Eye Position Stability in Amblyopia and in Normal Binocular Vision

Esther G. González,[1,2,4] *Agnes M. F. Wong,*[1–3] *Ewa Niechwiej-Szwedo,*[3] *Luminita Tarita-Nistor,*[1] *and Martin J. Steinbach*[1,2,4]

Purpose. We investigated whether the sensory impairments of amblyopia are associated with a decrease in eye position stability (PS).

Methods. The positions of both eyes were recorded simultaneously in three viewing conditions: binocular, monocular fellow eye viewing (right eye for controls), and monocular amblyopic eye viewing (left eye for controls). For monocular conditions, movements of the covered eye were also recorded (open-loop testing). Bivariate contour ellipses (BCEAs), representing the region over which eye positions were found 68.2% of the time, were calculated and normalized by log transformation.

Results. For controls, there were no differences between eyes. Binocular PS ($\log_{10}$BCEA = –0.88) was better than monocular PS ($\log_{10}$BCEA = –0.59) indicating binocular summation, and the PS of the viewing eye was better than that of the covered eye ($\log_{10}$BCEA = –0.33). For patients, the amblyopic eye exhibited a significant decrease in PS during amblyopic eye ($\log_{10}$BCEA = –0.20), fellow eye ($\log_{10}$BCEA = 0.0004), and binocular ($\log_{10}$BCEA = –0.44) viewing. The PS of the fellow eye depended on viewing condition: it was comparable to controls during binocular ($\log_{10}$BCEA = –0.77) and fellow eye viewing ($\log_{10}$BCEA = –0.52), but it decreased during amblyopic eye viewing ($\log_{10}$BCEA = 0.08). Patients exhibited binocular summation during fellow eye viewing, but not during amblyopic eye viewing. Decrease in PS in patients was mainly due to slow eye drifts.

Conclusions. Deficits in spatiotemporal vision in amblyopia are associated with poor PS. PS of amblyopic and fellow eyes is differentially affected depending on viewing condition. (*Invest Ophthalmol Vis Sci.* 2012;53:5386–5394) DOI:10.1167/iovs.12-9941

From the [1]Vision Science Research Program, Toronto Western Hospital, Toronto, Ontario, Canada; [2]Ophthalmology and Vision Sciences, University of Toronto, Toronto, Ontario, Canada; [3]Ophthalmology and Vision Sciences, The Hospital for Sick Children, Toronto, Ontario, Canada; and the [4]Centre for Vision Research, York University, Toronto, Ontario, Canada.

Supported by the Natural Sciences and Engineering Research Council of Canada (NSERC A7664); the Sir Jules Thorn Charitable Trust; the Vision Science Research Program, Toronto Western Hospital; an anonymous donor; Canadian Institutes of Health Research (CIHR MOP 106663); Leaders Opportunity Fund from the Canadian Foundation for Innovation; and the Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children.

Submitted for publication March 28, 2012; revised May 23 and June 26, 2012; accepted July 10, 2012.

Disclosure: **E.G. González,** None; **A.M.F. Wong,** None; **E. Niechwiej-Szwedo,** None; **L. Tarita-Nistor,** None; **M.J. Steinbach,** None

Corresponding author: Esther G. González, Vision Science Research Program, Toronto Western Research Institute, 399 Bathurst Street, FP 6-212, Toronto, Ontario M5T 2S8, Canada; esther.gonzalez@utoronto.ca.

Amblyopia is a developmental spatiotemporal visual impairment caused by early abnormal vision. It is frequently associated with early childhood strabismus (ocular misalignment), anisometropia (unequal refractive error), or form deprivation. Amblyopia cannot be optically corrected immediately and it is not caused by an obvious change or defect in the eyes. Strabismic and anisometropic amblyopia are produced by a disruption of binocular input during the critical period in the development of binocularity.[1,2] A large body of research[3–10] has been dedicated to the sensory characteristics of patients with amblyopia, which include deficits in visual acuity, contrast sensitivity, form and motion perception, spatial and temporal crowding, and stereopsis. Deficits in saccadic eye movements and visuomotor behavior have recently been investigated.[11–14]

During normal fixation, the eyes exhibit a series of involuntary movements ranging in amplitude from high-frequency tremors and microsaccades to slow drifts, the combination of which determines the precision or stability of the eyes.[15,16] A fourth kind of oscillatory eye movement of low amplitude (< 0.02°) and lower frequency (0.04–0.1 Hz) than any other movement has been recently discovered,[17] but requires long fixation trials to be detected. In patients with amblyopia, the stability of the eyes during attempted steady fixation has been shown to differ depending on the viewing eye, instructions, and viewing conditions. Ciuffreda and associates[18] have examined the fixation stability of patients with and without strabismus and/or amblyopia but without specific instructions to hold the gaze steadily. For the amblyopic eye, they found an increase in saccadic intrusions, which are associated with strabismus but not with amblyopia.[19] They also found drifts accounting for 75% of the total fixation time in amblyopia without strabismus, 50% of the total fixation time in constant strabismus amblyopia, and only 20% of the total fixation time in intermittent strabismus. From these data, they conclude that amblyopia rather than strabismus is the necessary condition producing an increase in drifts as people attempt to fixate.[20] In all three studies, references to normal viewing involves the fellow (nonamblyopic) eye, binocular viewing, or previous research findings with people with normal binocular vision, but no control group data were obtained under the same testing conditions.

During fixation of a stationary target, slips of retinal images stimulate the brain to generate eye movements that counter the slips in order to hold the gaze steady. This response to retinal image drifts caused by gaze instability during active fixation has been referred to as slow-control, or field-holding reflex.[21,22] Sporadic saccades away from fixation and their corrective counterparts (square-wave jerks) are also known to happen in pathological conditions and, in smaller numbers, in normal observers.[23] In this study, we use the term open loop as it is used in control theory where it refers to the removal of the visual feedback loop.[24] In this study, we used a quantitative measure of fixation stability in patients with amblyopia and in people with normal binocular vision, tested under binocular

Investigative Ophthalmology & Visual Science, August 2012, Vol. 53, No. 9

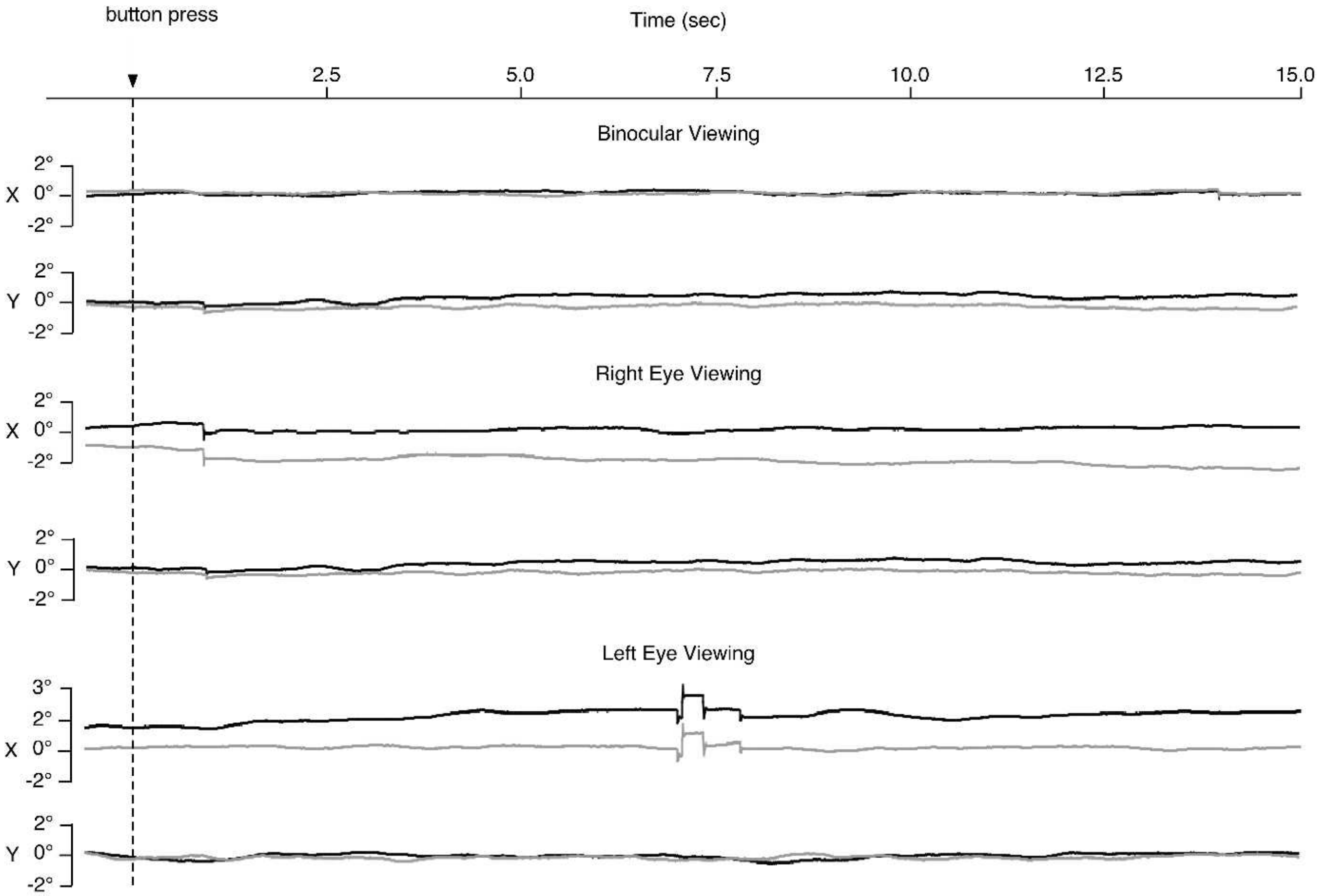

Figure 1. Horizontal (*X*) and vertical (*Y*) eye positions for a control participant. The *black lines* correspond to the right eye and the *grey* to the left. Negative values are leftward (*X*) or downward (*Y*).

and monocular viewing conditions and under instructions to hold the gaze steadily. We hypothesized that the sensory impairments associated with amblyopia should be reflected in the patients' fixation control during binocular and monocular viewing with the fellow and amblyopic eye. The present study also differed from the previous literature in that we recorded the movements of the two eyes concurrently, even during monocular viewing; in other words, we recorded the covered eye's position behind the occluder in an open-loop condition. This method allowed us to obtain measures of the magnitude of binocular summation and offered some insight into the mechanisms that control eye position in the absence of corrective visual feedback. We use the term open loop as it is used in control theory where it refers to the removal of the visual feedback loop.[24]

Methods

Participants

Two groups of volunteers (amblyopia and control) were recruited from advertisements posted at the University of Toronto Web site and from the Vision Science Research Program. Informed consent was obtained from all participants and the research was approved by the University Health Network Research Ethics Board and conducted in accordance with the tenets of the Declaration of Helsinki.

Amblyopia Group. The criterion for amblyopia was an interocular acuity difference equal to or greater than 2 logMAR lines. Strabismic amblyopia was defined as amblyopia in the presence of eye misalignment at distance and/or near fixation. Anisometropic amblyopia was defined as a difference in refractive error between the two eyes equal to or greater than 1 diopter of spherical or cylindrical power. Mixed amblyopia was defined as amblyopia in the presence of a combination of strabismus and anisometropia. Participants with a visual acuity between 0.2 and 1.0 logMAR (20/32–20/200 Snellen) in the amblyopic eye, 0.1 logMAR (20/25 Snellen) or better in the fellow eye, and an interocular acuity difference equal to or greater than 0.2 logMAR were recruited. Patients with severe amblyopia were excluded to ensure more homogeneity. Those with latent nystagmus were also excluded to avoid the confounding effect of the nystagmus on fixation stability.

Thirteen people (11 women; mean age = 31.5 ± 10.7 years) with a confirmed diagnosis of mild to moderate amblyopia participated. Five of them had strabismic, four had anisometropic, and four had mixed amblyopia. All patients underwent a standard orthoptic assessment, including visual acuity with a Snellen chart, a prism cover test, refractive error, and stereoacuity with the Fly Stereotest (provided in the public domain by http://www.stereooptical.com/). The Table shows the clinical data for this group.

Control Group. Twenty people (11 women; mean age = 30 ± 12.7 years) with normal or corrected to normal visual acuity and stereopsis of at least 40 seconds, as measured with the Fly Stereotest, participated. Seven of these control participants (35%) had experience in eye movement experiments.

Apparatus

Eye position was recorded with a desktop remote EyeLink 1000 eyetracker (SR Research Ltd., Mississauga, Ontario, Canada) at a

Table. Clinical Characteristics of the Patients in the Amblyopia Group

Type of Amblyopia	Age (y)	Acuity		Stereo (arc s^{-1})	Deviation (PD)		Comments
		RE	LE		Near	Distance	
Strab	28	−0.10 (20/15)	0.30 (20/40)	400	LET8,ET+E10	LET2,ET+E4	LMS
	29	0 (20/20)	0.30 (20/40)	400	LET2,ET+E14	LET2	LMS
	30	0 (20/20)	0.40 (20/50)	-	LHT8	LHT8; ET12	
	30	−0.10 (20/15)	0.18 (20/30)	-	ET35	ET35	
	47	0.10 (20/25)	0.48 (20/60)	-	XT40	XT35	
Aniso	18	0 (20/20)	0.48 (20/60)	200	LET2	LET2	
	25	0 (20/20)	0.40 (20/50)	120	E4	E1	
	26	0.70 (20/100)	−0.10 (20/15)	3000	RXT2,XT+X8	RXT2	
	57	0.70 (20/100)	0 (20/20)	-	RXT2	RXT2	
Mixed	18	0 (20/20)	0.40 (20/50)	3000	LET4,ET+E18	LET4,ET+E6	LMS
	31	−0.10 (20/15)	0.70 (20/100)	3000	LET2,ET+E4	LET2,ET+E4	Partially accomm
	33	−0.10 (20/15)	0.10 (20/25)	-	LET20	LET25	
	37*	−0.10 (20/15)	0.40 (20/50)	-	LXT10,L hypo	25LXT8,L hypo20	

Example: LET4,ET+E12 means that the manifest/tropia part of the deviation is 4 PD (measured by simultaneous prism cover test), but that the total dissociated deviation (measured by the alternate prism cover test) is 12 PD. In other words, deviation increases with dissociation, and in this instance, the patient can control 8 PD of esophoria. Aniso, anisometropic amblyopia; bil, bilateral; DVD, dissociated vertical deviation; E, esophoria; hypo, hypotropia; LET, left esotropia; LMS, left monofixation syndrome; LXT, left exotropia; Mixed, mixed amblyopia; RET, right esotropia; RXT, right exotropia; Strab, strabismic amblyopia; X, exophoria.

* Unable to record fellow eye when covered.

sampling rate of 250 Hz. Calibration was performed with binocular viewing by using a standard 5-point grid with a modified calibration target: a nine-cycle square-wave radial grating subtending 4.3° of visual angle, the properties of which have been described elsewhere.[25] During calibration, participants were instructed to fixate the middle of the radial grating and to keep their eyes as steady as possible. A recording trial was initiated only when the eyetracker classified the calibration and its subsequent validation as "good" for each eye; otherwise, new calibration and validation procedures were initiated.

Stimuli were presented on a Samsung monitor (Sync Master 900 NF; Samsung, Seoul, South Korea) with a useful field of view of 34.4 × 26 cm at a viewing distance of 60 cm. Testing was done in a well-illuminated room and participants sat with their chin and forehead steadied by a headrest. All participants wore their optical correction, if any was needed.

Procedure

The fixation target was a 3° red cross, presented in the middle of the monitor, on a white background with a luminance of 240 cd/m^2.

There were three viewing conditions: (1) binocular, (2) monocular with fellow eye viewing, amblyopic eye covered (right eye viewing for the controls), and (3) monocular with amblyopic eye viewing, fellow eye covered (left eye viewing for the controls). During both binocular and monocular viewing, eye position recordings were always made for the two eyes simultaneously. For the monocular viewing conditions, an infrared (IR) long-pass filter, which appeared black to the observer, allowed the eyetracker to record the movements of the covered eye (open-loop condition). In other words, regardless of whether viewing was binocular or monocular, each trial produced data for both the left and right eye.

The first trial was always a binocular viewing trial followed by a trial with either the amblyopic or the fellow eye viewing (left or right eye for the controls) in random sequence. Approximately 2 seconds after the beginning of a trial, the experimenter pressed a button to start a 15-second recording of eye position. Participants were instructed to fixate the center of the red cross, at the intersection of the vertical and horizontal lines, and to keep their eyes as steady as possible.

For each of the three viewing conditions a trial was repeated until a good one was recorded. Control participants rarely required more than one test per condition, but several participants in the amblyopic group were tested more than once. In one case (patient 4 in the mixed amblyopia subgroup), even after two experimental sessions on separate days, we were unable to record the movements of the covered fellow eye during the amblyopic eye viewing condition (Table and Fig. 1). This was because, as soon as it was covered by the IR filter, the fellow eye quickly adopted an exophoric position that brought it out of the range of the eyetracker.

The test was not considered by the participants to be too long and it appears that even longer testing times can be easily tolerated. Research with élite shooters and inexperienced controls shows that fixation stability changes little in up to 60 seconds of recording time.[26]

Outcome Measures

The position stability of the eyes was measured in two ways: (1) with a global measure using a bivariate contour ellipse (BCEA), and (2) by analyzing the number and amplitude of the participants' microsaccades during the 15 seconds of recording time after the button press. Measures of binocular summation and of the importance of visual feedback were also computed.

Global BCEA. Using the horizontal and vertical eye positions recorded by the eyetracker, the BCEA[27] is given by the following formula:

$$\mathrm{BCEA} = \pi\chi^2\sigma_x\sigma_y\sqrt{1-\rho^2}$$

where σ_x and σ_y are standard deviations of the horizontal and vertical eye positions, ρ is their Pearson product-moment correlation, and $\chi^2 = 2.291$ is the chi-square value (2*df*) corresponding to a probability value of $P = 0.682$ (±1 standard deviation). The BCEA represents the region over which eye positions are found for a given percentage of the time, in our case 68.2%. A $\log_{10}$ transformation was used to normalize the resulting BCEAs.

Rate and Magnitude of Microsaccades and Blinks. Given that participants were instructed to hold the gaze steadily, any saccade-like movement was considered a microsaccade regardless of size, a definition used in previous research.[16] The rate and magnitude of the microsaccades and the presence of blinks were detected with a combination of the eyetracker's and in-house software, and visually examined. We used the standard EyeLink saccade detection algorithm with a combined velocity >22 deg/s and an acceleration criterion >4000 deg/s^2. For blinks, the data obtained 100 ms before and after the pupil's occlusion were removed from analysis.[28]

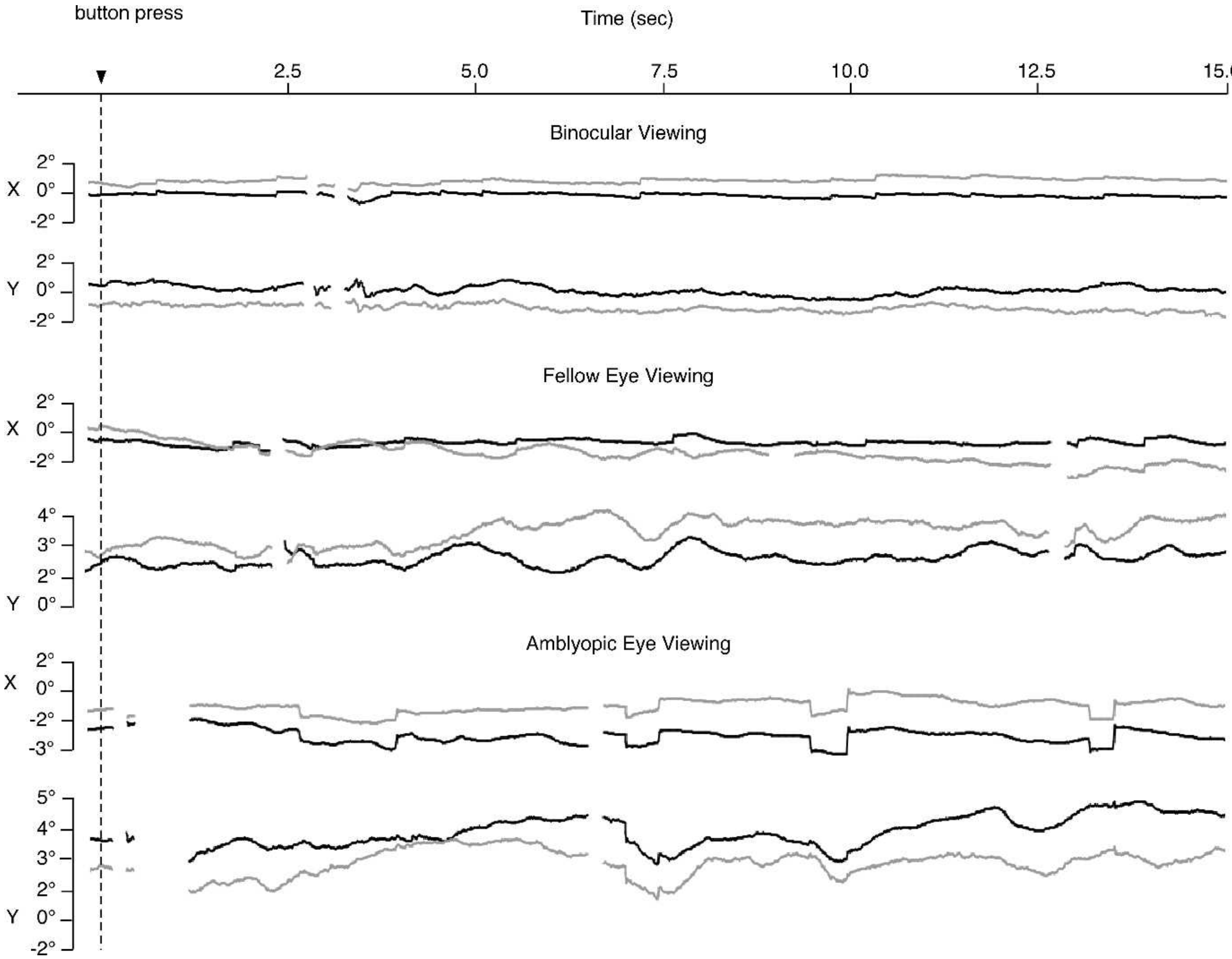

Figure 2. Horizontal (*X*) and vertical (*Y*) eye positions for a patient in the strabismic amblyopia subgroup. The *black lines* correspond to the fellow (right) eye and the *grey* to the amblyopic (left) eye. Blinks were deleted from the record. Negative values are leftward (*X*) or downward (*Y*). Compared with the control participant in Figure 1, more slow drifts are evident in the amblyopic patient.

Binocular Summation Ratios. For ease of comparison to other published results in which better performance is associated with larger values, binocular summation was calculated as the monocular minus the binocular $\log_{10}$BCEA for each participant. The group mean of the differences was then transformed into a linear scale, which is equivalent to the mean of the ratios ($BCEA_{monocular\ viewing}/BCEA_{binocular\ viewing}$).

Visual Feedback Ratios. Visual feedback ratios were calculated as the difference between the monocular position stability ($\log_{10}$BCEA) of the covered eye in the open-loop condition and the fixation stability ($\log_{10}$BCEA) of the viewing eye. The group mean of the differences was then transformed into a linear scale, which is equivalent to the mean of the ratios ($BCEA_{covered\ [open\ loop]\ eye}/BCEA_{viewing\ eye}$).

Data Analysis

For analysis, the data were rearranged into three viewing conditions: binocular, viewing eye during monocular viewing, and covered eye during monocular—that is, open loop—testing. Each viewing condition had two levels: fellow and amblyopic eye for the amblyopia group, and right and left eye for the controls (see Fig. 3).

Except for the count data (microsaccade rate and blinks), which were analyzed with nonparametric statistics, univariate analyses of variance (ANOVAs) with a Geisser-Greenhouse conservative *F* statistic were reported. An α level was set at 0.05 for all statistical tests and, for multiple comparisons, family-wise error was controlled by using a Holm's sequential Bonferroni approach.

Results

Representative eye position tracings from a control and a patient with strabismic amblyopia are shown in Figures 1 and 2. The amblyopic eye exhibited significantly lower fixation stability during both monocular and binocular viewing. The fixation stability of the fellow eye was comparable to that of the controls when it was the viewing eye and during binocular viewing. During monocular open-loop testing, the position stability of the amblyopic and fellow eyes was not significantly different.

Control Group

A 2 $\times$ 3 repeated-measures ANOVA of the logarithmically transformed BCEAs was used to determine whether there were any differences between the positional stability of the left and right eyes in the binocular, monocular for the viewing eye, and monocular for the covered eye (open loop) testing conditions. The analysis yielded no significant differences between the left and right eyes; a significant difference amongst testing

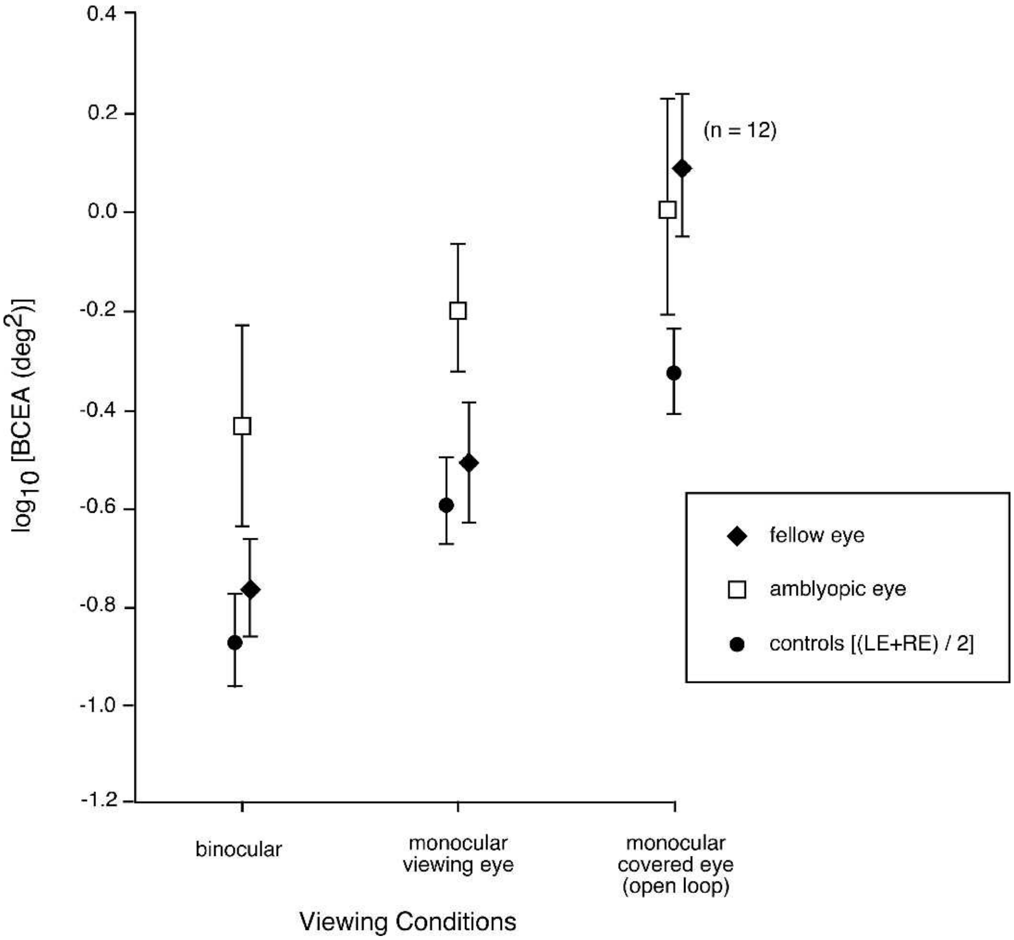

Figure 3. Fixation stability for the amblyopia ($n = 13$ for all except one condition) and control ($n = 20$) groups. For the control group, the displayed values are the pooled $\log_{10}$BCEAs from the left and right eye. *Error bars* represent the 95% inferential confidence intervals (ICIs)[29] about the mean, corrected for multiple comparisons. *Nonoverlapping error bars* are equivalent to a statistically significant test with $P < 0.05$ (see text). Symbols are shifted horizontally for clarity purposes.

conditions, $F_{(1.75,33.31)} = 46.68$, $P < 0.001$, partial $\eta^2 = 0.72$; and a nonsignificant interaction between eyes and testing conditions.

Multiple comparisons of the testing conditions showed that the best fixation stability was obtained during binocular viewing ($\log_{10}$BCEA = –0.88 ± 0.28), which was significantly better ($P < 0.001$) than the fixation stability of the viewing eye during monocular viewing ($\log_{10}$BCEA = –0.59 ± 0.26). The position stability of the viewing eye during monocular viewing, in turn, was better ($P < 0.001$) than that of the covered eye in the open-loop condition ($\log_{10}$BCEA = –0.33 ± 0.33). Figure 3 shows the results.

Mean binocular summation ($BCEA_{monocular\ viewing}/BCEA_{binocular\ viewing}$) was 1.91 (±0.27) and the measure of the importance of corrective visual feedback ($BCEA_{covered\ [open\ loop]\ eye}/BCEA_{viewing\ eye}$) produced a similar value of 1.84 (±0.20).

Amblyopia Group

To use all the patients' data in a repeated-measures ANOVA, the missing point from patient 4 in the mixed amblyopia subgroup (Table) was substituted for the mean value of the group. This procedure preserves the rank order of the data values and does not change the group mean.[30] A 2 × 3 ANOVA of the logarithmically transformed BCEAs yielded a significant difference between the eyes, $F_{(1,12)} = 5.82$, $P = 0.03$, partial $\eta^2 = 0.33$, a significant difference amongst testing conditions, $F_{(1.95,23.39)} = 26.68$, $P < 0.001$, partial $\eta^2 = 0.69$; and a significant interaction between eyes and testing conditions, $F_{(1.58,18.90)} = 7.34$, $P = 0.007$, partial $\eta^2 = 0.38$. Analysis of the significant interaction showed that the fellow eye exhibited significantly better fixation stability than the amblyopic eye during binocular viewing ($P = 0.008$) and when it was the viewing eye ($P = 0.001$); but there were no significant differences in position stability when the fellow and amblyopic eyes were covered in open-loop testing.

The fixation stability of the amblyopic eye did not exhibit binocular summation—the difference between binocular ($\log_{10}$BCEA = –0.44 ± 0.47) and monocular viewing ($\log_{10}$BCEA = –0.20 ± 0.30) failed to reach statistical significance. In contrast, the stability of the fellow eye exhibited binocular summation; that is, the fixation stability of the fellow eye was better ($P < 0.001$) during binocular viewing ($\log_{10}$BCEA = –0.77 ± 0.23) than with monocular viewing ($\log_{10}$BCEA = –0.52 ± 0.28), yielding a measure of binocular summation of 1.79 (±0.24) ($BCEA_{fellow\ eye\ viewing}/BCEA_{binocular\ viewing}$).

There was no statistically significant difference between the position stability exhibited by the amblyopic eye when it was the viewing eye ($\log_{10}$BCEA = –0.20 ± 0.30) and when it was the covered eye in open-loop testing ($\log_{10}$BCEA = 0.0004 ± 0.50). In contrast, the position stability of the fellow eye was significantly better ($P < 0.01$) when it was the viewing eye ($\log_{10}$BCEA = –0.52 ± 0.28) than when it was covered in open-

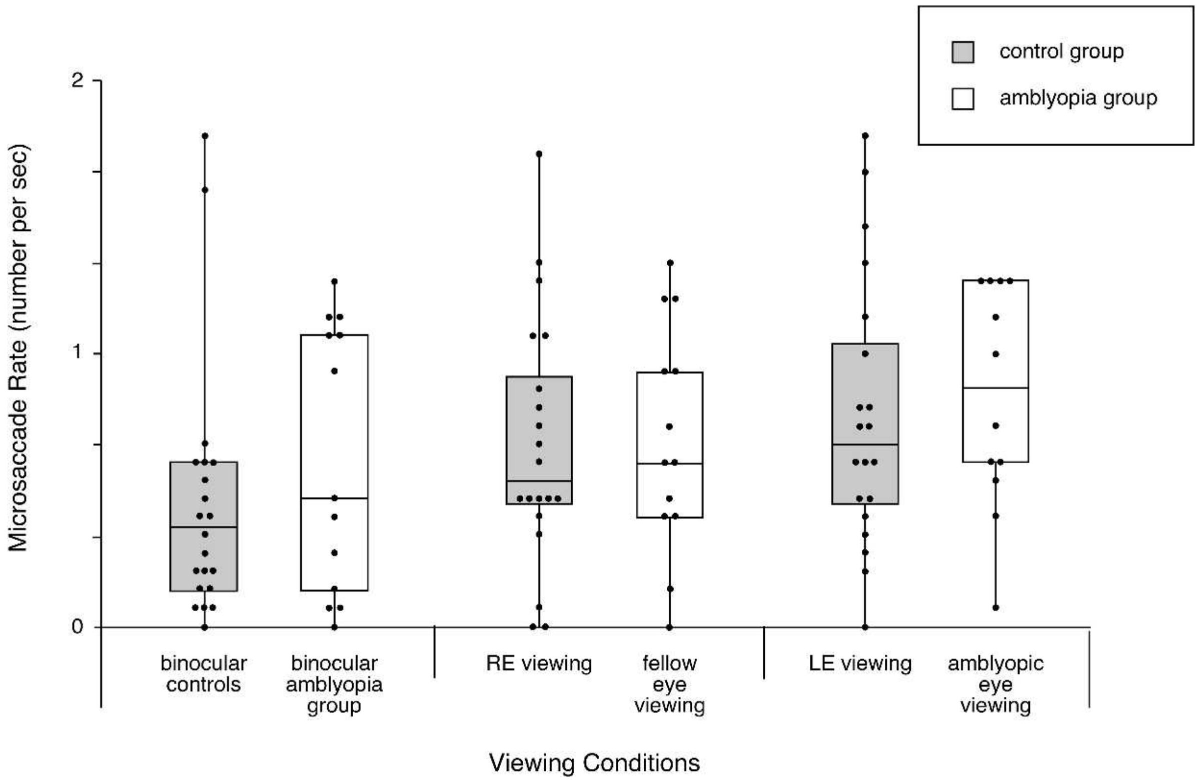

FIGURE 4. Box plots of microsaccade rates for the control and amblyopia groups. *Black discs* show individual data points shifted horizontally for clarity purposes.

loop testing ($\log_{10}$BCEA = 0.08 ± 0.33), yielding a measure of the importance of corrective visual feedback ($BCEA_{fellow\ eye\ covered\ [open\ loop]}/BCEA_{fellow\ eye\ viewing}$) of 4.01 (±30).

The fixation stability of the amblyopic eye was worse (higher mean BCEAs) than that of the control subjects in all three viewing conditions; that is, its 95% inferential confidence intervals (ICIs) fell above the 95% ICIs of the control group. During binocular viewing and also when it was the viewing eye, the mean fixation stability of the fellow eye of patients was comparable to that of the controls; that is, their 95% ICIs overlapped. In the open-loop condition, however, the position stability of the fellow eye was worse (higher mean BCEA) than that of the covered eye of the controls (Fig. 3).

For the amblyopia group, visual acuity and fixation stability did not exhibit significant correlations. However, the interocular difference in visual acuity (acuity deficit) did correlate significantly with the $\log_{10}$BCEA of the fellow eye during binocular viewing, $r(11) = -0.72$, $P = 0.003$, and moderately with the fellow eye's fixation stability during monocular viewing, $r(11) = -0.48$, $P = 0.046$. The interocular difference in visual acuity was not significantly related to the interocular difference in fixation stability or to the interocular difference in position stability during open-loop testing.

Fixational Eye Movements

Microsaccade Rate. For the control group, Friedman's two-way analysis of variance of the number of microsaccades per second in the three viewing conditions (binocular, monocular with right eye viewing, and monocular with left eye viewing) yielded a significant effect, χ^2_r (2) = 9.92, $P = 0.007$. Post hoc comparisons with a Wilcoxon matched-pairs signed rank test found that binocular viewing produced a significantly lower rate of microsaccades than monocular viewing with the right ($P = 0.01$) or with the left eye ($P = 0.002$), and that there were no significant differences between the two monocular conditions. The mean rate of microsaccades was 0.46 (±0.47)/s during binocular viewing and 0.72 (±0.44)/s during monocular viewing with either eye.

For the amblyopia group, Friedman's two-way analysis of variance of the rate of microsaccades in the three viewing conditions (binocular, monocular with fellow eye viewing, and monocular with amblyopic eye viewing) yielded a nonsignificant effect of viewing condition. The mean rate of microsaccades in the three viewing conditions were: binocular (0.62, ±0.49), monocular with fellow eye viewing (0.69, ±0.42), and monocular with amblyopic eye viewing (0.85, ±0.39).

Paired comparisons between the two groups, using a Mann-Whitney *U* test, in the binocular, right eye/fellow eye viewing, and left/amblyopic eye viewing conditions were not statistically significant. Figure 4 shows the data.

Microsaccade Amplitude. Because no significant differences between the right and left eyes were found, the controls' data were averaged and submitted to a one-way repeated-measures ANOVA of the three viewing conditions (binocular, monocular with right eye viewing, and monocular with left eye viewing). This analysis yielded a nonsignificant effect. The mean amplitude of the controls' microsaccades was 0.42 ± 0.16°.

For the amblyopia group, we also found no significant differences between the eyes. Their data were also averaged and analyzed with a one-way ANOVA of the three viewing conditions (binocular, monocular with fellow eye viewing, and monocular with amblyopic eye viewing), which yielded a nonsignificant effect. The mean amplitude of the microsaccades for the amblyopia group was 0.51 ± 0.13°.

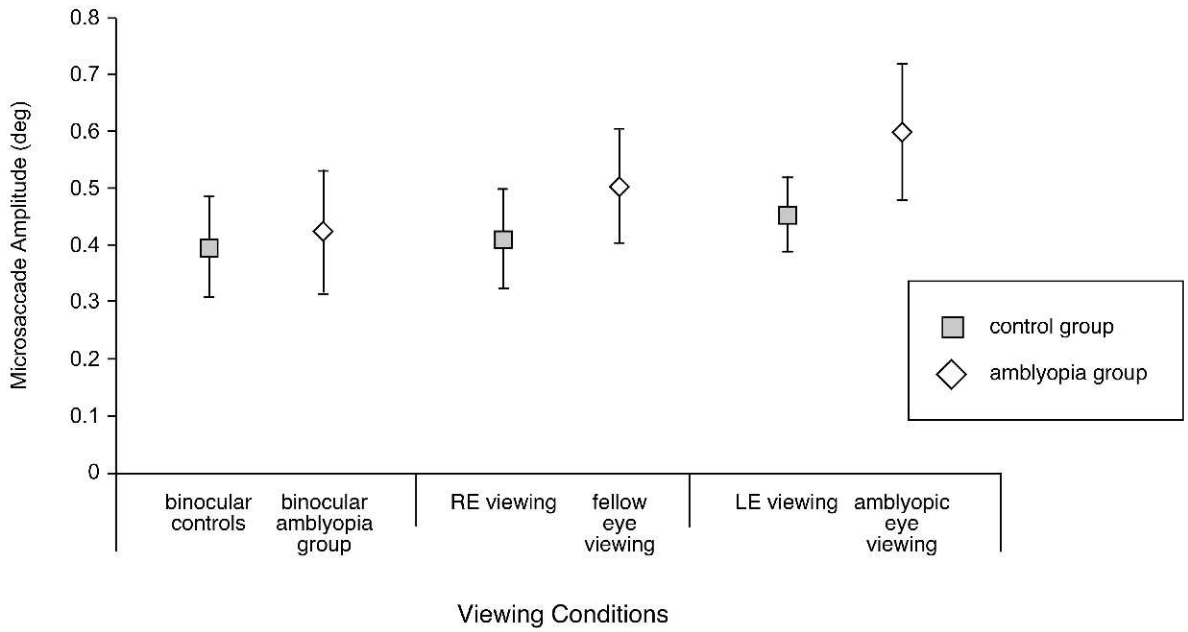

FIGURE 5. Microsaccade amplitude for the control and amblyopia groups with *error bars* representing the Bonferroni-corrected ICIs[29] around their means created for the between-groups comparisons in the binocular, monocular with fellow eye viewing (right eye for the controls), and monocular with the amblyopic eye viewing (left eye for the controls) conditions. The overlapping ICIs indicate no statistically significant differences.

There were no statistically significant differences between the amblyopia and control groups in terms of microsaccade amplitude. Figure 5 shows the results.

Blinks. For both groups, there were no significant differences amongst viewing conditions in the number of blinks. Pairwise comparisons between the two groups in the binocular, right eye/fellow eye viewing, and left eye/amblyopi eye viewing conditions were also not statistically significant. During the 15 seconds of testing, the control and amblyopia groups made an average of 1.45 (±1.34) and 2.01 (±2.70) blinks, respectively. Both groups showed a significantly reduced number of blinks compared to those made by binocularly normal people while reading (~4) or watching videos (~5.6).[31]

DISCUSSION

The four major findings of this study were as follows: (1) patients with amblyopia exhibited a significant decrease in fixation stability (higher mean BCEAs) in the amblyopic eye during binocular and monocular viewing; (2) the fixation stability of the fellow eye was dependent on viewing condition: fixation stability was comparable to that of normal controls during binocular viewing and during monocular viewing when it was the viewing eye, but its position stability decreased significantly when it was covered (i.e., when the amblyopic eye was the viewing eye); (3) patients exhibited binocular summation with the fellow but not with the amblyopic eye; and (4) because the amblyopia and control groups did not differ in terms of the rate or magnitude of intrusive microsaccades, the decrease in fixation stability in the amblyopia group can only be attributed to slow eye drifts.

We found that patients with amblyopia exhibit reduced fixation stability as a result of slow ocular drifts. For the amblyopic eye, this was evident during monocular and binocular viewing; for the fellow eye, it occurred during open-loop testing. It has been shown that patients with amblyopia exhibit increased random internal noise and positional uncertainty.[32–35] It is possible that because patients had difficulty localizing the target, the sensory signals used to trigger the field-holding reflex were degraded, resulting in increased ocular drifts. Although the precise mechanism for the decreased fixation stability remains to be elucidated, our findings provide further support that amblyopia is not only a visual/sensory disorder, it is also associated with abnormal ocular motor control[11] and altered visuomotor behaviors.[12–14]

Binocular summation is a measure of the advantage of binocular performance relative to monocular viewing. For the binocularly normal controls, we found a binocular summation of fixation stability ratio of 1.91, which is higher than the value of $\sqrt{2}$ (1.41) attributed to the physiological summation of the two monocular signals. The value we found is consistent with those found in binocular summation of acuity, contrast sensitivity, and motion detection.[24,36–38] For the amblyopia group, the fixation stability of fellow eye exhibited binocular summation close in magnitude to that of the controls (1.79). However, given that the difference between the binocular and monocular with the amblyopic eye viewing conditions was not statistically significant, we were unable to obtain a measure of binocular summation for the amblyopic eye.[39] One possible explanation is that the mechanisms of binocular summation are compromised in amblyopia. An alternate explanation, as Baker and associates demonstrated,[40] is that binocular summation is intact in amblyopia but that the contribution from the amblyopic eye during binocular stimulation is simply too weak to affect performance. Since we did not compensate for the different sensitivities between the eyes[40,41] in the present study, the effects of binocular summation on fixation stability remain to be explored in amblyopia, although dioptric blur and contrast have been shown to have very small effects on fixation stability in observers with normal binocularity.[42]

The difference between the monocular position stability of an eye when it is the viewing eye and when it is covered (open-loop condition) is a measure of the effectiveness of corrective visual feedback and perhaps also of the quality of the fixation control signals originating from the viewing eye. In the control group, this effect (1.84) was as strong as the measure of binocular summation, but for the fellow eye in the amblyopia group, this value (4.01) was significantly larger. For patients with amblyopia, the best fixation stability was produced by the fellow eye in the binocular condition, but when the same eye was patched and the amblyopic eye viewed the target, the fellow eye exhibited, invariably, the worst position stability of the three viewing conditions.

For the amblyopic eye, the data showed no statistically significant advantage of the availability of corrective visual feedback. In other words, there was no significant difference between the position stability of the amblyopic eye when it viewed the target and when it was covered by the IR filter in the open-loop condition. There are two possible, perhaps not exclusive, reasons for this. First, the fixation stability of the viewing amblyopic eye could be degraded by factors such as reduced acuity and contrast sensitivity (although in the present study the high-contrast target was above their visual threshold), neural undersampling, and deficits in global contour segregation and integration.[9,43–45] Second, it is also possible that the contribution to fixation control from the fellow eye is not as strong as that of people with normal binocular vision. Research in animals[46–48] and in humans with amblyopia[40,41,49–51] has shown that the reduced binocularity in amblyopia is due to the functional suppression of input from the affected eye rather than to loss of binocular cortical neurons. It is unknown whether fixation stability is related to the extent of binocularity.

Hering's law of equal innervation[52] states that there are separate neural controllers for conjugate and vergence gaze changes and that each eye receives an identical neural command from each controller. It is still a matter of debate[37,53] whether the covered eye's position stability is controlled by the innervation of the viewing eye according to Hering's law, or whether the two eyes have independent and learned neural controls, according to Helmholtz.[54] If its neural controls were independent, we would expect the covered fellow eye to have a position stability somewhat closer to that exhibited by the covered eyes of the control group; instead, for all the patients in the amblyopia group, the covered fellow eye exhibited the worst performance. In this sense, our fixation control data appear to support Hering's law.

Zhang and associates[55] have found significant correlations between visual acuity deficits and the relative deficits (normalized against the respective values obtained with controls with a 4-Hz–simulated nystagmus) in multifocal visual evoked potential, multifocal electroretinogram, and horizontal fixation stability. This suggests that the interpretation of neural or perceptual deficits in amblyopia should take into consideration the fixation instability of the eyes. In the present study, we only found a correlation between the patients' visual acuity deficit and the $\log_{10}$BCEA of the fellow eye viewing either binocularly or monocularly. That fixation stability and acuity show a significant relationship only for measures of the better eye has also been found in patients with age-related macular degeneration (AMD)[56] but, in contrast to the amblyopia group reported here, binocular fixation stability in AMD is determined by the better eye; that is, the fixation stability of the worse eye improves during binocular viewing, and the fixation stability of the better eye is the same regardless of whether viewing is monocular or binocular. The differences in etiology between AMD and amblyopia demonstrate that fixation stability is not a simple function of the reduction in acuity.

We showed that patients with mild to moderate amblyopia exhibited an ability to inhibit intrusive saccades comparable to that of people with normal binocular vision during fixation and under instructions to hold their gaze steadily, which is consistent with previous findings.[57–60] Blinks, which could have also affected fixation stability, were found to be insignificant in number. In agreement with previous research,[16,60,61] we conclude that the differences in fixation stability between people with normal binocular vision and patients with amblyopia viewing with their amblyopic eye are due mainly to slow drifts rather than to the rate or amplitude of intrusive saccades. Recent research[62] has shown that in normal observers, both trained and untrained, the speed of ocular drift is the best predictor of fixation precision.

Ciuffreda and associates[20] have found differences in the amplitude and velocity of slow drifts among patients with amblyopia without strabismus, with constant strabismic amblyopia, and with intermittent strabismus with or without mild amblyopia, but the number and amplitude of microsaccades (some as large as volitional saccades) varied across participants. One important difference between their study and the present one is that, in the present study, all participants were given instructions to maintain a steady gaze as opposed to simply fixate. In another study,[58] the same authors found that patients with strabismic or anisometropic amblyopia produced a significant suppression of fixational saccades when instructed to maintain a steady gaze.

The size of the amblyopia subgroups in our study precluded statistical analysis of any differences in fixation stability, rate or amplitude of microsaccades, or blinks. Visual inspection, however, showed no obvious trends. Future research should focus on the analysis of drifts during monocular viewing, their effects on fixation stability over time, and their relationship to the degree and subtype of amblyopia.[63,64]

Acknowledgments

Linda Colpa and Jennifer Sacco performed the orthoptist assessment of the patients. The authors are also grateful to Linda Lillakas and Linda Colpa for editorial and technical help, respectively.

References

1. American Academy of Ophthalmology. Amblyopia: preferred practice pattern. 2007. Available at: http://one.aao.org/CE/PracticeGuidelines/PPP.aspx?sid=d9939a8b-1675-4bf8-85fb-154652305795. Accessed January 18, 2012.
2. Lewis TL, Maurer D. Multiple sensitive periods in human development: evidence from visually deprived children. *Dev Psychobiol*. 2005;46:163–183.
3. Ciuffreda KJ, Levi D, Selenow A. *Amblyopia, Basic and Clinical Aspects*. Boston, MA: Butterworth-Heinemann; 1991.
4. Levi DM, Carkeet AD. Amblyopia: a consequence of abnormal visual development. In: Simons K, ed. *Early Visual Development, Normal and Abnormal*. New York, NY: Oxford University Press; 1993:391–408.
5. McKee SP, Levi DM, Movshon JA. The pattern of visual deficits in amblyopia. *J Vis*. 2003;3:380–405.
6. Barrett BT, Bradley A, McGraw PV. Understanding the neural basis of amblyopia. *Neuroscientist*. 2004;10:106–117.
7. Levi DM. Visual processing in amblyopia: human studies. *Strabismus*. 2006;14:11–19.
8. Kiorpes L. Visual processing in amblyopia: animal studies. *Strabismus*. 2006;14:3–10.
9. Sireteanu R, Baumer CC, Sarbu C, Iftime A. Spatial and temporal misperceptions in amblyopic vision. *Strabismus*. 2007;15:45–54.
10. Hou C, Pettet MW, Norcia AM. Abnormalities of coherent motion processing in strabismic amblyopia: visual-evoked potential measurements. *J Vis*. 2008;8:1–12.
11. Niechwiej-Szwedo E, Goltz HC, Chandrakumar M, Hirji ZA, Wong AM. Effects of anisometropic amblyopia on visuomotor behavior, I: saccadic eye movements. *Invest Ophthalmol Vis Sci*. 2010;51:6348–6354.

12. Niechwiej-Szwedo E, Goltz HC, Chandrakumar M, Hirji Z, Crawford JD, Wong AM. Effects of anisometropic amblyopia on visuomotor behavior, II: visually guided reaching. *Invest Ophthalmol Vis Sci*. 2011;52:795–803.
13. Niechwiej-Szwedo E, Goltz HC, Chandrakumar M, Hirji Z, Wong AM. Effects of anisometropic amblyopia on visuomotor behavior, III: temporal eye-hand coordination during reaching. *Invest Ophthalmol Vis Sci*. 2011;52:5853–5861.
14. Niechwiej-Szwedo E, Goltz HC, Chandrakumar M, Wong AM. The effect of sensory uncertainty due to amblyopia (lazy eye) on the planning and execution of visually-guided 3D reaching movements. *PLoS ONE*. 2012;7:e31075.
15. Ditchburn RW. Eye-movements in relation to retinal action. *Opt Acta (Lond)*. 1955;1:171–176.
16. Martínez-Conde S. Fixational eye movements in normal and pathological vision. In: Martínez-Conde S, Macknick SL, Martínez LM, Alonso J-M, Tse PU, eds. *Progress in Brain Research*. Vol 154. Part A. Amsterdam, The Netherlands: Elsevier; 2006:151–176.
17. Pansell T, Zhang B, Bolzani R, Ygge J. Slow oscillatory eye movement during visual fixation. *Exp Brain Res*. 2011;209:1–8.
18. Ciuffreda KJ, Kenyon RV, Stark L. Fixational eye movements in amblyopia and strabismus. *J Am Optom Assoc*. 1979;50:1251–1258.
19. Ciuffreda KJ, Kenyon RV, Stark L. Saccadic intrusions in strabismus. *Arch Ophthalmol*. 1979;97:1673–1679.
20. Ciuffreda KJ, Kenyon RV, Stark L. Increased drift in amblyopic eyes. *Br J Ophthalmol*. 1980;64:7–14.
21. Murphy BJ, Kowler E, Steinman RM. Slow oculomotor control in the presence of moving backgrounds. *Vision Res*. 1975;15:1263–1268.
22. Epelboim J, Kowler E. Slow control with eccentric targets: evidence against a position-corrective model. *Vision Res*. 1993;33:361–380.
23. Herishanu YO, Sharpe JA. Normal square wave jerks. *Invest Ophthalmol Vis Sci*. 1981;20:268–272.
24. Howard IP. *Seeing in Depth*. Vol 1. Thornhill, Canada: Porteus; 2002;95:418.
25. González E, Teichman J, Lillakas L, Markowitz SN, Steinbach MJ. Fixation stability using radial gratings in patients with age-related macular degeneration. *Can J Ophthalmol*. 2006;41:333–339.
26. Di Russo F, Pitzalis S, Spinelli D. Fixation stability and saccadic latency in élite shooters. *Vision Res*. 2003;43:1837–1845.
27. Steinman RM. Effect of target size, luminance, and color on monocular fixation. *J Opt Soc Am*. 1965;55:1158–1165.
28. Aguilar C, Castet E. Gaze-contingent simulation of retinopathy: some potential pitfalls and remedies. *Vision Res*. 2011;51:997–1012.
29. Tryon WW. Evaluating statistical difference, equivalence, and indeterminacy using inferential confidence intervals: an integrated alternative method of conducting null hypothesis statistical tests. *Psychol Methods*. 2001;6:371–386.
30. Tabachnick BG, Fidell LS. *Using Multivariate Statistics*. 5th ed. Boston, MA: Allyn & Bacon; 2007:67.
31. Gowrisankaran S, Nahar NK, Hayes JR, Sheedy JE. Asthenopia and blink rate under visual and cognitive loads. *Optom Vision Sci*. 2011;89:1–8.
32. Levi DM, Klein SA, Yap YL. Positional uncertainty in peripheral and amblyopic vision. *Vision Res*. 1987;27:581–597.
33. Xu P, Lu ZL, Qiu Z, Zhou Y. Identify mechanisms of amblyopia in Gabor orientation identification with external noise. *Vision Res*. 2006;46:3748–3760.
34. Levi DM, Klein SA, Chen I. The response of the amblyopic visual system to noise. *Vision Res*. 2007;47:2531–2542.
35. Levi DM, Klein SA, Chen I. What limits performance in the amblyopic visual system: seeing signals in noise with an amblyopic brain. *J Vis*. 2008;81:1–23.
36. Blake R, Sloane M. Further developments in binocular summation. *Percept Psychophys*. 1981;30:266–276.
37. Rose D. The binocular: monocular sensitivity ratio for movement detection varies with temporal frequency. *Perception*. 1980;9:577–580.
38. Meese TS, Georgeson MA, Baker DH. Binocular contrast vision at and above threshold. *J Vis*. 2006;6:1224–1243.
39. Pardhan S, Gilchrist J. Binocular contrast summation and inhibition in amblyopia: the influence of the interocular difference on binocular contrast sensitivity. *Doc Ophthalmol*. 1992;82:239–248.
40. Baker DH, Meese TS, Mansouri B, Hess RF. Binocular summation of contrast remains intact in strabismic amblyopia. *Invest Ophthalmol Vis Sci*. 2007;48:5332–5338.
41. Mansouri B, Thompson B, Hess RF. Measurement of suprathreshold binocular interactions in amblyopia. *Vision Res*. 2008;48:2775–2784.
42. Ukwade MT, Bedell HE. Stability of oculomotor fixation as a function of target contrast and blur. *Optom Vis Sci*. 1993;70:123–126.
43. Hess RF, Demanins R. Contour integration in anisometropic amblyopia. *Vision Res*. 1998;38:889–894.
44. Hess RF, McIlhagga W, Field D. Contour integration in strabismic amblyopia: the sufficiency of explanation based on positional uncertainty. *Vision Res*. 1997;37:3145–3161.
45. Levi DM, Yu C, Kuai S-G, Rislove E. Global contour processing in amblyopia. *Vision Res*. 2007;47:512–524.
46. Crewther SG, Crewther DP. Amblyopia and suppression in binocular cortical neurones of strabismic cat. *Neuroreport*. 1993;4:1083–1086.
47. Sengpiel F, Blakemore C, Kind PC, Harrad R. Interocular suppression in the visual cortex of strabismic cats. *J Neurosci*. 1994;14:6855–6871.
48. Malach R, Ebert R, Van Sluyters RC. Recovery from effects of brief monocular deprivation in the kitten. *J Neurophysiol*. 1984;51:538–551.
49. Bonneh YS, Sagi D, Polat U. Spatial and temporal crowding in amblyopia. *Vision Res*. 2007;47:1950–1962.
50. Huang CB, Zhou J, Lu ZL, Feng L, Zhou Y. Binocular combination in anisometropic amblyopia. *J Vis*. 2009;9:11–16.
51. Sengpiel F, Blakemore C. The neural basis of suppression and amblyopia in strabismus. *Eye (Lond)*. 1996;10:250–258.
52. Hering E. *The Theory of Binocular Vision*. New York, NY: Plenum Press; 1977.
53. King WM. Binocular coordination of eye movements—Hering's Law of equal innervation or uniocular control? *Eur J Neurosci*. 2011;33:2139–2146.
54. Helmholtz H. *Helmholtz's Treatise on Physiological Optics*. New York, NY: Dover; 1962.
55. Zhang B, Stevenson SS, Cheng H, et al. Effects of fixation instability on multifocal VEP (mfVEP) responses in amblyopes. *J Vis*. 2008;8:1–14.
56. Tarita-Nistor L, Brent MH, Steinbach MJ, González, EG. Fixation stability during binocular viewing in patients with age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2011;52:1887–1893.
57. Steinman RM, Cunitz RJ, Timberlake GT, Herman M. Voluntary control of microsaccades during maintained monocular fixation. *Science*. 1967;155:1577–1579.
58. Ciuffreda, KJ, Kenyon RV, Stark L. Suppression of fixational saccades in strabismic and anisometropic amblyopia. *Ophthalmic Res*. 1979;11:31–39.
59. Flom MC, Kirschen DC, Bedell HE. Control of unsteady, eccentric fixation in amblyopic eyes by auditory feedback of eye position. *Invest Ophthalmol Vis Sci*. 1980;19:1371–1381.
60. Schor CM, Hallmark W. Slow control of eye position in strabismic amblyopia. *Invest Ophthalmol Vis Sci*. 1978;17:577–581.
61. Bedell HE, Yap YL, Flom MC. Fixational drift and nasal-temporal pursuit asymmetries in stabismic amblyopes. *Invest Ophthalmol Vis Sci*. 1990;31:968–976.
62. Cherici C, Kuang X, Poletti M, Rucci M. Precision of sustained fixation in trained and untrained observers. *J Vis*. 2012;12:1–16.
63. McGivern RC, Gibson JM. Characterisation of ocular fixation in humans by analysis of saccadic intrusions and fixation periods: a pragmatic approach. *Vision Res*. 2006;46:3741–3747.
64. Poletti M, Listorti C, Rucci M. Stability of the visual world during eye drift. *J Neurosci*. 2010;30:11143–11150.