Acute Superior Oblique Palsy in Monkeys: III. Relationship to Listing's Law

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PURPOSE. To investigate the three-dimensional orientation of the eye and its relationship to Listing's Law in monkeys with acute acquired superior oblique palsy (SOP).

METHODS. The trochlear nerve was severed intracranially in two rhesus monkeys. Three-axis eye movements (horizontal, vertical, and torsion) were measured with binocular, dual search coils during fixation of targets in a 40° × 40° grid. Rotation vectors were calculated, and Listing's plane (LP) was determined by a least-squares planar fit of eye torsion as a function of horizontal and vertical position.

RESULTS. The main findings were: (1) In the paretic eye, there was an immediate and sustained rotation of the orientation plane by approximately 25° in the temporal direction; (2) the thickness of LP, defined as the torsional standard deviation (SD), increased little (by 0.13° in M1 and 0.08° in M2) after SOP, and (3) the SD of intrasaccadic torsion was slightly greater than that during fixation, but there was no change after SOP.

CONCLUSIONS. Acute SOP in rhesus monkeys leads to a temporal rotation of LP. This is consistent with a relatively increased extorsion in down gaze due to a loss of normal intorsion by the superior oblique muscle. The SD of torsion increased by only a small amount, implying that the validity of Listing's Law is not affected much by complete SOP, despite the large change in the orientation of LP. (*Invest Ophthalmol Vis Sci.* 2007;48: 2621–2625) DOI:10.1167/iovs.06-1319

In a series of papers, we present the ocular motor effects of acute superior oblique palsy (SOP), due to surgical section of the trochlear nerve. The first paper[1] describes the changes in static ocular alignment and their evolution over time. The second paper[2] shows the effect of SOP on three-dimensional (3-D) saccade dynamics. In the present paper, we address the effect of the lesion on the torsional orientation of the eye relative to its position in the orbit. There are two main questions: First, is Listing's Law still valid in acute SOP? Second, what is the effect on the horizontal (hPP) and vertical (vPP) components of the primary position?

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Listing's Law specifies the mathematical relationship between the torsional orientation of the eye and its horizontal and vertical position in the orbit. According to Listing's Law, if eye positions are expressed as 3-D rotations from a common reference position, the rotation vectors will lie in a plane. Listing's Law is likely determined by a combination of neural and mechanical factors.[3–6] One neural factor that may be involved is the innervation of the superior oblique (SO) muscle.[7] A lesion in the trochlear nerve leads to paresis of the SO muscle. The torsional action of the SO muscle is to rotate the upper pole of the eye toward the nose (intorsion). Thus, SOP leads to an abnormal extorsion of the paretic eye. This extorsion is greatest in down gaze, where the SO is normally most active. One might expect that this gaze-dependent change in torsion would alter the orientation of Listing's plane (LP) and possibly cause a violation of Listing's Law.

Previous studies in humans with chronic SOP have shown that LP is rotated temporally in the affected eye relative to the unaffected eye.[8,9] We asked whether the same is true in monkeys with an acute SOP created by section of the trochlear nerve. The primary advantage of the animal model over studies of humans with SOP is that both the site and the timing of the disease are known. In contrast, humans cannot easily be studied immediately after the onset of SOP, before adaptive mechanisms have been engaged. In fact, some individuals with acutely symptomatic SOP may actually have decompensation of more longstanding SOP. Others may have the basic phenotype of SOP but from a cause other than weakness of the SO muscle, such as heterotopy of the orbital pulleys.[10] Thus, our study provides the first investigation of the 3-D ocular kinematics of true SOP caused by complete severing of the trochlear nerve, both before and during subsequent adaptation.

METHODS

General Experimental Procedures

Details of the experimental procedures have been described in the first paper of the series.[1] Two female rhesus monkeys were used for the study. Dual search coils were implanted in each eye for measurement of 3-D eye positions by using a search coil system with three magnetic fields. Signals from each coil were demodulated by frequency detectors, filtered in hardware with a bandwidth of 0 to 90 Hz, sampled at 1000 Hz, and stored on computer for later analysis. All experimental and surgical procedures, including anesthesia and postoperative analgesia, were performed according to a protocol that was approved by the Institutional Animal Care and Use Committee (IACUC) of The Johns Hopkins University, in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Trochlear Nerve Section and Experimental Protocol

After training and recording of baseline data, the trochlear nerve was sectioned intracranially, as described in the first paper.[1] The left trochlear nerve was sectioned in M1 and the right trochlear nerve in M2. Before the monkey recovered from anesthesia, the paretic eye was covered with an opaque patch to prevent binocular viewing. This patched remained on for 6 days in M1 and 9 days in M2. Binocular viewing was then allowed for the remainder of the study, except during monocular viewing testing. Eye movements were recorded from both animals within the first 2 days after the lesion.

3-D eye orientations were determined from monocular viewing of a small target as it stepped in a pseudorandom sequence through an array of 81 target positions (± 20° horizontal and vertical with targets spaced every 5°). The target (0.3 × 0.3° square) was rear projected onto a tangent screen located 66 cm in front of the animal. The target jumped after the position of the eye of the animal had been in the fixation window (4°) for 250 ms. Eye movements were recorded before and nearly daily in the first 2 weeks after the lesion, and then three or four times a week. We report data from the first 30 postoperative days.

Data Analysis

Data analysis was performed with custom software (developed in MatLab; The MathWorks, Natick, MA). Raw coil signals were converted to rotation vectors, using straight-ahead fixation as the reference position.[11] Both before and after surgery when the to-be-paretic eye or the paretic eye, respectively, was habitually patched, we used the prepatch reference position for each eye. Once habitual binocular viewing was allowed, we updated the reference position daily. Signal gains were determined using a test coil, and offsets were minimized by shielding connectors with a magnetic shield (MuMetal; MuShield Co., Inc., Londonderry, NH). Angular eye velocity was computed from the rotation vectors.[12] Signs conform to the right-hand rule: positive positions and velocities are leftward, downward, and clockwise, from the perspective of the animal. To calculate the location of the primary position relative to the straight-ahead reference position, we performed for each eye a least-squares linear (i.e., planar) regression of the torsional component to the horizontal and vertical components of the individual rotation vectors, fitting the data to the equation:

$$r_x = a_0 + a_1 r_y + a_2 r_z$$

where r_x is the torsional component, r_y is the vertical component, and r_z is the horizontal component. The torsional offset is calculated from a_0, and the horizontal and vertical components of primary position from a_1 and a_2, respectively, relative to the original reference position.[13] We followed the convention of prior studies and defined the "thickness" of the plane by the standard deviations (SDs) of the torsional residuals from the regression.[14] Each regression fit was performed on the set of all instantaneous (sampled at 1 kHz) rotation vectors from a given recording session and condition (e.g., paretic eye viewing) that satisfied a specific velocity criterion. For fixation data, the magnitude of the angular velocity vector had to be 2°/s or less; for saccade data, it had to be 30°/s or greater.

For further analysis, the data were mirrored between the two eyes for M2, who had a right SOP. Thus, in both monkeys the paretic eye was considered to be the left eye and the normal eye the right eye. Statistical comparisons of primary position between before and after the lesion were performed with a Kruskal-Wallis nonparametric ANOVA.

Results

Acute SOP produced a vertical misalignment of the eyes, similar to that in humans with presumed SOP. The paretic eye was higher than the normal eye, and this misalignment was greatest in down gaze when the paretic eye was adducted (Fig. 1A). There was also an extorsion of the paretic eye (see the first paper[1] for details). Figure 1B shows the rotation vectors of both eyes in the side (*horizontal-torsion* plane; top) and top (*vertical-torsion* plane; bottom) view. Only the data from the viewing eye (the other eye was covered) were plotted, to ensure that the horizontal and vertical positions were restricted to a similar ocular motor range. Before the lesion, the data points formed a plane, in accordance with Listing's Law. The apparent increase in thickness after the lesion is largely due to horizontal and vertical tilts away from the coronal plane. This is seen more clearly in Figure 1C, which displays the same data as Figure 1B rotated in the horizontal direction (about the yaw axis) for the side view and in the vertical direction (about the pitch axis) for the top view, in such a way that the planes can be seen edge-on. Note that the thickness of the plane was similar before and after surgery, although there was a slight increase in curvature. The plane of the paretic eye, however, showed a large temporal rotation, as seen in the top view (Fig. 1, bottom right).

Validity of Listing's Law after SOP

The degree to which Listing's Law is obeyed is commonly assessed by determining the SD of eye torsion after a planar fit to the horizontal and vertical eye position.[14] By this measure, the smaller the torsional SD (the thickness of LP), the more precisely Listing's Law holds. Figure 2, left shows a comparison of the torsional SD during steady fixation before and after SOP, as a function of time. There are several important findings. First, there was a slight increase in SD when an eye was patched, even before the SOP, especially in M2. Second, after the lesion, there was a small increase (from 0.49° to 0.62° in M1, from 0.60° to 0.68° in M2) of the mean SD in the paretic eye, when it was viewing. There was no consistent change in the normal eye.

Primary Positions after SOP

As shown in Figure 1C, SOP produced a large temporal rotation of LP in both monkeys. It occurred immediately after the lesion and was sustained over time (Fig. 3, left). For M1, the horizontal component of primary position (hPP) in the paretic eye changed from 9.7° in the temporal direction to 34.8° in the temporal direction (mean of all values with that eye viewing). The results in M2 were similar (0.9° before, 26.9° after), but she also showed a small temporal rotation in the nonparetic eye (−0.1° before, −6.8° after). In both monkeys, a temporal rotation in hPP was apparent from the first postoperative recording, although in M2 there was a small further increase during the patching period. Once the patch was removed and viewing was permitted from the paretic eye, there was no further change in hPP for as long as 30 days after the induction of SOP.

The locations of the primary positions of the paretic eye before and after the SOP are shown in two-dimensional plots in Figure 4. In both monkeys, the range of the primary positions was tightly clustered during both the pre- and postlesion periods. While the temporal displacement of hPP was striking in both monkeys, the displacement of the vertical component was less, shifting upward by 6.3° in M2 and 2.8° in M1. When data from both monkeys were pooled, the shift in vPP was significant ($P < 0.01$).

Validity of Listing's Law and Measurement of Primary Position during Saccades

Our fixation paradigm presented fixation targets in a pseudorandom order. Hence, saccades between fixation targets covered a wide range of amplitudes and directions. This method allowed sampling of a wide range of eye positions and allowed us to determine the extent to which Listing's Law was obeyed during saccades, compared with fixation. We selected data points for which the magnitude of 3-D angular eye velocity was at least 30°/s and performed the same first-order planar fit of the torsional to the horizontal and vertical components of the rotation vectors as for the fixation data. The torsional SD is shown in the right panels of Figure 2, and hPP in Figure 3.

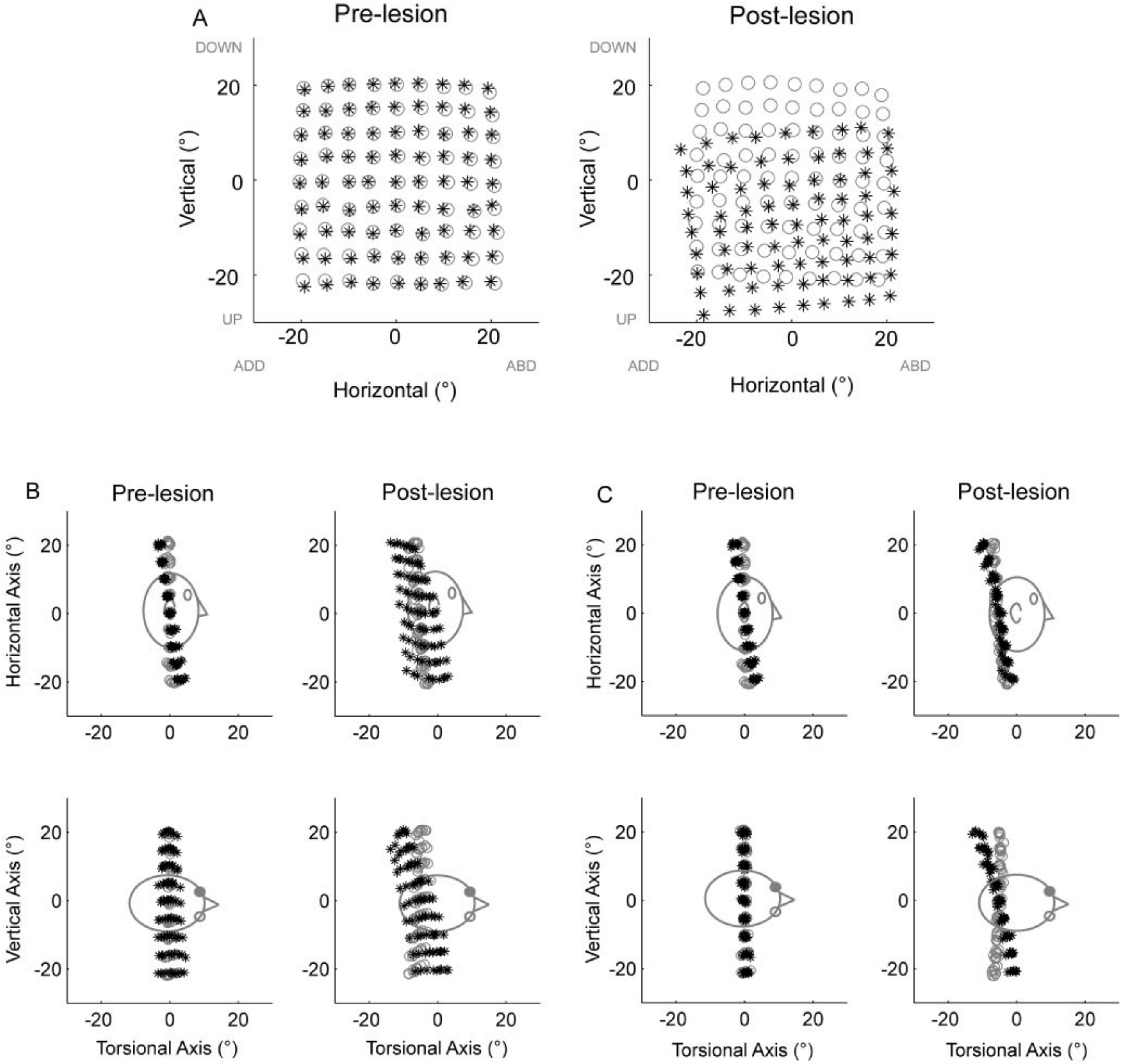

FIGURE 1. Median 3-D eye positions in M2 before and after SOP. Head-fixed rotation vectors have been converted to degrees[12] and are presented according to the right-hand rule (positive values are clockwise torsion, downward vertical, and leftward horizontal). (○) The normal eye; (✻) the paretic (or, if before SOP, the to-be-paretic) eye. For these data we used the prelesion reference position to show the actual change in torsion. (**A**) Horizontal and vertical eye alignment before and 2 days after SOP (normal eye viewing). After the lesion, there was a vertical misalignment (paretic eye higher) that was greatest in downward gaze and adduction. (**B**) Eye positions from the side (horizontal versus torsional rotation) and top (vertical versus torsional rotation) views. In contrast to (**A**), data are plotted from each eye when that eye was viewing. In the top view, the left eye (●) is the paretic one and the right eye (○) is the normal eye. (**C**) The data from (**B**) were rotated about the yaw axis (prelesion: PE 2.1° in the nasal direction, NE 0.1° in the temporal direction; postlesion: PE 27.5°, NE 4.9 ° both in the nasal direction) for the side view, and about the pitch axis (prelesion: PE 17.0 ° down, NE 3.5 ° down; postlesion: PE 23.7 ° down, NE 10.6 ° down) for the top view, so that the rotation of the plane about each axis could be visualized (i.e., so the planes could be seen edge-on). Note that the plane is rotated in the temporal direction in the paretic eye (*bottom right*). After surgery, there was also a negative (counterclockwise) torsional offset.

Comparing measurements during saccades with those during fixations, it can be seen that there was little difference in hPP, whether or not the eye was still or moving, both before and after the lesion. In contrast, the SD was slightly greater during saccades than during fixations, both before and after the onset of SOP and was similar in the normal and paretic eyes. Thus, there was no specific effect of the lesion on torsional SD during saccades.

DISCUSSION

In the present study, we investigated the effect of an acute trochlear nerve lesion on the 3-D orientation of the eye and its relationship to Listing's Law. Two key findings emerged. First, as in humans, in both animals there was a large temporal rotation of LP after SOP in the paretic eye. Second, despite this large change in the orientation of LP, there was only a small

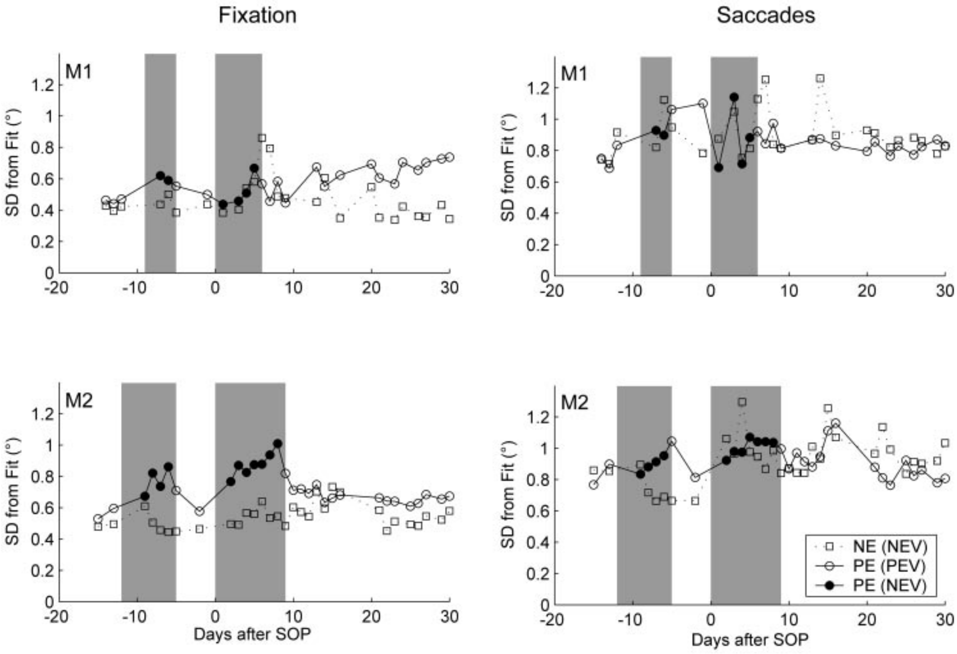

FIGURE 2. Thickness of Listing's plane, measured as the SD of the torsion residuals from the planar fit, as a function of time, in days, from the SOP. *Left*: data measured during visual fixations (angular velocity, ≤2°/s); *right*: data measured during saccades (≥30 °/s). (□) normal eye when viewing; (○) the paretic eye when viewing; and (●), the paretic eye during chronic patching with the normal eye viewing. *Shaded areas*: represent the periods during which the paretic (or to-be-paretic) eye was habitually patched.

increase in the thickness (the torsional SD) of LP for fixation, and little change in torsional SD for saccades. We will discuss these findings in relation to prior studies of patients with SOP and with respect to the possible role of the SO muscle in Listing's Law.

Comparison to Human Studies

Studies of humans with SOP have also shown a temporal rotation of the hPP in their affected eyes, compared with their normal eyes. Our findings in monkeys are most similar to those of Straumann et al.,[8] who found an approximately 20° temporal rotation of hPP compared with the unaffected eye as well as the eyes of normal subjects. Wong et al.[15] found a temporal rotation of hPP of similar magnitude, but they also found a smaller, temporal rotation in the nonparetic eye. Finally, Migliaccio et al.[9] found only approximately 6° of temporal rotation of hPP relative to control and nonparetic eyes. The reasons for the differences among these studies are not certain, but could include factors such as differences in site and size of the lesion, chronicity, and effects of adaptive processes.

In only one other study was the effect of SOP on 3-D eye positions during saccades examined.[15] In the strictest sense, Listing's Law does not apply during saccades because its definition is based on static eye orientations during steady fixation. Nonetheless, studies have shown that eye positions during saccades remain close to LP,[14] except for small deviations (torsional blips).[16] Wong et al.[15] reported two patients with acute (less than one month) SOP, in whom the torsional SD during saccades was very large (as much as 10°). They attributed this to very large torsional deviations at the time of each saccade, after which the eyes appeared to drift back to their

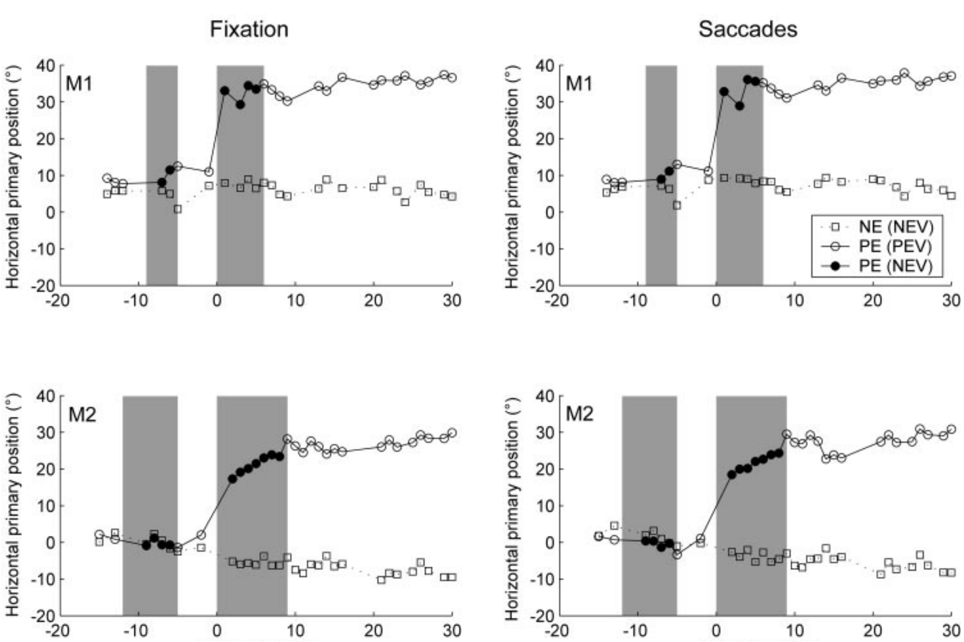

FIGURE 3. Horizontal primary position as a function of time (in days) after SOP. *Left*: values measured during steady fixation; *right*: values during saccades. *Shaded areas*: periods during which the paretic (or to-be-paretic) eye was habitually patched. Symbols are as described in Figure 2.

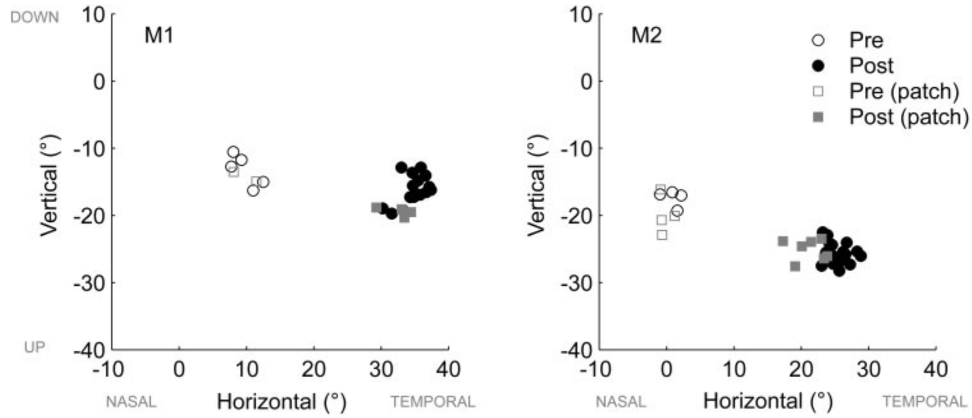

FIGURE 4. Two-dimensional plots of the vertical and horizontal components of primary eye position of the paretic eye before (*open symbols*) and after (*filled symbols*) SOP. All data are from the paretic eye. *Circles*: the paretic eye when it was viewing; *squares*: the paretic eye with the normal eye viewing, when the paretic eye was habitually patched. Each point represents the data from 1 day.

respective LP. Because they did not see this in patients with chronic SOP, they concluded that there must be a neural mechanism to restore the validity of Listing's Law. We did not observe such changes in torsional SD in our monkeys with acute SOP, though the site of the lesion in the human cases might be different. Although there were some changes in peak torsion during saccades (torsional blips),[2] SOP did not change the overall torsional SD during saccades.

Relationship to Torsional Alignment

The rotation of PP after SOP relates to the change in torsional alignment and its dependence on eye position.[1] The temporal rotation of hPP corresponds to an increased extorsion of the paretic eye in down gaze relative to the torsion in up gaze. This is expected, since the SO intorts the eye and is most strongly activated in down gaze. Similarly, the upward displacement of vPP reflects an increased extorsion of the paretic eye in abduction relative to the torsion in adduction.

What Is the Role of the Superior Oblique in Enforcing Listing's Law

There is general consensus that the implementation of Listing's Law is likely to involve a combination of neural and mechanical factors, that is, specific patterns of innervation acting through the orbital tissues,[7] an important component of which is the fibromuscular pulleys that determine the pulling directions of individual extraocular muscles.[6] In the present study, we investigated the contribution of the innervation of the SO muscle to ocular orientation and kinematics. We found, as demonstrated previously in humans,[8,9,15] that the SO muscle is critical for determining the orientation of LP. Whether this is due directly to the gradient of torsion produced by denervation of the SO muscle or to a secondary change in the configuration of the rectus muscle pulleys remains to be shown.[6] In contrast, our data show that complete denervation of the SO muscle has little effect on Listing's Law, per se, and does not substantially alter the shape of the torsional surface, as measured by the SD of the planar fit (see also Fig. 1).

Finally, there was little change in Listing's Law behavior over time, despite considerable changes in vertical misalignment during and after the postlesion period of habitual monocular viewing. Thus, the mechanisms that control Listing's Law behavior appear distinct from those that alter static vertical alignment.

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