

Normal Correspondence of Tectal Maps for Saccadic Eye Movements in Strabismus

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ABSTRACT

The superior colliculus is a major brainstem structure for the production of saccadic eye movements. Electrical stimulation at any given point in the motor map generates saccades of defined amplitude and direction. It is unknown how this saccade map is affected by strabismus. Three macaques were raised with exotropia, an outwards ocular deviation, by detaching the medial rectus tendon in each eye at age one month. The animals were able to make saccades to targets with either eye and appeared to alternate fixation freely. To probe the organization of the superior colliculus, microstimulation was applied at multiple sites, with the animals either free-viewing or fixating a target. On average, microstimulation drove nearly conjugate saccades, similar in both amplitude and direction, but separated by the ocular deviation. Two monkeys showed a pattern deviation, characterized by a systematic change in the relative position of the two eyes with certain changes in gaze angle. These animals' saccades were slightly different for the right eye and left eye in their amplitude or direction. The differences were consistent with the animals' underlying pattern deviation, measured during static fixation and smooth pursuit. The tectal map for saccade generation appears to be normal in strabismus, but saccades may be affected by changes in the strabismic deviation that occur with different gaze angles.

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NEW AND NOTEWORTHY SECTION

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Electrical stimulation of the superior colliculus drives a rapid eye movement, or saccade, that is nearly identical in each eye. In strabismus, the eyes are offset, but microstimulation still generates similar saccades. Minor discrepancies in saccade amplitude and direction are sometimes present, which are likely due to altered downstream ocular motor pathways that also mediate smooth pursuit and steady fixation.

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INTRODUCTION

Most patients with divergent strabismus (exotropia) have normal visual acuity in each eye. They usually have a dominant eye, but can freely alternate fixation on visual targets. Objects are localized with surprising precision, by either eye or hand, despite the fact that images falling on each retina fail to land on locations that normally correspond (Agaoglu et al. 2014; Bucci et al. 2009; Das 2009; Das et al. 2004; Griffiths et al. 2011; Niechwiej-Szwedo et al. 2014). Diplopia is prevented by suppression of the peripheral temporal retina in each eye (Cooper and Record 1986; Economides et al. 2012; Herzau 1980; Joosse et al. 1997). Confusion is avoided by shifting the perceived location of objects sensed via the deviating eye (Cooper and Feldman 1979). Typically, the magnitude of the shift is equal to the ocular deviation, effectively cancelling it (Economides et al. 2012). The fovea of the deviating eye, by becoming perceptually realigned, acquires a common visual direction with a point in the temporal retina of the fixating eye (Herzau 1996). Consequently, the center of gaze of the deviating eye in retinotopic coordinates is mapped anomalously to a peripheral location in a body-centered frame of reference. Where this transformation is represented in the brain is unknown.

In normal monkeys, the superior colliculus contains binocular cells organized in a topographic map, with sensory cells in the superficial layers and sensory/motor cells in the deeper layers (Schiller 1984; Wurtz and Albano 1980). The latter cells have receptive fields activated by visual stimuli and movement fields encoding a saccade of corresponding size and direction. Electrical activation of a locus in the tectal map generates conjugate saccades (Robinson 1972). The superior colliculus plays a critical role in the guidance of eye-hand

movements, as well as in the selection of targets (Gandhi and Katnani 2011; Glimcher and Sparks 1992; Horwitz and Newsome 1999; Schall 2001; Song and McPeck 2015).

Few studies have been undertaken of the superior colliculus in strabismus, although it is a logical place to begin the effort to understand how the brain achieves accurate spatial localization despite ocular misalignment. In cats, after early transection of eye muscles, neurons in the superior colliculus have been reported to remain binocularly driven (Gordon and Gummow 1975; Gordon and Presson 1977). This result is surprising because strabismus has the opposite effect in striate cortex. Lack of concordant visual stimulation during early life leads to enhanced segregation of geniculate inputs within ocular dominance columns and to a striking loss of binocular neurons (Wiesel 1982). In the superior colliculus, eye input to the superficial layers is organized into separate zones that are analogous to the ocular dominance columns in striate cortex (Hubel et al. 1975). One would expect strabismus to increase the segregation of this direct retinal input. The indirect visual input that comes from striate cortex should also show reduced binocularity.

If neurons in the superior colliculus remain binocular despite strabismus, then the visual system faces a challenge when it comes to controlling which eye will acquire a visual target. For example, a stimulus situated between the fixation points in an exotropic subject falls on the temporal retina in the left eye and the nasal retina in the right eye (**Fig. 1**). The stimulus evokes sensory responses at two different loci within the superior colliculi (**Fig. 1B**). Given that neurons are binocular, the responses cannot be coded by eye, yet only one eye is destined to acquire the target. Somehow, the ocular motor system must disregard or suppress sensory

responses at one locus, and allow neurons at the other locus to generate an appropriate saccade. To explore this phenomenon, it would be interesting to compare neuronal activity at a given site during trials when a target was presented, and then subsequently fixated by either the left eye or the right eye.

We have recorded from single units in the superior colliculus of three macaques raised with alternating exotropia while they were engaged in alternating fixation onto targets. To localize the superior colliculus for recordings, electrical microstimulation was applied. This report describes the impact of strabismus on the eye movements obtained from electrical stimulation. A subsequent report will describe single unit observations.

METHODS

Animals

Three male macaques (*Macaca mulatta*) were reared with strabismus at the California National Primate Research Center, Davis, CA by performing a tenotomy of the medial rectus muscle in each eye at age 4 weeks. The muscle eventually re-attaches to the sclera, but fusion is disrupted during the critical period for binocular vision. This leads to an alternating exotropia without amblyopia, which shares many features of decompensated intermittent exotropia in humans (Economides et al. 2007). It differs, however, in some important respects. First, adduction remains reduced compared with normal animals, in part because the muscle insertion site may be abnormal. Second, the onset of exotropia is sudden and irrevocable, rather than occurring via a process of gradual decompensation. Other models of strabismus have been developed successfully, each with advantages and disadvantages (Crawford and von Noorden 1980; Das et al. 2005; Kiorpes 1992; Tychsen and Burkhalter 1997).

After the monkeys reached age 3 years, they were transferred to our laboratory at the University of California San Francisco. A titanium headpost and recording chamber were implanted, as described previously (Adams et al. 2007; Adams et al. 2011). The chamber was situated on the right side over medial parietal cortex, just anterior to the lunate sulcus, to avoid making penetrations through striate cortex. The right superior colliculus was stimulated in most experiments, but the left superior colliculus was stimulated in two monkeys by making penetrations near the medial edge of the chamber (**Fig. 1B**). All procedures were approved by

the Institutional Animal Care and Use Committee at UC Davis or UC San Francisco. Monkey 1 was referred to as “Monkey 2” in an earlier study (Economides et al. 2007); Monkey 2 and 3 have not been previously reported.

Video Eye Tracking and Target Presentation

Each monkey’s eye movements were recorded while head-restrained in a primate chair. Computer-generated targets (Cambridge Research Systems, Rochester, England) were rear-projected onto a tangent screen by a digital light projector (Hewlett Packard, Palo Alto, California) with a 60 Hz refresh rate. The tangent screen, subtending $\pm 45^\circ$ horizontally and vertically, was placed 57 cm in front of the monkey. Eye movements were monitored by two independent eye trackers, operating at 60 Hz, each using an infrared video camera (SensoMotoric Instruments, Teltow, Germany). The comparatively slow sampling rate limited the precision of saccade measurements, but should not affect comparisons between the eyes. The cameras were mounted overhead; a dielectric mirror that reflected infrared light was oriented obliquely to obtain video images of the animal’s eyes. Each eye was illuminated by a separate infrared light source, positioned laterally. This arrangement enabled us to track each eye over a wide range, from 20° nasally to almost 80° temporally. This was an important advantage, given the large exotropia present in the strabismic monkeys. The position of each eye and the location of visual stimuli on the tangent screen were sampled at 120 Hz by a Power1401 data acquisition and control system (Cambridge Electronics Design, Cambridge, England). To calibrate the eye

trackers the digital gain and offset were adjusted on-line to match eye and target locations while the monkey tracked a spot oscillating sinusoidally in a horizontal, and then vertical direction. Each eye was calibrated independently, with the other eye covered. The monkey was rewarded with food puree for accurate fixation within an adjustable window.

Superior Colliculus Stimulation

A plastic grid with holes 1 mm apart was placed inside the recording chamber. A 30-gauge guide tube was lowered to approximately 10 mm above the superior colliculus. Recordings were made with quartz-platinum/tungsten tetrodes (Thomas Recording, Giessen, Germany), having an impedance of 0.5 – 1.0 M Ω . As the tetrode was advanced, electrical stimulation was employed to locate the surface of the superior colliculus (MultiChannel Systems, STG 1001, Reutlingen, Germany). Typically, trains of biphasic square wave pulses, 500 μ s at 500 Hz, 20 – 400 μ A, were applied for 500-1,000 ms to generate repetitive saccades (Katnani and Gandhi 2012). After staircase saccades were observed, the tetrode was often advanced another 500 – 1,500 μ m to reduce the current required to elicit eye movements (20 – 200 μ A). Sites stimulated in each monkey are shown in **Fig. 1B**. The average number of stimulation trials at each site was 30.

For each monkey, saccade size and direction elicited by electrical stimulation were compared for the right eye and the left eye. Each staircase eye movement was comprised of saccades that were similar, but there was a tendency for successive saccades to diminish in

amplitude (Breznen et al. 1996; Stryker and Schiller 1975). Successive saccades also tended to be more variable than initial saccades. This was due to several factors, including intrusion of volitional saccades, limits in ocular excursion (a major issue in strabismic animals), and eye tracker inaccuracy at extreme gaze angles. For this reason, only the first saccade in each staircase was analyzed to compare right eye and left eye movements.

To obtain eye velocities, horizontal and vertical position signals for each eye were differentiated using a 3-point central difference algorithm (Spike 2, Cambridge Electronics Design, Cambridge, England). After stimulus onset, each saccade duration was defined as the period when the absolute value of the mean of all 4 velocity signals exceeded the steady state fixation baseline by 3 standard deviations (usually, about 75°/sec). To determine saccade amplitude and direction, position was compared at the beginning and end of each eye movement. Saccades under 3° were excluded, because of the limited resolution of the video eye trackers. The amplitude of saccades made by each eye were compared as follows (Walton et al. 2014):

$$\text{Amplitude Ratio} = \text{Saccade Amplitude}_{\text{left eye}} / \text{Saccade Amplitude}_{\text{right eye}}$$

When the left eye and right eye saccades are equal, the Amplitude Ratio = 1. Unequal saccades by the left eye and the right eye yield Amplitude Ratio values that are asymmetrical around 1. Therefore, Amplitude Ratios were normalized for statistical comparisons by using their common logarithm as the final measurement unit.

The difference between the polar angle of each eye's saccade was calculated as follows:

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234 $\text{Direction Difference} = \text{Polar Direction}_{\text{left eye}} - \text{Polar Direction}_{\text{right eye}}$

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236 For any given tectal site, Amplitude Ratio, log Amplitude Ratio, and Direction

237 Difference were calculated for each stimulation trial and then averaged to derive mean values \pm

238 sd for each parameter. For the log Amplitude Ratio and Direction Difference, confidence

239 intervals were calculated based on the critical value of the t-distribution for each sample size,

240 with an $\alpha = 0.05$.

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RESULTS

In all 3 exotropic monkeys, stimulation of the superior colliculus resulted in a series of consecutive saccades (**Fig. 2**). The cardinal finding was that for any given stimulus site, the saccades in each eye evoked by current application were approximately equal in size and direction. In other words, tectal stimulation elicited saccades that were essentially conjugate. The eyes moved in parallel, by the same amount, with their relative positions determined by the animal's underlying strabismic deviation. In the 3 monkeys, 48 tectal sites were stimulated in total. Stimulation of the right superior colliculus always produced leftwards movement of the eyes, and vice versa. Ipsiversive saccades were never evoked.

In each animal, we stimulated repeatedly the same site in the superior colliculus. **Fig. 3** shows data from Monkey 1, an animal with an alternating exotropia measuring 35-40°. His ocular deviation remained relatively constant with changes in gaze angle. The relative positions of Monkey 1's eyes over a 40° range of vertical and horizontal static fixations have been reported previously (see Fig. 9, Economides et al (2007)). The animal was rewarded for fixation with either eye on a target located at the center of the tangent screen (**Fig. 3A,B**). At this location he preferred to use his right eye. After fixation was acquired, current was delivered to drive a staircase of saccades. The Amplitude Ratio (saccade amplitude_{left eye} / saccade amplitude_{right eye}) was 1.00 ± 0.09 for trials initiated with the right eye ($n = 55$) and 0.90 ± 0.07 for trials initiated with the left eye ($n = 28$). The Direction Difference (polar direction_{left eye} - polar direction_{right eye}) was $2.2^\circ \pm 4.8^\circ$ for right eye trials and $2.1^\circ \pm 3.8^\circ$ for left eye trials. Regardless

of which eye fixated on the target at the origin, the saccades in each eye evoked by stimulation drove movements of the eyes that differed little in amplitude or direction.

When the location of the initial fixation point was moved along the horizontal meridian, the monkey's fixation behavior changed. He preferred to acquire targets on the left with the left eye, and on the right with the right eye (**Fig. 3C**). Combining the data for all fixation starting positions tested in **Fig. 3** ($n = 149$), the right eye saccades had a mean amplitude of $21.3^\circ \pm 3.9^\circ$ [95% CI, $20.7^\circ - 21.9^\circ$] and a mean polar angle of $124.7^\circ \pm 7.4^\circ$ [95% CI, $123.5^\circ - 125.9^\circ$]. The left eye saccades had an amplitude of $20.4^\circ \pm 3.6^\circ$ [95% CI, $19.8^\circ - 21.0^\circ$] and a polar angle of $127.9^\circ \pm 8.2^\circ$ [95% CI, $126.5^\circ - 129.2^\circ$]. The confidence intervals for saccade amplitude overlapped, indicating that there was no significant difference in the magnitude of saccades driven in each eye by electrical stimulation at this site in the superior colliculus. For saccade direction, however, there was a small yet significant difference (**Fig. 3D**).

In Monkey 1, 5 sites were stimulated, all in the right superior colliculus (**Fig. 1B**). For these sites, the mean Amplitude Ratio was 1.07 ± 0.16 . The mean log Amplitude Ratio was 0.017 ± 0.067 [95% CI, $-0.066 - 0.101$]. The 95% confidence interval included 0, indicating that there was no significant difference in the size of saccades for the left eye and right eye. The mean Direction Difference for saccades was $3.9^\circ \pm 7.7^\circ$ [95% CI, $-5.7 - 13.5$]. The Direction Difference was also not significant.

In Monkey 1 the superior colliculus was stimulated while the animal was being rewarded for fixating a target. In Monkey 2, we tested the effect of applying stimulation while free-

viewing either random dot noise patterns or natural scenes (**Fig. 4A**). The images were needed to maintain alertness, because no reward was being provided. Given that tectal stimulation was not linked to behavior, the eye of fixation was indeterminate and saccade starting points were widely scattered. Nonetheless, the saccades made by each eye were similar, with an Amplitude Ratio = 1.13 ± 0.13 and Direction Difference = $1.2^\circ \pm 9.2^\circ$ (**Fig. 4B**).

In Monkey 2 electrical stimulation was applied at 18 collicular sites, 16 on the right and 2 on the left (**Fig. 1B**), while either free-viewing or fixating a target. The mean Amplitude Ratio was 1.14 ± 0.17 . The mean log Amplitude Ratio was 0.038 ± 0.066 [95% CI, 0.005 – 0.071], indicating that saccades for the left eye were significantly larger than those for the right eye. The mean Direction Difference was $-3.6^\circ \pm 8.2^\circ$ [95% CI, $-7.6^\circ - 0.49^\circ$]. This difference was not significant.

The positions of the two eyes for Monkey 2 during static fixation by the left eye on a 9-point grid is shown in **Fig. 5A**. In addition to an alternating exotropia of $40-45^\circ$, the visual axis of the animal's left eye was nearly 20° above that of his right eye. This left hypertropia accounts for the fact that left eye vectors were always higher than corresponding right eye vectors (**Fig. 4A**). This vertical offset had a negligible impact on the conjugacy of saccades. However, the arrays of static fixations revealed another feature of this animal's strabismus that did affect the conjugacy of saccades. The horizontal separation of left eye positions and corresponding right eye positions increased with left gaze. For example, left eye positions on the vertical meridian (0° horizontal) matched right eye positions at a mean of 40.5° , whereas left eye positions at -40° matched right eye positions at a mean of 5.5° . Moving the left eye from primary gaze to -40°

increased the horizontal deviation of the eyes from 40.5° to 45.5° . This effect of gaze angle on the horizontal separation between the eyes was also evident during smooth pursuit (**Fig. 5B**). Tracking a target with the left eye that moved from the midline leftwards by 40° resulted in an increase in the exotropia. To summarize: shifts in gaze angle by the monkey towards the left side caused an increase in exotropia due to a 12% greater movement of the left eye compared with the right eye.

Monkey 3 had the largest alternating exotropia, measuring $60\text{--}65^\circ$ (**Fig. 6A**). He preferred to fixate with the right eye. He had an unusual pattern deviation: his exotropia decreased on upgaze. In addition, his left eye became hypertropic on left gaze, but hypotropic on right gaze. As a result, a 9-point grid of targets fixated by the right eye corresponded to a rotated grid of matching left eye positions (**Fig. 6A**). The left eye grid was rotated clockwise about 17° compared with the right eye grid. Note that the plot does not show that the left globe was cyclorotated, but rather, that the left eye array of static fixation positions was rotated.

When engaged in smooth pursuit (**Fig. 6B**) Monkey 3 usually employed his right eye, but switched to the left eye when the target moved far enough to the left side (**Fig. 6C**). The angle between the trajectory of right eye positions and left eye positions equaled about 16° during smooth pursuit of a target moving back and forth along the horizontal meridian (**Fig. 6B**). This rotation recapitulated the relative angle between right eye and left eye positions during static fixation.

Stimulation was delivered to 17 sites in the right superior colliculus and 8 sites in the left superior colliculus, while either free-viewing or fixating a target (**Fig. 1B**). For all 25 sites, the mean Amplitude Ratio was 1.00 ± 0.20 . The mean log Amplitude Ratio was -0.015 ± 0.084 [95% CI, $-0.050 - 0.020$]; there was no significant difference in the magnitude of saccades by the left eye and the right eye. The mean Direction Difference was $-15.0^\circ \pm 9.9^\circ$ [95% CI, $-19.1^\circ - -10.9^\circ$]. The polar direction of saccades was significantly different, with left eye saccades rotated clockwise relative to right eye saccades (**Fig. 7**). The mean Direction Difference was close to the difference observed in the angular alignment of the static fixation grids (**Fig. 6A**) and in the relative trajectory of eye movements during smooth pursuit (**Figs. 6B,C**).

The data for all stimulation sites in each monkey are shown in **Fig. 8**. As mentioned previously, the recording chamber was mounted on the right side of the head in each animal, causing us to stimulate the right superior colliculus more often than the left superior colliculus. Consequently, the majority of saccades were elicited to the left. There was variability in the log Amplitude Ratio from site to site in each animal, but only Monkey 2 showed a systematic bias (**Fig 8A**). The saccades made by his left eye were greater in amplitude than those made by his right eye, reflected by positive values for log Amplitude Ratio. For Direction Difference there was also variability among stimulation sites (**Fig. 8B**). The mean Direction Difference was close to 0, except in Monkey 3, the animal with a clockwise rotation of left eye saccades relative to right eye saccades.

Conjugacy of Saccades in Normal Subjects

It is possible that the rather small disconjugacies observed in these strabismic monkeys were due to measurement error by the eye trackers. It would be ideal to perform parallel experiments in normal monkeys, but we had none available in our laboratory. As an alternative, we tested 5 human subjects with normal visual function and orthotropic eye alignment, following a protocol approved by the UCSF Committee on Human Research. The eye movement recordings were made with the same apparatus used for the strabismic monkeys.

Subjects fixated a central target on a tangent screen at a distance of 57 cm. A peripheral target appeared briefly at a given eccentricity and polar angle. The subjects made a saccade to the peripheral target. Trials were repeated for a single peripheral target locus, before testing the next target locus. A total of 30 trials were analyzed for each target locus. **Fig. 9** shows examples of saccades to a target at 20° eccentricity made by two subjects. In the first subject, the Amplitude Ratios ranged between 0.92 and 1.08; the Direction Differences ranged between -1.3° and 5.4°. In the second subject, the Amplitude Ratios ranged between 0.93 and 1.11; the Direction Differences ranged between -1.5° and 4.4°. Many of the mean saccade vectors for each eye differed significantly in amplitude or direction.

One can assume the human subjects made saccades that were nearly perfectly conjugate (the change in vergence angle required to maintain eye alignment on a flat screen induces only minor disconjugacy). The discrepancy in the size and direction of saccades that was measured in our subjects is due mostly to inaccuracy in eye position measurements generated by each eye tracker. Although they are calibrated carefully, they are susceptible to errors from many sources,

379 such as fluctuations in eyelid height, pupil thresholding, pupil size, ocular surface reflectivity,
380 image quality, and subject position.

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DISCUSSION

Our main finding was that in strabismus, although the eyes' fixation points are offset in position, they move nearly conjugately when the superior colliculus is stimulated electrically. Stimulation did not cause a major change in the magnitude of the strabismic deviation or any tendency for the eyes to converge on a location in visual space. It has been reported that disconjugate movements related to shifts in vergence angle can be evoked by stimulation at the rostral pole (Chaturvedi and Van Gisbergen 2000; Van Horn et al. 2013). We did not stimulate this region, and hence, only conjugate eye movements were observed in our experiments.

At individual stimulation sites, there were often minor differences in the amplitude or direction of saccades evoked in each eye (**Fig. 8**). Some differences attained statistical significance, but they were not significant in biological terms. For example, the stimulation site illustrated in **Fig. 3** yielded saccades with a 3.2° Direction Difference. However, this Direction Difference fell within the range of Direction Differences encountered in normal subjects engaged in conjugate eye movements. The trackers monitoring each eye, even when calibrated fastidiously, yield small errors in position signals that can make conjugate saccades appear slightly disconjugate (**Fig. 9**). The problem is compounded in strabismic monkeys because of their large ocular deviations. For example, with extreme globe rotations, the tracking of position can be degraded by movement of the illuminator light reflex from the cornea to the sclera.

The discrepancies we measured in saccade size or direction from one stimulation site to another were usually small and variable in sign (**Fig. 8**). Most importantly, except as noted below, there was no systematic trend or pattern to the saccade asymmetries. For this reason, it seemed valid to average the data obtained from tectal stimulation sites in each animal.

In Monkey 2, the mean Amplitude Ratio compiled from all 18 stimulation sites was 1.14. This was a significant deviation from an equal amplitude ratio, and well outside the range of tracker error. Comparison of relative left eye and right eye positions during static fixation (**Fig. 5A**) and smooth pursuit (**Fig. 5B**) showed that with shifts in gaze angle to the left, the animal's exotropia increased by 12%. The most likely explanation is that the surgery on the medial rectus muscles performed during infancy resulted in asymmetrical weakness. If the right medial rectus were weaker than the left medial rectus, then the animal's exotropia would increase on left gaze, just as shown in **Fig. 5**. In this animal we happened to stimulate the right superior colliculus on 16/18 trials, driving the eyes to the left. Accordingly, tectal stimulation evoked 14% larger saccades in the left eye compared to the right eye.

In strabismus, a shift in the relative positions of the two eyes that occurs with changes in gaze angle is known as "incomitance". The increase in exotropia on left gaze in Monkey 2 represents a horizontal gaze incomitance. In Monkey 3 there was a different type of incomitance. Left eye saccades showed a mean clockwise shift of 15° relative to right eye saccades (**Fig. 8**). A clockwise shift was observed for saccades of all amplitudes, directions, and starting points. The most likely cause was an unusual property that this animal displayed, namely, a 16° – 17° rotation in the relative positions of the eyes' fixation points that occurred

with either vertical or horizontal changes in gaze angle (**Fig. 6A**). With upgaze, the animal's exotropia was reduced – a form of incomitance known as an A-pattern. An A-pattern produces a clockwise Direction Difference (i.e., left eye clockwise relative to right eye), but only for the vertical component of saccades (**Fig. 6A**, left inset). Consequently, the Direction Difference is maximal for pure vertical saccades and absent for pure horizontal saccades. In addition to an A-pattern exotropia, Monkey 3 had a left hypertropia on left gaze and a left hypotropia on right gaze. This change in relative vertical position of the eyes as a function of horizontal gaze induces a clockwise Direction Difference, but only for the horizontal component of saccades (**Fig. 6A**, right inset). The combination of a relative shift in the horizontal separation of the two eyes with vertical gaze (A-pattern), and a relative shift in the vertical separation with horizontal gaze, account for the 15° clockwise rotation of the left eye's saccades.

It is debated whether incomitant strabismus patterns are due to orbital mechanical forces, such as oblique muscle dysfunction, altered rectus muscle action, or displaced pulleys (Ghasia and Shaikh 2013; Ghasia et al. 2015; Hao et al. 2016; Kushner 2010; Narasimhan et al. 2007; Oh et al. 2002). The surgery performed to induce strabismus in Monkeys 2 and 3 caused changes in eye muscle mechanics that contributed to the incomitance of their ocular deviation. Monkey 3 also had a very large deviation, which would be likely to alter extraocular muscle action in different gaze positions. However, such pattern deviations have been observed in monkeys with a much smaller deviation, whose strabismus was induced without eye muscle surgery. For example, Das and Mustari (see their Monkey S1 in Figure 1, 2007) reported a pattern deviation in a monkey rendered strabismic by early bilateral visual deprivation that was nearly identical to the pattern in Monkey 3. Incomitance – especially A- and V- pattern deviations – is also encountered frequently in humans with strabismus who have not undergone eye muscle surgery

(Deng et al. 2013; Dickmann et al. 2012). This suggests that the incomitance in Monkeys 2 and 3 was not simply an odd feature of the surgical model of strabismus.

Recently, Fleuriet and colleagues (Fleuriet et al. 2016) have reported observations from electrical stimulation of the superior colliculus in strabismic monkeys. They also recorded small differences in the size and direction of saccades made by each eye. Our results are in close agreement with their findings. They suggested that in strabismus, activation of a single location in the superior colliculus is not interpreted by the saccade generator as the same desired displacement for each eye. This notion implies two separate, shifted motor maps in the superior colliculus, one for each eye. If offset monocular motor maps were present, it is interesting to consider the outcome of microstimulation applied to a single site. The result can be predicted from experiments stimulating at two different sites in the superior colliculus of normal animals. The ensuing saccade resembles the vector sum of the saccades driven at either site alone (Katnani et al. 2012; Noto and Gnadt 2009; Robinson 1972; Vokoun et al. 2014). The saccade is conjugate, because tectal output is conveyed to a saccade generator that drives yoked eye movements. For the same reason, one would expect microstimulation to drive conjugate saccades in strabismus, even if there were monocular maps comprised of separate populations of neurons encoding different vectors for each eye. It seems doubtful that the technique of microstimulation could reveal separate monocular maps in strabismus, even if present. This point holds true, regardless of which eye is engaged in fixation at the moment of microstimulation.

Fleuriet and colleagues (2016) found that the eyes' peak saccadic velocities remain equal, leading them to conclude that disconjugate saccades could not be fully explained by changes in the oculomotor plant. We previously compared adducting and abducting saccades after tenotomy of the medial rectus, and also found equal saccadic velocities (Economides et al. 2007). Saccadic velocity, however, is only one facet of muscle function. Other changes in muscle action could contribute to saccadic disconjugacy.

In Monkeys 2 and 3, evoked saccades were disconjugate, but smooth pursuit and static fixations also showed disconjugacy (**Fig. 5B, 6B,C**). This fact provides the strongest evidence that disconjugate saccades did not arise from plasticity of the map in the superior colliculus, but were a general property of all gaze angle shifts in these animals. Evidence has emerged that pattern deviations are due to cross-axis eye movements generated by the firing behavior of ocular motoneurons (Das 2011; Das and Mustari 2007; Walton et al. 2015; Walton et al. 2013). Even in normal animals, saccade metrics can adapt without any change in the tectal saccade map (Quessy et al. 2010). Incomitance in strabismus is likely due to perturbations downstream from the colliculus, at sites that control yoking of eye position in different gaze angles for saccades, smooth pursuit, and steady fixation.

In this context, Monkey XT1 in the report by Fleuriet and colleagues (2016) had a pattern deviation that was extremely similar to that recorded in our exotropic Monkey 3 (compare **Fig. 6A** to Walton et al. 2014, **Fig. 1 C, D**). Their Monkey XT1 had a -20° Direction Difference for saccades, similar to the value of -15° in Monkey 3. Reasons suggested for this Direction

Difference included: density of eye muscle innervation, site of medial rectus reattachment, altered vergence tone, and abnormalities in the saccade generator (Walton et al. 2014).

When strabismic subjects plan a saccade to a target, they must decide which eye to use and then calculate the appropriate saccade vector for that eye. Sometimes the target is detected by the same eye that acquires it, but strabismic subjects are also capable of perceiving the location of a target with one eye and then making a saccade to it with the other eye – a phenomenon known as a “crossover” saccade (Economides et al. 2014). It remains unclear how this process is controlled in the visual system. Any given visual target evokes a sensory response at two different locations in the superior colliculus, but motor activity to drive a saccade occurs at only one site. Single unit recordings, currently underway, should provide further insight into how sensory responses are gated to yield subsequent motor activation at a single site in the superior colliculus.

FIGURE LEGENDS

Fig. 1) Electrical stimulation of the superior colliculus of strabismic monkeys. **(A)** Diagram showing right eye (RE) fixating at the origin and exotropic left eye (LE). A target (black circle) presented between the eyes' fixation points could be acquired by the left eye with a 30° saccade or by the right eye with a 20° saccade. **(B)** Schematic map of the superior colliculus (SC), dorsal view, showing where sensory responses would be evoked by the target in **(A)** for the left eye (blue circle) and the right eye (red circle). Sensory responses at only one site would be followed by a motor discharge to bring an eye onto the target. Small circles denote sites where microstimulation was delivered in each monkey.

Fig. 2) Saccades elicited by stimulation of the superior colliculus are essentially conjugate. Representative eye position traces from three exotropic monkeys showing staircases of saccades after current application in the right superior colliculus. The ocular deviation remains constant in each animal as the eyes are driven to the left. Positive values = upgaze or rightgaze.

Fig. 3) Repeated stimulation at a single site in the superior colliculus yields saccades of similar size and direction in each eye of Monkey 1. **(A)** Saccade vectors for the right eye (red arrows) fixating at a target (green dot) at the screen center. Vectors were computed from initial saccades of staircases driven by electrical stimulation **(B)** Saccade vectors for the left eye fixating at the

origin. **(C)** Saccade vectors for intermediate target positions along the horizontal meridian. **(D)**
 Mean saccadic vectors (bold arrows) for all trials **(A-C)** are similar for the two eyes.

Fig. 4) Stimulation of the superior colliculus while free-viewing produces nearly conjugate
 saccades in Monkey 2. **(A)** Saccadic vectors for the left eye (blue arrows) and the right eye (red
 arrows), resulting from electrical stimulation applied every 5 sec while the animal looked at
 patterns on a tangent screen. **(B)** The amplitudes and directions of saccades made by each eye
 are similar, despite initiation from widely scattered positions in visual space.

Fig. 5) Horizontal incomitance in Monkey 2. **(A)** Eye positions during static fixations by
 Monkey 2 with the left eye (blue points) on a grid of 9 targets spaced 20° horizontally and
 vertically. The grid for the right eye (red points) is displaced downwards because the animal has
 a left hypertropia. The clouds of points are also elongated, because he has a small vertical
 pendular nystagmus. Note by comparing matching positions for the two eyes (brackets), that the
 exotropia increases when the animal moves the left eye from the midline to the left. **(B)** Smooth
 pursuit by the left eye shows an increase in the horizontal exotropia (black trace) from 40° to 45°
 when the eye moves leftwards from primary gaze to -40° . Note low gain, saccadic smooth
 pursuit, which is typical of strabismus.

Fig. 6) Pattern deviation in Monkey 3. **(A)** Static fixation by Monkey 3 with the right eye on a
 grid of points separated horizontally and vertically by 20° . The corresponding positions for the
 non-fixating left eye produce a grid rotated by $\sim 17^\circ$. The left inset depicts schematically an
 incomitant deviation known as an A-pattern. It results in a clockwise Direction Difference for

the vertical component of gaze shifts (compare orientation of lines connecting central 3 grid points). In addition to an A-pattern, the left eye of Monkey 3 moves down relative to the right eye as the animal looks to the right (the right inset shows this effect schematically). **(B)** Smooth pursuit by Monkey 3 of a target oscillating sinusoidally $\pm 30^\circ$ (green line). He tracks it with his right eye (red), except beyond 20° to the left. Note the sloping trajectory (blue line) of the non-fixating left eye positions, which forms a $\sim 16^\circ$ angle with the trajectory of the right eye's positions. **(C)** Monkey 3 switches to fixate with the left eye when the target in **(B)** moves more than 15° to the left of the midline. Note the corresponding rotation of the deviated right eye's positions.

Fig. 7) Saccades generated by tectal stimulation differ in polar angle because of pattern deviation. Families of saccades and their mean vectors obtained in Monkey 3 are shown for 4 representative stimulation sites. At all sites, left eye saccades were rotated clockwise in polar angle with respect to right eye saccades, due to the animal's combination of pattern deviations **(Fig. 6)**. Number in the polar plots represents scale for saccade amplitude.

Fig. 8) Data from tectal stimulation sites in 3 monkeys. **(A)** Log Amplitude Ratio plotted versus polar angle; most points are on the left side because the right superior colliculus was stimulated in 38/48 experiments. The points are scattered around 0 (dotted line), which equals an Amplitude Ratio of 1. Only Monkey 2 had a mean log Amplitude Ratio that differed significantly from 0. **(B)** Histograms of Direction Differences (10° bins) for stimulation sites in each monkey; Monkey 3 had a mean Direction Difference of -15.0° .

579 **Fig. 9)** Noise in video eye tracker measurements of saccade conjugacy. Examples from two
580 normal human subjects making 30 consecutive saccades to targets at an eccentricity of 20°
581 (circle) spaced every 45° . Bold blue (left eye) and red (right eye) vectors represent mean
582 saccades. Both subjects show non-systematic discrepancies in saccade amplitude (AR = left eye
583 saccade/right eye saccade) and direction (DD = left eye polar angle – right eye polar angle).
584 Asterisk denotes significant difference at 95% confidence level.
585

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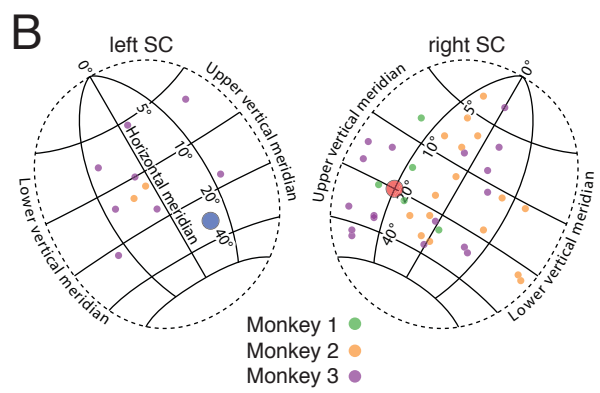
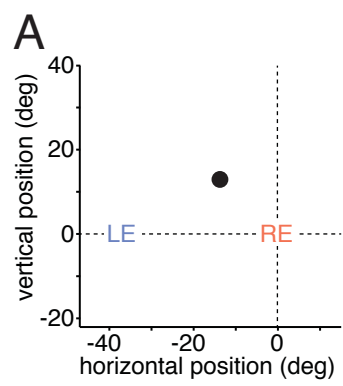
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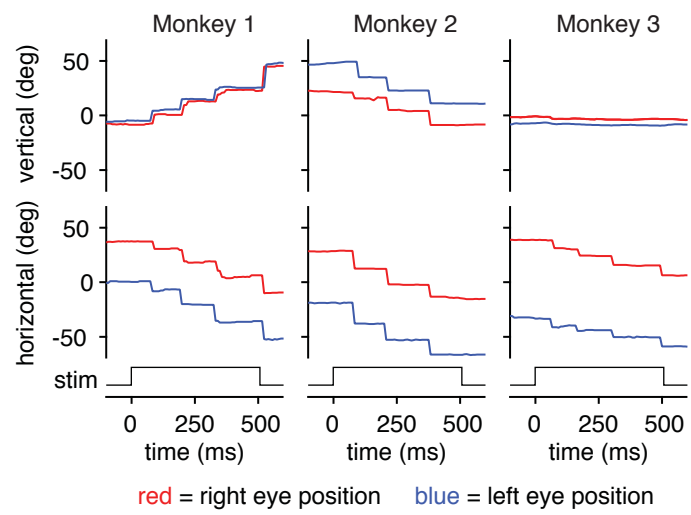
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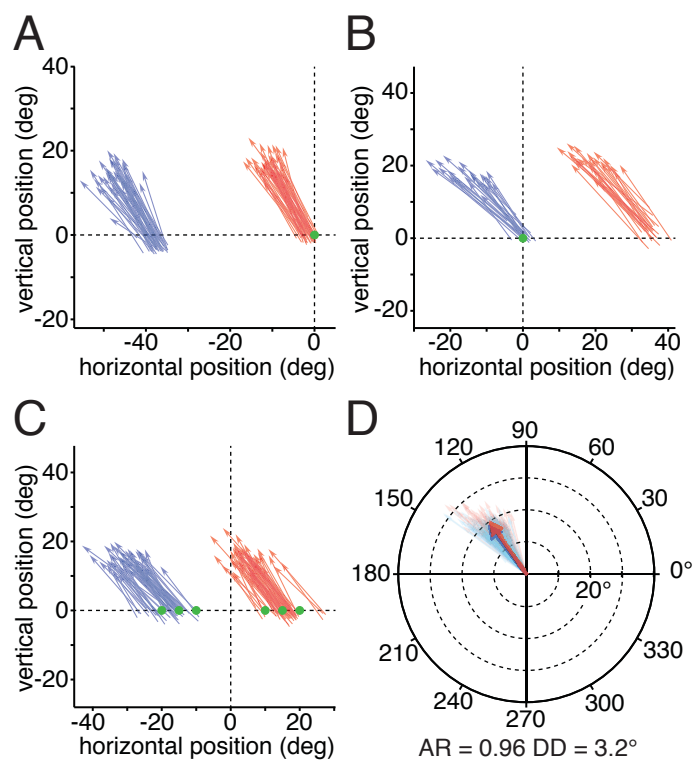
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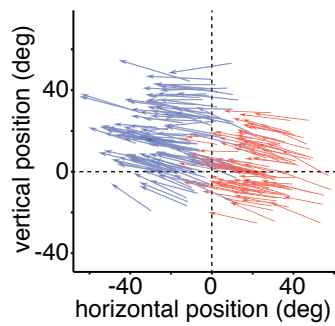
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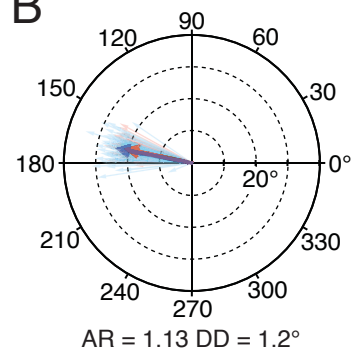


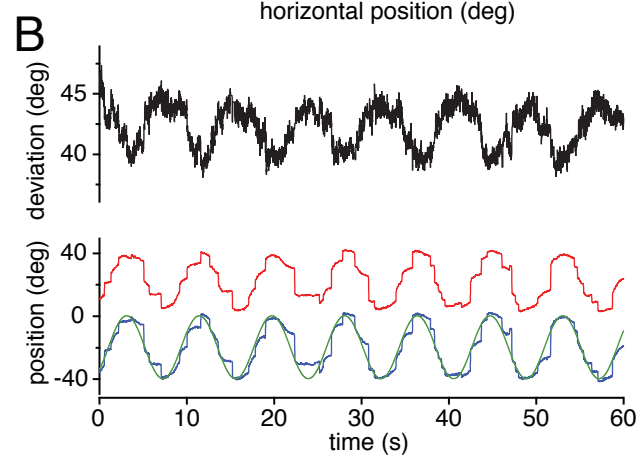
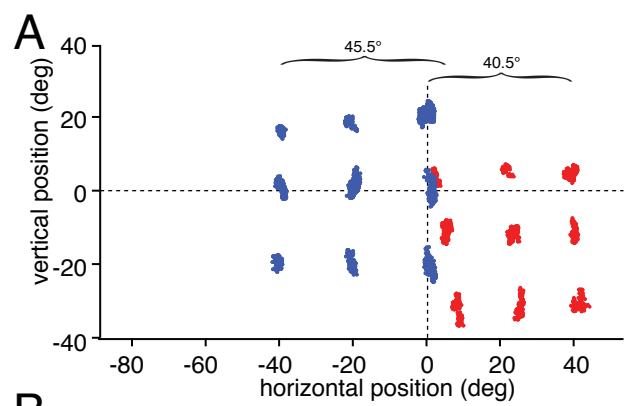


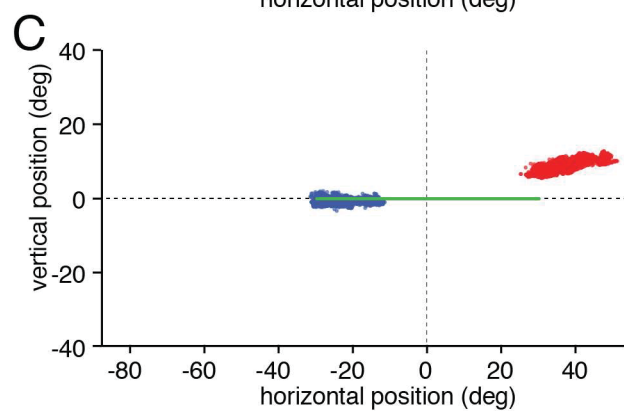
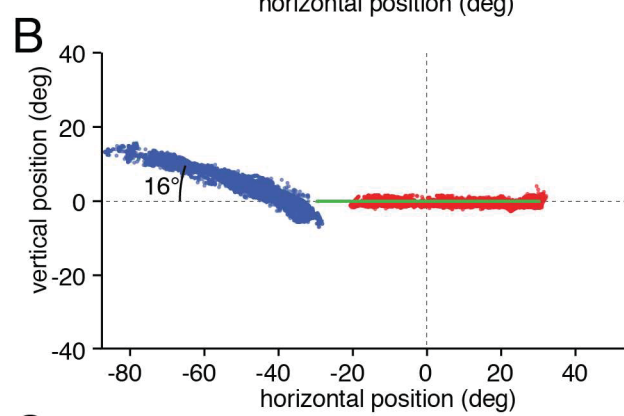
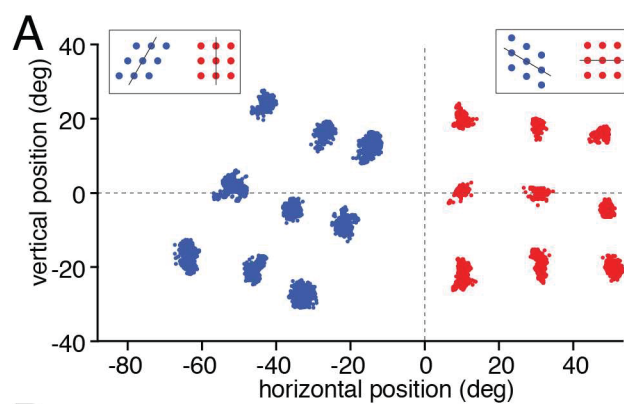
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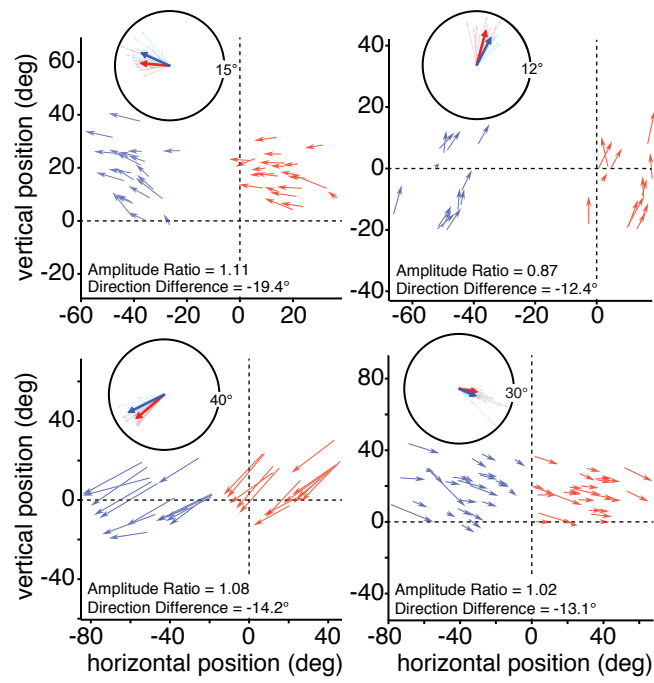


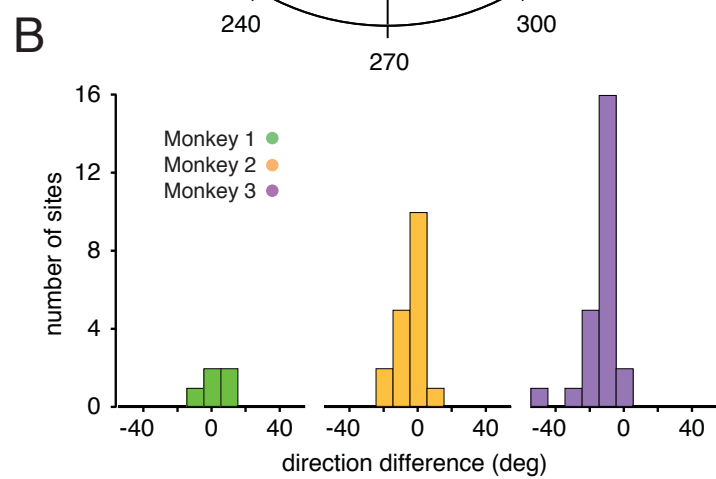
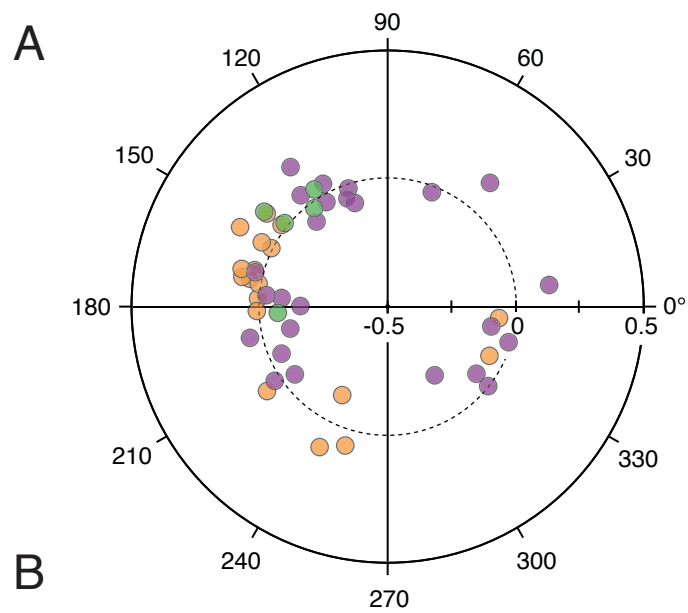
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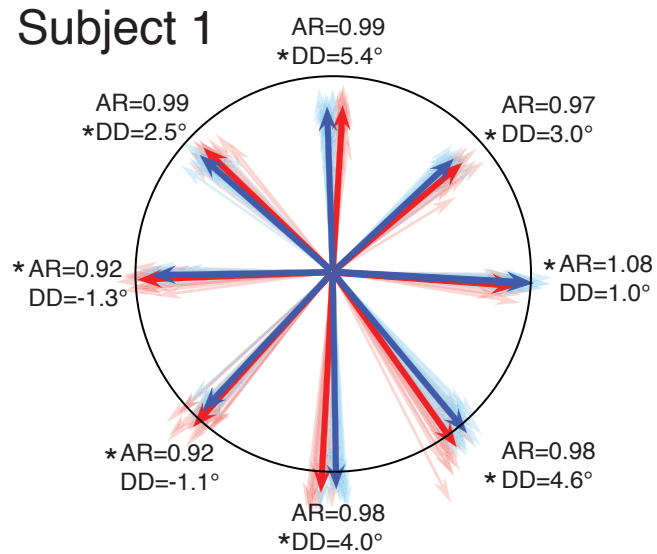








Subject 1



Subject 2

