

# Longitudinal Evaluation of Eye Misalignment and Eye Movements Following Surgical Correction of Strabismus in Monkeys

Mythri Pullela,<sup>1</sup> Brittany A. Degler,<sup>1</sup> David K. Coats,<sup>2</sup> and Vallabh E. Das<sup>1</sup>

<sup>1</sup>College of Optometry, University of Houston, Houston, Texas, United States

<sup>2</sup>Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, United States

Correspondence: Vallabh E. Das, College of Optometry, University of Houston, 4901 Calhoun Road, Houston, TX 77204, USA; vdass@central.uh.edu.

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**PURPOSE.** Strabismus correction surgery is well documented in both the literature and practice with varying levels of success and permanence. Our goal was to characterize longitudinal changes in eye alignment and eye movements following strabismus correction surgery in a monkey model for developmental strabismus.

**METHODS.** We studied two juvenile rhesus monkeys with exotropia previously induced via an optical prism-rearing paradigm in infancy. Eye misalignment was corrected via a resection-recession surgery of the horizontal rectus muscles of one eye. Binocular search coils were used to collect eye movement data during smooth-pursuit, saccades, and fixation tasks before surgical treatment, immediately after surgery, and through 6 months after treatment.

**RESULTS.** Both animals showed an immediate ~70% reduction in misalignment as a consequence of surgery that regressed to a 20%–40% improvement by 6 months after treatment. Significant changes were observed in saccade and smooth-pursuit gain of the nonviewing eye after surgery, which also reverted to presurgical values by 6 months. A temporary improvement in fixation stability of the nonviewing eye was observed after surgery; naso-temporal (N/T) asymmetry of monocular smooth-pursuit remained unchanged.

**CONCLUSIONS.** Surgical realignment is followed by plastic changes that often lead to reversal of surgery effects. Immediate improvement in misalignment and changes in eye movement gains are likely a result of contractility changes at the level of the extraocular muscle, whereas longer-term effects are likely a combination of neural and muscle adaptation.

**Keywords:** strabismus, resection, recession, eye movements, nonhuman primate

Strabismus is a developmental disorder that affects ~2%–4% of children worldwide.<sup>1–3</sup> Although the exact etiology of strabismus is often unknown, a disruption of binocular vision in infancy likely leads to eye misalignment, a strategy also used to develop animal models for strabismus.<sup>4–6</sup> These animal models have been very effective in replicating a variety of strabismus oculomotor features beyond just horizontal misalignment, such as dissociated deviations, latent nystagmus, fixation instability, fixation switch behavior, naso-temporal asymmetry of horizontal smooth-pursuit and optokinetic nystagmus, and disconjugate eye movements.<sup>6–16</sup> Studies in animal models have also led to several insights into neural strabismus mechanisms including identifying neural substrates for misalignment, A/V patterns, and saccade disconjugacies. For example, previous studies from our laboratory and others have shown that, in monkeys with a sensory strabismus, an innervational drive from the motor nuclei (oculomotor and abducens nucleus) plays a critical role in setting the state of ocular misalignment on a moment-to-moment basis.<sup>17,18</sup>

In addition to investigating basic mechanisms for strabismus, the availability of appropriate animal models provides an outstanding opportunity to study treatment strategies for the disorder. One of the most prevalent approaches to correct misalignment is to surgically alter extraocular muscle (EOM) function. Strabismus correction surgery is well documented in

the literature and practice with varying levels of permanence.<sup>19,20</sup> By various accounts, approximately 40% of the subjects that undergo surgery, develop significant recurrent strabismus, and may have to undergo multiple surgeries.<sup>21,22</sup> Although it is not known why surgery sometimes works and sometimes does not, it is possible to speculate that both central (brain) and peripheral (muscle) factors influence the outcome of strabismus correction surgery. Muscle remodeling has been shown to occur on surgical manipulation of EOM in rabbits and in monkey.<sup>23–25</sup> For example, sarcomeres are added or removed when eye muscles were detached from the globe and attached to the orbit for a few days.<sup>25,26</sup> It is also likely that eye movement properties and visual experience following surgery influences the outcome of surgery via neural mechanisms.<sup>27–30</sup> Similarly proprioceptive feedback following surgery could also influence central oculomotor circuits that are innervating the EOM.<sup>31,32</sup>

Thus, the overall goal of our research is to gain insight into neural and muscle adaptive mechanisms that are triggered following strabismus correction surgery. In this particular study, we examined the longitudinal changes in eye alignment and eye movements that occur in monkey models of strabismus following a typical strabismus correction surgery to provide a framework to interpret future neural investigation.

## METHODS

### Subjects and Rearing Paradigms

Behavioral and neurophysiological data were collected from two exotropic juvenile rhesus (*Macaca mulatta*) monkeys (~6 years, ~9–10 kg). Strabismus was previously induced by optical prism-rearing of infant monkeys.<sup>4</sup> During the prism-rearing paradigm, infant monkeys wore a helmet like device that housed a horizontally oriented Fresnel prism in front of one eye and a vertically oriented Fresnel prism (20 prism diopter) in front of the other eye, starting from day 1 after birth to 4 months of age, after which they were allowed unrestricted vision. Disruption of binocular vision due to prism-viewing during this initial period leads to strabismus as it is the critical period for development of eye alignment, stereopsis, and binocular sensitivity.<sup>33–35</sup>

### Surgical Procedures

All procedures were carried out in accordance with National Institutes of Health guidelines and were approved by the University of Houston Institutional Animal Care and Use Committee. Prior to commencement of experiments associated with this study, each juvenile monkey (~4 years of age) underwent surgical procedures carried out under aseptic conditions under isoflurane anesthesia (1.25%–2.5%). In the first surgery, a head stabilization post was implanted to restrict head movements during the experiments.<sup>36</sup> In subsequent procedures, scleral search coils were implanted in both eyes, using the technique of Judge et al.,<sup>37</sup> to record binocular eye movements.

After collecting baseline data, each animal underwent an extraocular muscle resection-recession procedure to reduce their angle of misalignment (strabismus correction surgery). Strabismus correction surgery was performed by an expert strabismus surgeon (also one of the study authors) on the left eye only of M1 and right eye only of M2 and consisted of lateral rectus recession (weakening the lateral rectus muscle) and medial rectus resection (strengthening the medial rectus muscle) to improve their strabismus angle. We specifically chose to operate on only one eye, because we wanted to use the fellow eye as a control.

### Data Acquisition and Experimental Procedures

The goal of data acquisition and analysis was to perform a longitudinal evaluation of eye alignment and eye movements following strabismus correction surgery. Therefore, binocular eye movement data were collected as the animals performed central and eccentric fixation, saccades ( $\pm 15^\circ$  horizontal and vertical target locations), and smooth pursuit (0.3 Hz,  $\pm 15^\circ$ ) tasks under monocular left eye or right eye viewing conditions. Alignment data were collected at five time points: before surgical treatment (Pre), the day after surgical treatment (P1d), 1 week after treatment (P1w), 1 month after treatment (P1m), and 6 months after treatment (P6m). Saccade and smooth-pursuit data were analyzed at Pre, P1w, P1m, and P6m because there were not enough experimental trials collected at P1d.

Binocular data were collected using the magnetic search coil technique (Primelec Industries, Regensdorf, Switzerland). Eye coils were calibrated at the beginning of each experiment by rewarding the animal with small amounts of juice as they looked at a series of targets projected along the horizontal or vertical meridian on a screen at distance of 57 cm. Calibration of each eye was performed independently during monocular viewing forced by occluding one or the other eye using liquid crystal shutter goggles (Citizen Fine Devices, Nagano, Japan)

that were under computer control. Visual stimuli were generated using the BITS# visual stimulus generator (Cambridge Research Systems, Cambridge, United Kingdom) and Psychtoolbox 3<sup>38</sup> operated under computer control and presented using a DepthQ projector running at a 120-Hz frame rate (Lightspeed Design, Inc., Bellevue, WA, USA). The target and binocular eye position signals were passed through anti-aliasing filters with a cutoff of 400 Hz before digitization 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 (FIR) low-pass filter with a cutoff of 80 Hz prior to further analysis.

### Data Analysis

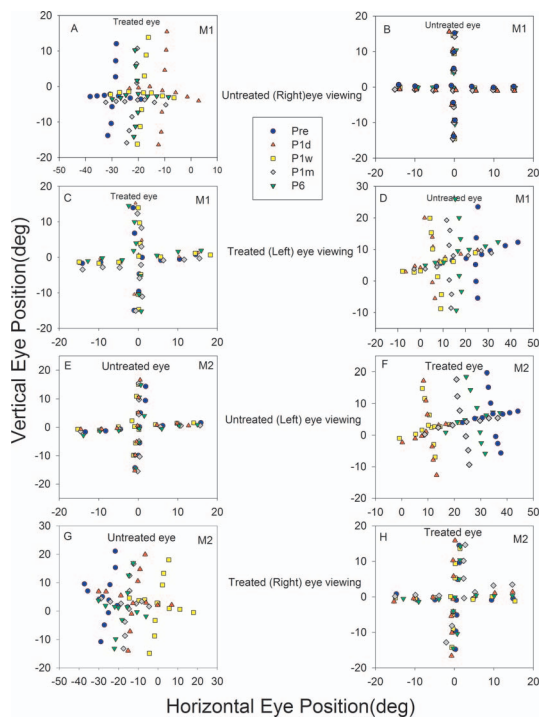
Analysis of eye movement data was partially automated using custom scripts written in MATLAB (Mathworks, Natick, MA, USA). For saccade data, the computer displayed target position, binocular eye position, eye velocity, and eye acceleration traces of a single saccade trial. Velocity and acceleration signals were generated by digital differentiation of the position signal using a central difference algorithm. The investigator viewed the traces and decided whether the saccade trial was to be accepted or rejected. Trials that were rejected were usually those in which the animal was not fixating prior to the target step, the saccade did not appear to be directed toward the target, or the saccade did not occur within 400 ms of the target step. Once a decision to accept the trial was made, mean and SD of control eye acceleration prior to the saccade were calculated over a 70-ms fixation period. Saccade onset was automatically determined by the software as the first time point at which eye acceleration was greater than 5 SDs away from the control eye acceleration, and saccade offset was determined as the last time point at which eye deceleration was less than 5 SDs away from the same mean eye acceleration. Although detection of saccade onset and offset was automated, the investigator visually examined the velocity and acceleration traces of every saccade and had the option of either accepting or changing the computer selection, although this was not typical. Once the saccade was identified, saccade metric parameters (amplitude and peak velocity) for each eye were calculated. Main sequence plots of horizontal saccade peak velocity versus horizontal saccade amplitude were fitted with the following exponential rise to maximum equation:

$$PV = PV_{\max} \times \left(1 - e^{(-A \times \text{amp})}\right) \quad (1)$$

where amp = horizontal saccade amplitude, PV = horizontal saccade peak velocity, A = constant determining exponential rise, and  $PV_{\max}$  = asymptotic peak velocity.

For sinusoidal smooth-pursuit analysis, the eye movement data were first desaccaded by a custom MATLAB program. Then smooth-pursuit gain (ratio of peak eye velocity to peak target velocity) was calculated separately for nasalward and temporalward movements. To assess fixation stability, ~10 seconds of eye movement data from both the eyes were analyzed as the monkey maintained monocular fixation at a stationary straight-ahead target. Trials were checked manually offline to make sure the animal was fixating the target. Fixation stability was quantified by calculating the bivariate contour ellipse area (BCEA), a metric that quantifies the area of the region over which eye positions are dispersed during attempted fixation. Therefore, a smaller value for BCEA is indicative of greater fixation stability. The BCEA encompassing 68.2% of fixation points was calculated using the following equation<sup>39</sup>:

$$BCEA = 2.291 \times \pi \times \sigma_x \times \sigma_y \times \sqrt{(1 - p^2)}, \quad (2)$$



**FIGURE 1.** Longitudinal change in eye misalignment following strabismus treatment in M1 (A–D) and M2 (E–H). Plots show eye positions of the viewing eye (B, C, E, H) and the nonviewing eye (A, D, F, G) during monocular viewing of a series of targets along the horizontal or vertical meridian. A, B (M1) and E, F (M2) show data collected when the animal viewed with their untreated eye (treated eye under cover) and C, D (M1) and G, H (M2) show data collected when the animals viewed with their treated eye. Each plot shows data collected at five time points: before surgery (pre; blue circle), 1 day after surgery (P1d; red triangle), 1 week after surgery (P1w; yellow square), 1 month after surgery (P1m; gray diamond), and 6 months after surgery (P6; green inverted triangle). Pairs of plots on each row show that the viewing eye is on target while the covered eye is abducted. The angle of misalignment is reduced on P1d and gradually reverses by P6. Rightward and upward eye positions are positive and leftward and downward eye positions are negative.

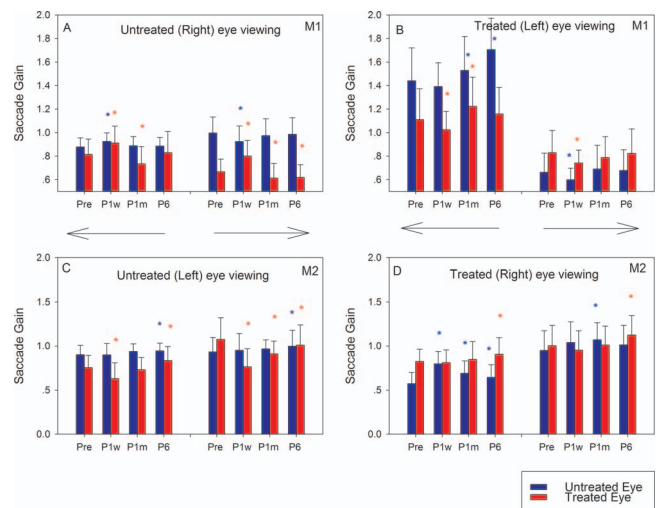
where  $\sigma_x$  = SD of horizontal eye position,  $\sigma_y$  = SD of vertical eye position, 2.291 is the  $\chi^2$  value (2 df) corresponding to a probability of 0.68, and  $p$  is the Pearson product moment correlation coefficient of horizontal and vertical eye positions.

For statistical analysis, the Kruskal-Wallis 1-way ANOVA on ranks was performed, and if significant, post hoc testing was performed using Dunn’s method.

## RESULTS

### Longitudinal Changes in Eye Alignment

Prior to treatment, both monkeys had an exotropia whose magnitude depended on the eye of fixation, a likely indicator for dissociated horizontal deviation. M1 had an exotropia of  $\sim 27^\circ$  under left eye viewing (LEV) conditions and  $\sim 30^\circ$  under right eye viewing (REV) conditions. M2 had an exotropia of  $\sim 37^\circ$  under LEV conditions and  $\sim 30^\circ$  during REV. Immediately after surgery (P1d), exotropia in M1 reduced to  $\sim 10^\circ$  (LEV) and  $\sim 11^\circ$  (REV) and exotropia in M2 reduced to  $\sim 9^\circ$  (LEV) and  $\sim 12^\circ$  (REV). The initial decrease in strabismus angle after the surgery was followed by a gradual reversal toward presurgical exotropia by the end of 6 months. Figure 1 is a Hess plot showing alignment at different time points as the animals



**FIGURE 2.** Longitudinal change in horizontal saccade gains in M1 (A, B) and M2 (C, D) following strabismus treatment. Bars in each plot show means and SDs of saccade gains of the untreated eye (blue) and treated eye (red) at four time points (Pre, P1w, P1m, and P6). Leftward and rightward saccades were analyzed separately and are represented by the directional arrows. The asterisks represent statistically significant difference compared with presurgical values (blue, untreated eye; red, treated eye).

fixated a series of targets along the horizontal and vertical meridian. By 6 months after the correction surgery, M1 showed an exotropia of  $\sim 20^\circ$  during either eye viewing and M2 showed an exotropia of  $\sim 30^\circ$  (LEV) and  $\sim 20^\circ$  (REV). Thus, it appeared that both animals reverted to  $\sim 60\%$ – $80\%$  of their original strabismus angle within 6 months. Alignment was also examined at 10 months, and the strabismus angle for M1 was still  $\sim 20^\circ$  but had changed slightly in M2 to  $\sim 24^\circ$  (LEV) and  $\sim 18^\circ$  (REV).

### Longitudinal Changes in Saccade Parameters

We analyzed 4021 saccades at four time points (Pre [1449 saccades], P1w [648 saccades], P1m [1103 saccades], and P6m [821 saccades]). Because the numbers of saccadic trials acquired on any single day were relatively low, data from multiple days near the indicated time point were pooled for analysis. Average saccade gains (ratio of saccade amplitude to target amplitude) of the two animals are summarized in Figure 2. In general, saccade gain of the viewing eye, irrespective of whether the animal viewed with the treated or untreated eye, did not show significant longitudinal changes (blue bars in 2A, 2C; red bars in 2B, 2D), as might be expected if the goal of the viewing eye is to saccade onto the target. Longitudinal changes in the nonviewing eye were more striking, although slightly different in the two monkeys. When M1 viewed with the untreated eye (Fig. 2A), the treated eye (red bars) showed a significant increase in saccade gain after surgery that decreased to presurgery values by P6. On the other hand, when M2 viewed with the untreated eye (Fig. 2C), there was an immediate drop in saccade gain in the treated eye (red bars) following surgery that reverted to presurgical values by P1m–P6. These findings were generally true for both rightward and leftward saccades. Note that when viewing with the untreated eye, any changes in treated eye saccade gains can be attributed to adaptive muscle or neural changes. Complementary findings were observed when the animals viewed with their treated eye (Figs. 2B, 2D). Thus, in M1 (Fig. 2B), the untreated eye saccade gain (blue bars) decreased or remained the same at P1w and

thereafter increased by P6 (greater than Pre values for leftward saccades). In M2 (Fig. 2D), the untreated eye saccade gain increased at P1w and thereafter decreased by P6 for leftward saccades only. Note that when viewing with the treated eye, any changes in untreated eye saccade gains are likely driven by a combination of Hering's law based neural mechanisms and adaptive untreated eye muscle or neural changes triggered by the surgical intervention of the treated muscle.

Main sequence plots are shown in Figure 3 along with the mean and 95% upper and lower confidence limits. Significant differences were visually identified as those where 95% confidence limits do not overlap. Longitudinal changes in saccade peak velocity were relatively small and difficult to identify because of the large variability in saccade velocities. There was a slight but significant increase in saccade velocity in both the treated and untreated eyes as animal M1 viewed with the treated eye (Figs. 3C, 3D) as P6 velocities are higher than they were before surgery. A similar, albeit greater magnitude, increase in peak saccade velocity was observed in both the treated and untreated eyes as animal M2 viewed with the treated eye (more so for rightward than leftward saccades; Figs. 3G, 3H). Estimated fit parameters for  $PV_{max}$  and A from Equation 1 for each of the eyes under the different viewing conditions and at the different time points are provided in the Table.

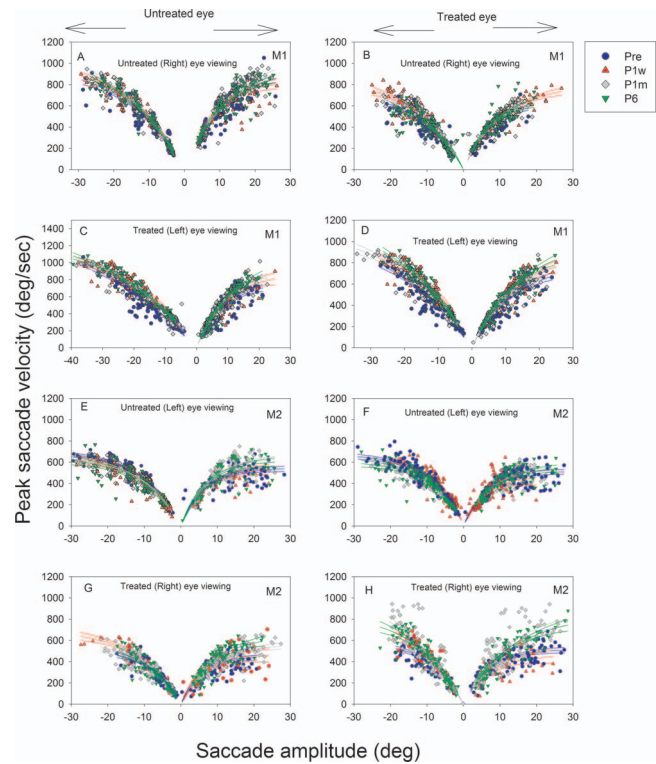
**Longitudinal Changes in Smooth-Pursuit Behavior**

Summary of leftward and rightward smooth-pursuit gains obtained from 15 to 30 cycles of smooth pursuit at each time point for each monkey are shown in Figure 4 along with results of statistical comparison to presurgical smooth-pursuit gains. Longitudinal trends in smooth-pursuit gains reflected the trends observed for saccadic eye movement gains. Gains of viewing eye remained more or less constant following surgery (blue bars in Figs. 4A, 4C; red bars in Figs. 4B, 4D). When M1 viewed with the untreated eye (Fig. 4A), there was a significant increase in gain in the treated eye (red bars) for both rightward and leftward pursuit that was mostly reversed by P6. On the other hand, when M2 viewed with the untreated eye (Fig. 4C), there was a significant decrease in gain in the treated eye that mostly reversed by P6. Smooth-pursuit gains calculated when the animals viewed with their treated eyes (Figs. 4B, 4D) also followed trends observed during saccade analysis.

Strabismic humans and monkeys often exhibit increased monocular viewing smooth-pursuit tracking gains for nasalward target motion compared with temporalward motion (naso-temporal asymmetry). Both the monkeys in the current study displayed a naso-temporal (N/T) asymmetry prior to surgery (except M2 during left eye viewing; Fig. 5B, blue bars), and we wondered whether surgical manipulation of EOM resulted in any modification of this feature of strabismus. A naso-temporal asymmetry index was calculated as the ratio of viewing eye temporalward smooth-pursuit gain to viewing eye nasalward smooth-pursuit gain for both treated and untreated eyes viewing conditions. In M1, the naso-temporal asymmetry persisted mostly unchanged following surgery during either untreated or treated eye viewing. In M2 also, N/T asymmetry was unchanged during treated (right) eye viewing. During untreated (left) eye viewing, there was a slight improvement in the index (reduced asymmetry) that reversed by P1m.

**Longitudinal Changes in Fixation Stability**

Fixation stability was assessed by calculating the BCEA metric for each eye during monocular viewing (Fig. 6). In both monkeys, when the untreated eye was viewing, fixation stability of the nonviewing treated eye (red bars) improved



**FIGURE 3.** Longitudinal changes in horizontal saccade dynamics following strabismus treatment. Main sequence plots (M1, A–D; M2, E–H) showing horizontal saccade amplitude on the x-axis and horizontal saccade peak velocity on the y-axis. Negative saccade amplitudes indicate leftward saccades and positive amplitudes indicate rightward saccades. Left column panels (A, C, E, G) show data from the untreated eye and right column panels (B, D, F, G) show data from the treated eye. Each data point represents a single saccade, and lines represent exponential rise-to-maximum fits to the main sequence data. For these plots, viewing and nonviewing eyes' data were collected when the animals viewed monocularly with either treated eye or untreated eye. Data points and curve fits are overlaid for saccade data collected at Pre (blue circle), P1w (red triangle), P1m (gray diamond), and P6 (green inverted triangle).

(lower BCEA) after surgery but regressed by P6 in M2 only. These data were not amenable for statistical testing because each bar represents a single data point for the fixation stability metric. Fixation stability of the viewing untreated eye (blue bars) did not show consistent longitudinal effects. When the treated eye was viewing, fixation stability of nonviewing untreated eye (blue bars) showed postsurgical improvement that regressed by P6 in M1 but no consistent longitudinal effects in M2. Fixation stability of the viewing treated eye also did not show consistent longitudinal effects. Fixation stability of the nonviewing eye was worse at all time points for both the monkeys, consistent with previous studies of fixation stability in strabismic monkeys.<sup>8</sup>

**DISCUSSION**

To our knowledge, this study is the first attempt at investigating longitudinal changes in eye alignment and eye movements following strabismus treatment surgery in either humans or animal models. In addition to providing a detailed account of time course of change in alignment and movements, these data will provide a basis for examination of neural and muscle plasticity that is likely to occur as a consequence of correction surgery. Our main findings in this study were that 1)

TABLE. Fit Parameters for Main Sequence Plots Derived From Equation 1

Animal and Viewing Condition	Untreated Eye				Treated Eye			
	Leftward		Rightward		Leftward		Rightward	
	PV <sub>max</sub>	A	PV <sub>max</sub>	A	PV <sub>max</sub>	A	PV <sub>max</sub>	A
Untreated eye viewing								
M1								
Pre	1005	0.06	975	0.08	800	0.07	672	0.11
P1w	1037	0.06	849	0.11	848	0.08	795	0.09
P1m	1141	0.05	1009	0.08	797	0.09	685	0.12
P6	1125	0.05	940	0.09	679	0.09	677	0.13
M2								
Pre	709	0.09	542	0.16	674	0.11	516	0.15
P1w	668	0.09	540	0.16	635	0.14	466	0.17
P1m	667	0.09	645	0.13	597	0.12	552	0.13
P6	654	0.09	618	0.14	607	0.11	555	0.13
Treated eye viewing								
M1								
Pre	1889	0.02	772	0.11	1245	0.03	791	0.08
P1w	1237	0.04	868	0.10	982	0.07	862	0.08
P1m	1282	0.04	907	0.10	1179	0.04	791	0.08
P6	1402	0.03	1035	0.08	1302	0.04	1007	0.06
M2								
Pre	518	0.12	452	0.17	580	0.12	516	0.14
P1w	695	0.09	469	0.16	768	0.09	457	0.14
P1m	552	0.11	565	0.12	1206	0.05	809	0.08
P6	620	0.09	645	0.11	865	0.07	842	0.07

A, constant determining exponential rise to maximum; PV<sub>max</sub>, asymptotic horizontal saccade peak velocity (deg/s<sup>2</sup>).

eye misalignment reduced as a consequence of resect-recess surgery, but regressed to close to presurgical values by 6 months after surgery; 2) patterns of longitudinal changes in saccadic and smooth-pursuit eye movements also showed immediate postsurgical changes that reversed by 6 months after treatment, although naso-temporal asymmetry of smooth-pursuit tracking was unchanged; 3) there were only small

changes in fixation stability; and 4) the overall similarity in response to human strabismus surgical treatment validates our monkey model for strabismus. Below we discuss each of our findings in the context of longitudinal adaptive changes occurring in strabismus.

### Eye Alignment

Compared with dynamic changes in eye movements that were generally small but significant (discussed later), longitudinal changes in eye alignment were clear and consistent in the two monkeys in our study. Both the exotropic monkeys in the study responded to treatment in that exotropia was reduced significantly to ~30% of presurgical misalignment immediately after surgery as might be expected following a resect-recess procedure in a human patient. At P1d (day after surgery), the reduction in misalignment observed when viewing with the untreated eye is possibly only a consequence of the change in contractility of the treated EOM as the brain may not have had sufficient time to adapt. In other words, the neural signals to the EOM of viewing (untreated) and nonviewing (treated) eyes are expected to be the same as in the presurgical condition. When the monkey is forced to view with the treated eye, the brain must alter the neural signals to the treated muscles to compensate for their altered contractility to allow fixation of the target, for example, increase in neuronal drive of recessed lateral rectus from ipsilateral abducens nucleus. The anatomical connection between abducens nucleus and contralateral oculomotor nucleus (underpinnings of Hering's law) leads to increase in neuronal drive to the medial rectus muscle of the contralateral (in this case untreated) eye. Note that the change in neural drives to the treated and untreated eye EOM when the monkey is forced to view with the treated eye is not a long-term adaptive response per se as the brain is only momentarily adjusting signals to enable target acquisition and fixation.

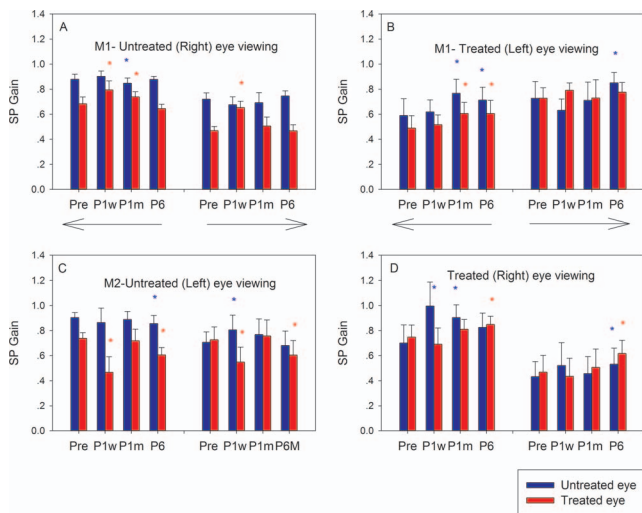
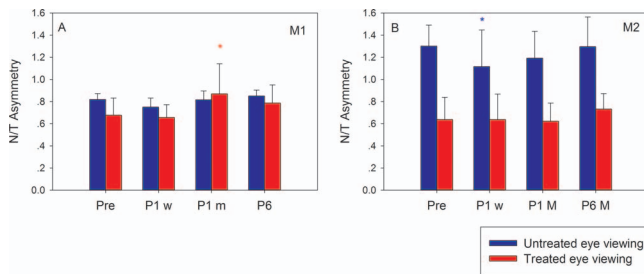


FIGURE 4. Longitudinal change in horizontal smooth-pursuit gains in M1 (A, B) and M2 (C, D) following strabismus treatment. Bars in each plot show mean smooth-pursuit gains and SDs of the untreated eye (blue) and treated eye (red) at four time points (Pre, P1w, P1m, and P6). Leftward and rightward smooth-pursuit was analyzed separately and are represented by the directional arrows. The asterisks represent statistically significant difference compared with presurgical values (blue, untreated eye; red, treated eye).

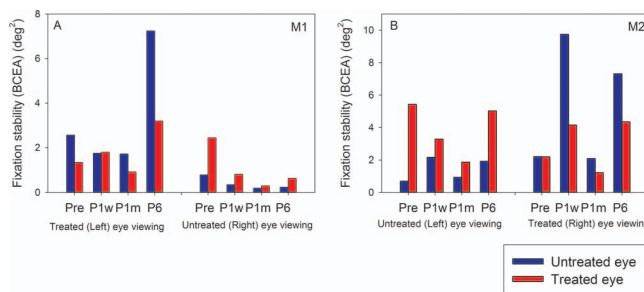


**FIGURE 5.** Longitudinal change in naso-temporal asymmetry following strabismus treatment. *Bars* in each plot show a naso-temporal asymmetry index calculated as the ratio of the viewing eye temporal-ward and nasalward smooth-pursuit gain for M1 (**A**) and M2 (**B**) at four time points (Pre, P1w, P1m, P6). N/T asymmetry index during untreated eye viewing is shown in *blue* and during treated eye viewing is shown in *red*.

Following the initial reduction in strabismus angle, the angle of misalignment reverted to ~60%–80% of presurgical value by 6 months after treatment, again similar to outcomes of strabismus correction surgery in a proportion of human subjects whose initial procedure resulted in undercorrection.<sup>22</sup> The lack of persistence of the initial improvement in strabismus angle may be because improvement in alignment in the two study animals was beyond the range at which central binocular factors would be expected to be able to maintain the initial improvement in postsurgical ocular alignment. Therefore, remodeling at the level of the EOM<sup>23,24</sup> along with central neural adaptation could act to “fight” the intent of surgery (Pullela M, et al. *IOVS* 2015;ARVO E-Abstract 5221) (Agaoglu MN, et al. *IOVS* 2015;ARVO E-Abstract 5222). Additional studies directly examining neural drives and muscle contractility are needed to understand the relative plastic changes due to these two factors. These studies are underway in our laboratory. Another point to note is that the patterns of alignment change following treatment, which are similar to human strabismus response to treatment, also help to validate the monkey model as suitable for investigating strabismus mechanisms.

**Saccadic and Smooth-Pursuit Eye Movements**

There were significant small magnitude changes in saccadic and smooth-pursuit gains following treatment, although it was different in the two monkeys (Figs. 2, 4). Thus, saccade and smooth-pursuit gain for M1 showed an increase after surgery, whereas saccade and smooth-pursuit gains for M2 showed a decrease after surgery, although both animals regained presurgical gain values by P6. Analysis of performance of saccades in human subjects after undergoing strabismus surgery showed an improvement after surgery that was



**FIGURE 6.** Longitudinal change in fixation stability (M1, **A**; M2, **B**) following strabismus treatment. *Bars* in each plot show BCEA values of the treated eye (*red*) and untreated eye (*blue*) during either eye viewing at the four time points (Pre, P1w, P1m, P6m).

proposed as due to improvement in binocular coordination because eye misalignment is corrected.<sup>29</sup> Because full alignment was not achieved in our monkeys even on P1d, we suggest that the observed changes in saccade and smooth-pursuit gain in our monkeys are more likely to be driven by brain and muscle adaptation that is unrelated to binocular coordination. It is unclear why eye movement gains increased after surgery in M1 but decreased after surgery in M2. We suggest that this likely represents variability that might be observed in humans as well and could represent differences in relative adaptation (neural or muscle related) of the recessed versus the resected muscle. Confirmation of this hypothesis would come from future studies that measure neuronal drive to the treated and untreated muscles.

Longitudinal changes in saccade peak velocities were unremarkable. The most likely explanation is that saccade velocities are inherently too variable to show significant changes following surgical treatment. We also did not find any consistent changes in N/T asymmetry during monocular smooth-pursuit. Because N/T asymmetry is fundamentally due to developmental loss of binocular inputs from sensory areas such as middle temporal/middle superior temporal (MT/MST) to brainstem areas such as the nucleus of the optic tract (NOT), any longitudinal changes in N/T asymmetry would have been, in fact, surprising.<sup>14,15,40,41</sup>

**Fixation Stability**

Fixation stability or lack thereof in strabismic animals and humans is usually influenced by presence of visual problems such as amblyopia<sup>42–46</sup> and oculomotor instabilities such as nystagmus and excessive drifts. In addition, fixation stability may be influenced by several visual factors such as target size, shape, contrast, and luminance.<sup>8</sup> We measured fixation stability in these animals at the different time points and found a temporary improvement in fixation stability of the treated eye when the untreated eye was viewing but no consistent changes when the treated eye was viewing. Dell’Osso et al. suggested that tenotomy surgery reduces nystagmus intensity<sup>47,48</sup> in humans with infantile nystagmus syndrome due to manipulation of proprioceptive receptors in the EOM. It is possible that similar mechanisms are at play following the strabismus correction surgery that we performed, although another study that attempted tenotomy surgery in monkeys with infantile nystagmus showed inconsistent results.<sup>49</sup>

**Implications for Plasticity Mechanisms**

Our data suggest that surgical treatment of strabismus is followed by an adaptive response that evolves and reaches a steady state within the first 6 months after treatment. Because adaptation can either oppose or facilitate the outcome of treatment strategies, the fundamental challenge to improving treatment methods is to identify and devise methods that will induce adaptation that promotes desired treatment effects while avoiding adaptation that may oppose or reverse desired treatment effects. As suggested earlier, both central neural adaptation and peripheral muscle remodeling are likely driving the final state of alignment and ultimately determining the success or failure of the surgical treatment. Immediate (P1d) changes in alignment and eye movements are likely driven by changes in muscle contractility alone, but later changes (P1m and P6m) are likely due to a combination of neural plasticity and peripheral remodeling.<sup>23,24</sup> Behavioral studies alone cannot readily differentiate between these mechanisms, but they are critical to develop a framework to conduct and interpret neural recording studies or studies that evaluate

muscle function. Such neuronal studies are underway in our laboratory in the same animals as in this study.

### Limitations of Study

Major portions of the strabismus treatment literature are accounts of outcomes following different types of surgical procedures. The strength of these studies is the relatively large number and large variety (e.g., esotropes versus exotropes and patients with residual binocularity versus patients with no binocularity) of patients that are evaluated. Due to several (mostly practical) reasons, monkey studies cannot hope to replicate the large  $n$  of human studies. However, the strength of our monkey investigation lies in the ability to perform careful and controlled longitudinal evaluation in the same animals that is not possible in human clinical investigation. Moreover, these behavioral studies provide a framework to perform a neurophysiologic investigation that is not possible in humans and is critical to extend our understanding of strabismus mechanisms including plasticity.

It is possible that outcomes in the current study might have been different (i.e., monkeys' exotropia does not revert) if we had chosen to treat both eyes and attempted to more closely align the eyes, given the general notion that surgical realignment in the vicinity of 5–10 PD helps facilitate binocularity. However, on the other hand, both the animals in this study were reared with prisms from the first day of birth, likely preventing the development of even coarse stereopsis.<sup>50</sup> Also, we purposely did not attempt a second procedure on the fellow eye because we wanted to be able to use the fellow eye as a control and thereby ascertain the nature and time course of plasticity that follows surgical treatment. Using the fellow eye as an untreated control is also quite critical when evaluating neural responses that are driving the extraocular muscles.

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