PURPOSE. Visually guided saccades are disconjugate in human and nonhuman strabismic primates. The superior colliculus (SC) is a region of the brain topographically organized in visual and motor maps where the saccade goal is spatially encoded. The present study was designed to investigate if a site of stimulation on the topographic motor map was evoking similar or different saccade vectors for each eye.

METHODS. We used microelectrical stimulation (MS) of the SC in two strabismic (one esotrope and one exotrope) and two control macaques under binocular and monocular viewing conditions. We compared the saccade amplitudes and directions for each SC site and each condition independently of the fixating eye and then between each fixating eye. A comparison with disconjugacies of visually guided saccades was also performed.

RESULTS. We observed different saccade vectors for the two eyes in strabismic monkeys, but conjugate saccades in normal monkeys. Evoked saccade vectors for the left eye when that eye was fixating the target were different from those of the right eye when it was fixating. The disconjugacies evoked by the MS were not identical but similar to those observed for visually guided saccades especially for the dominant eye.

CONCLUSIONS. Our results suggest that, in strabismus, the saccade generator does not interpret activation of a single location of the SC as the same desired displacement for each eye. This finding is important for advancing understanding of the development of neural circuits in strabismus.

Keywords: superior colliculus, strabismus, disconjugacy, saccades

OBJECTIF. Les saccades visuellement guidées de chaque œil sont asymétriques chez le primate humain et non humain strabique. Le colliculus supérieur (CS) est une région sous-corticale topographiquement organisée en cartes visuelles et motrices où le but de la saccade est spatialement codé. La présente étude a été menée afin de tester si la stimulation électrique de la carte topographique motrice du CS évoquait des vecteurs saccadiques similaires pour chaque œil.

MÉTHODES. Nous avons utilisé des micro-stimulations électroniques (MS) du CS chez deux singes strabiques (un esotrope et un exotrope) et deux macaques témoins en vision binoculaire et monocular. Nous avons comparé les amplitudes et directions des saccades pour chaque site et condition indépendamment de l’œil utilisé lors de la fixation puis, entre chaque œil utilisé lors de la fixation. Une comparaison avec les asymétries observées au niveau des saccades visuellement guidées a également été réalisée.

RÉSULTATS. Nous avons observé différents vecteurs saccadiques pour chaque œil chez les singes strabiques, mais des saccades identiques chez les singes sains. Les vecteurs de saccades évoqués pour l’œil gauche lorsque cet œil fixait la cible étaient différents de ceux de l’œil droit lorsque ce dernier fixait. Les asymétries évoquées par la MS n’étaient pas identiques mais similaires à celles observées lors de saccades visuellement guidées et particulièrement pour l’œil dominant.

CONCLUSIONS. Nos résultats suggèrent que, dans le cas d’un strabisme, le générateur saccadique n’interprète pas la stimulation d’un site du CS comme le même déplacement désiré pour chaque œil. Ce résultat est important pour la compréhension du développement des circuits neuronaux lors d’un strabisme.
Everyday actions require orienting our gaze toward a spatial region of interest. An approaching object, a pedestrian crossing the street or keeping an eye on progeny activates brain circuits to generate quick saccadic gaze shifts. The saccade goal, where we need to look, is shaped from the retinotopic location of the target’s image. Once the saccade goal is identified, an oculomotor command can then be generated in the brainstem saccade generator.1 Visually guided saccades will then be directed toward the visual target conjugately, providing the new target and the initial fixing point are equidistant. In contrast, strabismic human and nonhuman primates produce disconjugate saccades toward targets presented on a tangent screen2–5 possibly related to functional abnormalities in saccadic structures as the parmedian pontine reticular formation (PPrF).6,7

In recent years, numerous studies have used a nonhuman primate model of infantile strabismus to investigate possible neural correlates of oculomotor symptoms. Compelling evidence has emerged to show that, regardless of the method used to induce strabismus, the loss of binocular vision during a sensitive period early in postnatal life leads to abnormalities of brainstem oculomotor structures.6–12 The superior colliculus (SC) constitutes the last visuomotor relay in the brainstem carrying a spatial representation of the saccade goal.13,14 Neurons in the superficial and intermediate layers (iSC) are topographically organized and have, respectively, visual and motor maps that are aligned.15,16 Single unit recordings in the motor map of the iSC show neurons evincing a burst of action potentials 20 to 30 ms preceding a saccade.17,18 Electrical microstimulation (MS) at a specific iSC recording site evokes a saccade of short latency (~30 ms) with amplitude and direction matching the location of the electrode on the topographic map.15,16,19,20 According to many models of the saccadic system, a desired displacement command from the SC serves as the input to a local feedback loop, which controls saccade dynamics.1,19,21,22 During the saccade, the current displacement is constantly compared with this command. When the difference between these two signals reaches zero, the movement ends. Because human and nonhuman primates with strabismus are able to perform accurate saccades with either eye fixating, and even perform accurate “crossover” saccades,23,24 the desired eye displacement and the control loop processes described above must be achieved accurately.

It is currently unknown if the iSC specifies a common saccade goal for each eye on its topographic map or if one site is specifying two different saccade goals according to which eye is used. To address this gap in knowledge, we electrically evoked saccades from the iSC in strabismic and normal monkeys in binocular and monocular viewing conditions. We then compared the evoked saccades with visually guided saccades. This methodology has several goals: (1) testing if disconjugate saccades can be evoked from the iSC in strabismic monkeys, (2) testing if the saccade vectors are dependent on which eye is used and therefore if the common topography for each eye is broken, and (3) testing if the pattern of disconjugacies is similar to those reported for visually guided saccades.

**Materials and Methods**

**Monkey Preparation**

Four monkeys (Macaca mulatta) were used as subjects. We performed electrical microstimulation at 48 sites in the intermediate layers of the SC of one female exotrope (XT1; 7 years of age, 6.5 kg) and one female esotrope (ET1; 5 years of age, 7 kg), and 24 sites in one normal female (N1; 7 years of age, 6.6 kg) and one normal male (N2; 5 years of age, 9.1 kg). Strabismus was created early in life by two different approaches. Monkey ET1 wore prism goggles for the first 3 months of life, resulting in incomitant esotropia (typically ~15° but could range from ~25° esotropia to 2° of exotropia). Monkey XT1 underwent a bilateral medial rectus tenotomy during the first week of life, which resulted in a strong “A” pattern exotropia (25° when fixating with the right eye and 35°–40° when fixating with the left eye). To prepare for neurophysiological experiments, the monkeys were equipped with a scleral search coil on each eye for measurement of eye movements (CNC Engineering, Seattle, WA, USA) and recording chambers. Detailed descriptions of our surgical procedures can be found in previous reports.25,26 All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington (Seattle, WA, USA).

**Eye Movement Recordings**

The eye position data were sampled at 1 kHz. Calibration of each eye was performed under monocular viewing conditions by requiring the animal to fixate the target as it was stepped systematically from ~20° to 20°, in 5° increments, horizontally and vertically. Eye position signals and target position feedback signals were passed through an antialiasing, 6-pole Bessel filter (200 Hz). These signals were then digitized at 1 kHz with 16-bit precision using CED-Power1401 hardware (Cambridge Electronic Designs, Cambridge, England).

**Electrical Microstimulation**

Electrical microstimulation was performed using glass-coated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA, USA) with impedances ranging from 1 to 5 megaOhms (MΩ). Monophasic current pulses (0.1 ms, 7–40 μA, 400 Hz, 200-ms train duration) were delivered during fixation of static targets. Stimulation was applied only at sites where visuomotor neurons were identified, approximately 1.2 to 2 mm below the surface of the superior colliculus. For a given site, the intensity of the delivered current was adjusted to the lowest value needed to consistently evoke a staircase of saccades. The train duration of 200 ms evoked a staircase of at least two saccades per trial.

**Data Analysis**

Spike 2 was used for data acquisition and preliminary offline analyses. Data were then imported into MATLAB (Mathworks, Natick, MA, USA) and analyzed further using custom software. To avoid contamination by posttectonic drifts, saccade onsets and offsets were measured using a combination of velocity and acceleration criteria. Saccade offset was defined as the first point in time at which either of two conditions was met: (1) the eye velocity dropped below 50°/s, or (2) eye velocity dipped below 100°/s and the absolute value of acceleration dropped below 10,000°/s/s. This algorithm successfully detected the occasional large posttectonic drift in strabismic monkeys that reaccelerated the eye before the velocity dipped below the 50°/s threshold. For each eye, saccade onsets and offsets were measured separately for the horizontal and vertical components. The amplitudes and durations of each component were defined with respect to these time points. The conjugacy of the horizontal and vertical components was quantified using the following equation:
Amplitude ratio \(= \frac{RA_{\text{Left Eye}}}{RA_{\text{Right Eye}}}\) (1)

Where RA represents the radial amplitude of the saccade. The differences in saccade direction were computed as:

\[
\text{Direction Difference} = \text{Polar direction}_{\text{Left Eye}} - \text{Polar direction}_{\text{Right Eye}} \quad (2)
\]

For saccade directions falling between 270° and 90°, there was a risk of finding very large differences in saccade direction. For example, if saccade directions were 20° and 330° for the left and right eyes respectively, the direction difference would be −310°. In order to avoid this error, if one evoked saccade was slightly down, its direction was transformed by subtracting 360° from its direction. For our given example, the directions were then 20° and 150° resulting in a direction difference of 50°.

Due to large variability in eye positions and evoked saccade amplitudes, four sites were excluded for monkey XT1. The final number of sites was 32 sites for this monkey and 44 sites for the strabismic monkeys.

In binocular conditions, we first computed the two conjugacy parameters without taking into account which eye was fixating the target. Then we calculated the same parameters but between the left eye fixating and the right eye fixating in binocular and monocular conditions as defined by the following equations:

Amplitude ratio \(= \frac{\text{geomean}(RA_{\text{Left Eye Fixating}})}{\text{geomean}(RA_{\text{Right Eye Fixating}})}\) (3)

and

\[
\text{DirectionDifference} = \frac{\text{mean}(\text{PolarDirection}_{\text{Left Eye Fixating}})}{\text{mean}(\text{PolarDirection}_{\text{Right Eye Fixating}})} \quad (4)
\]

In the binocular condition, our algorithm first checked if at least one eye was within 3° of the target. Then the fixating eye was defined as the closest to the target. In this analysis, eight sites were included for monkey ET1 and 22 sites for monkey XT1 in binocular viewing condition. In monkeys XT1 and ET1, eight and seven sites, respectively, were tested in monocular viewing condition. In monkeys N1 and N2, six and seven sites were tested, respectively.

During free binocular viewing, we noticed that monkeys XT1 and ET1 each had a preferred eye that we defined as the dominant eye (left eye for XT1 and right eye for ET1). The influence of the fixation on the dominant eye or the nondominant eye was analyzed by computing the amplitude ratios and saccade direction differences when each eye was fixating versus not fixating during binocular and monocular viewing. The following equations defined our quantification for the dominant eye but the same procedure was used for the nondominant eye:
AmplitudeRatioDomEye
\[
= \frac{\text{geomean}(\text{RADomEyeFixating})}{\text{geomean}(\text{RADomEyeNotFixating})} \tag{5}
\]

and

Direction Difference DomEye
\[
= \frac{\text{mean}(\text{Polar directionDomEyeFixating})}{\text{mean}(\text{Polar directionDomEyeNotFixating})} \tag{6}
\]

To compare the evoked saccade amplitudes to the visually guided saccade amplitudes, we proceeded in two steps. The first step was to create a pool of visually guided saccade amplitudes matching the evoked ones. The criteria for selection were defined by drawing an ellipse around the dispersion of the evoked saccade amplitudes of the fixating eye for each site. The positions of the center of this ellipse was defined as:

\[x_0 = \frac{\left( (Q_{h3} + 1.5*Iqr(\text{hor.ampl}.) - (Q_{b1} - 1.5*Iqr(\text{hor.ampl}.) \right)}{2} + (Q_{b1} - 1.5*Iqr(\text{hor.ampl}.) \right) \tag{7} \]

and

\[y_0 = \frac{\left( (Q_{v3} + 1.5*Iqr(\text{vert.ampl}.) - (Q_{v1} - 1.5*Iqr(\text{vert.ampl}.) \right)}{2} + (Q_{v1} - 1.5*Iqr(\text{vert.ampl}.) \right) \tag{8} \]

Where Qh1 and Qv1 are the first quartiles of the horizontal and vertical evoked amplitudes, respectively, and Qh3 and Qv3 the third quartiles. Iqr is the interquartile range. The ellipse border was then determined by:

\[x_s = x_0 + \left( \frac{\left( (Q_{h3} + 1.5*Iqr(\text{hor.ampl}.) - (Q_{b1} - 1.5*Iqr(\text{hor.ampl}.) \right)}{2} * \cos(\theta) \right) \tag{9} \]

and

\[y_s = y_0 + \left( \frac{\left( (Q_{v3} + 1.5*Iqr(\text{vert.ampl}.) - (Q_{v1} - 1.5*Iqr(\text{vert.ampl}.) \right)}{2} * \sin(\theta) \right) \tag{10} \]

where \( \theta \) is an independent parameter increasing from 0 to 2\( \pi \).

By using the MATLAB function inpolygon(), we then searched for matching visually guided saccades for the same eye taken from a dataset of visually guided saccades used for a previous study. The distribution of selected visually guided saccades was controlled manually to verify any accumulation of points close to an edge of the ellipse. For only three sites (two in monkey XT1 and one for monkey ET1) the radius of the ellipse was reduced to one SD. For rostral sites, an additional criterion was used to exclude amplitudes of visually guided saccades less than 0.5°.

Once the visually guided saccade population was selected, the second step consisted of drawing a second ellipse based on the dispersion of the evoked saccade amplitudes of the fellow eye (nonfixating eye). This second hull was drawn following...
Finally, we counted the number of visually guided saccades for which the fellow eye fell inside this second ellipse, using the MATLAB function inpolygon().

A matching percentage (MP) was then calculated by the following equation:

$$MP = \frac{n_2}{n_1} \times 100$$

where \(n_1\) is the number of saccades selected with our first step procedure and \(n_2\) the number of saccades for which the fellow eye fell inside the second hull.

From these two populations (evoked versus visually guided saccades), we also calculated the mean deviations in terms of amplitude ratios and saccade direction differences of each eye between the two conditions with these two equations:

$$Amplituderatio = \frac{\text{geomean}(RA_{VisuallyGuidedSaccades})}{\text{geomean}(RA_{EvokedSaccades})}$$

and

$$\text{Direction Difference} = \frac{\text{mean}(\text{Polar direction VisualGuidedSaccades})}{\text{mean}(\text{Polar direction EvokedSaccades})}$$

All statistical analyses and confidence intervals (CI) were computed with freeware R (in the public domain, www.r-project.org). Confidence intervals were calculated through a bootstrap method. Direction differences have been represented by negative and positive values. The means, CI, and statistical tests were computed on absolute values. All CIs are 95% CI.

RESULTS

Figure 1 illustrates eye movement data from example sites for one normal (N1, Fig. 1A) and two strabismic macaques (ET1 and XT1, Figs. 1B, 1C, respectively) under binocular viewing. For these three cases, we found a standard pattern of staircase-like saccadic eye movements evoked by left iSC stimulation. The first evoked saccades of each staircase in normal monkeys display a quasiperfect conjugacy between the two eyes (left eye, blue; right eye, red). As we can see in the polar representations of the first evoked saccades, the two vectors were similar in amplitude (mean ± SD: 13.76 ± 0.8° and 14.09 ± 1.6° for left and right eye, respectively) and direction (mean ± SD: 14.27 ± 2.82° and 14.97 ± 2.22° for left and right eye, respectively). In contrast, MS in strabismic monkeys evoked saccades with both amplitude and direction differences, as reported for visually guided saccades in human2,5 and nonhuman primates.3,4 In monkey ET1 (Fig. 1B), the evoked saccades for the two eyes differed in both amplitude (mean ± SD: 5.79 ± 0.91° and 4.42 ± 0.56° for left and right eye, respectively) and direction (mean ± SD: 278.1 ± 2.7° and 268.25 ± 4.1° for left and right eye, respectively). Some disconjugacies are also illustrated for monkey XT1 in Figure 1C with differences in radial amplitudes (mean ± SD: 8.6 ± 1.26°;
10.5 ± 1.38° for left and right eye, respectively) and directions (mean ± SD: 343.71 ± 5.94°; 2.43 ± 4.19° for left and right eye, respectively).

Figure 2 represents the relation between the initial eye positions and the evoked saccade amplitudes for horizontal and vertical components at the same sites illustrated in Figure 1 for monkeys ET1 (Figs. 2A, 2B) and XT1 (Figs. 2C, 2D) under binocular viewing. In monkey ET1, we checked if nonmatching initial orbital positions were associated with changing the evoked saccade in amplitude. This control was made for each site. For monkey XT1 though, this control was not possible due to large exotropia. However, the possible effect of initial eye positions on evoked saccades was unlikely to be a factor because each eye was not limited by initial eye position based on what has been observed in behavior previously and by using high frequency MS (>300 Hz). The left eye position limits in monkey XT1 for goal-directed saccades ranged between −30 and +35° whereas it was between 0 and +35° for the right eye.

To quantify the disconjugacies across stimulation sites, the amplitude ratios and the direction differences between the two eyes were calculated (see Methods) and plotted. This allowed us to illustrate, for the first time, saccade disconjugacies evoked by microstimulation of SC in strabismic monkeys. The mean amplitude ratios and direction differences were calculated subsequently between the fixating eyes. We analyzed the possible influence of fixation on the evoked saccades for each eye (dominant and nondominant eye). Finally, comparison with matching visual saccades was made in the two different fixation conditions.

**Comparison of Evoked Saccade Conjugacies Between Normal and Strabismic Monkeys Under Binocular Viewing**

If the evoked saccades are conjugate for each stimulation site, then the amplitude ratios and direction differences should be near the conjugacy values (1 for the amplitude ratios and 0° for the direction differences). In Figures 3A and 3B, we show a wider dispersion of these two indicators around the conjugacy values for the two strabismic monkeys confirming preliminary results (Fleuriet, et al., *IOVS* 2013;54:ARVO E-Abstract 1930). Sites from monkey ET1 had large saccade direction differences (10.71°, CI: 7.25°–15.36°, N = 12) and likewise for monkey XT1 (17.93°, CI: 15.78°–20.03°, N = 32). The geometric means of the amplitude ratios were not far from 1 on average for these two monkeys (ET1: 1.09, CI: 0.97–1.21, N = 12; XT1: 0.93, CI: 0.88–0.99, N = 32) but only a small percentage fell within the range of 0.9–1.1 (ET1: 8% [1/12]; XT1: 31% [10/32]).

Microelectrical stimulation of the iSC in normal monkeys did not evoke disconjugate saccades. Sites from monkey N1 were associated with small saccade direction differences (1.53°, CI: 0.82°–2.48°, N = 11) similar to the sites from monkey N2 (1.7°, CI: 1.1°–2.69°, N = 13). Amplitude ratios are very close to 1 on average (N1: 1, CI: 0.99–1.02, N = 11; N2: 0.99, CI: 0.95–1.03, N = 13) and almost exclusively concentrated within the range of 0.9 to 1.1 (N1: 100% [11/11]; N2: 92% [12/13]).

Altogether, the mean of the absolute direction deviations for strabismic monkeys was 15.96° (CI: 13.64°–17.92°, N = 44), in contrast with the normal monkeys (mean: 1.62°; CI: 1.17°–2.33°, N = 24) as illustrated in Figure 3D. These saccade direction differences between normal and strabismic monkeys were significantly different (P < 0.001, Kolmogorov-Smirnov test). The mean amplitude ratios were similar for normal and strabismic monkeys (geometric mean: 0.99 and 0.97; CI: 0.97–1.02 and 0.92–1.03 for normal versus strabismic monkeys, respectively), but were distributed differently (Fig. 3C).

Indeed, while 96% (23/24) of sites in normal monkeys showed an amplitude ratio between 0.9 and 1.1, only 25% (11/44) of sites were in this range in strabismic monkeys. A significant difference was found between the two amplitude ratio populations (P < 0.01, Kolmogorov-Smirnov test).
Figure 4 shows the main sequences of all the evoked saccades for the two strabismic monkeys on each component (horizontal and vertical) and each eye. It is clear that the two eyes move at a similar speed for similar amplitude in the range of amplitudes tested. This indicates that the disconjugacies described above could not be fully explained by a change in the muscles or motor plant.

Comparison of Saccade Vectors for Each Fixating Eye Under Binocular Viewing

We then compared amplitude and saccade direction associated with each attending eye. For example, we compared evoked saccades for the left eye when the left eye was fixating with evoked saccades for the right eye when the right eye was fixating (see Methods).

In Figure 5A, we illustrate our analysis for one example site in monkey XT1. In this example, we separated the trials where XT1 fixated with the right eye versus the left eye during binocular viewing. By comparing the two eyes in the two fixation conditions (same comparison as in Fig. 3), we found the evoked movements were similar. When the left eye was fixating, the amplitude ratio was 1.1 and the direction difference 16.2°. When the right eye was fixating, the amplitude ratio was 0.98 and the direction difference 20.3°. When we compared only the vectors obtained for each fixating eye (represented by an asterisk in Fig. 5A), the amplitude ratio was 1 and the direction difference 14.5°. This observation shows that, for a given site in SC, MS will evoke different vectors regardless of which eye is fixating.

Across all of the sites we tested in this condition (Fig. 5B), the two evoked vectors for each fixating eye were displaced far from the expected conjugacy values in terms of both amplitude ratios and directions for each strabismic monkey. During the binocular viewing condition, only 13% (1/8) and 36% (8/22) of sites were in the amplitude ratio range of 0.9 to 1.1 for monkeys ET1 and XT1, respectively. Saccade direction differences were consistently found between the two fixating eyes, with a mean absolute deviation of 8.12° for ET1 (CI: 3.28°–13.71°, N = 8) and 16.07° for XT1 (CI: 12.84°–19.62°, N = 22).

No significant difference was found between the amplitude ratios and direction differences for the same sites represented in Figures 3A and 3B for both strabismic monkeys (P > 0.05, Kolmogorov-Smirnov test) indicating that fixation has no significant effect on these values.

Comparison of Saccade Vectors for Each Fixating Eye Under Monocular Viewing

To ensure that these differences were not due to visual influences coming from the nonfixating eye, we explicitly suppressed the visual sensory influence of this eye by placing an opaque occluder in front of that eye. The same example site
described in Figure 5A for monkey XT1 is illustrated in Figure 6A under this monocular viewing configuration. The direction difference is $21^\circ$ and the amplitude ratio 1.02 between the fixating eyes (represented by asterisks). This shows that the same differences exist between the fixating eyes but with a larger direction difference when vision was prevented for one eye.

Across the sites tested under this monocular viewing condition in strabismic monkeys, the saccade direction differences we found were large, averaging $11.33^\circ$ (CI: 7.76$^\circ$–14.86$^\circ$, $N = 7$) for monkey ET1 and $16.8^\circ$ (CI: 13.3$^\circ$–19.6$^\circ$, $N = 8$) for monkey XT1. The amplitude ratios also showed some differences in conjugacy values. On average, this ratio was of 1.02 (CI: 0.92–1.14, $N = 7$) for monkey ET1 and 0.99 (CI: 0.74–0.98, $N = 8$) for monkey XT1. Only 57% (4/7) and 50% (4/8) of these amplitude ratios were within the range of 0.9 to 1.1 in monkeys ET1 and XT1, respectively.

In normal monkeys N1 and N2 though, both direction differences and amplitude ratios were close to the conjugacy values as indicated by the colored circles on each graph (Fig. 6B). The saccade direction differences were, on average, 2.56$^\circ$ (CI: 1.32$^\circ$–3.82$^\circ$, $N = 6$) for monkey N1 and 1.54$^\circ$ (CI: 0.82$^\circ$–2.37$^\circ$, $N = 7$) for monkey N2. Amplitude ratios were 0.99 (CI: 0.96–1.02, $N = 6$) and 0.99 (CI: 0.95–1.02, $N = 7$) for monkeys N1 and N2, respectively.

Altogether, the amplitude ratios for the two normal monkeys were inside the range of 0.9 to 1.1 in 100% of cases (13/13). In contrast, only 53% (8/15) of the amplitude ratios were inside this range in strabismic monkeys. However, no significant difference was found between the two amplitude ratio populations ($P > 0.05$, Kolmogorov-Smirnov test). Saccade direction differences were small in normal monkeys because 77% (10/13) of sites had a difference of less than 3$^\circ$, and the maximum difference found was only 4.5$^\circ$. In strabismic monkeys, 67% (10/15) of sites produced direction differences greater than 10$^\circ$. These saccade direction differences were significantly different between normal and strabismic monkeys ($P < 0.001$, Kolmogorov-Smirnov test).

No significant differences were found between the amplitude ratios and direction differences for the same sites represented in Figures 3A and 3B for both strabismic monkeys ($P > 0.05$, Kolmogorov-Smirnov test).

**Comparison of Saccade Vectors for Each Eye in Different Fixation Conditions**

We then analyzed the influence of fixation on the saccade vector evoked for each eye (when that eye was fixating versus when it was not) in monocular and binocular conditions, as summarized in Figure 7. In the binocular condition (solid lines), the evoked saccades of the dominant (in black) or the nondominant (in gray) eye were only slightly affected by fixation. For monkey ET1, the amplitude ratios (Fig. 7A) were very close to 1 for the dominant eye (mean: 1.01, CI: 0.99–1.04, $N = 8$) and for the nondominant eye (mean: 0.99, CI:
The amplitude ratios for the dominant eye and nondominant eye of monkey XT1 (Fig. 7B) were also very close to 1 even though the dominant eye showed a little more variability (dominant eye: 1.11, CI: 1.03–1.19, N = 18; nondominant eye: 1.03; CI: 0.96–1.02, N = 18). The saccade direction deviations (Figs. 7C, 7D) were mostly less than 5° for monkeys ET1 (dominant eye: 88% [7/8]; nondominant eye: 72% [13/18]; nondominant eye: 56% [10/18], Fig. 7D). In this binocular viewing condition (solid lines), the two eyes (dominant versus nondominant eye in black and gray, respectively) had similar sensitivities to the fixation because we did not find any significant difference between their amplitude ratios and direction differences in monkey ET1 (P > 0.05, Kolmogorov-Smirnov test, N = 8). In monkey XT1, only the amplitude ratios were significantly different between the dominant and nondominant eyes (P < 0.05, Kolmogorov-Smirnov test).

In the monocular viewing condition, the dominant eyes (black dashed lines) for each monkey still showed small changes in their amplitudes. The amplitude ratios were close to 1 for ET1 (mean: 1.05, CI: 0.9–1.21, N = 7, Fig. 7A) and XT1 (mean: 0.95, CI: 0.86–1.04, N = 8, Fig. 7B). The saccade direction deviations were again mostly less than 5° (ET1: 86% [6/7]; XT1: 63% [5/8]). It was however the nondominant eye (dashed lines in gray) of each monkey that seemed to be the most sensitive in this condition. For monkey ET1, 57% (4/7) of saccade direction deviations were greater than 5° and, for monkey XT1, the amplitudes ratios were mostly greater than 1 (mean: 1.35, CI: 1.15–1.5, N = 8). As for the binocular viewing condition, the two eyes (dominant versus nondominant eye in black and gray, respectively) had similar sensitivities to the fixation because only the amplitude ratios were significantly different between the dominant and nondominant eyes in monkey XT1 in this monocular viewing condition (P < 0.01, Kolmogorov-Smirnov test).

Comparison of Evoked Versus Visually Guided Saccades in Different Fixation Conditions

To compare evoked with visually guided saccades, we first selected visually guided saccades from one eye matching evoked saccades when this same eye was fixating, using a first hull (ellipse). Then we calculated for how many visually guided saccades from this group, the other eye was inside the second hull (see Methods). Figure 8 shows one example site from monkey XT1 (Fig. 8A, 8B). In Figure 8A, we have drawn ellipses around the evoked saccades of each eye. The black hull is the one drawn for the fixating eye (dominant eye, in black) and was used to select the visually guided saccades. The group of visually guided saccades selected by this method is represented in Figure 8B in black. From the nondominant eye evoked saccades dispersion on the two amplitude components (horizontal and vertical), a second hull was drawn. We then calculated the percentage of visually guided saccades for which the nondominant eye fell inside the gray hull. In this example, we can see that a majority of visually guided saccades (69%, 53/77) are inside this ellipse (Fig. 8B) even though a greater
6) and XT1 (matching percentage (MP, see methods) for each eye (right eye) in gray fixating the target with the dominant eye (left eye). (A) In this example site, monkey XT1 is fixing the target with the dominant eye (left eye). (A) Represented evoked saccades of the dominant eye in black and of the nondominant eye (right eye) in gray. The black and gray ellipses are the windows used in this example to select the visually guided saccades (black ellipse) and to calculate the matching percentage (gray ellipse). (B) Plotted the selected visually guided saccades with the same color code. The same ellipses are plotted. The green stars represent the visually guided saccades for the nondominant eye not matching inside the gray ellipse. (C, D) Histograms of the matching percentage (MP; see methods) for each eye (black and gray bars for dominant and nondominant eyes, respectively) in monkey ET1 (C, N = 6) and XT1 (D, N = 8).

Dispersion can also be observed (see green stars representing visually guided saccades outside the gray ellipse).

When we applied this method for our tested sites (8 in XT1 and 6 in ET1), a tendency emerged with a higher matching percentage (MP, see methods) when the dominant eye was used for fixation during MS or to foveate a peripheral target percentage (MP, see methods) when the dominant eye was used. When the nondominant eye was used though, only one site (1/8) showed a MP superior to 60% (in gray). In monkey ET1 (Fig. 8C), the MPs were in general lower than for monkey XT1. When the dominant eye (in black) was used, 67% (4/6) presented a MP greater than 45% and no case with a percentage inferior to 35%. For the nondominant eye (in gray), 50% (3/6) presented a percentage greater than 45% and the other half presented a low percentage of matching (<30%). However, this trend was less obvious than for monkey XT1 and no significant difference was found between the two populations (dominant versus non dominant eyes; P > 0.05, Kolmogorov-Smirnov test) and no higher probability to get a better matching percentage was found for the dominant eye (P > 0.05, Fisher’s exact test).

Finally, in Figures 9 and 10 we show the mean deviations in terms of amplitude ratios and direction difference for each eye between the evoked and the selected visually guided saccades. Due to the methods we used to select the visually guided saccades, the eye serving as a reference for the saccade selection showed small differences, supporting our selection methods (dominant eye in Figs. 9A and 9C and nondominant eye in 9B and 9D). In both animals this eye serving as a reference presented no deviation in direction greater than 10° with the selected visually guided saccades. In monkey XT1 (Fig. 9) no sites (0/8) presented an amplitude ratio outside the range 0.9 to 1.1. In monkey ET1 (Fig. 10) only one site (1/6) was outside this range for the dominant eye.

The fellow eyes (the eye not fixating the target) in monkey XT1 (Fig. 9) showed significantly different deviations in amplitude ratios for both eyes (P < 0.05, Kolmogorov-Smirnov test) but only for the dominant eye in direction (P < 0.05, Kolmogorov-Smirnov test) in comparison with the values obtained when the same eye was fixating (and then used as a reference). In monkey ET1 though (Fig. 10), only the amplitude ratios when the dominant eye was not fixating eye showed a significant difference (P < 0.05, Kolmogorov-Smirnov test). Besides, we observed some values close to a ratio of one and a direction deviation of 0° in both monkeys, showing some similarities to the visually guided saccades. In monkey XT1 (Fig. 9), for example, only 25% (2/8) showed a saccade direction differences with visually guided saccades greater than 10°, regardless of which eye was fixating/targeting the visual target. For monkey ET1 (Fig. 10), these differences were greater than 10° for only 17% (1/6) when the dominant eye was used as the reference, and 50% (3/6) when it was the nondominant eye. Finally, some more marked differences were observed in the amplitude ratios. When the dominant eye was used as a reference in monkey XT1 (Fig. 9A), 25% (2/8) were inside the range of 0.9 and 1.1 and 38% (3/8) in case of the nondominant eye (Fig. 9B). In ET1 (Fig. 10A), 33% (2/6) were outside this range for the dominant eye.
DISCUSSION

Electrical MS of iSC has been extensively used in normal monkeys to map and understand the topographic organization and the integration mechanisms of the iSC.15,16,19,20,28,29 In the present study, we provide the first evidence of large and significant differences in the conjugacy of evoked saccades (amplitude ratios and direction differences) between strabismic and normal monkeys (Figs. 1 and 3). These results do not seem to be due to abnormalities in the motor plant because the main sequences of both eyes were overlapping (Fig. 4). Similar disconjugacies were reported previously for visually guided saccades.2–5 In our study, we wanted to test if a same locus of activity in the iSC was coding for a common desired displacement for both eyes. Because some functional abnormalities have been found at the level of the PPRF,6,7 the disconjugacies could be due to unbalanced drives for the two eyes but, depending on which eye was used, the evoked vector from the iSC could still be the same. To test this possibility, we compared the evoked saccade vectors of each eye when that eye was fixating. The vectors observed for each fixating eye were different (Fig. 5) and even when vision was prevented for one eye (Fig. 6). Comparison of the disconjugacy values (amplitude ratios and direction differences) for the same sites (sites in Fig. 3 versus in Fig. 5 and sites in Fig. 3 versus in Fig. 6) did not show significant differences suggesting that the fixating eye in both viewing condition has few influence on these values. Also, a monocular analysis showed that evoked saccade vectors were nearly identical regardless of the fixation condition (Fig. 7).

These observations are particularly interesting because human and nonhuman primates with strabismus are able to perform accurate saccades with either eye fixating, and even perform accurate "crossover" saccades.23,24 Because either eye can be used to perform accurate saccades (even though a preference could be given to the dominant eye), the desired eye displacement and the control loop processes must be achieved accurately. If we consider that the SC is still providing the desired displacement in strabismic monkeys, different populations of neurons in the SC have to be recruited to accurately bring the chosen eye to the target according to our results. The SC is indeed a key structure along with the frontal eye field (FEF) in saccade generation in normal monkeys.30–32 This FEF-SC pathway also informs the brain of where the eye is going after a saccade. Perturbation studies have indeed shown that saccades evoked from MS of the SC33,34 or the FEF35 before an interceptive saccade (the intended saccade) are compensated. This compensation mechanism has not been found when the evoked saccade was triggered from the caudal fastigial nucleus (cFN),36 the only output nucleus of the cerebellum projecting to the saccade generator. The FEF-SC pathway plays an essential role in the construction of the saccade plan. The hypothesis of two desired displacements implemented in the iSC would presuppose the development of two different topographic maps in strabismic monkey, as studies in other mammals have already shown that normal visual experience is crucial to the development of normal motor maps in the SC.37,38 The FEF and perhaps other brain regions could activate these populations according to which eye was used for foveation of the visual target. However, the way to control which eye was used in our experiments was by determining the fixating eye in binocular condition, and the nonoccluded eye in monocular condition. There is a possibility that, in strabismus, the SC could function as a switch to evoke saccades for only one eye. In normal monkeys, in some very specific experimental conditions, the...
site of activity on the topographic map of the SC could be momentarily dissociated from the actual saccade after adaptation, suggesting that the locus of activity from the SC is read out differently by distal structures including nucleus reticularis tegmenti pontis (NRTP), cerebellum, and PPRF. If a similar mechanism is at play during development of strabismus in early life then, for any given saccade, the SC might be uninformed of the desired displacement for one of the two eyes. From our results, the evoked saccades of the dominant eye were more predictive of the disconjugacies observed in visually guided saccades (Figs. 8–10). The probability to get a better matching percentage with the dominant eye was significant for monkey XT1 but not for monkey ET1 though. In this case, the locus of activity for a defined saccade amplitude and direction could be the same for both eyes in the iSC but read out differently by other structures for the nondominant eye. The cerebellum, via the cFN, could steer saccades differently, according to which eye is used to accomplish the desired displacement. This hypothesis is plausible, as the cerebellum seems to influence saccade velocity and duration in normal monkeys. If this alternative is supported by future experiments, it will invalidate the hypothesis of two different topographic maps in the iSC. However, the final result would be that the neural circuits, as a whole, are indirectly using two different functional maps through different structures.

Elucidating the neural pathways that code the saccade goal for each eye presents an important and interesting challenge that will answer questions about how the visuo-oculomotor system develops and adapts to eye misalignment in early life. Determining if the separate maps are implemented in the SC could be answered using single-unit recordings of neurons at specific sites in the iSC. Different firing rates for the same saccade vector should emerge for each eye. Perturbation studies evoking a saccade before a target interception, as described above, could also help to determine if only one or both eyes compensate and then inform us if the saccade plan for each eye was built from the same neural pathway.

Acknowledgments
The authors thank R. Koepke and B. Congdon for technical assistance, and A. Pallus, PhD, and L. Bakst for helpful comments on the manuscript. Supported by National Institutes of Health, National Eye Institute Grants EY06069 (MJM), EY024848 (MMGW), EY019266 (SO); NIH, Office of Research Infrastructure Programs (ORIP) P51OD010425; Research to Prevent Blindness (New York, USA); Philippe Foundation (Paris, France).

Disclosure: J. Fleuriet, None; M.M.G. Walton, None; S. Ono, None; M.J. Mustari, None

References
Stimulation of SC in Strabismic Monkeys


