectively.[26,28,69] In the current paper, we examined the fixation stability, fast and slow FEM characteristics, and eye alignment under monocular (i.e. amblyopic eye viewing [AEV] and fellow eye viewing [FEV]), both eye viewing (BEV) and dichoptic viewing (DcV) with varying FE contrasts. We hypothesize that fixation stability, fast and slow FEMs of the FE and AE will be differentially modulated per the viewing conditions in patients with amblyopia with and without nystagmus. We also hypothesize that the eye alignment will be differentially affected in patients with and without nystagmus under different viewing conditions.

Methods

The experiment protocols complied with the tenets of the Declaration of Helsinki, and were approved by the Cleveland Clinic Institutional Review Board. Informed consent was obtained from the study participants/parents or legal guardians on behalf of the minors/children. We recruited 34 subjects with amblyopia and 7 healthy controls. All the subjects had a comprehensive eye examination.

Visual and Stereo-Acuity and Eye Movement Measurements

Psykinematix (KyberVision) software was used to generate test stimuli, which were displayed on a monitor with a resolution of 1280 × 800 at 60 Hz with a white luminance of 111 cd/m2 at a distance of 3.1 m in a dark room. Monocular distant visual acuity was assessed while the non-viewing eye was occluded. Subjects viewed one randomly selected Early Treatment Diabetic Retinopathy Study (ETDRS) optotype with crowding bars – the size was adjusted per a 2-down-1-up staircase with 6 reversals.[70] The thresholds were taken to be the arithmetic mean of the reversals and converted into logMAR. The Titmus Stereoacuity Test was used to measure stereo-acuity in log arcsec. Patients with no stereo-acuity were assigned a value of 3.85 log arcsec.

A high-resolution video-based eye tracker (EyeLink 1000) was used as described previously to measure binocular horizontal and vertical eye positions.[52,56] Each subject's head was supported on a chin-rest, 84 cm away from the LCD screen. An infrared permissive filter was used to block visible light while allowing the non-viewing eye to be tracked. Monocular calibration and validation of each eye was done using a 5-point constellation with best-corrected vision in a dark room.

The subjects fixated their gaze on a white circular target (0.5 degrees visual angle) projected against a black background on the LCD 30 inch monitor with a resolution of 2560 × 1600 at 60 Hz with brightness of 350 cd/m^2. The recordings were obtained under BEV, FEV, and AEV. Recordings were also obtained under DcV, where the dot is presented independently but coincidently to each eye. To deliver different images to the right and left eyes, we used interleaved polarization: every even line was visible only to one eye, and every odd line was visible only to the other eye owing to opposite polarization. During the development of the program, calibrations were performed to ensure that the displayed images (to the right and left eyes) were identical at the pixel level. Subjects viewed a target on a 3D LCD 32 inch monitor with a resolution of 1920 × 1080 at 120 Hz with a brightness of 111 cd/m^2. Each session began with both horizontal and vertical alignment of the dichoptic nonius lines. Subjects were shown a PowerPoint presentation on how the cross should appear for each eye and both eyes together, and they were asked to draw the image as observed. The image to one eye was the bottom and left side of the cross, whereas the image to the other eye was the top and right side of the cross. For subjects who were not able to view the entire cross, the contrast of the image of the nonius cross that was presented to the FE were reduced. With proper alignment, the image was a cross with a square cut out of the center, surrounded by four additional squares and a high contrast border that was visible in both eyes. The above stimulus was presented to all subjects. We found that the perception of the portion of the nonius cross that was presented to the AE was frequently transient despite lowering the contrast of the image of the FE, particularly in strabismic subjects. Subjects with strabismic amblyopia (including older participants) frequently verbalized the transient perception and

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fleeting location of the portion of the nonius cross as seen by the AE as they attempted the alignment procedure. The primary goal of the current study was to evaluate FEMs of the FE and AE eye as the FE contrast was varied. Thus, due to the transient visibility with the variable location of the nonius cross as perceived by several strabismic subjects, we did not shift the target dot location per the nonius cross measurements for consistency of the visual stimulus in the current experiment. Thus, a total of four trials were done under DcV. The target dot (0.5 degrees visual angle) was presented at the center of the screen – the contrast of the target presented to the AE was kept at 100% contrast for all trials, whereas the FE contrast varied from 100% (trial 1), 50% (trial 2), 25% (trial 3), and 10% (trial 4). Each trial lasted for 45 seconds. We used longer fixation times compared to most studies evaluating fixation instability in amblyopia in the literature.[12,71] Our choice of longer fixation times allowed us to optimize micro-saccade detection and provide an adequate sample size for drift and microsaccade analysis. Our and other groups studying FEMS have utilized a similar trial duration to allow ample data collection for FEM analysis.[53,54,72–74] It also balances the amount of experiment time, so it does not appear to be too long, especially for our younger participants, whereas providing enough data for analysis of both eye alignment and FEMs. To maintain the subject's attention, we used a fixation window of 4 × 4 degrees and an auditory alert if the subject's gaze left the area of the fixation window for >500 msec.

Data Analysis

The eye positions were analyzed using MatLab (MathWorks). Fixational saccades and quick phases of nystagmus were identified using the Engbert and Kleigl algorithm.[75–77] We pooled together the quick phases and fixational saccades as they share the same dynamic properties. We computed the frequency (Hertz) of fast FEMs (fixational saccade in controls and patients without nystagmus or quick phase in patients with nystagmus), as described in our previous work.[52,56] Drifts and slow phases were defined as epochs between fixational saccades and quick phases in patients without and with nystagmus, respectively, as described previously.[52,56] The composite amplitude (degrees) and median velocity(degrees/s) of fast and slow FEMs for each eye was calculated as Composite = $[\text{Horizontal}^2 + \text{Vertical}^2]^{1/2}$.

We computed the 25th, 50th, 75th, and 90th percentiles of the composite amplitude and velocity of the FE and AE for each subject obtained during each trial.

The fixation stability was quantified by calculating the bivariate contour ellipse area (BCEA) encompassing 95% of fixation points[12,71,78] and a $\log_{10}$ transformation was applied. Vergence BCEA values were also calculated for all subjects (left XY position-right XY position) on data obtained under monocular, binocular, and dichoptic viewing.[79]

The eye position data were filtered with a moving average filter (window size 1.5 seconds) to remove fast eye movements. The filtered data were then used to compute the composite eye position for each eye. The difference in eye positions between the two eyes were used to calculate the eye alignment (degrees). A histogram of eye alignment for each trial/subject was plotted and the 95% lower and upper bounds were computed.

The eye position traces obtained during BEV, FEV, and AEV were evaluated to categorize patients per their FEM waveforms (Fig. 1).[52] Like controls, patients without nystagmus exhibited inter-saccadic drifts between fixational saccades. Patients with nystagmus were evaluated for the presence of FMN – a marker of disruption of binocularity in early infancy, defined as having a nasally directed slow phase under monocular viewing with the reversal in the direction of the quick phase toward the uncovered eye.[80,81] Patients with jerk nystagmus/nystagmus-like movements with dynamic overshoots who did not exhibit this direction reversal were characterized as nystagmus without

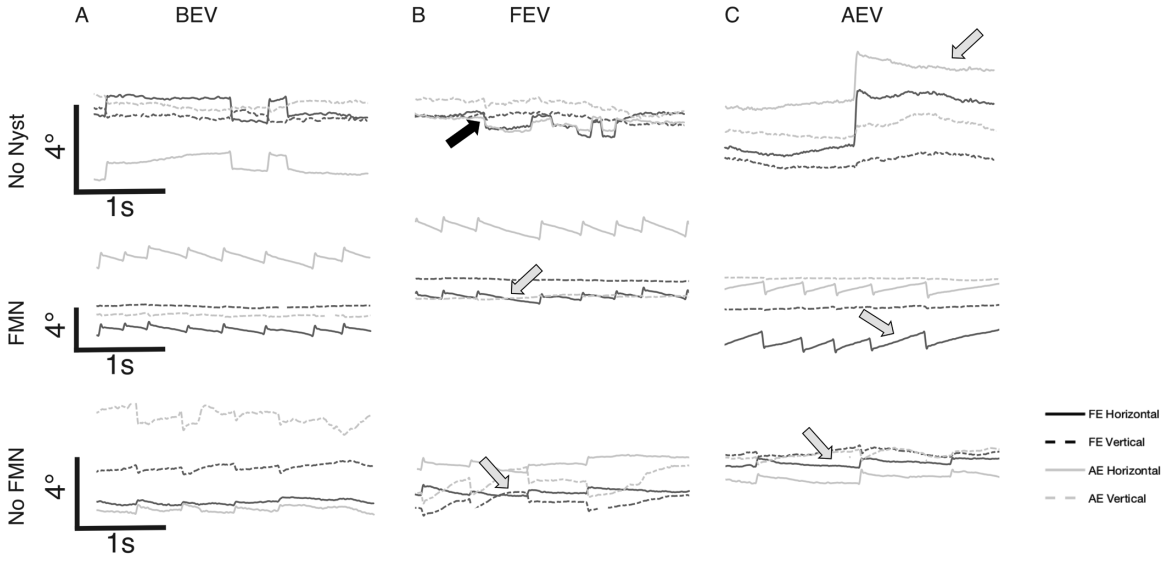

Figure 1. Examples of fixation eye movements during a 3 second epoch under conditions of (**A**) both eye viewing (BEV), (**B**) fellow eye viewing (FEV), and (**C**) amblyopic eye viewing (AEV) from a subject without nystagmus (*top row*), subject with fusion maldevelopment nystagmus (FMN) (*middle row*), and subject with nystagmus without FMN (*bottom row*). The x-axis represents time and the y-axis represents horizontal (*solid line, black*: fellow eye, and *grey*: amblyopic eye) and vertical (*dotted line, black*: fellow eye, and *grey*: amblyopic eye) positions. The *black arrow* represents the fast fixation eye movements (FEMs), whereas the *grey arrows* represent slow FEMs. Rightward and upward movements correspond to positive vertical axis.

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Table 1. Clinical and Demographic Characteristics of Participants

Subject ID	Gender	Age	Type	FEM	VA FEV (LogMAR)	VA AEV (LogMAR)	Stereopsis (Logarc sec)	Refraction OD	Refraction OS	Strabismus Distance (Prism Diopters)
1	M	11	A	None	0.0	1.0	2.3	+2.25 + 1.00 × 75	Plano	Ortho
2	F	6	A	None	0.1	0.3	2.3	+3.50 + 0.50 × 056	+0.25 Sphere	Ortho
3	M	13	A	None	0.0	0.8	2.3	+4.25 +1.25 × 045	+0.25 + 0.50 × 089	Ortho
4	M	6	A	None	0.0	0.2	1.9	+5.25 Sphere	+4.25 Sphere	Ortho
5	F	5	A	No FMN	0.1	0.4	1.8	+7.00 + 1.25 × 085	+7.25 + 1.00 × 092	Ortho
6	F	11	A	None	0.1	0.3	2.1	+4.75 + 0.75 × 090	+5.50 + 1.25 × 080	Ortho
7	F	15	A	None	0.0	0.5	1.9	-0.25 + 1.00 × 085	-2.25 + 6.00 × 085	Ortho
8	F	9	A	None	0.0	0.2	2.3	+2.50 + 2.25 × 087	Plano +2.0 × 080	Ortho
9	F	5	A	None	-0.1	0.1	1.8	Plano +0.75 × 080	Plano +2.5 × 095	Ortho
10	M	15	A	No FMN	-0.1	0.2	2.0	-0.25 + 0.50 × 070	+3.25 + 1.75 × 110	Ortho
11	M	6	A	No FMN	0.0	0.1	2.0	+2.75 + 0.50 × 085	-0.50 + 0.75 × 080	Ortho
12	F	9	A	None	0.0	1.0	1.9	+0.25 + 0.25 × 031	+3.00	Ortho
13	M	8	A	None	0.0	0.3	2.3	+2.0 Sphere	Plano +0.50 × 080	Ortho
14	F	39	M	No FMN	-0.1	0.7	3.5	+3.50 + 0.50 × 030	+1.75	Ortho with glasses
15	M	7	M	None	0.1	0.4	1.8	+0.75 + 1.25 × 105	+3.00 + 1.50 × 081	Ortho with glasses
16	M	8	M	No FMN	0.0	0.3	2.0	+3.25 Sphere	+4.75 Sphere	Ortho with glasses
17	M	6	M	None	0.1	0.4	2.0	+4.25 + 0.50 × 083	+3.25 + 0.50 × 082	RE(T)4
18	F	6	M	None	0.0	0.9	2.6	-8.75	-0.75	RE(T)16
19	M	10	M	No FMN	-0.1	1.0	3.8	+4.0 + 2.0 × 066	-0.25 + 0.75 × 087	Ortho with glasses
20	M	35	M	FMN	0.3	0.8	3.8	-2.50 + 2.75 × 090	-0.50	X(T) 8LH(T)20
21	M	3	M	None	0.2	0.5	3.5	+4.25 + 0.25 × 162	+3.0 + 0.25 × 020	RE(T)8
22	F	7	M	No FMN	-0.1	0.1	3.5	+3.25 + 1.00 × 075	+4.75 + 0.50 × 113	8LE(T)
23	F	34	S	FMN	0.1	0.3	3.8	+0.75 + 2.75 × 090	+0.25 + 2.50 × 090	LX(T)20
24	F	9	S	FMN	0.0	0.2	2.3	+5.75 + 1.5 × 085	+5.25 + 1.25 × 100	Ortho with glasses
25	M	17	S	FMN	0.0	0.2	3.8	+2.5 + 1.0 × 015	+2.5 + 0.5 × 170	Flick DVD
26	M	5	S	No FMN	0.0	0.2	3.9	+2.50 + 2.5 × 070	+2.25 + 0.25 × 020	RE(T)40
27	F	54	S	FMN	0.0	0.2	3.8	Plano +2.50 × 084	+0.50 + 2.00 × 115	RX(T)18-20
28	F	49	S	FMN	0.1	0.2	3.5	-0.50 + 0.75 × 155	-1.0 + 0.50 × 060	X(T)2-4
29	F	33	S	None	0.0	0.4	2.6	-1.25 + 0.75 × 010	-1.75 + 1.25 × 165	X(T)4
30	M	8	S	No FMN	0.0	0.3	3.8	+1.50	+1.50	X(T)12
31	F	6	S	No FMN	0.0	0.3	2.3	+4.00 + 0.75 × 085	+4.75 + 0.75 × 090	RE(T) 4, RHT
32	F	15	S	FMN	0.0	0.2	3.8	+2.5 + 1.75 × 090	+1.00 + 1.25 × 101	X(T)6RHT6
33	M	5	S	No FMN	-0.1	0.2	3.8	+1.5	+1.75	Ortho with glasses
34	F	22	S	FMN	0.0	0.2	3.8	Plano	+0.75 + 0.25 × 150	LE(T)4

A anisometrope; at least one of the following criteria must be met: (a) ≥0.50 D difference between eyes in spherical equivalent or ≥1.50 D difference between eyes in astigmatism in any meridian. ***S*** strabismic; at least one of the following criteria must be met, and criteria are not met for mixed amblyopia: (a) heterotropia at distance (with or without spectacles), (b) history of strabismus surgery, (c) history of strabismus that has resolved with glasses and/or surgery. ***M*** mixed; both of the following criteria must be met: (a) criteria for strabismus (see above), (b) ≥1.00 D difference between eyes in spherical equivalent or ≥1.50 D difference between eyes in astigmatism in any meridian.

F, female; M, male; C, control; FMN, fusion maldevelopment nystagmus; FEM, fixational eye movement; BEV, both eye viewing; FEV, fellow eye viewing; AEV, amblyopic eye viewing; DVD, dissociated vertical deviation; Ortho, Orthotropia; () = intermittent deviation; XT, exotropia; ET, esotropia; HT, hypertropia (preceded by L – left and R – right).

FMN. The slow phase velocities were decreasing or constant, in contrast to the increasing velocity seen in infantile nystagmus. In addition, patients with nystagmus without FMN did not have the dissociated vertical deviation frequently seen in FMN.

We categorized the subjects per the clinical amblyopia type based on Pediatric Eye Disease Investigator Group (PEDIG) studies[63] and FEM waveforms (Table 1). There were 13 anisometropic (10 = no nystagmus and 3 = nystagmus no FMN), 9 mixed (4 = no nystagmus, 4 = nystagmus no FMN, and 1 = FMN), and 12 strabismic (4 = nystagmus no FMN and 8 = FMN) patients with amblyopia. We wanted to examine the effects of different viewing conditions on changes in FEMs and eye alignment in patients with and without FMN. Thus, for analysis, the subjects were divided into four groups: controls (group 0), anisometropic (group 1), mixed/strabismic without FMN (group 2), and mixed/strabismic with FMN (group 3). Thirty percent of the amblyopic subjects had absent stereopsis (group A), 38% had intermediate level with some stereopsis defined as 3500 arc sec to worse than 140 sec arc (group B), and 32% had good stereopsis defined as 140 sec arc or better (group C). None of the subjects in group 1 had absent stereopsis whereas none of the subjects in group 3 had good stereopsis.

The statistical analysis was performed using SPSS. Normality of data was evaluated using the Kolmogorov–Smirnov test. For all the tests, the Levene's test and the Mauchly's test of sphericity indicated that the assumption of homogeneity of variance and sphericity were met. FEM characteristics (log 95% BCEA, vergence BCEA, percentile data of composite amplitude and velocity, and frequency of fast FEMs) and eye alignment were analyzed separately using 2-way repeated-measures ANOVA (one within viewing conditions and one between subject factors – groups 0–3). We also analyzed the fast and slow FEMs and vergence fixation instability in amblyopic subjects per their stereo-acuity deficits using 2-way repeated-measures ANOVA (one within viewing conditions and one between subject factors) and have included the results in supplementary tables. Three-way repeated-measures ANOVA was used to examine the effects of varied contrasts during DcV on FE and AE fixation stability. Visual acuity and stereopsis across the four groups were compared using 1-way ANOVA. Post hoc analyses were conducted on significant main effects and interactions using Bonferroni correction. Mann-Whitney *U* test was used to compare the age between controls versus amblyopic subjects. All statistical tests had a critical alpha value of 0.05.

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Results

We examined the effects of monocular, binocular, and dichoptic viewing on fast and slow FEMs, fixation stability, and eye alignment across the four groups. There were no differences in age (years) between controls (age range = 6–44 years) versus amblyopes (controls =14.6 ± 13.3, amblyopes = 16.4 ± 13.3, $P = 0.7$). There was no difference in the AE visual acuity (logMAR) across the amblyopes (group 1 = 0.41 ± 0.31, group 2 = 0.39 ± 0.33, and group 3 = 0.27 ± 0.21, F(2,33) = 0.5, $P = 0.5$). The stereopsis (log arcsec) was significantly worse in group 3 (group 1 = 2.0 ± 0.2, group 2 = 3.0 ± 0.8, and group 3 = 3.5 ± 0.5, F(2,33) = 18.7, $P < 0.0001$, Bonferroni correction group 1 vs. 2 $P = 0.001$, and group 1 vs. 3 $P < 0.0001$).

Monocular and Binocular Viewing

Fast and Slow FEMs

Table 2 depicts the frequencies of fast FEMs obtained during BEV, FEV, and AEV and were greatest in group 3. There was no effect of viewing conditions on frequencies F(2,70) = 0.96, $P = 0.38$, $\eta_p^2 = 0.02$ with no interaction between viewing conditions and groups, F(6,70) = 0.29, $P = 0.93$, $\eta_p^2 = 0.02$. Figure 2 summarizes the normalized cumulative sum histogram of the composite amplitude of fast FEMs of the FE (top) and AE (bottom) during monocular and binocular viewing in groups 0 and 1 (Figs. 2A, 2C) and in groups 0, 2, and 3 (Figs. 2B, 2D). There is a rightward shift of the distribution in the amplitude of AE during AEV than BEV particularly for patients in group 3, which was significant for the 25th and 50th percentiles (Table 3). We analyzed the percentile data as a function of stereopsis deficits obtained under FEV, AEV, and BEV. We found that for the 25th percentile the amplitude of the AE was greater during AEV than during BEV (Supplementary Table S1).

Figure 3 summarizes the normalized cumulative sum histogram of the composite velocity of slow FEMs of the FE (top) and AE (bottom) during monocular and binocular viewing in groups 0 and 1 (Figs. 3A, 3C) and groups 0, 2, and 3 (Figs. 3B, 3D). There is a rightward shift of the distribution of the velocity of the AE during AEV than BEV in patients in group 3. We analyzed the percentiles and found that the velocity of the AE is greater during AEV than BEV in group 3 with a significant interaction for the 90th percentile (see Table 3). We analyzed the percentile data of the composite velocity as a function of stereopsis deficits and did not see any statistical significance across groups and viewing conditions (see Supplementary Table S1).

Table 2. Frequency of Fast FEMs During Monocular and Binocular Viewing Across the Four Groups

Groups	BEV (Hz)	FEV (Hz)	AEV (Hz)
Group 0	0.91 ± 0.44	0.86 ± 0.44	1.0 ± 0.45
Group 1	1.0 ± 0.49	1.0 ± 0.40	1.0 ± 0.54
Group 2	1.1 ± 0.59	1.0 ± 0.56	1.1 ± 0.49
Group 3	1.7 ± 1.1	1.7 ± 0.81	1.9 ± 0.98

BEV, both eye viewing; FEV, fellow eye viewing; AEV, amblyopic eye viewing.

Group 0 = controls; group 1 = anisometropic amblyopia; group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus; group 3 = mixed/strabismic amblyopia with fusion maldevelopment nystagmus.

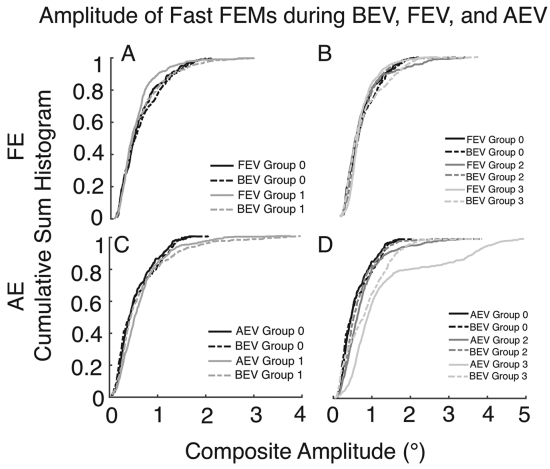

Figure 2. Cumulative sum histogram of composite amplitude (degrees) of fast fixation eye movements of fellow eye obtained during fellow eye viewing and both eye viewing in groups 0 vs. 1 (**A**) and groups 0, 1, and 2 (**B**) and amblyopic eye obtained during amblyopic eye viewing and both eye viewing in groups 0 vs. 1 (**C**) and groups 0, 1, and 2 (**D**).

Fixation Stability

We compared the log BCEA of the FE during FEV (group 0 = 0.38 ± 0.35, group 1 = 0.52 ± 0.35, group 2 = 0.64 ± 0.53, and group 3 = 0.37 ± 0.18) and BEV (group 0 = 0.4 ± 0.41, group 1 = 0.53 ± 0.358, group 2 = 0.50 ± 0.35, and group 3 = 0.36 ± 0.26). There was no effect of viewing conditions on log BCEA of FE, F(1,36) = 0.04, $P = 0.83$, $\eta_p^2 = 0.001$ with no interaction between viewing conditions and groups, F(3,36) = 1.5, $P = 0.2$, $\eta_p^2 = 0.11$. Similarly, we found no difference in log BCEA of the AE during AEV (group 0 = 0.39 ± 0.44, group 1 = 0.73 ± 0.41, group 2 = 0.6 ± 0.36, and group 3 = 0.61 ± 0.35) and BEV (group 0 = 0.4 ± 0.45, group 1 = 0.72 ± 0.49, group 2 = 0.79 ± 0.42, and group 3 = 0.69 ± 0.43) F(1,36) = 1.67, $P = 0.21$, $\eta_p^2 = 0.04$ with no interaction between viewing conditions and groups F(3,36) = 0.50, $P = 0.68$, $\eta_p^2 = 0.04$. The log BCEA of the AE is higher than FE and controls.[12,53,55]

We analyzed the log vergence BCEA and found that the values were greater during AEV (group 0 = 0.73 ± 0.48, group 1 = 0.91 ± 0.54, group 2 = 0.94 ± 0.45, and group 3 = 1.06 ± 0.27) and FEV (group 0 = 0.65 ± 0.48, group 1 = 0.73 ± 0.42, group 2 = 0.91 ± 0.29, and group 3 = 0.99 ± 0.22) than during BEV (group 0 = 0.47 ± 0.36, group 1 = 0.74 ± 0.48, group 2 = 0.63 ± 0.37, and group 3 = 0.76 ± 0.35) for all groups, F(2,72) = 8.0, $P = 0.001$, $\eta_p^2 = 0.18$ with no interaction between viewing conditions and groups, F(6,72) = 0.56, $P = 0.75$, $\eta_p^2 = 0.04$. This is in agreement with prior studies which have shown higher vergence BCEA in patients with amblyopia than controls.[52]

We also analyzed log vergence BCEA as a function of stereoacuity deficits and found a similar result where the values were greater during AEV (group A = 0.97 ± 0.30, group B = 1.19 ± 0.47, group C = 0.65 ± 0.35, and group D = 0.73 ± 0.48) and FEV (group A = 0.95 ± 0.27, group B = 0.97 ± 0.30, group C = 0.65 ± 0.37, and group D = 0.65 ± 0.48) than during BEV (group A = 0.77 ± 0.31, group B = 0.82 ± 0.39, group C = 0.48 ± 0.45, and group D = 0.47 ± 0.36) for all groups, F(2,72) = 7.5, $P = 0.001$, $\eta_p^2 = 0.17$ with no interaction between viewing conditions and

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Table 3. Percentile of Composite Amplitude and Velocity of Fast and Slow Fixation Eye Movements of Fellow Eye and Amblyopic Eye During Monocular and Binocular Viewing Across the Four Groups

	Composite Amplitude (Degrees) of Fast FEMs					
Percentile	**Group 0**	**Group 1**	**Group 2**	**Group 3**	**Main Effect**	**Interaction**
25th	0.39 ± 0.20 (0.45 ± 0.24) 0.45 ± 0.24 (0.40 ± 0.20)	0.36 ± 0.14 (0.39 ± 0.20) 0.48 ± 0.28 (0.38 ± 0.14)	0.40 ± 0.19 (0.37 ± 0.15) 0.46 ± 0.15 (0.38 ± 0.12)	0.42 ± 0.15 (0.40 ± 0.28) 0.97 ± 1.0 (0.50 ± 0.36)	F = 0.15, $P = 0.71$, η_p^2 = 0.004; **F = 8.6, $P = 0.006$, $\eta_p^2 = 0.19$**	F = 0.83, $P = 0.49$, η_p^2 = 0.064; F = 2.4, $P = 0.082$, $\eta_p^2 = 0.17$
50th	0.56 ± 0.26 (0.60 ± 0.29) 0.58 ± 0.29 (0.60 ± 0.28)	0.50 ± 0.23 (0.56 ± 0.30) 0.68 ± 0.35 (0.60 ± 0.32)	0.61 ± 0.40 (0.56 ± 0.20) 0.65 ± 0.22 (0.54 ± 0.17)	0.54 ± 0.19 (0.55 ± 0.37) 1.1 ± 1.1 (0.65 ± 0.40)	F = 0.18, $P = 0.68$, η_p^2 = 0.005; **F = 5.6, $P = 0.023$, $\eta_p^2 = 0.14$**	F = 0.53, $P = 0.66$, η_p^2 = 0.042; F = 2.2, $P = 0.11$, $\eta_p^2 = 0.15$
75th	0.72 ± 0.34 (0.83 ± 0.40) 0.75 ± 0.32 (0.81 ± 0.38)	0.69 ± 0.34 (0.81 ± 0.47) 0.92 ± 0.46 (0.89 ± 0.53)	0.94 ± 0.66 (0.79 ± 0.27) 1.0 ± 0.52 (0.81 ± 0.30)	0.71 ± 0.26 (0.74 ± 0.45) 1.4 ± 1.2 (0.89 ± 0.50)	F = 0.21, $P = 0.65$, η_p^2 = 0.006; F = 2.6, $P = 0.14$, $\eta_p^2 = 0.068$	F = 1.2, $P = 0.34$, $\eta_p^2 = 0.088$; F = 1.4, $P = 0.27$, $\eta_p^2 = 0.10$
90th	0.95 ± 0.40 (1.1 ± 0.49) 0.89 ± 0.33 (0.99 ± 0.41)	0.91 ± 0.46 (1.1 ± 0.60) 1.3 ± 0.62 (1.3 ± 0.88)	1.5 ±1.2 (1.0 ± 0.33) 1.4 ± 0.83 (1.1 ± 0.43)	0.95 ± 0.37 (0.95 ± 0.46) 1.7 ± 1.3 (1.2 ± 0.53)	F = 0.64, $P = 0.80$, η_p^2 = 0.002; F = 2.0, $P = 0.17$, $\eta_p^2 = 0.053$	F = 1.8, $P = 0.17$, $\eta_p^2 = 0.13$; F = 1.1, $P = 0.38$, $\eta_p^2 = 0.081$
Composite velocity (degrees/s) of slow FEMs						
25th	0.27 ± 0.17 (0.33 ± 0.13) 0.25 ± 0.090 (0.27 ± 0.094)	0.35 ± 0.14 (0.32 ± 0.22) 0.35 ± 0.23 (0.37 ± 0.23)	0.30 ± 0.15 (0.26 ± 0.092) 0.33 ± 0.066 (0.34 ± 0.15)	0.63 ± 0.40 (1.1 ± 1.7) 2.9 ± 5.1 (1.2 ± 1.7)	F = 0.97, $P = 0.33$, η_p^2 = 0.026; F = 2.3, $P = 0.14$, $\eta_p^2 = 0.061$	F = 0.98, $P = 0.41$, η_p^2 = 0.076; F = 2.3, $P = 0.094$, $\eta_p^2 = 0.16$
50th	0.50 ± 0.18 (0.56 ± 0.24) 0.44 ± 0.12 (0.54 ± 0.20)	0.61 ± 0.21 (0.55 ± 0.32) 0.55 ±0.33 (0.66 ± 0.38)	0.54 ± 0.38 (0.43 ± 0.14) 0.50 ± 0.13 (0.54 ± 0.15)	1.1 ± 1.1 (1.4 ± 2.0) 3.4 ± 5.7 (1.7 ± 2.0)	F = 0.12, $P = 0.74$, η_p^2 = 0.003; F = 1.8, $P = 0.19$, $\eta_p^2 = 0.048$	F = 0.74, $P = 0.54$, η_p^2 = 0.058; F = 2.5, $P = 0.079$, $\eta_p^2 = 0.17$
75th	0.79 ± 0.25 (0.91 ± 0.43) 0.81 ± 0.27 (0.98 ± 0.45)	1.0 ± 0.66 (0.90 ± 0.66) 0.93 ± 0.54 (1.1 ± 0.53)	0.91 ± 0.91 (0.74 ± 0.18) 0.84 ± 0.29 (0.90 ± 0.31)	1.6 ± 1.6 (1.7 ± 2.2) 4.2 ± 6.3 (2.1 ± 2.2)	F = 0.031, $P = 0.86$, η_p^2 = 0.001; F = 1.7, $P = 0.20$, $\eta_p^2 = 0.046$	F = 0.34, $P = 0.80$, η_p^2 = 0.027; F = 2.9, $P = 0.051$, $\eta_p^2 = 0.19$
90th	1.2 ± 0.30 (1.3 ± 0.64) 1.3 ± 0.44 (1.4 ± 0.55)	1.5 ± 1.1 (1.3 ± 0.96) 1.4 ± 0.90 (1.8 ± 1.2)	1.7 ± 2.5 (1.1 ± 0.25) 1.2 ± 0.49 (1.4 ± 0.60)	2.1 ± 2.0 (2.0 ± 2.3) 5.2 ± 6.9 (2.5 ± 2.3)	F = 0.37, $P = 0.55$, η_p^2 = 0.010; F = 1.7, $P = 0.20$, $\eta_p^2 = 0.045$	F = 0.40, $P = 0.75$, η_p^2 = 0.033; **F = 3.8, $P = 0.019$, $\eta_p^2 = 0.24$***

Top row = fellow eye and bottom row = Amblyopic eye. All values in parenthesis represent data obtained during both eye viewing.

Group 0 = controls, group 1 = anisometropic amblyopia, group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus, and group 3 = mixed/strabismic amblyopia with fusion maldevelopment nystagmus.

Post hoc Bonferroni correction was performed with $P < 0.05$ indicated by * = group 0 versus group 3.

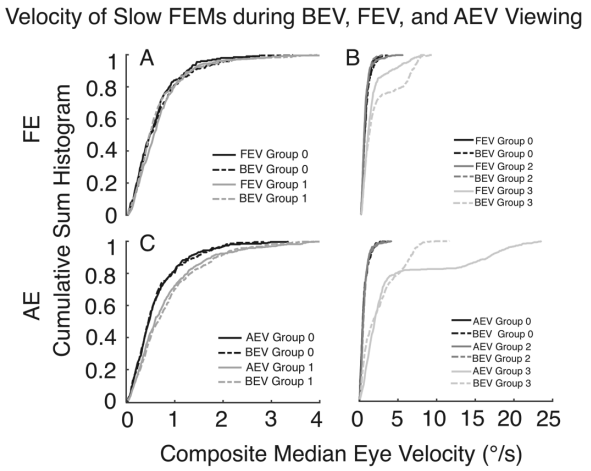

Figure 3. Cumulative sum histogram of composite eye velocity (degrees/sec) of slow fixation eye movements of fellow eye obtained during fellow eye viewing and both eye viewing in groups 0 vs. 1 (**A**) and groups 0, 1, and 2 (**B**) and amblyopic eye obtained during amblyopic eye viewing and both eye viewing in groups 0 vs. 1 (**C**) and groups 0, 1, and 2 (**D**).

groups, F(6,72) = 0.43, $P = 0.85$, $\eta_p^2 = 0.03$. In agreement with previous studies, we found that amblyopic subjects with good stereopsis had lower vergence BCEA suggestive of less vergence instability than those with some or absent stereopsis.[52]

Eye Alignment

Figure 4 depicts the scatter plot of eye positions in a subject from group 3 during BEV, FEV, and AEV. The bottom panel depicts the histogram of composite eye position differences of the right and left eyes from the same subject. The 95% upper and lower bounds of the histogram are greatest during FEV. We calculated the 95% upper and lower bounds of the histogram of eye alignment obtained from each subject/trial and pooled the values across the 4 groups (Table 4). We found that the eye misalignment was greatest during FEV for all amblyopic subjects with most increases in group 3 (lower bound: main effect F(2,70) = 3.9, $P = 0.02$, $\eta_p^2 = 0.1$), pairwise comparison BEV versus FEV: 0.009, interaction (F(6,70) = 2.2, $P = 0.049$, $\eta_p^2 = 0.16$), pairwise comparison group 0 vs. 3: $P = 0.007$; upper bound: main effect (F(2,70) = 3.4, $P = 0.03$, $\eta_p^2 = 0.09$), pairwise comparison BEV versus FEV: 0.005, interaction (F(6,70) = 2.1, $P = 0.06$, $\eta_p^2 = 0.15$), pairwise comparison group 0 vs. 3: $P = 0.006$).

Table 4. Eye Alignment (Degrees) During Monocular and Binocular Viewing Across the Four Groups

Groups	BEV (degrees)	FEV (degrees)	AEV (degrees)
Group 0	0.75 ± 0.82	0.67 ± 0.75	0.78 ± 0.68
	2.17 ± 0.91	2.6 ± 1.0	3.4 ± 1.3
Group 1	2.0 ± 2.4	2.5 ± 3.1	1.9 ± 3.3
	5.1 ± 4.2	5.0 ± 3.9	4.6 ± 5.1
Group 2	1.7 ± 1.9	4.4 ± 3.8	4.5 ± 4.4
	3.8 ± 2.7	7.6 ± 4.1	7.5 ± 6.3
Group 3	6.2 ± 6.1	9.8 ± 8.9	5.6 ± 6.0
	9.6 ± 8.6	13.5 ± 8.6	9.1 ± 5.8

The 95% lower bound (top row) and 95% upper bound (bottom row) of the histogram of composite eye position difference between the right and left eyes.

BEV, both eye viewing; FEV, fellow eye viewing; AEV, amblyopic eye viewing.

Group 0 = controls, group 1 = anisometropic amblyopia, group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus, and group 3 = mixed/ strabismic amblyopia with fusion maldevelopment nystagmus.

Dichoptic Viewing at Varied FE Contrasts

Fast and Slow FEMs

Table 5 depicts the frequencies of fast FEMs obtained during DcV and were greatest in group 3. There was no effect of

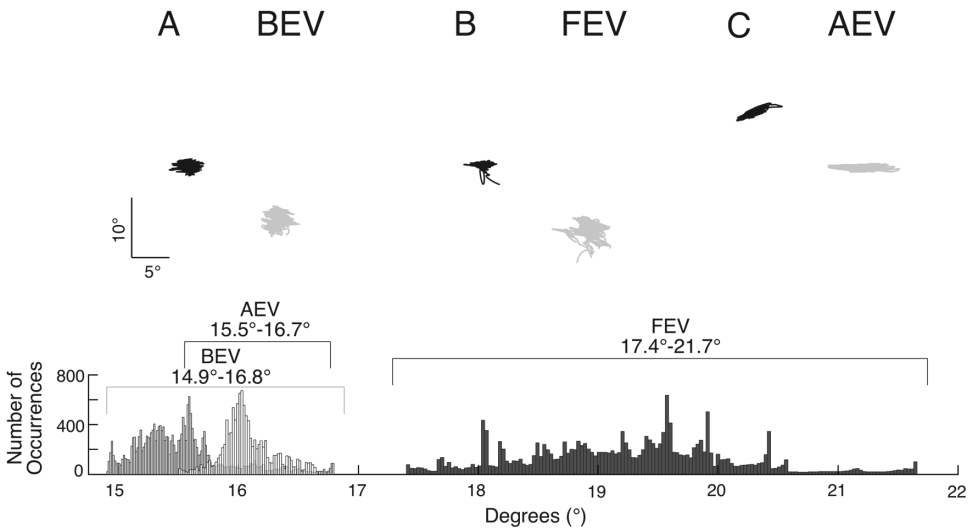

Figure 4. *Top*: Horizontal and vertical eye positions of fellow eye (*black*) and amblyopic eye (*grey*) during both eye viewing (BEV), fellow eye viewing (FEV), and amblyopic eye viewing (AEV). Rightward and upward movements correspond to positive vertical axis. Notice the right hypotropia and exotropia during BEV and FEV condition and left exotropia and left hypertropia during AEV. *Bottom*: Histogram showing range of composite eye position difference during BEV (*grey*), FEV (*black*), and AEV (*white*).

Table 5. Frequency of Fast Fixation Eye Movements (Hertz) During Dichoptic Viewing Across the Four Groups

Groups	FE Contrast 100%	FE Contrast 50%	FE Contrast 25%	FE Contrast 10%
Group 0	0.74 ± 0.32	0.86 ± 0.40	0.96 ± 0.35	0.95 ± 0.50
Group 1	1.1 ± 0.42	0.95 ± 0.35	0.93 ± 0.33	0.94 ± 0.34
Group 2	1.1 ± 0.54	1.2 ± 0.53	1.2 ± 0.55	1.2 ± 0.42
Group 3	2.2 ± 1.3	2.1 ± 1.2	1.9 ± 0.79	1.9 ± 1.3

FE = fellow eye contrast varied at 100%, 50%, 25%, and 10% while amblyopic eye contrast was at 100% for all dichoptic viewing trials.

Group 0 = controls, group 1 = anisometropic amblyopia, group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus, and group 3 = mixed/strabismic amblyopia with fusion maldevelopment nystagmus.

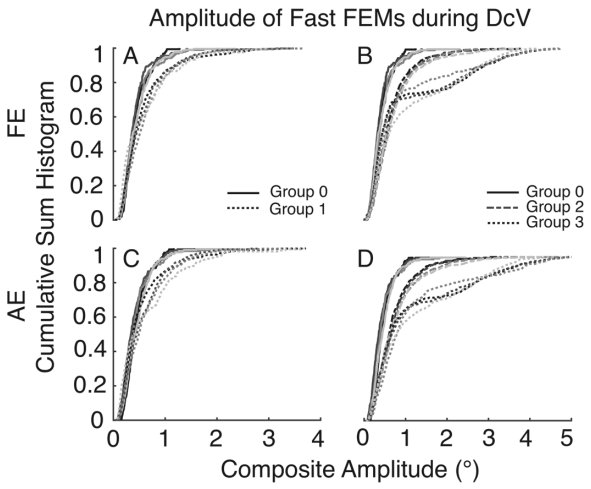

Figure 5. Cumulative sum histogram of composite amplitude (degrees) of fast fixation eye movements of fellow eye and amblyopic eye obtained during dichoptic viewing across fellow eye contrasts of 100% (*black*), 50% (*darkest grey*), 25% (*lighter grey*), and 10% (*lightest grey*) in groups 0 vs. 1 (**A, C**) and groups 0, 1, and 2 (**B, D**).

DcV at varied FE contrasts on frequencies of FEMs F(3,9) = 0.25, $P = 0.85$, $\eta_p^2 = 0.008$ with no interaction between FE contrasts and groups, F(9,90) = 1.5, $P = 0.16$, $\eta_p^2 = 0.13$. Figure 5 summarizes the normalized cumulative sum histogram of the composite amplitude of fast FEMs of the FE (top) and AE (bottom) during DcV at varied FE contrasts in groups 0 and 1 (see Figs. 5A, 5C) and in groups 0, 2, and 3 (see Figs. 5B, 5D). There is a rightward shift of the distribution of amplitude of FE and AE of all amblyopes than controls which was most pronounced in group 3. For all percentiles, the amplitude of the FE and AE is greater when FE contrast is 10% vs. 100% and for the 50th and 75th percentiles when FE contrast is 25% vs. 100%. There was no statistically significant interaction across groups as a function of various FE contrasts (Table 6). We also analyzed the percentile data of the FE and AE as a function of stereopsis deficits during DcV. We found that for 25th, 50th, and 75th percentiles, the amplitude of the FE and AE were greater when FE contrast is 10% vs. 100% and for the 50th percentiles when FE contrast is 25% vs. 100%. There was no statistically significant interaction across groups as a function of various FE contrasts (Supplementary Table S2).

Figure 6 summarizes the normalized cumulative sum histogram of the composite velocity of slow FEMs of the FE (top) and AE (bottom) during DcV in groups 0 and 1 (see Figs. 6A, 6C) and groups 0, 2, and 3 (see Figs. 6B, 6D). There is a rightward shift of the distribution of velocity of FE and AE of all amblyopes than controls which was most pronounced in group 3. We computed the percentiles of the composite velocity of the FE and AE for each subject obtained at various FE contrasts and found no significant differences across the four groups (Table 7). We also computed the percentiles of the composite velocity of the FE and AE for each subject obtained at various FE contrasts under DcV per the stereopsis deficits and found no significant differences across the groups (Supplementary Table S3).

Fixation Stability

Table 8 depicts fixation stability of the right eye (100% contrast) and the left eye (varied contrasts) in group 0 and FE (varied contrasts) and AE (100% contrast) in group 1. We conducted a 3-way ANOVA evaluating the effects of FE contrasts on FE and AE fixation stability. We found a statistically significant three-way interaction between FE contrasts, eye in groups 0 vs. 1, F(3,48) = 2.8, $P = 0.04$, $\eta_p^2 = 0.15$. There was a significant simple two-way interaction between FE contrast and eye for group 0, F(3,18) = 6.61, $P = 0.015$, $\eta_p^2 = 0.52$ but not for group 1, F(3,30) = 0.32, $P = 0.8$, $\eta_p^2 = 0.03$. The simple main effect of varying contrast for group 0 in the left eye was statistically significant, F(318) = 4.5, $P = 0.015$, $\eta_p^2 = 0.43$ but not in the right eye, F(3,18) = 0.33, $P = 0.8$, $\eta_p^2 = 0.05$. The results suggest the differential effects on fixation stability of the two eyes in group 0, where the fixation instability of the left eye whose contrast was varied increased without any change in the fixation instability of the right eye. The fixation instability of both the FE and AE was greater in group 1 and increased further with reduced FE contrast particularly at 25% and 10%.

The log vergence BCEA was higher in patients with amblyopia compared to controls during DcV (Table 9). There was an effect of various FE contrasts under DcV on the vergence BCEA, F(3,90) = 3.0, $P = 0.03$, $\eta_p^2 = 0.09$ with no interaction between viewing conditions and groups, F(9,90) = 0.58, $P = 0.80$, $\eta_p^2 = 0.05$. The log vergence BCEA was higher in amblyopic patients particularly those with absent and some stereopsis during DcV (Supplementary Table S4). There was an effect of various FE contrasts under DcV on the vergence BCEA, F(3,90) = 3.3, $P = 0.03$, $\eta_p^2 = 0.09$ with no interaction between viewing conditions and groups, F(9,90) = 1.3, $P = 0.24$, $\eta_p^2 = 0.11$.

Eye Alignment

Figure 7 plots eye positions of the FE and AE from 2 subjects (left- subject 1 = group 2 and right-subject 2 = group 3). In subject 1, the AE picked up fixation during DcV at 25% and 10% FE contrast (fixation switch) (i.e. the AE attends to presented target). In subject 2, the FE fixates with an increase in eye misalignment at lower FE contrasts (arrows) with no fixation switch (i.e. the AE did not attend to the target). We found that 5 of 12 patients in group 2 vs. 1 of 9 patients in group 3 exhibited a fixation switch during DcV at lower FE contrasts.

Figure 8 depicts the scatter plot of eye positions (right panel) in the same subject as used in Figure 4 during DcV.

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Table 6. Percentile Composite Amplitude (Degrees) of Fast Fixation Eye Movements of Fellow Eye and Amblyopic Eye during Dichoptic Viewing Across the Four Groups

Percentile	FE contrast	Group 0	Group 1	Group 2	Group 3	Main effect	Interaction
25th	100%	0.37 ± 0.15 (0.35 ± 0.09)	0.32 ± 0.13 (0.31 ± 0.15)	0.41 ± 0.14 (0.41 ± 0.16)	0.72 ± 0.95 (0.79 ± 0.98)	F = 5.1, $P = 0.003$, $\eta_p^2 = 0.15$ * (F = 2.9, $P = 0.04$, $\eta_p^2 = 0.08$) † * = 100% vs. 10%, $P = 0.01$, † = 100% vs. 10%, $P = 0.02$	F = 1.8, $P = 0.096$, $\eta_p^2 = 0.15$ (F = 0.87, $P = 0.54$, $\eta_p^2 = 0.080$)
	50%	0.33 ± 0.13 (0.35 ± 0.20)	0.29 ± 0.10 (0.30 ± 0.11)	0.49 ± 0.18 (0.48 ± 0.17)	0.66 ± 0.84 (0.73 ± 0.86)		
	25%	0.35 ± 0.14 (0.35 ± 0.18)	0.37 ± 0.12 (0.35 ± 0.13)	0.49 ± 0.17 (0.49 ± 0.17)	0.66 ± 0.77 (0.75 ± 0.79)		
	10%	0.37 ± 0.14 (0.37 ± 0.13)	0.38 ± 0.18 (0.38 ± 0.20)	0.54 ± 0.25 (0.50 ± 0.20)	0.80 ± 0.80 (0.84 ± 0.82)		
50th	100%	0.48 ± 0.17 (0.45 ± 0.12)	0.46 ± 0.18 (0.43 ± 0.22)	0.60 ± 0.26 (0.59 ± 0.24)	0.89 ± 1.1 (1.0 ± 1.2)	F = 5.2, $P = 0.004$, $\eta_p^2 = 0.15$ * (F = 5.6, $P = 0.002$, $\eta_p^2 = 0.16$) † * = 100% vs. 10%, $P = 0.01$ 100% vs. 25%, $P = 0.01$ † = 100% vs. 10%, $P = 0.005$ 100% vs. 25%, $P = 0.004$	F = 1.1, $P = 0.39$, $\eta_p^2 = 0.098$ (F = 1.0, $P = 0.43$, $\eta_p^2 = 0.093$)
	50%	0.49 ± 0.31 (0.46 ± 0.27)	0.44 ± 0.17 (0.45 ± 0.19)	0.68 ± 0.25 (0.68 ± 0.26)	0.83 ± 1.0 (0.97 ± 1.1)		
	25%	0.48 ± 0.24 (0.47 ± 0.23)	0.57 ± 0.22 (0.58 ± 0.25)	0.68 ± 0.23 (0.68 ± 0.24)	0.97 ± 1.2 (1.2 ± 1.4)		
	10%	0.47 ± 0.17 (0.48 ± 0.16)	0.57 ± 0.32 (0.55 ± 0.31)	0.75 ± 0.32 (0.75 ± 0.31)	1.0 ± 0.91 (1.1 ± 0.91)		
75th	100%	0.60 ± 0.23 (0.60 ± 0.19)	0.72 ± 0.47 (0.69 ± 0.49)	0.83 ± 0.40 (0.87 ± 0.38)	1.1 ± 1.3 (1.3 ± 1.4)	F = 3.4, $P = 0.02$, $\eta_p^2 = 0.10$* (F = 3.7, $P = 0.01$, $\eta_p^2 = 0.11$) † * = 100% vs. 10%, $P = 0.02$ 100% vs. 25%, $P = 0.01$ † = 100% vs. 10%, $P = 0.04$ 100% vs. 25%, $P = 0.03$	F = 0.57, $P = 0.82$, $\eta_p^2 = 0.054$ (F = 0.55, $P = 0.80$, $\eta_p^2 = 0.052$)
	50%	0.63 ± 0.36 (0.57 ± 0.28)	0.79 ± 0.38 (0.73 ± 0.35)	0.96 ± 0.41 (0.98 ± 0.38)	1.1 ± 1.3 (1.3 ±1.4)		
	25%	0.62 ± 0.29 (0.58 ± 0.28)	0.86 ± 0.33 (0.81 ± 0.35)	1.0 ± 0.39 (1.1 ± 0.45)	1.3 ± 1.6 (1.5 ± 1.7)		
	10%	0.65 ± 0.24 (0.65 ± 0.22)	0.82 ± 0.46 (0.81 ± 0.48)	1.1 ± 0.56 (1.1 ± 0.61)	1.4 ± 1.0 (1.5 ± 1.0)		
90th	100%	0.71 ± 0.22 (0.76 ± 0.20)	1.1 ± 0.81 (1.0 ± 0.76)	1.1 ± 0.53 (1.2 ± 0.54)	1.4 ± 1.5 (1.7 ± 1.7)	F = 3.0, $P = 0.03$, $\eta_p^2 = 0.09$* (F = 2.9, $P = 0.04$, $\eta_p^2 = 0.08$) † * = 100% vs. 10%, $P = 0.05$ †= 100% vs. 10%, $P = 0.03$	F = 0.79, $P = 0.60$, $\eta_p^2 = 0.074$ (F = 0.57, $P = 0.80$, $\eta_p^2 = 0.054$)
	50%	0.71 ± 0.36 (0.68 ± 0.33)	1.2 ± 0.69 (1.2 ± 0.80)	1.3 ± 0.61 (1.6 ± 1.7)	1.4 ± 1.6 (1.6 ± 1.7)		
	25%	0.74 ± 0.35 (0.72 ± 0.34)	1.2 ± 0.55 (1.2 ± 0.63)	1.4 ± 0.63 (1.5 ± 0.63)	1.6 ± 2.0 (2.0 ± 2.3)		
	10%	0.83 ± 0.35 (0.82 ± 0.35)	1.2 ± 0.54 (1.3 ± 0.80)	1.6 ± 0.97 (1.7 ± 0.96)	1.7 ± 1.1 (2.0 ± 1.0)		

FE = Fellow eye contrast varied at 100%, 50%, 25%, and 10% while amblyopic eye contrast was at 100% for all dichoptic viewing trials.

All the values in parenthesis are for amblyopic eye. Post hoc Bonferroni correction was performed with $P < 0.05$ indicated by * for FE and † for AE data in parenthesis. Group 0 = controls, group 1 = anisometropic amblyopia, group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus, and group 3 = mixed/strabismic amblyopia with fusion maldevelopment nystagmus.

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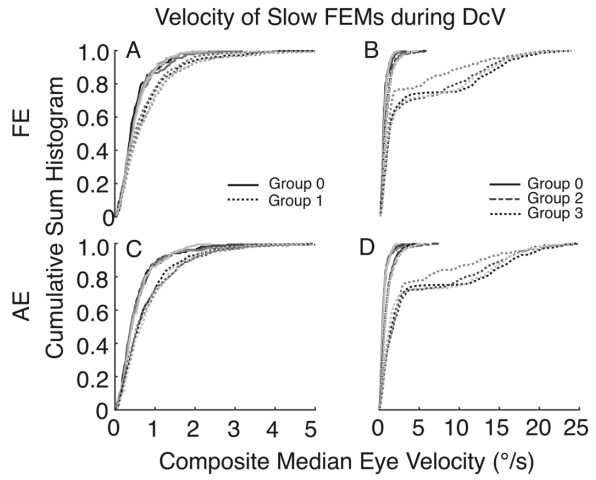

Figure 6. Cumulative sum histogram of composite velocity (degrees/sec) of slow fixation eye movements of fellow eye and amblyopic eye obtained during dichoptic viewing across fellow eye contrasts of 100% (*black*), 50% (*darkest grey*), 25% (*lighter grey*), and 10% (*lightest grey*) in groups 0 vs. 1 (**A, C**) and groups 0, 1, and 2 (**B, D**).

Table 8. Bivariate Contour Ellipse Area of FE and AE During Dichoptic Viewing in Controls and Patients With Anisometropic Amblyopia

Groups	FE Contrast 100%	FE Contrast 50%	FE Contrast 25%	FE Contrast 10%
Group 0	0.22 ± 0.33	0.35 ± 0.34	0.32 ± 0.43	0.49 ± 0.45
	0.30 ± 0.25	0.36 ± 0.32	0.35 ± 0.36	0.28 ± 0.40
Group 1	0.32 ± 0.55	0.37 ± 0.54	0.49 ± 0.49	0.52 ± 0.56
	0.40 ± 0.59	0.39 ± 0.53	0.54 ± 0.43	0.56 ± 0.47

FE = Fellow eye (right eye for controls) contrast varied at 100%, 50%, 25%, and 10% while amblyopic eye (left eye for controls) contrast was at 100% for all dichoptic viewing trials. The values are Log BCEA 95% (log 10[BCEA (deg^2)])

Top row = fellow eye, and bottom row = amblyopic eye.

Group 0 = controls and group 1 = anisometropic amblyopia.

The left panel depicts the histogram of composite eye position differences of the right and left eyes for each trial. We calculated the 95% upper and lower bounds of the histogram of eye alignment obtained from each subject at various FE contrasts and pooled the values across the 4 groups (Table 10). There was no statistically significant difference

Table 7. Composite Velocity of Slow Fixation Eye Movements of Fellow Eye and Amblyopic Eye During Dichoptic Viewing Across the Four Groups

Percentile	FE contrast	Group 0	Group 1	Group 2	Group 3	Main effect	Interaction
25th	100%	0.22 ± 0.10	0.30 ± 0.14	0.34 ± 0.15	2.8 ± 5.2	F = 1.9, P = 0.14, η_p^2	F = 2.2, P = 0.11, η_p^2
		(0.23 ± 0.11)	(0.32 ± 0.13)	(0.40 ± 0.17)	(3.3 ± 6.0)	= 0.058 (F = 2.2, P	= 0.18 (F = 1.7, P =
						= 0.15, η_p^2 = 0.067)	0.17, η_p^2 = 0.15)
	50%	0.29 ± 0.12	0.27 ± 0.17	0.39 ± 0.17	2.3 ± 4.1		
		(0.29 ± 0.11)	(0.30 ± 0.13)	(0.41 ± 0.18)	(2.8 ± 4.7)		
	25%	0.30 ± 0.11	0.33 ± 0.18	0.43 ± 0.12	1.7 ± 2.6		
		(0.25 ± 0.097)	(0.29 ± 0.15)	(0.44 ± 0.15)	(2.1 ± 3.2)		
	10%	0.29 ± 0.088	0.30 ± 0.21	0.42 ± 0.13	2.6 ± 4.5		
		(0.28 ± 0.12)	(0.30 ± 0.21)	(0.48 ± 0.19)	(2.9 ± 5.3)		
50th	100%	0.41 ± 0.15	0.50 ± 0.20	0.57 ± 0.25	3.5 ± 6.2	F = 0.67, P = 0.47, η_p^2	F = 2.1, P = 0.098, η_p^2
		(0.39 ± 0.18)	(0.53 ± 0.18)	(0.68 ± 0.29)	(4.0 ± 6.9)	= 0.022 (F = 1.0, P	= 0.17 (F = 1.2, P =
						= 0.36, η_p^2 = 0.033)	0.31, η_p^2 = 0.11)
	50%	0.46 ± 0.20	0.47 ± 0.20	0.63 ± 0.25	3.1 ± 5.4		
		(0.47 ± 0.24)	(0.55 ± 0.23)	(0.70 ± 0.30)	(3.6 ± 5.9)		
	25%	0.48 ± 0.17	0.61 ± 0.26	0.71 ± 0.22	2.8 ± 4.7		
		(0.43 ± 0.16)	(0.50 ± 0.18)	(0.72 ± 0.29)	(3.5 ± 5.6)		
	10%	0.42 ± 0.16	0.53 ± 0.29	0.70 ± 0.21	3.2 ± 5.4		
		(0.40 ± 0.18)	(0.51 ± 0.28)	(0.79 ± 0.32)	(3.7 ± 6.2)		
75th	100%	0.64 ± 0.29	0.85 ± 0.32	1.0 ± 0.59	4.2 ± 7.3	F = 1.4, P = 0.25, η_p^2	F = 1.3, P = 0.29, η_p^2
		(0.61 ± 0.31)	(0.84 ± 0.37)	(1.2 ± 0.55)	(5.0 ± 8.3)	= 0.046 (F = 0.71, P	= 0.11 (F = 2.0, P =
						= 0.50, η_p^2 = 0.023)	0.076, η_p^2 = 0.17)
	50%	0.79 ± 0.39	0.87 ± 0.43	1.1 ± 0.47	3.8 ± 6.5		
		(0.79 ± 0.47)	(0.99 ± 0.50)	(1.2 ± 0.42)	(4.5 ± 7.3)		
	25%	0.71 ± 0.35	0.96 ± 0.35	1.2 ± 0.44	4.3 ± 7.4		
		(0.70 ± 0.29)	(0.81 ± 0.24)	(1.2 ± 0.49)	(5.2 ± 8.4)		
	10%	0.65 ± 0.28	0.84 ± 0.40	1.2 ± 0.42	3.9 ± 6.4		
		(0.72 ± 0.32)	(0.92 ± 0.40)	(1.3 ± 0.52)	(4.5 ± 7.1)		
90th	100%	0.98 ± 0.42	1.4 ± 0.65	1.6 ± 1.0	5.3 ± 8.8	F = 0.21, P = 0.77, η_p^2	F = 0.91, P = 0.49, η_p^2
		(1.0 ± 0.59)	(1.5 ± 0.70)	(1.8 ± 0.91)	(6.0 ± 9.6)	= 0.007 (F = 0.32, P	= 0.083 (F = 1.0, P
						= 0.81, η_p^2 = 0.011)	= 0.42, η_p^2 = 0.094)
	50%	1.2 ± 0.57	1.5 ± 1.2	2.0 ± 1.3	4.5 ± 7.5		
		(1.3 ± 0.91)	(1.7 ± 1.3)	(1.9 ± 0.84)	(5.3 ± 8.3)		
	25%	0.97 ± 0.42	1.5 ± 0.67	1.8 ± 1.0	5.3 ± 9.0		
		(1.2 ± 0.81)	(1.3 ± 0.37)	(2.0 ± 0.84)	(6.2 ± 9.8)		
	10%	0.91 ± 0.44	1.4 ± 0.74	1.8 ± 0.91	4.9 ± 7.4		
		(1.1 ± 0.42)	(1.4 ± 0.55)	(2.0 ± 0.94)	(5.6 ± 8.5)		

FE = Fellow eye contrast varied at 100%, 50%, 25%, and 10% while amblyopic eye contrast was at 100% for all dichoptic viewing trials. All the values in parenthesis are for Amblyopic Eye.

Group 0 = Controls, Group 1 = Anisometropic amblyopia, Group 2 = Mixed/Strabismic amblyopia without Fusion Maldevelopment Nystagmus, Group 3 = Mixed/Strabismic amblyopia with Fusion Maldevelopment Nystagmus.

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Table 9. Vergence Bivariate Contour Ellipse Area During Dichoptic Viewing Across the Four Groups

Groups	FE Contrast 100%	FE Contrast 50%	FE Contrast 25%	FE Contrast 10%
Group 0	0.41 ± 0.27	0.34 ± 0.28	0.47 ± 0.35	0.45 ± 0.49
Group 1	0.34 ± 0.53	0.39 ± 0.54	0.50 ± 0.48	0.43 ± 0.43
Group 2	0.65 ± 0.66	0.78 ± 0.42	0.89 ± 0.42	0.93 ± 0.52
Group 3	0.58 ± 0.43	0.43 ± 0.23	0.72 ± 0.46	0.73 ± 0.36

FE = Fellow eye contrast varied at 100%, 50%, 25%, and 10% while amblyopic eye contrast was at 100% for all dichoptic viewing trials.

Group 1 = anisometropic amblyopia, group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus, group 3 = mixed/strabismic amblyopia with fusion maldevelopment nystagmus. The values are Log vergence BCEA 95% (log 10[vergence BCEA (deg^2)]).

across the four groups (lower bound: main effect F(3,81) = 1.5, $P = 0.19$, $\eta_p^2 = 0.05$), interaction (F(9,81) = 0.44, $P = 0.9$, $\eta_p^2 = 0.04$), and (upper bound: main effect (F(3,81) = 2.3, $P = 0.09$, $\eta_p^2 = 0.08$), and interaction (F(9,81) = 0.96, $P = 0.47$, $\eta_p^2 = 0.09$).

Discussion

We categorized patients with amblyopia per the clinical type and the FEM waveforms and examined FEMs and eye alignment under different viewing conditions. The main findings were (1) FEMs are differentially affected with increased amplitude of quick phases of FMN observed during AEV than BEV and incomitance of eye misalignment with increased strabismus angles observed during FEV than BEV, (2) reduction of contrasts as used during DcV results in increase fixation and vergence instability, (3) there was a significant increase in the amplitude of quick phases with reducing the contrast of the FE during DcV in patients with FMN, and (4) strabismic/mixed amblyopia patients without FMN demonstrate a fixation switch where the AE attends to the target presented during DcV at lower FE contrasts, whereas patients with FMN were less likely to demonstrate the fixation switch.

Vision is used to optimize eye movements. Thus, eye movements elicited during a target's fixation reflect the visual sensory and motor functions. They constitute an excellent quantitative end point of a final common pathway. Abnormalities of fast (increased saccadic intrusions) and slow (increased inter-saccadic drifts) FEMs are seen in patients with low vision, such as those with age-related macular degeneration.[82–85] Studies from our and other laboratories have found that fixation instability in amblyopia limits the visual acuity.[56,53–55,86] We have described features per the FEM waveforms that reflect the severity of visual acuity and stereo acuity deficits.[52] We have also found that patients with amblyopia with nystagmus have greater impairment of visual functions with increased amplitude of quick phase, eye position variance, and velocities of the slow phases compared to patients without nystagmus.[52,65,87] These collectively suggest that increased fixation instability with increased amplitude and velocities of fast and slow FEMs in amblyopia are associated with worse visual function deficits.

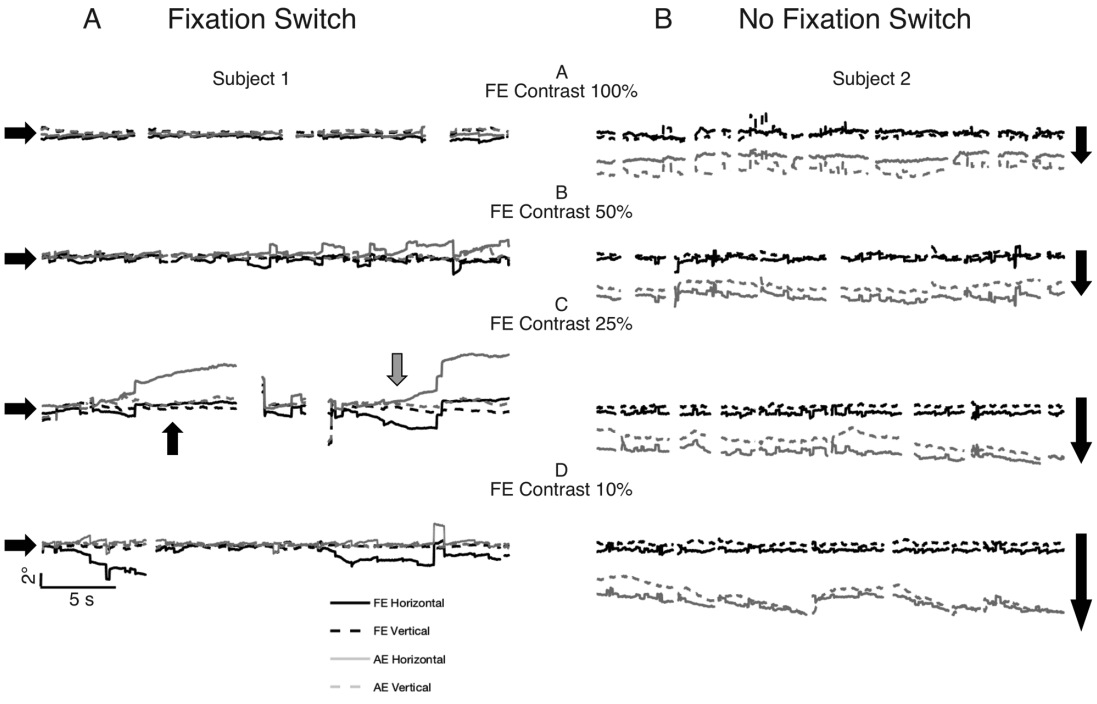

Figure 7. Horizontal and vertical eye position traces (vertical axis) and time (horizontal axis) in 2 subjects obtained during dichoptic viewing at fellow eye contrasts of (**A**) 100%, (**B**) 50%, (**C**) 25%, and (**D**) 10% contrast. To the left is a subject with mixed/strabismic amblyopia without fusion maldevelopment nystagmus (FMN) that demonstrates a fixation switch indicated by the *black arrow* when the fellow eye is fixing at FE contrast of 25% and the *grey arrow* that represents the amblyopic eye picks up with fixation. The *horizontal arrows* on the far left represent the primary position. To the right is a subject with mixed/strabismic amblyopia with fusion maldevelopment nystagmus (FMN). Notice that through all the trials **A** to **D** the fellow eye fixates in primary position with increase in eye misalignment at lower fellow eye contrasts indicated by the *vertical black arrows* on the far right.

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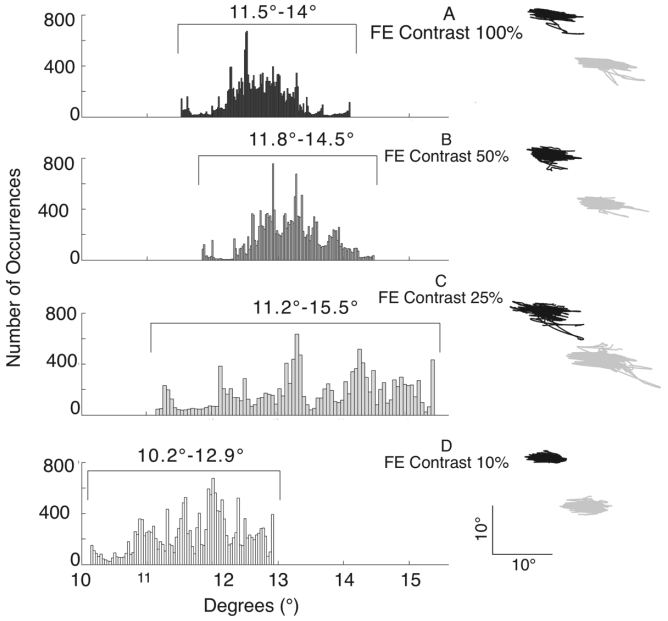

Figure 8. *Right*: Horizontal and vertical eye positions of fellow eye (*black*) and amblyopic eye (*grey*) obtained from the same subject shown in Figure 4 during dichoptic viewing across fellow eye contrasts of 100% (**A**), 50% (**B**), 25% (**C**), and 10% (**D**). Rightward and upward movements correspond to positive vertical axis. Notice the right hypotropia and exotropia during all dichoptic viewing conditions. *Left*: Histogram showing range of composite eye position difference during dichoptic viewing across the fellow eye contrasts of 100% (**A**), 50% (**B**), 25% (**C**), and 10% (**D**).

Table 10. Eye Alignment (Degrees) During Dichoptic Viewing Across The Four Groups

Groups	FE Contrast 100%	FE Contrast 50%	FE Contrast 25%	FE Contrast 10%
Group 0	0.80 ± 0.42	0.72 ± 0.56	0.96 ± 0.66	1.4 ± 0.67
	2.0 ± 0.41	2.1 ± 0.76	2.3 ± 0.83	2.9 ± 0.98
Group 1	0.72 ± 0.55	0.38 ± 0.47	0.70 ± 0.97	0.63 ± 0.79
	2.4 ± 1.7	2.2 ± 1.7	2.2 ± 1.7	2.2 ± 1.6
Group 2	1.9 ± 1.8	2.0 ± 2.2	2.1 ± 2.2	2.2 ± 2.7
	4.7 ± 3.3	4.7 ± 3.2	5.7 ± 3.7	5.7 ± 4.6
Group 3	4.3 ± 4.3	3.7 ± 3.2	3.8 ± 2.9	4.3 ± 3.5
	6.5 ± 4.7	5.4 ± 3.6	5.9 ± 3.6	6.7 ± 4.2

FE = Fellow eye contrast varied at 100%, 50%, 25%, and 10% while amblyopic eye contrast was at 100% for all dichoptic viewing trials.

The 95% lower bound (top row) and 95% upper bound (bottom row) of the histogram of composite eye position difference between the right and left eyes.

Group 0 = controls, group 1 = anisometropic amblyopia, group 2 = mixed/strabismic amblyopia without fusion maldevelopment nystagmus, group 3 = mixed/strabismic amblyopia with fusion maldevelopment nystagmus.

In the current study, we found that the FEM abnormalities are differentially affected per the viewing condition, with greater amplitude of the fast FEMs of the AE during AEV than BEV for all patients with amblyopia, which was most pronounced for patients with FMN. The slow phase velocity of AE of patients with FMN increased substantially under AEV than BEV. Thus, we found better fixation stability under binocular viewing than monocular viewing in agreement with previous studies.[12,52,71] Incomitance of strabismus angle during right eye versus left eye viewing has been reported in patients with intermittent exotropia without paralytic strabismus.[8] We also found that eye misalignment (esotropia/exotropia) is greater during FEV than BEV. Engagement of vergence and fusion mechanisms with binocular summation could result in better fixation stability and eye alignment during BEV.[50,88] Thus, collectively, the results suggest that visual input is a key factor affecting fixation stability and eye alignment control.

Reducing the contrast of stimuli presented to the FE is thought to overcome the suppression of AE and aid the visual recovery and is the basis of dichoptic treatments.[2,89,90] Thus, we would expect the fixation instability of the AE to improve while lowering the FE contrast during DcV as it reduces suppression. We found that the reduction of contrast is associated with increased BCEA values suggestive of greater fixation instability of that eye in controls and FE of patients with anisometropic amblyopia. Patients with anisometropic amblyopia also exhibited an increase in the amplitude of AE at lower FE contrasts reflected by an increase in the instability of the AE. Thus, contrary to our expected results of reduced FEM abnormalities in the AE, we saw an increase in FEM abnormalities as the FE contrasts are reduced. We have previously shown that FEM abnormalities of the non-viewing eye under monocular viewing are driven primarily by the viewing eye (i.e. the FEM abnormalities of the FE are increased when the AE is viewing and the FEM abnormalities of the AE are reduced when the FE is viewing).[53] Thus, the worsening of FE fixation stability at lower FE contrasts likely drives the increased AE fixation instability under DcV. This is in agreement with a prior report examining fixation stability in adult patients with anisometropic amblyopia and controls during DcV.[91]

In strabismic amblyopia, reduced AE fixation instability appears to be associated with a loss of foveation and suppression, both of which degrade fusion and vergence.[91,92] Thus, we expect the AE fixation instability to improve when rebalancing the inter-ocular contrast

overcomes and reduces the suppression. Our study found that several patients with strabismic/mixed amblyopia were unable to overcome suppression with contrast rebalancing between the two eyes.[92] Subjects with strabismus who were able to perceive the nonius cross frequently verbalized that the perception was transient and the location of the nonius cross would vary as they attempted the alignment procedure. This is in agreement with previous studies that have shown that in patients with strabismic/mixed amblyopia where the balance point can be determined, there is a transient improvement in AE fixation stability with subsequent drifting of the eye away from foveal alignment.[92] In our cohort, we found an overall increase in amplitude of FE and AE in patients with mixed/strabismic amblyopia with and without FMN, particularly at lower FE contrasts. We also found a similar change in amplitude of FE and AE in all patients with amblyopia at lower FE contrasts irrespective of their stereoacuity deficits. An increase in the amplitude of the AE may be driven by the changes in the FE fast FEMs amplitude at lower FE contrasts. The collective increase in the FE and AE instability would also affect the vergence instability, which was seen in all subjects particularly in subjects with greater stereo-acuity deficits.

The DcV recordings were performed without offsetting the target per the subjective nonius cross alignment due to the nonius cross's transient perception and fleeting location in the patients with strabismic/mixed amblyopia. We found a small and variable change in eye alignment under DcV at varied FE contrasts. Interestingly, we also found that patients with strabismic/mixed amblyopia without FMN were more likely to demonstrate a fixation switch where the AE fixates on the presented target during DcV than those with FMN. Thus, the current experiments provide novel evidence that the AE can overcome the suppression at lower FE contrasts and attend to the presented target, as demonstrated by the fixation switch on eye movement recordings. NHP studies have shown that loss of horizontal cells in area V1 due to disruption of binocularity is the necessary and sufficient cause for FMN development and contributes to inter-ocular suppression and stereopsis deficits.[40,47,93] We have shown that patients with FMN had worse stereopsis deficits than patients without FMN.[70] In the current study, most of the patients with FMN had absent stereopsis. We examined the inter-ocular suppression using dichoptic motion coherence, and preliminary analysis demonstrates that patients with FMN had greater inter-ocular suppression for a given level of visual acuity deficits. Thus, we speculate that the poor binocularity and greater inter-ocular suppression in patients with FMN could be a contributing factor to lack of fixation switch seen during DcV.

We have previously examined characteristics of FEMs during binocular viewing in school-aged children versus adults and did not find differences in frequency of fixational saccades or inter-saccadic drifts. The amplitude of fixational saccades was slightly higher in children than adults [median fixation saccade amplitude in adults was 0.4 degrees; 25 and 75 percentiles = 0.26–0.6 degrees, whereas in children it was 0.55 degrees (25 and 75 percentiles = 0.36–0.84 degrees)].[74] The current study is the largest cohort of patients with amblyopic with FEM measurements obtained under DcV at various contrasts, monocular and binocular viewing. The majority of patients with amblyopia (27/34) were <18 years of age, thus limiting our ability to analyze differences in changes in FEM abnormalities as a function of age. Future studies systematically investigating the interactions of age and viewing conditions on FEM abnormalities in patients with amblyopia with and without nystagmus are warranted.

The central research emphasis in the field is to create new amblyopia treatments, however; both conventional and emerging treatments have produced mixed results.[4–6,59,60] Most amblyopia therapies take effect through changing viewing conditions. We have found that fixation instability and FEM abnormalities are sensitive to viewing conditions (i.e. the high-speed eye trackers can capture the modulation of FEMs in response to transient changes in the viewing conditions). Increased fixation instability or greater FEM abnormalities during a given viewing condition can affect visual functions and could potentially render the amblyopia treatment less effective.[94,95] Similarly, changes in strabismus angle across various viewing conditions should be considered while planning strabismus repair and determining the likelihood of developing diplopia with amblyopia treatments.[6,96] Development of treatment protocols designed per the optimal conditions when FEM abnormalities are minimal could improve outcomes. Thus, future studies quantifying FEMs before and during treatment are critical. It may serve as a missing link to understand the mechanisms responsible for varying therapeutic efficacy seen with current treatment regimens.

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