Fixational Saccades and Their Relation to Fixation Instability in Strabismic Monkeys

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PURPOSE. To evaluate the contribution of fixational saccades toward fixation instability in strabismic monkeys.

METHODS. Binocular eye movements were measured as six experimental monkeys (five strabismic monkeys and one monkey with downbeat nystagmus) and one normal monkey fixated targets of two shapes (Optotype, Disk) and two sizes (0.5°, 2°) during monocular and binocular viewing. Fixational saccades were detected using an unsupervised clustering algorithm.

RESULTS. When compared with the normal monkey, amplitude and frequency of fixational saccades in both the viewing and nonviewing eye were greater in 3 of 5 strabismic monkeys (1-way ANOVA on ranks $P < 0.001$; median amplitude in the normal monkey viewing eye: 0.33°; experimental animals: median amplitude range 0.20–0.82°; median frequency in the normal monkey: 1.35/s; experimental animals: median frequency range 1.3–3.7/s). Increase in frequency of fixational saccades was largely due to quick phases of ongoing nystagmus. Fixational saccade amplitude was increased significantly (3-way ANOVA; $P < 0.001$) but by small magnitude depending on target shape and size (mean difference between disk and optotype targets = 0.02°; mean difference between 2° and 0.5° targets = 0.1°). Relationship between saccade amplitude and the Bivariate Contour Ellipse Area (BCEA) was nonlinear, showing saturation of saccade amplitude. Fixation instability in depth was significantly greater in strabismic monkeys (vergence BCEA: 0.63 deg^2–2.15 deg^2) compared with the normal animal (vergence BCEA: 0.15 deg^2; $P < 0.001$).

CONCLUSIONS. Increased fixational instability in strabismic monkeys is only partially due to increased amplitude and more frequent fixational saccades. Target parameter effects on fixational saccades are similar to previous findings of target effects on BCEA.

Keywords: strabismus, nonhuman primate, fixation, saccades, microsaccades

O ur eyes are continuously moving even when consciously fixating on a stationary object.[1–3] Such fixational eye movements can be broadly categorized into two components: quick, rapid flicks of the eye that are termed microsaccades or fixational saccades and a much slower drift component that occurs in between fixational saccades.[4–7] A third kind of fixational eye movement, tremor, is too small to be physiologically relevant.[8] Fixational eye movements are necessary to prevent retinal fading and also to help gather information about features of the object of regard.[7,9,10] On the other hand, excessive fixational eye movements lead to fixation instability and are detrimental to visual function (e.g., reading).[11] Among fixational eye movements, we know the most about fixational saccades.[12–14] These saccadic movements are also called microsaccades but we prefer the term fixational saccades because the term microsaccades implies an arbitrary amplitude limit. These movements are fundamentally tiny saccades that are generated by the same neural circuitry as that of the large saccade.[15,16] Fixational saccades follow the same main-sequence relationships as larger saccades and may be used to reorient gaze between closely spaced objects just like a large saccade is used to reorient gaze between widely spaced objects.[10,15] Although the object of regard is already on the

fovea, fixational saccades are subject to adaptive control in the face of consistent retinal error, similar to large saccades.[17]

Instability in fixation (i.e., larger than normal fixational eye movements) is often a hallmark of many forms of visual system pathology, such as in strabismus and amblyopia or macular degeneration.[18–21] One of the many quantitative measures used to quantify fixation instability is the Bivariate Contour Ellipse Area (BCEA), which is a metric that measures area over which eye position is dispersed during fixation.[22] Because this is a measure of dispersion of eye position, it takes into account both fixational saccade and drift components. Using the BCEA metric, we have recently shown that strabismic monkeys have significantly greater fixation instability compared with normal monkeys. We also showed that fixation instability was influenced by target parameters such as size and shape of the target, in both normal and strabismic monkeys.[23] The goal of the current study was to further investigate the components that contribute to larger fixation instability in strabismus. Specifically, we were interested in investigating the contribution of fixational saccades toward fixation instability in strabismic monkeys, while also investigating the influence of target parameters, such as target shape and size on fixational saccades. Some of these data have appeared before in abstract

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form and in our previous publication on fixation instability in strabismic monkeys (Upadhyaya S, et al. *IOVS* 2017;58:ARVO E-Abstract 3441; Pullela M, et al. *IOVS* 2017;58:ARVO E-Abstract 751).[23]

METHODS

Subjects, Rearing Paradigms, and Surgical Procedures

We examined fixational instability and fixational saccades in seven adult rhesus macaque (*Macaca mulatta*) monkeys of which two had normal ocular alignment (NM, PM) and the rest exhibited ocular misalignment as a consequence of either optical prism rearing (SM1, SM4, SM5) or daily alternating monocular occlusion (AMO; SM2, SM3). In all strabismic monkeys, strabismus was induced in infancy by disrupting binocular vision starting from day 1 after birth for the first 4 months of life. In the optical prism paradigm, the infant monkeys wore lightweight helmets fitted with either a base-in or base-out prism in front of one eye and a base-up or base-down in front of the other eye until they were 4 months old, after which they were allowed unrestricted viewing.[24,25] The AMO rearing consisted of the infant animals wearing an opaque contact lens in front of one eye while the fellow eye was unrestricted. The opaque lens was alternated between each eye every day until the age of 4 months.[26] Both rearing paradigms disrupt binocular vision during the critical period for visual development, thus resulting in strabismus.[27] One animal (PM) was also specially reared using optical prism methods but did not develop ocular misalignment. However, this animal developed a consistent nystagmus and therefore could not be categorized as either normal or strabismic. Data from this animal are presented as a separate entity.

When the animals were approximately 4 years old, they underwent a surgical procedure carried out under aseptic conditions with isoflurane anesthesia (1.25%–2.5%) to implant a head stabilization post.[28] Later in a second surgery, a scleral search coil was implanted in one eye using the technique of Judge and colleagues[29] and, in a third surgery, a scleral search coil was implanted in the fellow eye. All procedures were performed according to National Institutes of Health guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Houston.

NM and SM1 to 3 were previously part of a published study from our laboratory that examined influence of target parameters on fixation instability in normal and strabismic monkeys.[23] In that study, we examined fixation instability using the BCEA metric. A subset of the fixation data that were collected for the previous study from NM and SM1 to 3 are now analyzed to quantify fixational saccades. In addition, we have collected and analyzed new data from SM4, SM5, and PM.

Experimental Paradigms, Data Acquisition, and Analysis

Movements of both eyes were recorded as the monkeys, trained previously on a variety of oculomotor tasks, fixated a stationary target back-projected onto a tangent screen at a distance of 114 cm using a DepthQ LCD projector (Lightspeed Design, Inc., Bellevue, WA, USA) running at 120-Hz frame rate. Eye movements were calibrated as the monkey monocularly viewed targets at ±15° horizontally and vertically. During experiments, each fixation trial was developed from 1 of 12 different conditions: two target shapes (optotype: "%" sign or disk), two target sizes (0.5° or 2°), and three viewing conditions (monocular right eye or left eye viewing and binocular viewing). Our target choices for this study were motivated from our recently published study that showed these target parameters produced maximal differences in fixation instability in both normal and strabismic monkeys.[23,30] An additional motivation for using the "%" optotype was that this target is typically used in our laboratory for training the animal on oculomotor tasks. All the fixation targets were white (luminance 470 cd/m²) on a black background (luminance 0.5 cd/m²). Monocular and binocular viewing was facilitated by occluding an eye using liquid crystal shutter goggles (Citizen Fine Devices, Nagano, Japan) under computer control. Five fixation trials (60 seconds' duration each) were obtained for each combination of target shape, size, and viewing conditions. Fixation trails were interleaved with saccade and smooth pursuit tasks so as to avoid after-images and adaptation across target parameters.

Eye movements of both eyes of all animals were recorded using the magnetic search coil technique,[29] except in SM3 that had a functional scleral coil in only the left eye. Advantages of using the scleral search coil technique include high resolution and precision and the ability to measure movements of both eyes during both monocular and binocular viewing. Eye movement data were processed with anti-aliasing filters at 400 Hz before sampling at 2.79 kHz with 12-bit precision (AlphaLab SNR system; Alpha-Omega Engineering, Nazareth, Israel). All eye movement data were additionally calibrated offline and filtered using a software finite impulse response low-pass filter with a pass-band of 0 to 80 Hz. Epochs of fixation were selected by visual inspection of data. Saccades, blinks, and any sections of data that the monkey was not looking at the target (readily apparent on visual inspection of data) were not a part of selected fixation periods. Nystagmus (e.g., latent nystagmus), drifts, and fixational saccades would not be removed by our selection method.

We used an unsupervised clustering algorithm published by Otero-Millan and colleagues[31] to detect fixational saccades (MATLAB; Mathworks, Natick, MA, USA). Briefly, this method detected saccade-like events using a velocity and acceleration threshold of 8°/s and 100°/s², respectively, and organized these events into clusters by principal component analysis. In a minor deviation from the published method, any detected event smaller than 0.05° was removed and considered to be noise. Also, saccade events from the viewing and nonviewing eyes of all animals were independently detected by the clustering program during both monocular and binocular viewing conditions in the strabismic monkeys (SM1–5) and during monocular viewing in the normally aligned monkeys (NM, PM). In conditions when the normally aligned monkeys viewed binocularly, the clustering program for detection of saccade events used an average of right and left eye data. Once fixational saccades were identified, radial amplitude, direction, and peak velocity of the saccade were calculated. Frequency of fixational saccades in each trial was calculated as the number of fixational saccades per second over the length of the trial. BCEA, a metric that quantifies the area over which eye positions are dispersed during attempted fixation, was also calculated as an overall measure of fixation instability. A smaller value of BCEA indicates lower fixation instability. The BCEA encompassing 68.2% of fixation points was calculated using the following equation:

$$BCEA = 2.291 * pi * \sigma x * \sigma y * \sqrt{(1 - p^2)},$$

Where σx is the SD of horizontal eye position, σy is the SD of vertical eye position, 2.291 is the χ^2 value (2 df) corresponding to a probability of 0.68, and p is the Pearson

TABLE 1. Properties of Strabismus in Study Animals

| Monkeys | Age, y | Strabismus Angle, deg | | Refractive Error, Diopter | | Strabismus Properties | High-Frequency Cutoff in Contrast Sensitivity Function, cyc/deg | | Fixation Times(s), Mean ± SD |
		RE View	LE View	RE	LE		RE	LE	
NM	8	ortho	ortho	+0.75	+1.25		15	12	31.4 ± 8.5
PM	6.5	ortho	ortho	+2	+1.75	N			34.2 ± 10.2
SM1	5	20°–25° XT	10° XT	Plano	−1.50	DVD, DHD, N	17	10	44.7 ± 5.9
SM2	8	10° XT	10° XT	+2.75	+4.75	DVD, N			32.2 ± 10.7
SM3	9	15° XT	15° XT	+8.00	+4.25	DVD, N			38.1 ± 8.9
SM4	7	5° ET–15°XT	1°ET–12°XT	+4.50	+0.75	DHD, N	8	15	45.3 ± 7.2
SM5	6	25° XT	25° XT	Plano	Plano	DVD, N	16	11	42.8 ± 7.0

Contrast sensitivity testing was performed only in animals NM, SM1, SM4 and SM5. DHD, dissociated horizontal deviation; DVD, dissociated vertical deviation; ET, esotropia; LE, left eye; N, nystagmus; RE, right eye; XT, exotropia.

product moment correlation coefficient of horizontal and vertical eye positions.

MATLAB and Sigmaplot 12.0 (Systat, Inc., San Jose, CA, USA) were used for statistical analysis. A Kruskal-Wallis ANOVA on ranks test was used to test differences in amplitude and frequency of fixational saccades between the strabismic and normal monkeys and a 3-way ANOVA followed by Holm-Sidak post hoc testing was used to test the main effect of target shape, target size, and viewing condition on fixational saccades.

RESULTS

Properties of Strabismus

Table 1 summarizes the alignment properties of the animals used in this study. Along with ocular misalignment, the strabismic monkeys also showed nystagmus and dissociated deviations as indicated in Table 1. PM showed no misalignment, but a robust downbeat nystagmus was present. Table 1 also shows high-frequency thresholds obtained during monocular contrast sensitivity testing using a psychophysical method that we have described previously,[32] in four monkeys and the

mean fixation duration within each 60-second trial in each monkey.

In Figure 1, we show eye position traces of the left eye over a duration of 10 seconds while the normal monkey and one of the strabismic monkeys (NM and SM5) viewed a 2° disk-shaped target with their left eye (right eye occluded). Stable fixation was observed in the viewing eye of the normal monkey with fixational saccades on the order of approximately 0.5° in amplitude and small amplitude drifts. The strabismic monkeys showed significant instability due to large fixational saccades, nystagmus, and increased drifts. In each plot, fixational saccades as identified by the automated clustering method are marked.

Fixational Saccade Amplitude and Frequency

In all, we analyzed approximately 6000 fixational saccades from NM, approximately 47,000 fixational saccades from SM1 to 5, and approximately 11,000 fixational saccades from PM. Figure 2 shows the distribution of fixational saccade radial amplitudes in the normal and one of the strabismic monkeys (SM2). Overall, the pattern of amplitude distribution was similar in normal and strabismic monkeys. The median amplitude of fixational saccades in the normal monkey was 0.33° and is similar to that previously published.[15] The median
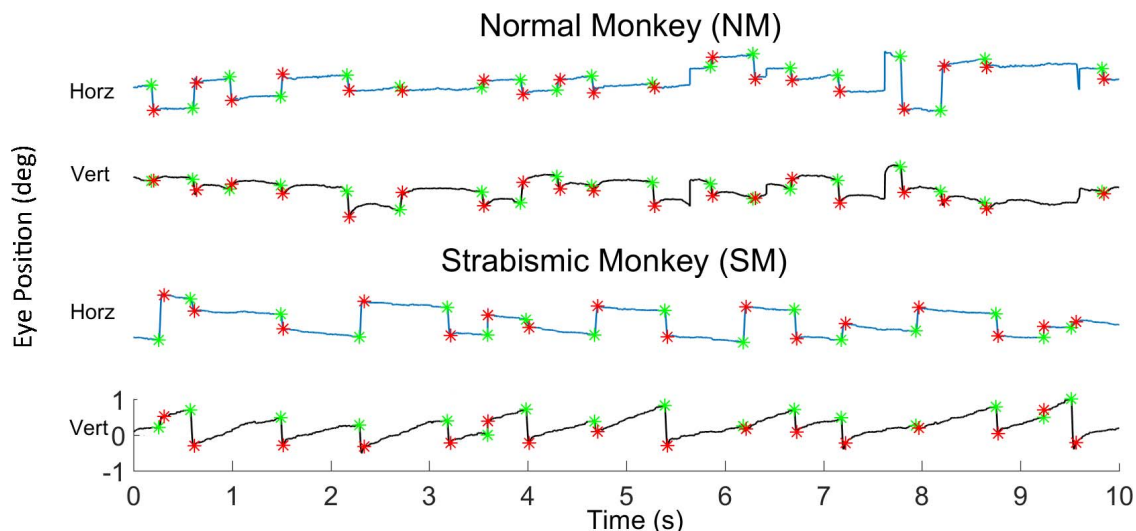

FIGURE 1. Raw data showing horizontal (*blue*) and vertical (*black*) left eye position of a normal monkey and a strabismic monkey (SM5) while fixating a 2° disk target with their left eye. *Green* and *red asterisks* denote start and end of saccade events identified by the automated clustering algorithm that include quick phases of nystagmus and other fixational saccades. Positive values indicate rightward and upward eye positions.
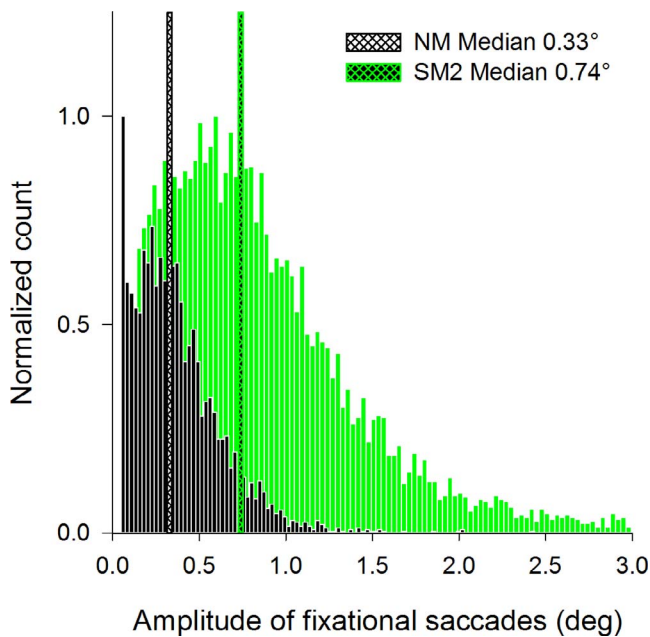
FIGURE 2. Histogram showing amplitudes of all fixational saccades in viewing eye of NM (*black*), and a strabismic monkey (SM2, *green*).

amplitude of fixational saccades in the strabismic monkey was 0.74° (dotted line), which is significantly greater than that of the normal monkey. Figures 3A, 3B show a box-plot summary of fixational saccade amplitude across all targets and monocular/binocular viewing conditions in the viewing and nonviewing eye of each animal. Note that for this and other plots there were no data included for the nonviewing eye during binocular viewing in normally aligned animals NM and PM. In all strabismic animals, the viewing eye during binocular viewing is the eye that is fixating the target and the nonviewing eye is the deviated eye. Median amplitudes of fixational saccades from the viewing eye of three strabismic monkeys were larger than that of the normal monkey (Kruskal-Wallis ANOVA on ranks H[6] = 9624.03, $P < 0.001$; Dunn's method for post hoc testing $P < 0.05$). Fixational saccades in the nonviewing eye of all the strabismic monkeys were larger than that of the normal monkey (Kruskal-Wallis ANOVA on ranks H[5] = 4510.70, $P < 0.001$; Dunn's method for post hoc testing

$P < 0.05$). Although PM did not show eye misalignment, fixational saccade amplitude in this animal was also significantly greater than that of NM.

We also calculated the frequency of fixational saccades in the viewing and nonviewing eyes of the animals, and these data are summarized in Figure 4. Frequency of fixational saccades was increased in three of five strabismic monkeys in comparison with the normal monkey in the viewing eye (Kruskal-Wallis ANOVA on ranks H[6] = 196.69, $P < 0.001$; Dunn's test $P < 0.05$) and one monkey in the nonviewing eye (Kruskal-Wallis ANOVA on ranks H[5] = 201.82, $P < 0.001$; Dunn's test $P < 0.05$). Fixational saccade frequency in PM was significantly higher than NM and in many cases even higher than in the strabismic monkeys.

Influence of Nystagmus on Estimates of Fixational Saccade Amplitude and Frequency

As seen in Figure 1, strabismic monkeys usually show significant nystagmus during fixation. We wondered whether the nystagmus quick-phase components could be driving the increased amplitude and/or frequency of fixational saccades in SM1 to SM5 and PM. In our sample of animals, we observed that although nystagmus quick phases tended to be oriented in a specific direction that was different for an individual monkey, they all showed a downward component (see SM5 data in Fig. 1 for example). Therefore, to compare fixational saccade amplitude and frequency in normal and strabismic monkeys without the influence of nystagmus, we simply compared fixational saccades with an upward component in the animals. Upward fixational saccade amplitude data from the viewing and nonviewing eyes is shown in Figures 5A, 5B and frequency data is shown in Figures 5C, 5D.

Median amplitudes following the removal of nystagmus quick phases were slightly smaller than when the nystagmus was included in all the strabismic monkeys in both the viewing and nonviewing eyes (Figs. 5A, 5B). Despite the reduction, saccade amplitudes in the strabismic monkeys were still higher than in the normal monkey in three of five strabismic monkeys in the viewing eye and in all the strabismic monkeys in the nonviewing eye (Kruskal-Wallis ANOVA on ranks viewing eye H[6] = 2777.93, $P < 0.001$; nonviewing eye H[5] = 1210.921 $P < 0.001$; Dunn's method for post hoc testing $P < 0.05$). The two animals whose fixational saccade amplitudes following removal of nystagmus were not significantly different from the normal were the same two animals that showed small
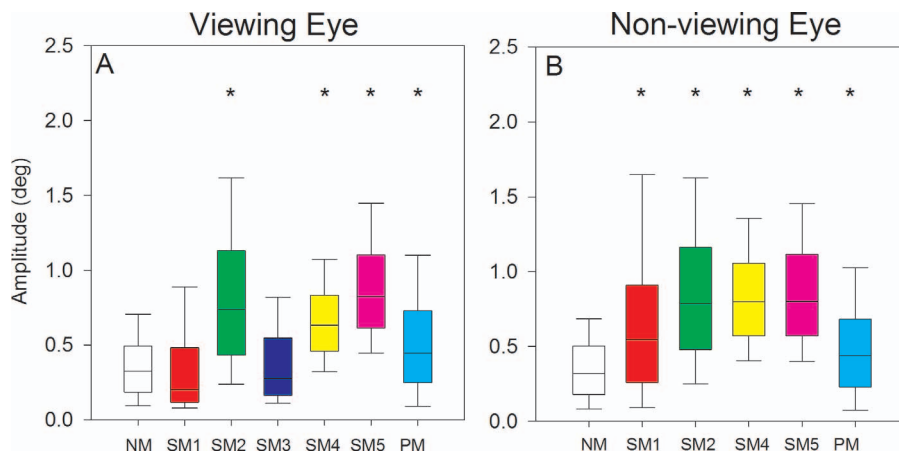

FIGURE 3. Box plots of amplitude of fixational saccades in viewing eye (**A**) and nonviewing eye (**B**) of each monkey in the study pooled across all experimental conditions. *Asterisks* indicate significant difference from the NM as determined by 1-way ANOVA on ranks followed by post hoc testing using Dunn's method. SM3 had only one functional eye coil and therefore no data from the nonviewing eye were acquired.
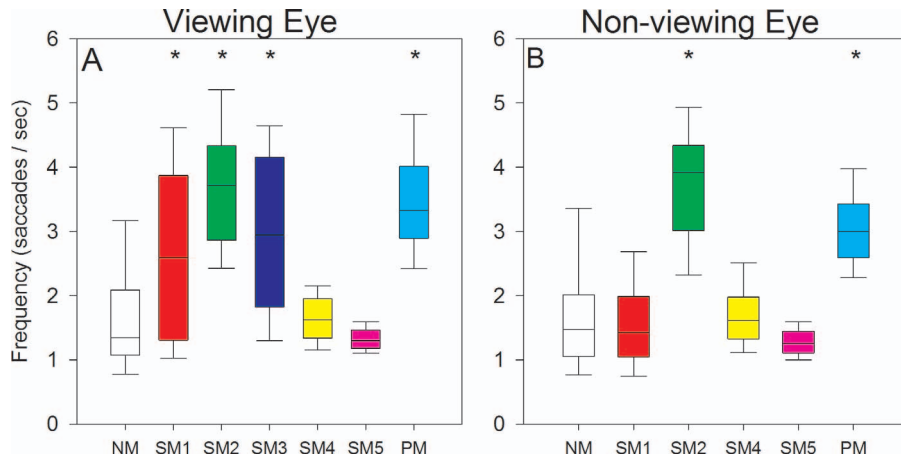
Viewing Eye / Non-viewing Eye

FIGURE 4. Box plots of frequency of fixational saccades in viewing eye (**A**) and nonviewing eye (**B**) of each monkey in the study. *Asterisks* indicate significant difference from the NM.

amplitude fixational saccades to begin with (Figs. 3A, 3B; SM1, SM3). Our findings suggest that even after accounting for nystagmus, larger fixational saccades could drive larger fixation instability in strabismic monkeys. Amplitude of fixational saccades in PM also did not significantly change after removing quick phase of nystagmus and was still greater than that of the normal monkey.

Only considering fixational saccades with an upward component in the normal and strabismic monkeys significantly impacted frequency estimates (Figs. 5C, 5D). Percentage reductions in median frequency estimates in the viewing eye were approximately 63% for NM, approximately 60% for SM1, approximately 78% for SM2, approximately 70% for SM3, approximately 74% for SM4, and approximately 91% for SM5. Note that a reduction of greater than 50% suggests that
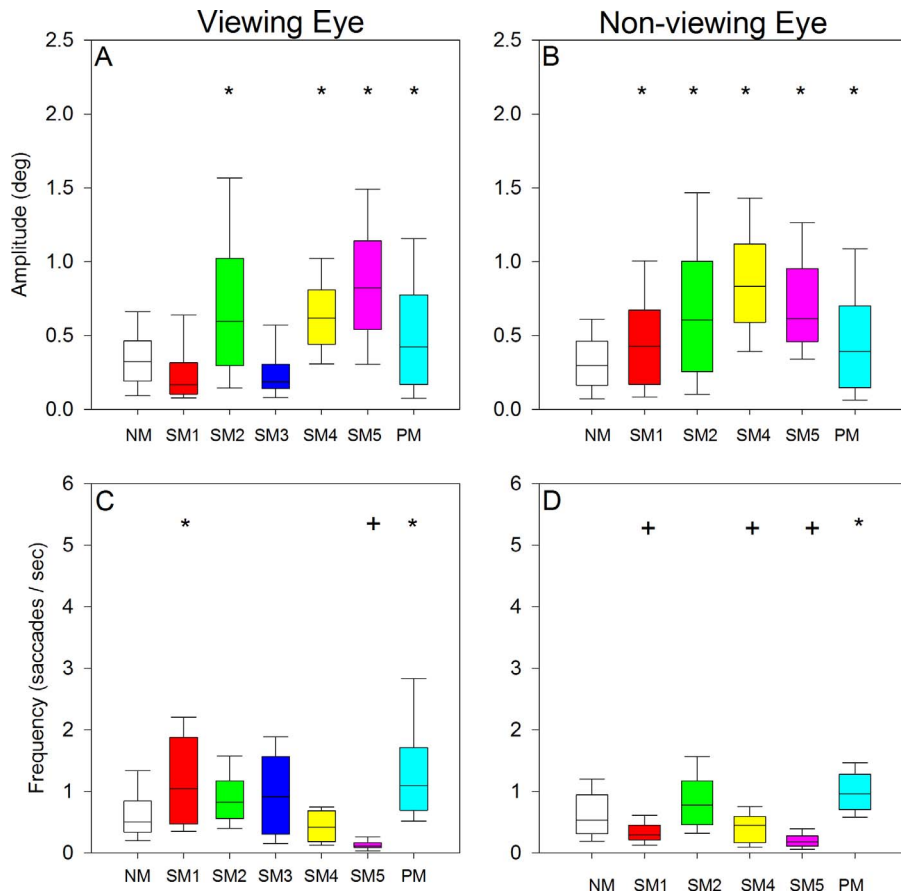

FIGURE 5. Box plots of amplitude (**A, B**) and frequency (**C, D**) of only upward-directed fixational saccades in the viewing eye (**A, C**) and nonviewing eye (**B, D**) of each monkey in the study. *Significantly higher values than NM; +Significantly lower values than NM.

downward components (including nystagmus quick phases) biased the estimates of frequency when all fixational saccade events are included. Further, frequency of fixational saccades in the strabismic monkeys was either similar to or less than in the normal monkey for four of five strabismic monkeys in the viewing eye and all strabismic monkeys in the nonviewing eye (Kruskal-Wallis ANOVA on ranks viewing eye H[6] = 179.79, $P < 0.001$; nonviewing eye H[5] = 167.21, $P < 0.001$; Dunn's method for post hoc testing $P < 0.05$). There was significant reduction in the frequency estimate in PM (approximately 68%), although still higher than that of NM in both the viewing and nonviewing eye.

Relationship Between Fixational Saccade Amplitude and BCEA

Fixation instability in the viewing and nonviewing eyes of all five strabismic monkeys and PM, as quantified using the BCEA metric, was increased (larger BCEA) compared with fixation instability in the normal monkey. To further understand how fixational saccades might affect stability of fixation, we sought to determine a relationship between fixational saccade amplitude and the BCEA metric. We plotted median amplitude of fixational saccades from the viewing eye along with the corresponding BCEA for each of the 60 experimental trials and found that an exponential rise to maximum model provided the best fit to the data for each monkey (Fig. 6). Note that for each animal, we also attempted linear regression between fixational saccade amplitude and BCEA and found that the exponential rise to maximum fit yielded better coefficients of determination in all monkeys except SM3. The plateauing of fixational saccade amplitude for larger BCEA suggests that factors other than fixational saccades (likely drift) contribute significantly toward increased BCEA conditions in the monkeys.

Fixational Stability in Depth

Although, historically, fixation instability has been investigated for each eye individually, it is also reasonable to consider fixation instability in depth (vergence fixation instability) in strabismic patient populations because their binocular vision capabilities are compromised. To interpret our data from a vergence standpoint, we calculated a vergence (left eye position − right eye position) BCEA for our study cohort (Fig. 7A). Vergence BCEA for the normal monkey was small (median = 0.15 deg^2) and less variable as opposed to the strabismic monkeys (range of medians: 0.63–2.15 deg^2). These findings suggest that fixation in depth is stable in the normal animal but is not in the strabismic population (Kruskal-Wallis ANOVA on ranks H[5] = 265.47, $P < 0.001$). Interestingly, monkey PM, who showed significant fixation instability due to nystagmus, was relatively stable in depth (median = 0.42 deg^2). We also calculated a versional (left eye position/2 + right eye position/2) BCEA and these data are plotted in Figure 7B. Our data show that versional fixation stability is also disrupted in the strabismic animals (Kruskal-Wallis ANOVA on ranks H[5] = 196.56, $P < 0.001$).

Influence of Target Parameters and Viewing Conditions on Amplitude of Fixational Saccades

In a previous study, we showed that target shape and size parameters significantly influenced fixation instability as measured by the BCEA in normal and strabismic monkeys. Changes in BCEA were however generally small in magnitude. One of the objectives of this study was to examine the influence of target parameters (those deemed significant in the

previous study) on amplitude of fixational saccades. Three-way ANOVA followed by Holm-Sidak post hoc testing was used to assess the main effects of target size, target shape, and viewing condition in the viewing eye (Figs. 8A, 8C, 8E) and nonviewing eye (Figs. 8B, 8D, 8F) of the animals. Table 2 summarizes the main effects outcomes of the 3-way ANOVA and post hoc testing for the viewing and nonviewing eyes.

Amplitude of fixational saccades was significantly larger for the disk-shaped target than for the optotype in the normal monkey and three of five strabismic monkeys in the viewing eye and two of four strabismic monkeys in the nonviewing eye. Fixational saccade amplitudes were also significantly larger for larger targets (2° vs. 0.5°) in the normally aligned monkeys (NM and PM) as well as in all strabismic monkeys (except SM1) in the viewing and nonviewing eyes. Amplitude of fixational saccades was larger for one of the monocular viewing conditions for five of six monkeys, including the normally aligned monkey. Binocular viewing was idiosyncratic but generally the same as the "better" monocular viewing condition. Although statistically significant, the magnitudes of the changes in fixational saccade amplitudes are generally small and not likely to be functionally significant. Consequently, analysis of interaction effects among the ANOVA variables was not pursued. Note that these findings are largely in line with our earlier observations of influence of target parameters on the BCEA.

DISCUSSION

In this study, we examined fixational saccades and their contribution toward fixation instability in strabismic monkeys and also considered how target parameters and viewing conditions affect fixational saccades. The main findings of our study were as follows:

1. Amplitude and frequency of fixational saccades were larger than those in the normal monkeys; nystagmus quick phases significantly influenced fixational saccade frequency but only slightly influenced fixational saccade amplitude.
2. Relationship between overall fixation instability and fixational saccade amplitude was nonlinear and showed a saturation of fixational saccade amplitudes.
3. Strabismic monkeys show significantly larger fixation instability in depth (vergence fixation instability) compared to the normal animal.
4. Target shape, size and viewing conditions affects amplitude of fixational saccades in both normal and strabismic monkeys.

Below we discuss the implication of each of these findings in detail.

Fixational Saccade Metrics in Normal and Strabismic Monkeys

Strabismic monkeys tended to exhibit larger and more frequent fixational saccades when compared to normal animals. Nystagmus quick phases affected estimates of fixational saccade amplitude only marginally but significantly reduced estimates of frequency toward normal levels. A previous study by Gonzalez and colleagues[19] also did not find differences in microsaccade amplitudes between amblyopic and fellow eyes in a cohort of strabismic and anisometropic amblyopes. One way to interpret our data is that fixation instability in strabismic monkeys is at least partially due to larger fixational saccades and when nystagmus is present, more frequent fixational saccade type events. Studies that have examined the frequency
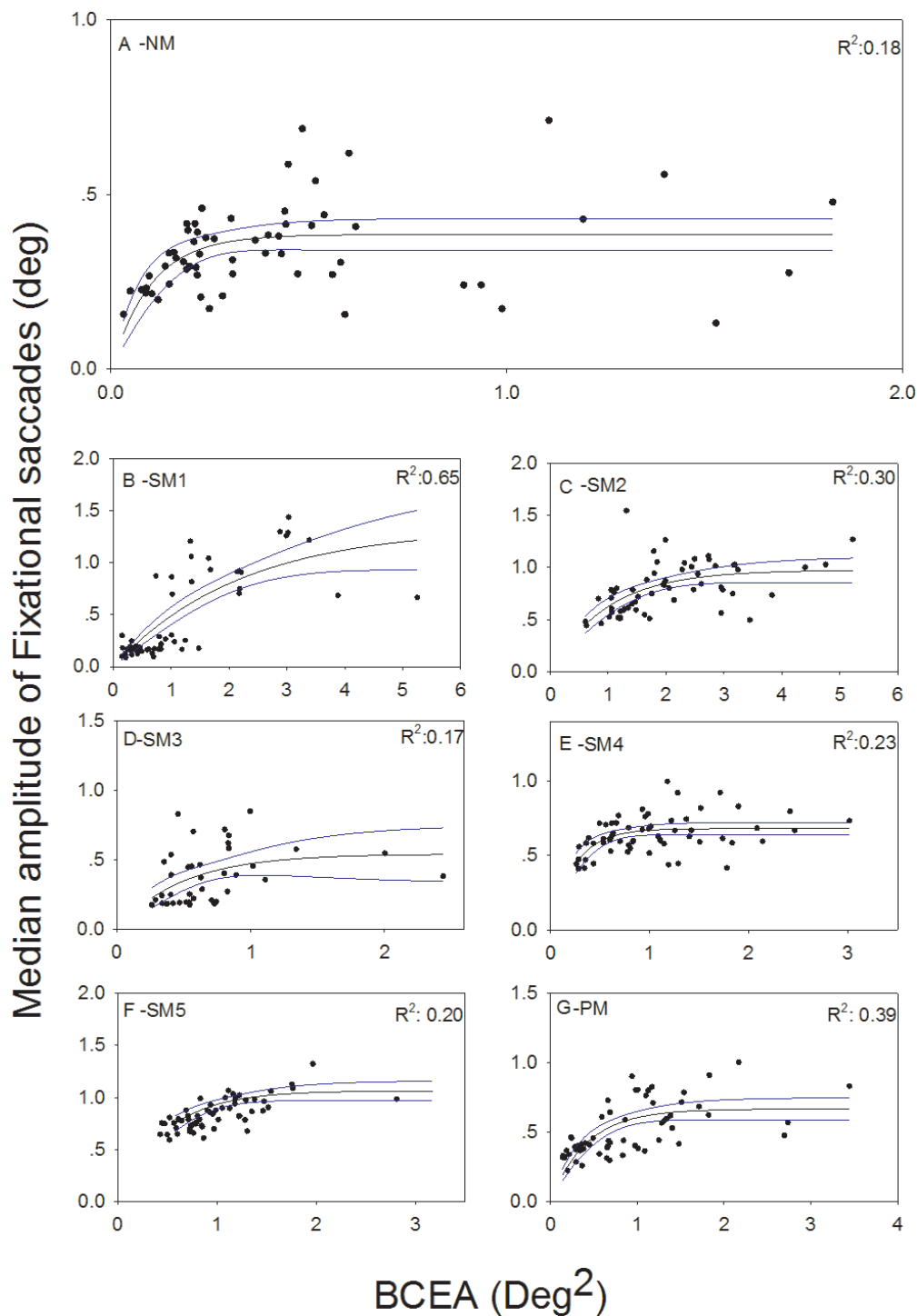
FIGURE 6. Relationship between median amplitude of fixational saccades and overall fixation instability as measured by the BCEA metric in each monkey. Each *data point* represents the calculated BCEA and median amplitude measures from the viewing eye data during a single experimental trial. The *continuous lines* in each panel are the exponential rise to maximum fit and 95% confidence intervals.

of fixational saccades in disease conditions have yielded inconsistent results. Thus, Shaikh and colleagues[33] and Ghasia and colleagues[34] suggested that the frequency of fixational saccades was decreased in strabismic and anisometropic amblyopes, whereas Gonzalez and colleagues[19] found that frequency of fixational saccades was similar to controls, and Ciuffreda and colleagues[18] and Chung and colleagues[35] suggested that frequency of fixational saccades was increased

in strabismic amblyopes. In AMD patients, it appears that the frequency of fixational saccades is similar to controls.[20] From our data analysis, we suggest that the inconsistency could be due to the presence or absence of nystagmus. The presence of nystagmus quick phases could present as an increase in frequency, when in fact the frequency of fixational saccades after accounting for nystagmus quick phases is actually the same as controls or even decreased. As shown by the data from
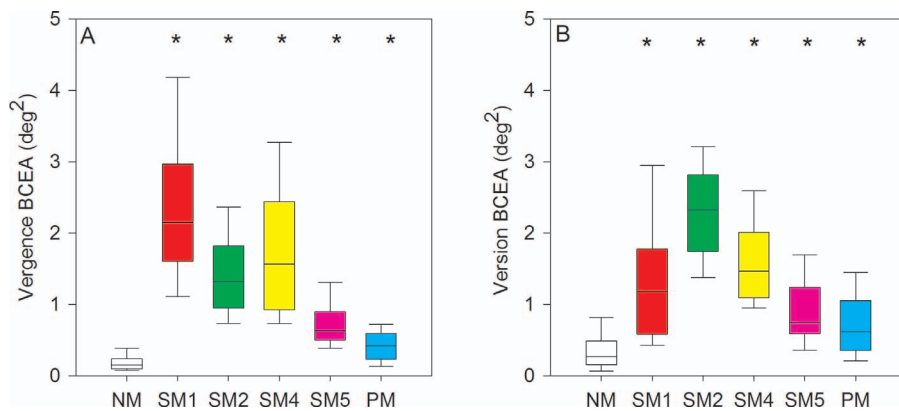
Figure 7. Box plots of vergence (left eye position − right eye position) BCEA (**A**) and version (left eye position/2 + right eye position/2) BCEA (**B**) of each monkey in the study. *Asterisks* indicate significant difference from the NM.

PM, the presence of nystagmus, even in the absence of strabismus, can significantly influence estimates of fixational saccade frequency.

An issue for debate is the relative role of drift in fixation instability and whether larger fixational saccades can completely account for increased instability in strabismic monkeys. A previous study on fixational eye movements in human strabismic and anisometropic amblyopes suggested that fixational saccades were the limiting factor that determined fixation instability.[35] We took advantage of the different viewing conditions and multiple trials in our experimental design to investigate this issue. Our analysis showed that fixation instability (BCEA value) and amplitude of fixational saccades could be related via an exponential rise to maximum model fit (Fig. 6). The implication of this relationship is that amplitude of fixational saccades is a primary driver for BCEA when stability is relatively high (low BCEA); however, there is a saturation effect of fixational saccade amplitudes and therefore

other factors (most likely drift) must drive fixation instability at higher BCEA.

Fixation Instability in Depth

The analysis of fixation instability and fixational eye movements has generally been uniocular, for example investigating right eye stability and left eye stability or instability of the viewing and nonviewing eye in the case of strabismus, or in the case of amblyopia, instability of the amblyopic eye and that of the fellow eye.[18-20] These analyses have been partially driven by considerations of relationship between instability on the fovea and visual acuity. However, fixation must also be relatively stable in depth to maintain the image within Panum's fusional area and therefore maintain clear and single vision. For a human with interpupillary distance of 6 cm, the size of Panum's fusional area for a straight-ahead object is approximately 20 minutes of arc horizontally and approximately 8

TABLE 2. Three-Way ANOVA Analysis of Fixational Saccade Amplitude

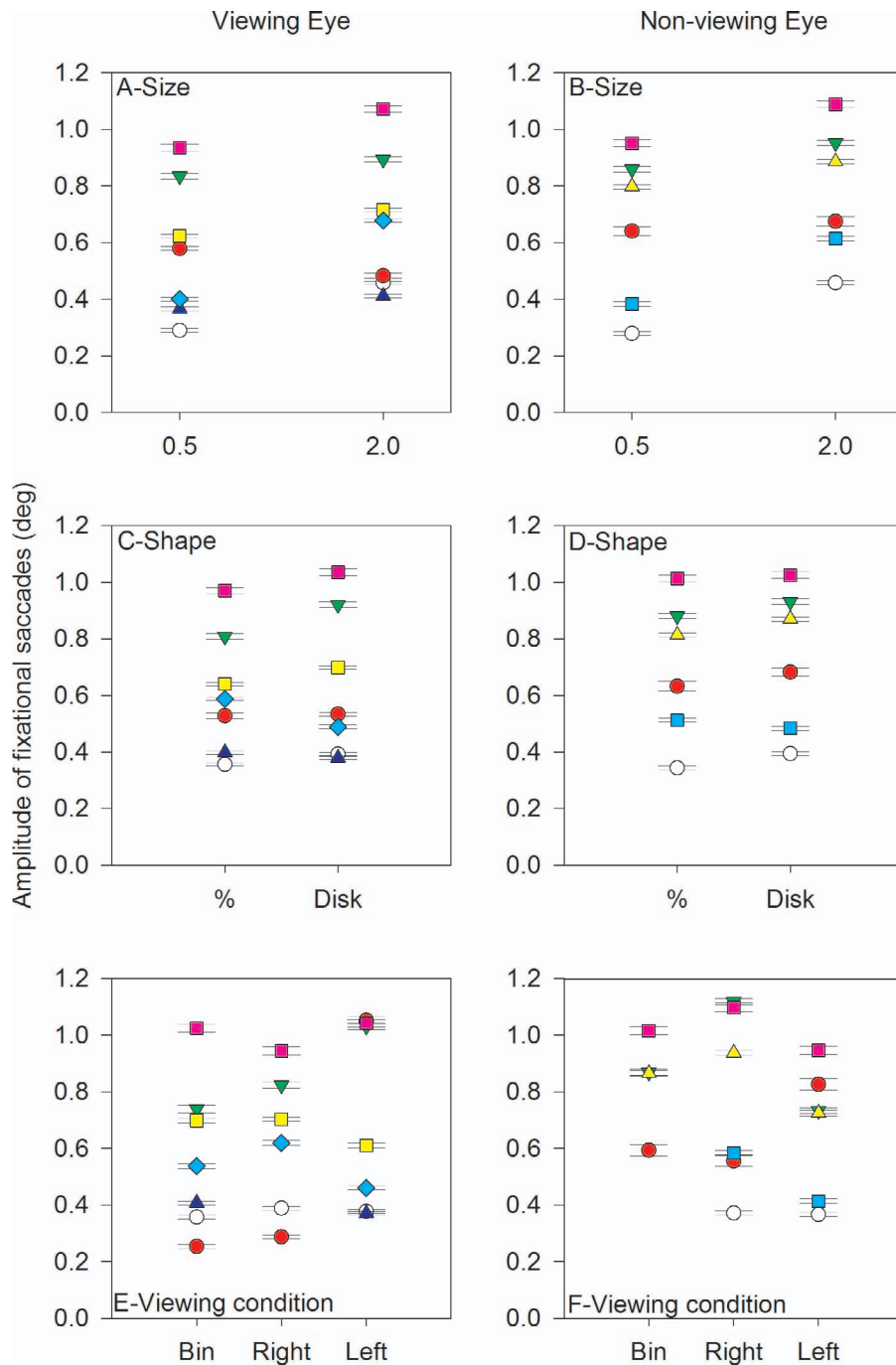| Factors	Monkey	Viewing Eye		Nonviewing Eye	
		ANOVA Results, (df Effect, df Error) = F Value, P	**Post Hoc Tests, Larger Amplitude**	**ANOVA Results (df Effect, df Error) = F Value, P**	**Post Hoc Tests, Larger Amplitude**
Shape	NM	$F_{1,3449} = 18.38$, <0.001	Disk	$F_{1,2391} = 26.03$, <0.001	Disk
	SM1	$F_{1,7066} = 0.24$, NS		$F_{1,3014} = 2.38$, NS	
	SM2	$F_{1,7827} = 65.87$, <0.001	Disk	$F_{1,7800} = 14.36$, <0.001	Disk
	SM3	$F_{1,4978} = 4.41$, <0.05	%		
	SM4	$F_{1,4529} = 42.69$, <0.001	Disk	$F_{1,4501} = 24.42$, <0.001	Disk
	SM5	$F_{1,3165} = 15.98$, <0.001	Disk	$F_{1,3117} = 0.12$, NS	
	PM	$F_{1,7249} = 116.94$, <0.001	%	$F_{1,4027} = 7.04$, <0.008	%
Size	NM	$F_{1,3449} = 411.96$, <0.001	2	$F_{1,2391} = 341.54$, <0.001	2
	SM1	$F_{1,7066} = 63.54$, <0.001	0.5	$F_{1,3014} = 1.30$, NS	
	SM2	$F_{1,7827} = 18.61$, <0.001	2	$F_{1,7800} = 49.67$, <0.001	2
	SM3	$F_{1,4978} = 25.41$, <0.001	2		
	SM4	$F_{1,4529} = 108.09$, <0.001	2	$F_{1,4501} = 58.62$, <0.001	2
	SM5	$F_{1,3165} = 70.19$, <0.001	2	$F_{1,3117} = 14.49$, <0.001	2
	PM	$F_{1,7249} = 948.3$, <0.001	2	$F_{1,4027} = 445.04$, <0.001	2
Viewing condition	NM	$F_{2,3449} = 4.40$, <0.05	Right eye viewing	$F_{1,2391} = 0.26$, NS	
	SM1	$F_{2,7066} = 1378$, <0.001	Left eye viewing	$F_{2,3014} = 232.71$, <0.001	Left eye viewing
	SM2	$F_{2,7827} = 155.95$, <0.001	Left eye viewing	$F_{2,7800} = 301.53$, <0.001	Right eye viewing
	SM3	$F_{1,4978} = 16.58$, <0.001	Binocular viewing		
	SM4	$F_{2,4529} = 42.38$, <0.001	Right eye viewing	$F_{2,4501} = 117.04$, <0.001	Right eye viewing
	SM5	$F_{2,3165} = 13.18$, <0.001	Left eye viewing	$F_{2,3117} = 27.43$, <0.001	Right eye viewing
	PM	$F_{2,7249} = 108.4$, <0.001	Right eye viewing	$F_{1,4027} = 243.5$, <0.001	Left eye viewing

FIGURE 8. Main effect plots of amplitude of fixational saccades in viewing eye (**A**, **C**, **E**) and nonviewing eye (**B**, **D**, **F**) of all the monkeys as a function of target size (**A**, **B**), target shape (**C**, **D**), and viewing condition (**E**, **F**). (**A–D**) include data from both monocular and binocular viewing conditions. *Colors* represent monkeys and are same as in other plots. REV, right eye viewing; LEV, left eye viewing; Bin, binocular viewing.

minutes of arc vertically. A recent study has shown that fixation in depth in healthy adults and children is quite stable and remains within these limits.[36]

In our data, we found that indeed the vergence BCEA of the normal monkey was quite stable and comparable to that of healthy human subjects (Fig. 6). In other words, the stability of fixation in depth for the normal monkey is adequate to maintain a clear image. We found significantly greater vergence BCEA values in the strabismic monkeys when compared to the normal. The absence of disparity information in the strabismic monkeys could lead to poorer vergence control and vergence instability indicated by the larger vergence BCEA. Alternatively, developmental disruption of binocular vision also leads to disruption of accommodation control; increased accommodation instability in these strabismic animals leads to the increased vergence instability due to cross links between the vergence and accommodation systems. We did not measure accom-

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modation in our studies and therefore cannot directly comment on instability of accommodation. However, other studies have suggested that accommodation is unstable (increased accommodative microfluctuations) in strabismus populations[37] (Joshi AC. *IOVS* 2016;57:ARVO E-Abstract 4577). It has been shown that office-based accommodative/vergence therapy improves control of accommodation and it would be interesting to investigate whether these therapies have a direct effect on improving accommodation instability and vergence fixation instability in strabismic patients.[38] Note that, in the strabismic monkeys, fixation stability is not rooted only in the vergence system. The increased versional BCEA (Fig. 7B) shows that there is instability driven via conjugate oculomotor pathways as well.

Influence of Target Parameters on Fixational Saccades

In a previous study, we found that fixation instability, as measured by the BCEA, was significantly influenced by target shape and size.[23] A disk-shaped target resulted in greater instability compared with the optotype, and larger targets resulted in greater instability compared with the smaller target. In this study, we found that fixational saccade amplitude also changed, similar to the BCEA, depending on the target parameters. A larger-sized target and a disk-shaped target resulted in larger fixational saccades in both the strabismic group and normal. Because the target parameter mediated changes in BCEA (previous study) and fixational saccade amplitude (this study) are small in magnitude and occur in the presence of large ongoing instability, we suggest that there is little functional utility to the target-mediated effects that are observed. The influence of monocular versus binocular viewing on fixational saccade amplitudes were also similar to those found previously on the BCEA (i.e., binocular viewing did not result in better stability than the "better" monocular viewing condition).

Conclusions

In summary, larger and more frequent fixational saccades and intersaccadic drifts contribute detrimentally to fixation in strabismic monkeys. Fixational saccade parameters are influenced by target parameters similar to target parameter effects on overall measures of fixational stability. These studies provide a framework for future neurophysiological investigation into the neural substrate for fixation instability and for quick phases of nystagmus in monkey models for strabismus and nystagmus.

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