FIXATION STABILITY IN MONKEYS WITH STRABISMUS.

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Abstract

Purpose: To assess the effect of target parameters on fixation stability in strabismic monkeys.

Methods: Eye movements were recorded in one normal and three strabismic monkeys during 72 fixation conditions (4 shapes; 3 sizes; 2 backgrounds; OD, OS or OU viewing), each repeated 5 times. Fixation stability was quantified using the Bivariate Contour Ellipse Area (BCEA). Influence of target parameters was assessed using 4-way ANOVA.

Results: BCEA was greater in the strabismic monkeys compared to the normal. In strabismus, BCEA of the deviated eye was significantly greater than BCEA in the fixating eye. Target shape and size significantly influenced fixation stability in both normal and strabismic monkeys. Background effects were idiosyncratic.

Conclusions: Target parameters that influence fixation stability in a normal, also affects fixation stability in disease conditions such as strabismus. Target parameter influences likely function via conjugate mechanisms since proportional effects were observed in both viewing and covered eyes.

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Chapter 1: Introduction

The main focus of my thesis research is to examine fixation stability in monkeys with strabismus. The goal of this chapter is to introduce, to the reader, the three main subject areas of the research – 1) Oculomotor features of strabismus including monkey models for human strabismus 2) Fixational eye movements and 3) Eye movements in strabismus with specific emphasis on fixational eye movements.

1.1 Strabismus – General Introduction

Strabismus is defined as a misalignment or relative deviation of the visual axes of the two eyes. Strabismus is a common visual developmental problem affecting 2-5% of human infants (Govindan, Mohney et al. 2005). The exact cause of strabismus is unknown; however various conditions such as monocular refractive errors, amblyopia, unilateral congenital cataracts, extraocular muscle paralysis etc., during early life results in developmental disruption of binocular vision that can eventually lead to strabismus (von Noorden and Campos 2002).

1.1.1 Describing the Strabismic State

Strabismus is broadly divided into two main classes: latent deviations (heterophorias) and manifest deviations (heterotropias). Heterophoria appears when fusion is broken and the two eyes are no longer looking at the same object whereas heterotropia is a misalignment of the two eyes when a subject is looking with both eyes uncovered. In addition, there are many keywords used to describe the state of eye misalignment including direction of the deviation, comitance, constancy, time of onset, state of accommodation and vergence system, eye involved; the etiology of strabismus etc. (von Noorden and Campos 2002).

Based on the direction of deviation, strabismus may be classified as esotropia (inward deviation of the eye – convergent misalignment), exotropia (outward deviation of the eye – divergent misalignment), hypotropia (downward deviation of the eye – vertical misalignment), and hypertropia (upward deviation of the eye – also vertical misalignment). Also a misalignment of one or both eyes around the line of sight due to clockwise or counterclockwise rotations of the globe is called cyclotropia (Torsional misalignment).

Strabismus is described as comitant when the angle of deviation is constant at different gaze locations or incomitant when the angle of deviation changes significantly in different positions of gaze. Incomitant strabismus is generally evidence of a paretic muscle.

In some patients due to inadequate fusion, the eyes are always deviated. This is called as constant deviation or constant strabismus whereas in other patients the fusion mechanism functions well in some but not in all circumstances. In this case, the deviation is manifest only at certain times, for example when the patient is tired, ill, or under stress. This type of misalignment is called an intermittent deviation or intermittent strabismus (von Noorden and Campos 2002).

The accommodation and vergence systems play an important role in determining the relative position of the visual axes. For example, in accommodative esotropia the act of accommodation (due to looking at a near target for example) has a major influence on the deviation, whereas in non-accommodative esotropia it does not. The accommodation system influences the vergence system and vice-versa due to cross-links whose strength is determined by the AC/A and CA/C ratios (Schor and Kotulak 1986). If an esodeviation is greater at near than at distance, it is called Convergence Excess; whereas if an exodeviation is greater at near than at distance fixation, then it is referred to as a Convergence Insufficiency. Similarly, if an exodeviation is greater at distance than at near, it is called as Divergence Excess; if an esodeviation is greater at distance than at near, it is referred as Divergence Insufficiency. Although the exact mechanisms are not yet clear, these forms of strabismus are believed to be due to either too large or too small AC/A or CA/C ratios (von Noorden and Campos 2002).

A deviation noted at birth or in the first months of life is termed congenital. However this terminology has been replaced by infantile, which includes all forms of strabismus with an onset during the first 6 months of life. If the deviation arises after first 6 months of life, it is called acquired (von Noorden and Campos 2002).

If the patient habitually fixates with a specific eye and the other eye is always deviated, then it is labeled as a Unilateral Strabismus. For example, if the patient fixates with his left eye and the right eye is deviated, it is called right eye heterotropia. However, if the turning eye is sometimes the right eye and other times the left eye then it is called an alternating strabismus (von Noorden and Campos 2002). A unilateral strabismus is usually associated with amblyopia in the habitually non-fixating eye.

Strabismus can also be described by its cause. For example paralytic strabismus is when action of one or a group of muscles is impaired, resulting in paralysis or paresis. This could be due to cranial nerve palsies or a lesion in the brain. Cranial nerves (III, IV, VI) responsible for eye movement can be weak or palsied and cause strabismus. Some examples of paralytic strabismus include third nerve palsy where all extraocular muscles supplied by the third cranial nerve are paralyzed, the paralysis is termed a complete oculomotor palsy; if one or more extraocular muscles are spared, the oculomotor palsy is partial. Other causes include retinopathy of prematurity (ROP) (Kushner 1982), uncorrected refractive error, anisometropia (Bremer, Palmer et al. 1998), congenital cataract (Spanou, Alexopoulos et al. 2011) and Congenital cranial disinnervation disorders (CCDD) (Oystreck, Engle et al. 2011).

CCDDs are primarily due to neurogenic disturbances of brainstem or cranial nerve development. The most common example of CCDDS is Duane retraction syndrome and congenital fibrosis of EOM (CFEOM) (Oystreck, Engle et al. 2011;

Andrews CV 2011). CFEOM is an either autosomal dominant or autosomal recessive disorder resulting from mutation of PHOX2A and KIF21A depending upon its type (Engle 2007). However CCDDs are rare compared to other form of strabismus.

1.2 Monkey Models for Strabismus

For our research we chose strabismic monkeys as they are excellent models for the human visual and oculomotor system. Below I describe some of the methods used to develop monkey models for strabismus.

1.2.1 Surgically induced strabismus:

Strabismus can be induced by recession of one particular muscle and resection of its antagonist muscle. Kiorpes and colleagues induced esotropia in infant monkeys by recession of the lateral rectus and resection of the medial rectus (Kiorpes, Walton et al. 1996). Economides and colleagues induced exotropia in 4wk old monkeys by surgical tenotomy of both medial recti (Economides, Adams et al. 2007). An advantage of the surgical method is the ease of the procedure. The surgical technique can be performed in a sterile surgery suite in less than 1hr and the recovery for the animal has minimal if any complications. However the disadvantage is the cut muscles frequently reattach and the strabismus that is produced tends to quite variable. Another potential disadvantage of this method is it can create an incomitant strabismus with large angles of deviation and disruptions to ocular motility that might limit the scope of behavioral study (Kiorpes, Walton et al. 1996; Crawford and Harwerth 2004).

1.2.2 Alternate Monocular Occlusion:

During an alternate monocular occlusion (AMO) procedure, an occluding patch (opaque goggles or contact lens) is placed in front of one eye and the patch is alternated daily for a period of 4-6 months (Tusa, Mustari et al. 2002). Therefore, during AMO rearing, the monkey's binocular vision is severely disrupted during the first few months of life, the critical period during which eye alignment, stereovision, and binocular sensitivity normally develop in the brain (Boothe, Dobson et al. 1985)

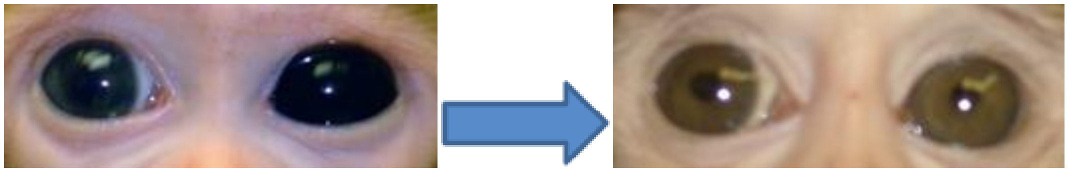

Figure 1.1: Alternate monocular occlusion paradigm: Picture on the left shows an infant monkey fitted with an opaque contact lens on left eye, which is alternated on a daily basis. Picture on the right shows another monkey with strabismus induced due to the AMO procedure.

1.2.3 Optically induced Strabismus:

In the optical prism viewing paradigm, infant monkeys view through a horizontal prism placed in front of one eye and vertical prism placed in front of the other eye. These horizontal and vertical Fresnel prisms are fitted in a lightweight helmet-like device which the animal wears for the first four months of life starting from 1-2 days after birth. This method induces concomitant strabismus at least in the horizontal gaze positions (Crawford and von Noorden 1980).

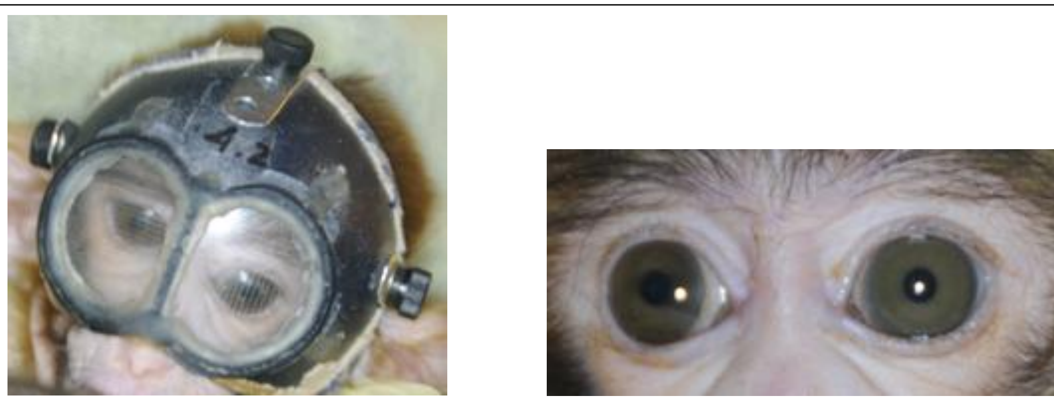

Figure 1.2: Optical prism viewing paradigm: The picture on the left shows an infant monkey fitted with helmet containing horizontal prism in front of right eye and vertical prism in front of left eye. The picture on the right shows the same monkey photographed approximately 6 months later with an exotropia.

1.2.4 Lid suture:

Monkeys reared with bilateral lid suturing with and without tarsal plates during the first 25-40 days of life have permanent strabismus, optokinetic nystagmus deficits during monocular viewing and latent nystagmus (Tusa, Mustari et al. 2002). However the oculomotor deficits (nystagmus) tend to be severe making these animals difficult to work with for behavioral studies.

1.2.5 Toxin injections:

Strabismus can also be induced by injection of *Botulinum A* neurotoxin into the extraocular muscles. For example, Kiorpes and colleagues reported esotropic. strabismus by botox injections into the lateral rectus muscle of the left eye and injection of antitoxin into the nasal, superior orbit. However a problem they encountered was that the monkey's initial esotropia resolved into an exotropia with time (Kiorpes, Walton et al. 1996).

1.3 Fixational eye movements (FEM):

Fixational eye movements (FEM) are involuntary eye movements produced during attempted visual fixation. Fixational eye movements are necessary to overcome vision loss due to uniform stimulation of retinal receptors (Martinez-Conde, Macknik et al. 2004). They play an important role in the correct discrimination of briefly presented low contrast stimuli (Rucci and Desbordes 2003). FEMs consist of microsaccades, drifts, tremors and the recently discovered slow oscillatory eye movements.

1.3.1 Microsaccades:

Microsaccades are involuntary, small, fast, jerky eye movements that occur during voluntary fixation. They are the largest and fastest of the fixational eye movements. The amplitude ranges from 1 arc minute to 2 degrees**.** The frequency is 3-4 per second and the duration is ~25 milliseconds. (Otero-Millan, Troncoso et al. 2008; Martinez-Conde, Macknik et al. 2009).

Microsaccades cannot be defined based on amplitude alone, as the amplitude of voluntary saccades can be as small as that of fixational microsaccades. One feature that differentiates microsaccades from regular saccades is that microsaccades are produced involuntarily while the subject is attempting to fixate (Martinez-Conde, Macknik et al. 2009). Microsaccades are generally binocular, conjugate movements with comparable amplitudes and directions in both eyes (Moller, Laursen et al. 2002). The relationship between microsaccade velocity and amplitude follows the main sequence, i.e., microsaccade peak velocity is linearly related to microsaccade amplitude (Otero-Millan, Troncoso et al. 2008; Martinez-Conde, Macknik et al. 2009; Di Stasi, McCamy et al. 2013; McCamy, Najafian Jazi et al. 2013).

The role of microsaccades is to correct the displacement of the eye produced by drifts during fixation. The likelihood of occurrence, direction and amplitude of microsaccades is related to previous displacement of image over the retina (Pritchard 1961). For example, if drifts carry the fixation target image to the right of the fovea, a leftward microsaccade will take place to bring it back towards the fovea.

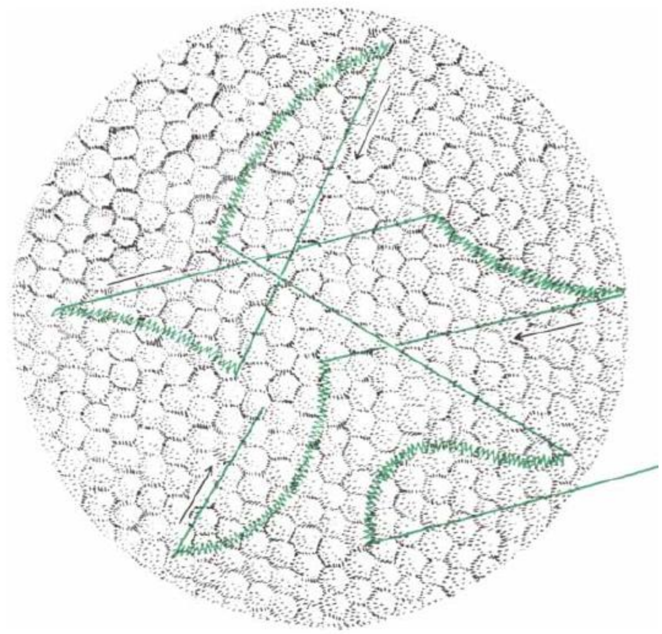

Figure 1.3: Fixational eye movements: The Diameter of the patch of the fovea shown here is only 0.5 mm. (Pritchard 1961)

Recent neurophysiological studies verify that microsaccades can generate neural responses in almost every visual area: the lateral geniculate nucleus, MT, the extrastriate area V2 and V4, the primary visual cortex (Martinez-Conde, Macknik et al. 2004). In the Lateral geniculate nucleus (LGN) and visual cortex (V1), microsaccades can move a stationary stimulus in and out of a neuron's receptive field, thereby producing transient neural responses (Martinez-Conde, Macknik et al. 2004). Hafed and associates reported that the superior colliculus plays an important role in microsaccade generation (Hafed, Goffart et al. 2009). Microsaccades might account for much of the response variability of neurons in visual area V1 of the awake monkey (Martinez-Conde, Macknik et al. 2004).

Microsaccades have received lot of interest in the past few years and have been investigated using behavioral and neurophysiological methods to understand their role in perception, attention and fixation. Various studies have assessed the dynamics of microsaccades including magnitude, rate, duration and peak velocity, and the main-sequence relationship during fixation tasks with a variety of targets.

McCamy and colleagues (McCamy, Najafian Jazi et al. 2013) have shown that the microsaccade rate and preference for horizontal direction decreased linearly and that microsaccade magnitude increased linearly with fixation target size. However these parameters did not show any significant change with luminance.

Otero-Milan and colleagues (Otero-Millan, Troncoso et al. 2008) have shown that microsaccade dynamics are equivalent across all fixation conditions, irrespective of the background image, e.g., blank or natural scene, picture puzzle etc. On the other hand, microsaccade dynamics vary considerably across free-viewing conditions, perhaps due to differences in task or the visual scene presented.

1.3.2 Drifts:

Drift refers to slow movement of the eye that occurs between microsaccades during attempted steady fixation (Ciuffreda, Kenyon et al. 1979; Martinez-Conde, Macknik et al. 2004). Drifts occur simultaneously with tremors and it can be conjugate or non-conjugate (Ditchburn and Ginsborg 1953). Initially, drifts were thought to be random motion of the eyes generated by instability within the oculomotor system. Later, these eye movements were found to have a compensatory role in the absence of microsaccades (St Cyr and Fender 1969). The direction of drifts depends upon the fixation target and can vary from subject to subject (St Cyr and Fender 1969). Amplitude ranges from 1 - 30 minutes of arc with the occurrence of 80-90% of fixation time. Duration ranges from 0.2 - 1 second (Martinez-Conde, Macknik et al. 2004).

1.3.3 Tremor:

Tremor refers to an aperiodic wave-like motion of the eye with high frequency (30-70 Hz) and small amplitudes (about the diameter of a cone in the fovea) (Ratliff and Riggs 1950). The amplitude ranges from 5 seconds of arc to 2 minutes of arc (Martinez-

Conde, Macknik et al. 2004). Tremor is superimposed on slow drifts and generally thought to be independent in the two eyes. It is the smallest of all eye movements and very difficult to record accurately as its amplitude and frequencies are usually in the range of recording system noise. To the best of my knowledge, no physiological studies have been conducted to analyze neuronal responses to tremor in the primate visual system.

1.3.4 Slow Oscillatory eye movement:

These fixational eye movements were recently discovered by Pansell and associates (Pansell, Zhang et al. 2011). The frequency of these oscillations ranged from 0.04-0.10 HZ and amplitude is less than 0.2°. These eye movements are conjugate in the vertical direction. The influence of these eye movements on visual function is not known.

1.4 Recording fixational eye movement

An ideal eye tracking system should measure a wide range of horizontal, vertical and torsional rotations of both eyes simultaneously at good spatial and temporal resolution. For measuring fixational eye movements, the requirement is to measure small eye movements with precision. Fixational eye movements can be recorded invasively by using the magnetic scleral search coil or non-invasively by using an eye tracking system that is based on the limbus, pupil, purkinje image reflections or retinal landmarks. This section describes fixational eye movement recording techniques and their advantages and disadvantages. Here, I briely describe the magnetic scleral search coil technique, dual purkinje image eye tracker, infrared video tracker, limbal eye tracker, microperimeter, and the scanning laser ophthalmoscope.

1.4.1 Magnetic scleral search coil:

This method is a highly accurate and widely used for measuring eye movements in humans and nonhuman primates (Bair and O'Keefe 1998; van der Geest and Frens 2002). This method is capable of measuring large and small eye movements. It has high spatial (< 1 arc minute) resolution and therefore is well suited to study fixational eye movements.

When a coil of wire moves in a magnetic field, a voltage is induced (Faraday's law)(Robinson 1963; Collewijn, van der Mark et al. 1975). This voltage is proportional to the sine of the angle between the plane of the coil and the magnetic field. When the coil moves in the magnetic field, the angle between the coil and field changes and hence induced voltage changes depending upon the amplitude of the movement. If the coil is attached to the eye, then a signal of eye position will be produced. In order to measure human eye movements, small coils of wire are embedded in a modified contact lens or silastic annulus which is inserted into the eye where it adheres to the sclera of the subject. In nonhuman primates these coils are implanted surgically beneath the conjunctiva. The subjects' (monkey or human) sit in the center of the field and perform ocular motor tasks. Horizontal and vertical fields produce two signals (one for vertical and the other horizontal eye movements). These signals are sorted out using appropriate phase detectors, enabling measurement of horizontal and vertical eye movements simultaneously. If the eye coil is of an appropriate design, then torsional movements can also be recorded.

The invasive nature of this method produces discomfort in human subjects even if the eye is anaesthetized and therefore the experimental time is limited generally to about 30-45 minutes. However this is not the case in non-human primates because the coils are implanted beneath the conjunctiva and experiments can be conducted for longer duration. This method is rarely used clinically, but is an extremely useful research tool, especially in animals (Bair and O'Keefe 1998).

1.4.2 Dual Purkinje image (DPI) eye tracker:

These eye trackers are based on the Purkinje image reflection from the front of the cornea and back of the lens which move by the same amount with eye translation but differentially when the eye rotates (Crane and Steele 1985). A DPI eye tracker can measure eye movements with an accuracy of 1 min of arc (Zhang, Stevenson et al. 2008). However the DPI tracker is not effective for measuring large eye movements due to a restricted field. DPI allows measurement of both monocular as well as binocular fixational eye movement in human and nonhuman primates (Snodderly and Kurtz 1985). Its effectiveness is limited in subjects with miotic pupils due to difficulty in identifying the fourth Purkinje image.

1.4.3 Video eye tracker:

There are various video based eye trackers, for example those designed by SMI and SR instruments, that have been used to record fixational eye movements (Crossland and Rubin 2002; Valsecchi and Turatto 2007) Most of these systems are head mounted and contain miniature cameras that record both eyes and head position depending upon the manufacturer. Eye positions are recorded using corneal or dark pupil reflection techniques (van der Geest and Frens 2002). Eye positions can be recorded either monocularly or binocularly. These systems sample the data at 250 or 500 Hz (Crossland and Rubin 2002; van der Geest and Frens 2002; Dimigen, Valsecchi et al. 2009) and have a spatial resolution of 0.01° RMS. Eye recordings are controlled using specific hardware and software provided by the manufacturer. Compensation for head motion can be made to calculate eye position. The software supplied with these eye tracker records a timestamp, x and y position of each eye, and pupil diameter.

1.4.4 Limbal eye tracker:

This instrument is rarely used to measure fixational eye movements. It measures only horizontal eye position accurately during fixation. It consists of photocells that detect the change in reflected light when the eye turns. It cannot measure torsional eye movement and vertical range is limited. Bandwidth of the recording system is 75Hz and resolution is approximately 12 min arc (Ciuffreda, Kenyon et al. 1979). Usually a chinrest and headrest are used to stabilize the head. A bite bar covered with dental impression material can also be used for accurate measurement of eye movement.

1.4.5 Microperimeter:

Nidek MP-1 (Nidek Technologies, Padova, Italy), is widely used for fixation assessment due to its ability to determine the retinal loci used for fixation. It allows fixation to be assessed during a microperimetric examination or as a separate assessment using an auto tracking system. It also measures fixation stability, retinal sensitivity, and can obtain color fundus images concurrently to study structure-function correlations. It comprises an infrared fundus camera and a liquid crystal display (LCD) that presents fixation stimuli to the observer. Options for fixation targets include a 'Single Cross', 'Circle' and 'Multiple Crosses' which consist of 4 'X' symbols laid out in a rhomboidal fashion. The size of the fixation target varies from 0.5 to 20.0 degree. These fixation targets are displayed at the center of the LCD screen, and subjects are asked to view the center of the fixation target. Standard fixation measurement includes the selection and tracking of reference image where first, a reference image of the fundus is captured, and a reference area where a high-contrast retinal features is selected. During the examination, inbuilt software (MP-1 SW 1.7) tracks this reference area, calculating any shift in its position between the reference image and subsequent frames within the image at a frequency of 25 Hz (Tarita-Nistor, Gonzalez et al. 2008; Dunbar, Crossland et al. 2010), producing x and y coordinates of retinal position in degrees of visual angle.

The MP-1 also reports the total time of a fixation trial and the tracked time; therefore, the amount of time during which tracking fails is known. Tracking reliability and robustness depend upon a good choice of the reference region, i.e., the higher the level of details present in the reference image, the more reliable and robust the tracking will be.

The MP-1 uses the Fixation Stability Score (described later) to quantify fixation stability (Chen, Patel et al. 2011). This particular metric is not use commonly due to certain limitations (described in section 1.5.2). However, raw data from the MP-1 can be exported into Microsoft Office Excel 2003 (Microsoft Corporation) to calculate the Bivariate Contour Ellipse Area (BCEA – described later). (Dunbar, Crossland et al. 2010; Chen, Patel et al. 2011). The MP-1 only allows monocular assessment of fixation stability.

1.4.6 Scanning Laser Ophthalmoscope:

Previous studies have shown that a scanning laser ophthalmoscope (SLO-101; Rodenstock GmbH) is particularly useful for the examination of fixation in subjects with eye disease (Crossland and Rubin 2002; Dunbar, Crossland et al. 2010). It comprises a helium-neon laser of wavelength 632.8 nm that produces the stimuli and an infrared laser of 780 nm that simultaneously images the fundus according to a confocal principle. Multiple images can be captured on a professional digital video recorder at a resolution of 768 X 576 pixels (model BR-DV600E; JVC, Yokohama, Japan) and a sampling frequency of 12.5 or 25 Hz (Crossland and Rubin 2002; Dunbar, Crossland et al. 2010). In-built SLO software (scotometry module, Rodenstock, Germany) produces a fixation target that is generally a red cross of different possible sizes. During measurement of fixation stability, subjects are asked to view the center of the cross until relatively blink free data trials are collected. The digital video recorder simultaneously records fundus images throughout the trial. These video images can be analyzed retrospectively using software that automatically tracks the fundus features and provides x and y axis positions of the eye in terms of pixels. These values are converted into degrees of visual angle using conversion factors obtained from calibration and finally BCEA is calculated.

1.5 Quantification of fixation stability

This section discusses different ways to quantify fixational eye movements. Among the most commonly used methods are the bivariate contour ellipse area, fixation stability score, isolines and microsaccades assessment.

1.5.1 Bivariate contour ellipse area (BCEA):

If the measured eye positions during fixation are assumed to have a bivariate normal distribution then the dispersion of these positions can be represented by an ellipse whose area is analogous to the standard deviation of a univariate distribution (Steinman 1965). This metric is called the bivariate contour ellipse area (BCEA). The area measured is expressed in $(\text{min arc})^2$ or $(\text{degree})^2$ of solid angle subtended at the eye by an ellipse projected on a plane surface parallel to Listings plane. BCEA can be calculated using the following equation:

$BCEA= 2k*pi*\sigma_x*\sigma_y* \sqrt{(1-p^2)}$,(Crossland, Sims et al. 2004)

where σ_x = Standard deviation of horizontal eye position,

σ_y = standard deviation of vertical eye position,

p is the pearson product moment correlation coefficient of horizontal and vertical eye position.

The value selected for ‘k’ sets up the confidence limit for the ellipse; the probability of fixation positions falling within the ellipse is given by $P = 1-e^{-k}$ where e is the base of natural logarithm. When k= 1, BCEA consists of 63.2% of fixation points.

This formula was modified to,

BCEA= x^2*pi* σ_x*σ_y* $\sqrt{(1-p^2)}$, (Timberlake, Sharma et al. 2005)

where 2k was replaced by x^2. x^2 represents the chi-squared variable with two degrees of freedom**.** Most commonly this x^2 is set to 2.291 which correspond to P value of 68%. However different authors have used different P values, such as 63.2% (White and Bedell 1990), 68% (Crossland, Sims et al. 2004), 95% (Amore, Fasciani et al. 2013) and 99% (Amore, Fasciani et al. 2013). A s*maller value of BCEA indicates more stable fixation whereas for unstable fixation, the BCEA will be larger.*

In order to determine the fixation stability in eyes with multiple preferred retinal loci during one fixation trial, the concept of local BCEAs was introduced by Crossland and colleagues (Crossland, Sims et al. 2004). The first step is to assess the number of local modes of the probability density function using kernel density estimation. In the second step, parameters of these local modes are estimated using an expectation-maximization algorithm that gives standard deviations along the x and y axes and correlation estimates. Finally these parameters are used to calculate local BCEA. In this case, fixation stability will be defined as the sum of the local BCEAs. A study by Crossland and colleagues (Crossland, Sims et al. 2004) showed that the sum of these local BCEAs was less than the global BCEA. For example in one subject with two preferred retinal loci, the global BCEA was 15900 minute arc^2 whereas the sum of the local BCEAs was 7950 (5260 + 2690) minute arc^2.

The BCEA measure has been used extensively in studies of patients with ocular disease such as AMD (Bellmann, Feely et al. 2004; Crossland, Sims et al. 2004). A study by Amore and associates (Amore, Fasciani et al. 2013) in subjects with macular diseases reported mean BCEA values with P values of 68%, 95% and 99% as 5.48$^{\circ 2}$ (68%), 14.8$^{\circ 2}$ (95%), and 26.4$^{\circ 2}$ (99%). BCEA values were smaller (more stable fixation) in subjects with retinitis pigmentosa with mean BCEAs of 2.01$^{\circ 2}$ (68%), 5.1$^{\circ 2}$ (95%), and 9.8$^{\circ 2}$ (99%). An example of another application of the BCEA metric is that it has been used as a measure to evaluate reading ability in normal and AMD subjects. (Crossland, Culham et al. 2004; Amore, Fasciani et al. 2013)

1.5.2 Fixation stability score:

Various studies of fixation stability using the Nidek MP-1 microperimeter instrument have also used a metric called the 'fixation stability score' to assess fixation. The Nidek MP-1 software calculates the proportion of fixation loci within 2 and 4° circles centered at the gravitational center of all fixation loci (so called centroid) and uses these proportions to classify the stability into three grades of stability. This grading system has been used to report fixation stability outcomes (Chen, Patel et al. 2011; Amore, Fasciani et al. 2013). The fixation was classified as (S1) stable if more than 75% of the fixation points fall inside the 2° diameter circle; (S2) relatively unstable if fewer than 75% of the fixation points were located within a 2°-circle, but more than 75% of the fixation points were located within a 4°-circle, and (S3) unstable if fewer than 75% of the fixation points are inside the 4° diameter circle.

In addition this system also classifies the 'location of fixation' which is defined as (L1) predominantly central when more than 50% of the preferred fixation points were located within a 2°-diameter circle centered on the fovea. Eyes with fewer than 50% but more than 25% of the preferred fixation points located within the 2°-circle were classified as (L2) poor central fixation and eyes with fewer than 25% of the preferred fixation points located within the 2°-circle were classified as (L3) predominantly eccentric fixation.

This system is valid for non-normal distributions; however it does not provide the typical elliptical nature of fixation distribution. Second, this method cannot be used effectively in people with macular disease who exhibit multimodal fixation patterns. Finally, the selection of 2 and 4° is arbitrary and is therefore not used commonly.

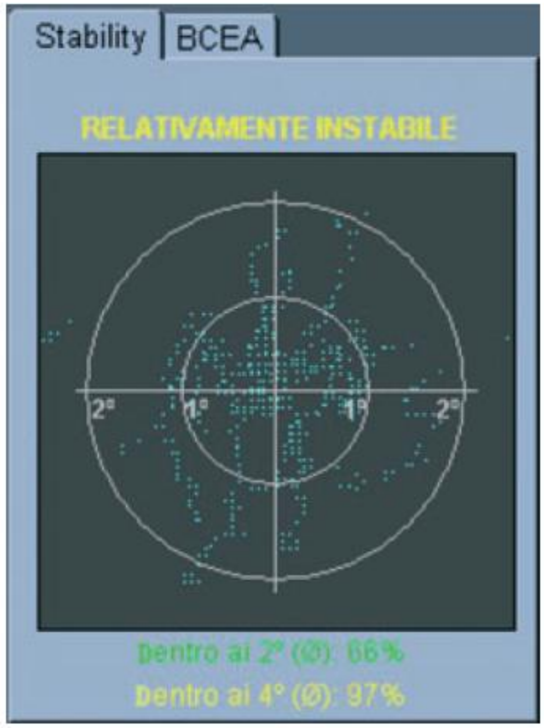

Figure 1.4: Example of a microperimeter display which considers fixation points within two and four degree circles for calculating fixation stability score (Amore, Fasciani et al. 2013).

A study in subjects with macular degeneration reported median fixation loci within a 2°-circle was 66.5 % whereas 90.5% of fixation loci were within 4°-circle (Chen, Patel et al. 2011). Another study of subjects with macular diseases reported relatively unstable fixation in subjects with age-related macular disease with a mean percentage of fixation value within 2° equal to 53% and within 4° equal to 85.2% on the other hand subjects with retinitis pigmentosa exhibited stable fixation with a mean percentage of fixation points falling within 2° equal to 85.5% and a mean percentage within 4° equal to 96.4% (Amore, Fasciani et al. 2013).

1.5.3 Contour plot (kernel density estimation):

This method is also applicable to assess fixation stability in eyes with multiple loci (Crossland, Sims et al. 2004). As mentioned earlier **(section 1.5.1)** the first step is to estimate the probability density function corresponding to the bivariate data set using kernel density estimation (KDE). In a second step, a contour plot is fitted to the data points. This contour plot consists of separate clusters of data points. Finally these clusters are added in such way that total area contains a particular proportion of data points, e.g., 68%. Each contour plot contains separate clusters whose total area comprises 68% of data points. In this case the fixation stability will be defined as sum of the local areas.

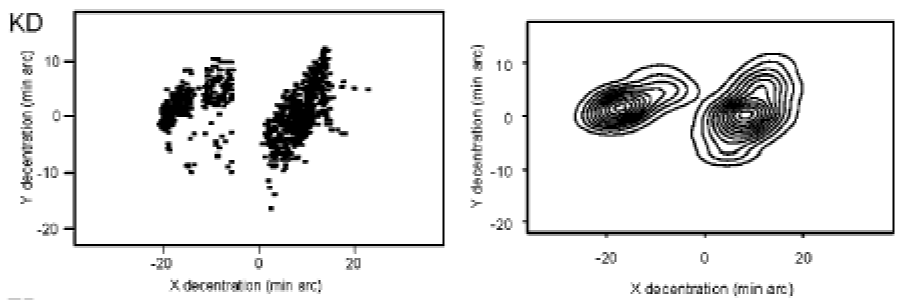

Figure 1.5: In the above figure the contour plot produced by KDE indicates two separate areas of fixation (Crossland, Sims et al. 2004)

An advantage of the contour plot is that it does not require a unimodal distribution of eye position. Also this method is not affected by distance between fixation positions or transient extreme shifts in eye position (outliers) (Crossland, Sims et al. 2004).

1.6 Factors affecting fixation stability

This section describes factors influencing fixational stability measures. Specifically, I focus on fixation target parameters such as shape, size, luminance, and eccentricity as these issues were investigated in my research project.

1.6.1 Fixation target parameters:

Manipulating the size, shape and luminance of the fixation target can alter both drifts and microsaccades during fixation and thereby fixation stability (Steinman 1965;

Thaler, Schutz et al. 2013). Previous research has shown that an increase in fixation target size can increase the fixation instability in both humans (Steinman 1965) and monkeys (Motter and Poggio 1984).

Thaler and colleagues (Thaler, Schutz et al. 2013) have shown that fixation target shape affects fixation stability in normal human subjects. Based on their initial survey of fixation target shapes, a set of seven targets that were circular, crosses and combinations of these two basic shapes were used for experiments where subject's primary task was to maintain fixation. They reported that a shape which looks like a combination of bull's eye and cross hair resulted in the lowest dispersion of eye positions and lowest microsaccade rate, i.e., best fixation stability. We use this same 'best' shape (combination of bullseye and cross hair) in our studies and the results are reported in Chapter 2.

Bellman and colleagues (Bellmann, Feely et al. 2004) also reported that fixation instability is significantly greater for pericentral fixation targets compared to targets that provide central fixation in normal subjects. Six different suprathreshold intensity target shapes that provide central and pericentral fixation were presented to normal and AMD subjects. The subject's task was to fixate the center of the target. They used a 1° cross, 1° solid circle, 1° letter 'X', small 4-point diamond, large 4-point diamond, and large-crossover whole-image diagonal with an open 1° center. In normal subjects, the lowest BCEA was found with a 1° cross and the highest with a large 4-point diamond whereas in AMD subjects the lowest BCEA was observed with a 1° letter 'X' and the highest with a small 4-point diamond. The difference between targets was significant for normal subjects but not in AMD subjects.

A study by McCamy and associates (McCamy, Najafian Jazi et al. 2013) assessed the effect of fixation target size and luminance on microsaccades. They presented circular target with nine different luminance levels and 6 different sizes and subjects were instructed to look at the center of the target. They found that microsaccade rate decreased linearly and microsaccade magnitudes increased linearly with target size. Target luminance did not affect microsaccade parameters significantly.

Steinman recorded fixational eye movements in two subjects as they maintained fixation at the center of concentric circles of varying sizes and luminance. Steinman observed that mean bivariate dispersion increased as a function of fixation target size and mean bivariate dispersion reduced slightly with an increase in the luminance of the fixation target (Steinman 1965).

Sansbury and colleagues (Sansbury, Skavenski et al. 1973) have shown that fixation instability increases with an increase in target eccentricity. In this experiment, the subjects' horizontal and vertical eye movements were recorded when they attempted to maintain their fixation at the center of 1.3° disk, at the center of two and four disk arrays separated by different distances (10°-29.5°) or in complete darkness. They found that fixation stability was better for the 1.3° disk and worsen as the separation between disks increased. However fixation stability with the most peripheral target was still better than with no target (complete darkness condition).

1.7 Fixation Stability in Strabismus and Amblyopia:

There have been relatively few studies that have examined properties of fixational eye movements in strabismus and amblyopia. Ciuffreda and associates (Ciuffreda, Kenyon et al. 1979; Ciuffreda, Kenyon et al. 1980) examined horizontal eye position during monocular and binocular fixation in subjects with and without strabismus and/or amblyopia. They reported abnormal fixational eye movements that included increased drift, saccadic intrusions, manifest nystagmus, and latent nystagmus. Drift was considered abnormal if the amplitude exceeded 12 min arc and/or the velocity exceeded 20 min arc/sec (Ciuffreda, Kenyon et al. 1979). The same study reported increased drift amplitude (up to 3-5 degrees) and velocity (up to 3 degrees per second) in amblyopic eyes during monocular fixation. However this was not present during binocular fixation or monocular fixation with the dominant eye. Increased drift was found 75% of the total fixation time in amblyopia without strabismus whereas it was found only 20% of the total fixation time in intermittent strabismus. From these data, they concluded that amblyopia rather than strabismus is the necessary condition producing an increase in drifts as people attempt to fixate (Ciuffreda, Kenyon et al. 1980). Errors created by abnormal drift were generally corrected by drift movements in the opposite direction rather than by microsaccades. In the dominant eye, nasal drift is corrected by temporally directed saccades. However, in the amblyopîa eye, slow temporal drift continued for several seconds without saccadic correction of the accumulated position error.

Gonzalez and colleagues (Gonzalez, Wong et al. 2012) assessed the microsaccade rate and amplitude in amblyopic subjects. In the amblyopic group, microsaccade rate did not show a significant difference between binocular and monocular viewing conditions (binocular, right eye monocular and left eye monocular). The viewing conditions did not affect microsaccade amplitude in either the normal or amblyopic groups and there was no significant difference found in microsaccade amplitude between normal and amblyopic group. However, they found higher BCEAs in the amblyopic eye during binocular and monocular viewing. Therefore the decrease in fixation stability in the amblyopia group can be attributed to slow eye drifts.

Subramanian (Subramanian, Jost et al. 2013) also studied the association between fixation instability and amblyopia in children and reported greater fixation instability (larger BCEA) with the amblyopic eye viewing as compared to non-amblyopic or normal eye viewing. The mean BCEAs for amblyopic eyes were 0.56 log deg^2, for fellow eyes it was 0.2 log deg^2, and for the right eyes of normal controls the BCEA was 0.12 log deg^2. The fixation instability was not associated with the type of amblyopia (strabismic, anisometropic, or combined mechanism); or its age of onset (infantile versus late onset). Also no significant difference in BCEA was found between children who underwent strabismus surgery compared to those who did not, in either the amblyopic group or non-amblyopic group. There was also no significant difference in the magnitude of fixation instability between children who had amblyopia treatment with a duration of less than 1 year, 13 months to 2 years, or >2 years. There was a statistically significant positive correlation between log stereo acuity and BCEA for amblyopic eyes. The authors concluded that the increase in fixation instability in amblyopic eyes of children with strabismus and/or anisometropia, and the associated poor stereoacuity probably is the consequence of decorrelated binocular experience early in life. A longer duration of decorrelated visual experience is associated with increased fixation instability, poorer stereoacuity, and more severe amblyopia therefore minimizing the duration of decorrelated visual experience may improve stereoacuity and decrease fixation instability.

In addition to amblyopia, poor visual acuity from other ocular abnormalities such as macular disease also is correlated with an increased BCEA (Tarita-Nistor, Gonzalez et al. 2008; Subramanian, Jost et al. 2013).

1.8 Research statement:

In the earlier sections, I discussed fixation stability in normal and abnormal visual conditions, its quantifications and measurement techniques and various fixation target parameters that influence fixational eye movements and fixation stability.

However it is unclear if these visual stimulus parameters exert a significant influence in disease conditions such as strabismus. Therefore a general goal of my research was to examine the effect of target parameters on fixational eye movements in both eyes of strabismic monkeys. I was also specifically interested in examining any binocular effects of target parameters, i.e., how target parameters change the stability of fixational eye movements in both the viewing and deviated eyes of the strabismic monkey.

In chapter 2, I reported detailed methods and results for following specific questions:

1) To assess whether manipulating fixation target shape, size and background affects fixation stability of the viewing and deviated eye in strabismic monkeys and compare these effects to effects observed in a normal monkey.

2) To assess whether fixation stability changes due to target parameters are proportional in the viewing and deviated eyes of the normal and strabismic monkeys

3) To assess fixation stability of the viewing and deviated eyes under monocular versus binocular viewing conditions in normal and strabismic monkeys.

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Chapter 2: Fixation Stability of Viewing and Deviated Eye in Monkeys with Strabismus

2.1 Introduction

Fixational eye movements are involuntary eye movements produced during attempted visual fixation. They comprise microsaccades, drifts, tremors and the recently discovered slow oscillatory eye movements (Martinez-Conde, Macknik et al. 2009; Pansell, Zhang et al. 2011). The primary role of fixational eye movements is perhaps to prevent visual fading that occurs as a neural adaptation to a stabilized image on the retina (Martinez-Conde, Macknik et al. 2006; Costela, McCamy et al. 2013). Rucci and Desbordes suggested that fixational eye movements are necessary for the correct discrimination of briefly presented low contrast stimuli (Rucci and Desbordes 2003). On the other hand, excessively large eye movements during fixation (unstable fixation or fixation instability) can lead to the fovea being drawn away from the object of regard. For example, unstable fixation interferes in tasks that require high acuity, such as reading (Crossland, Culham et al. 2004; Amore, Fasciani et al. 2013) or shooting clay pigeons (Di Russo, Pitzalis et al. 2003). Fixation stability in darkness is poor that fixation stability measured when viewing a target in the light, suggesting that both visual and oculomotor processes are playing a role in maintaining stable fixation (Sansbury, Skavenski et al. 1973).

Fixational instability is often a hallmark of visual disease. Thus unstable fixation has been reported in ocular abnormalities such as strabismus, amblyopia (Schor and Hallmark 1978; Ciuffreda, Kenyon et al. 1979; Gonzalez, Wong et al. 2012; Subramanian, Jost et al. 2013) and macular diseases (Crossland and Rubin 2002; Bellmann, Feely et al. 2004; Dunbar, Crossland et al. 2010). Increased drifts, saccadic intrusions, manifest and latent nystagmus in strabismus and amblyopia have also been reported (Schor and Hallmark 1978; Ciuffreda, Kenyon et al. 1979).

Parameters of the visual stimulus are known to influence fixation stability in normal humans and monkeys (Steinman 1965; Motter and Poggio 1984; McCamy, Najafian Jazi et al. 2013; Thaler, Schutz et al. 2013). For example, an increase in fixation target size can increase fixation instability in both humans (Steinman 1965) and monkeys (Motter and Poggio 1984). Bellmann and colleagues (Bellmann, Feely et al. 2004) and Thaler and colleagues (Thaler, Schutz et al. 2013) showed that the shape of the fixation target could affect fixation stability in normal human subjects. McCamy and colleagues (McCamy, Najafian Jazi et al. 2013) reported that target luminance does not affect fixational eye movement particularly, microsaccades. Steinman (Steinman 1965) also reported little effect of target luminance on fixation stability. Ukwade and Bedell (Ukwade and Bedell 1993) reported a small effect of blur and no effect of contrast on fixation stability in normal subjects.

The overall goal of this study was to consider whether factors that appear to influence fixation stability in normals (target parameters for example), would also exert similar influence in strabismics. One possibility is that the influence of fixation target parameters on fixation stability in strabismus is significant and perhaps similar to that observed in normals. Alternatively, in the strabismic subject, the ongoing drifts and nystagmus eye movements could mask any potential influence of fixation target parameters. Our studies were performed in strabismic monkeys as they are excellent models for the human visual and oculomotor system and strabismus in monkeys shows behavioral and neurophysiological properties that are very similar to human strabismus (Tychsen, Leibole et al. 1996; Das 2009; Joshi and Das 2011; Das 2012). Our first aim was to assess whether manipulating fixation target shape, size and background affects fixation stability of the viewing and deviated eye in strabismic monkeys and to compare these effects to the effects observed in a normal monkey. Secondly, we assessed whether changes in fixation stability due to target parameters are proportional in the viewing and deviated eyes of the normal and strabismic monkeys. Lastly, we compared fixation stability of the viewing and covered eyes under monocular and binocular viewing conditions in the normal and strabismic monkeys.

2.2 Methods:

2.2.1 Subjects and rearing paradigm:

Fixation stability data were collected from three strabismic (SM1, SM2, and SM3) and one normal (NM) rhesus monkey (*Macaca mulatta*). Monkeys were made strabismic using one of two sensory methods - Optical Prism viewing (S1) or daily Alternating Monocular Occlusion (SM2 and SM3) (Crawford and von Noorden 1980; Tusa, Mustari et al. 2002; Watanabe, Bi et al. 2005; Das 2009). In the optical prism viewing procedure, infant monkeys viewed through a horizontal 20D prism placed in front of one eye and vertical 20D prism placed in front of the other eye. These horizontal and vertical Fresnel prisms are fitted in a lightweight helmet-like device which the animal wears for the first four months of life starting from 1-2 days after birth. In the daily alternating monocular occlusion procedure, an occluding patch (opaque goggles or contact lens) is placed in front of one eye. The following day, the patch is switched to other eye and thereafter the patch is alternated daily for a period of four months. Both these rearing paradigms disrupt the monkey’s binocular vision during the critical period of visual and oculomotor development (binocular decorrelation in prism monkey and binocular deprivation in AMO). Disruption of binocular vision during this initial period leads to strabismus as it is the critical period for proper development of eye alignment, stereopsis and binocular sensitivity (Boothe, Dobson et al. 1985).

2.2.2 Surgical procedures:

After special rearing, the animals were allowed to grow normally until they were approximately 4 years of age before starting behavioral experiments. Sterile surgical procedures performed under aseptic conditions using isoflurane anesthesia (1.25%–2.5%) were used to stereotaxically implant a head stabilization post (Adams, Economides et al. 2007). In a second surgery, a scleral search coil was implanted in one eye in a procedure developed by Judge and colleagues (Judge, Richmond et al. 1980). Later in a third surgery, a second scleral search coil was implanted in the fellow eye. All procedures were performed in strict compliance with NIH guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at University of Houston.

2.2.3 Construction and presentation of experimental stimuli:

Fixation targets that allow stable central fixation and that are commonly used for fixation research were constructed using Matlab and the Cambridge Research Systems (CRS) toolbox (Bellmann, Feely et al. 2004; Thaler, Schutz et al. 2013). Visual stimuli were generated with the ViSaGe visual stimulus generator operated under computer control (Cambridge Research Systems, Cambridge, UK). Four target shapes (central cross, solid circle, optotype – '%' and a combination of a bullseye and crosshair - Figure 2.1), each in three sizes (0.5°, 1.0°, 2.0°) were presented against two backgrounds (white target on black background or black target on white background). Both monocular and binocular viewing conditions were tested (OD, OS, OU). Further, each stimulus condition was repeated 5 times yielding a total of 360 presentations. During testing, each stimulus, selected in random order, was presented for 60 seconds in the center of a tangent screen at distance of 114 cm from the monkey. After each fixation trial, monkeys were presented with a saccade stimulus (4-5 saccade trials of 2 secs each) to keep them alert and to avoid adaptation to the fixation target position. All animals were behaviorally trained in standard oculomotor tasks for several years in the lab prior to their participation in this study.

-Insert Figure 2.1 near here-

2.2.4 Eye movement measurement, data acquisition and analysis

Binocular eye position was measured using the magnetic scleral search coil technique. This method provides high spatial (<0.01°) resolution and therefore is well suited to study fixational eye movements (Robinson 1963; Collewijn, van der Mark et al. 1975). Eye coil signals were calibrated before each experimental session by rewarding the monkey with a small amount of juice for looking at a 1° optotype (solid circle) target projected at different horizontal and vertical positions. Calibration of each eye was performed independently during monocular viewing. During monocular viewing, the fellow eye was occluded with LCD shutter goggles (Micron Technology, Boise, ID) that were under computer control. At the time of the study, SM3 only had a functional coil in the left eye and so only data from this eye could be analyzed.

Eye position data were processed with anti-aliasing filters (Krohn-Hite, Brockton, MA) at 400Hz prior to sampling at a frequency of 2.79 KHz with 12-bit precision (AlphaLab SnR, AlphaOmega Industries, Nazareth, Israel). Eye position data were further filtered with a finite impulse response software digital filter with a passband of 0-60 Hz prior to analysis of fixation. Epochs of fixation (a minimum 10s for each trial) were selected by visual inspection of data thereby excluding saccades (>2° for normal monkey and >4° for strabismic monkey), blinks and any sections of data that the monkey was not looking at the target. Fixation data were analyzed using custom MATLAB programs that estimated the Bivariate Contour Ellipse Area (BCEA). The BCEA is a metric that estimates the area of the region over which eye positions fall during attempted fixation. Therefore, a smaller value for BCEA is indicative of greater fixation stability. The BCEA encompassing 68.2% of fixation points was calculated using the following equation (Timberlake, Sharma et al. 2005)

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

σx = Standard deviation of horizontal eye position,

σy = standard deviation of vertical eye position,

2.291 is the X^2 value (2df) corresponding to a probability of 0.68,

p is the Pearson product moment correlation coefficient of horizontal and vertical eye positions.

2.2.5 Statistical data analysis:

Statistical analysis was performed in Minitab 16 statistical software. For each monkey, multifactorial ANOVA was used to test the main effects of target shape, size, background and viewing conditions on BCEA and horizontal and vertical standard deviation of both the viewing and deviated eyes. In addition to examination of main effects, interactions effects between the different target parameters were investigated. Post-hoc comparison of BCEA was done using Tukey HSD test (95% confidence interval) in all monkeys. Paired t-tests were performed to compare BCEA values of the viewing and non-viewing eye.

2.3 Results:

Properties of strabismus in SM1-3 are provided in Table 2.1. Also included in table 2.1 are the means and ranges of fixation times that were analyzed in each 60sec trial. Figures 2.2 – 2.5 showed eye movements in each monkey during different viewing conditions. Stable fixation is observed in both eyes of the normal monkey (Figure 2.2). Eye movement amplitude is less than 0.5 degree. During binocular viewing, conjugate microsaccades are observed. In the section of data that is shown, the microsaccade is in vertical direction with an amplitude less than a degree. Fixational eye movements are larger in the strabismic monkey than in the normal. Fixational eye movements are also larger in the deviated eye compared to the fixating eye. In SM1 (Figure 2.3), fixating eye stability is worst during left eye viewing condition. A predominant vertical nystagmus is (downbeat nystagmus) is also observed in this condition. Fixation stability in SM2 is influenced by both horizontal and vertical nystagmus (Figure 2.4). In SM3 also we noticed vertical downbeat nystagmus (Figure 2.5). Compared to the other strabismic monkeys, perhaps the horizontal fixation stability is better but worse than normal monkey.

- Insert Figures 2.2 – 2.5 near here -

-Insert Table 2.1 near here-

Figure 2.6 shows raw horizontal (top panels) and vertical (bottom panels) eye position data in the normal (Fig 2.6A, C) and strabismic monkey (SM2; Fig 2.6B, D) during a fixation task in which the animals viewed a 1° solid circle against a black background. In these trials, the right eye viewed the target (left eye covered; left eye also deviated in the case of the strabismic monkey). The exotropia in the strabismic monkey is evident as the left eye is shifted to the left (more negative) in Fig 2.6B. Also evident are the increased horizontal gaze instability in the strabismic monkey compared to the normal and the increased instability in the covered eye compared to the viewing eye in the strabismic monkey. Fig 2.7 shows the corresponding two-dimensional dispersion of eye positions during fixation. Also shown is the ellipse that contains 68.2% of the fixation points. The BCEA value is the area of the plotted ellipse. For the normal monkey, the viewing eye BCEA was 0.12 degree^2 (Fig 2.7A) and the non-viewing eye BCEA was 0.20 degree^2 (Fig 2.7B). For the strabismic monkey, the viewing eye BCEA was 1.48 degree^2 (Fig 2.7C) and the non-viewing eye BCEA was 3.87 degree^2 (Fig 2.7D). In normal monkey the corresponding horizontal and vertical standard deviations for the viewing eye were 0.12 and 0.13 degrees respectively (Figure 2.7 A) whereas in the covered eye, the horizontal standard deviation was 0.16 degrees and vertical standard deviation was 0.19 degrees (Figure 2.7 B). Similarly in strabismic monkey (SM2) the horizontal and vertical deviation for the viewing eye were 0.31 and 0.66 degree respectively (Figure 2.7 C)suggesting more dispersion in vertical meridian whereas in covered eye the horizontal standard deviation was 0.70 degree and vertical standard deviation was 0.78 degree (Figure 2.7 D).

A fundamental observation was that BCEA values were greater in the three strabismic monkeys compared to the normal and that BCEA values in the deviated eye of the strabismic monkeys were significantly greater than BCEA in the fixating eye (paired t-test $p < 0.001$ for both SM1 ($t = -29.08$) and SM2 ($t = -18.71$)). In the normal monkey, the BCEAs in the fixating and covered eyes were not significantly different (paired t-test; $p = 0.162$, ($t = -1.40$)). The BCEA (mean ± SD) across all conditions in normal monkey was 0.46 ± 0.46 in the viewing eye and 0.50 ± 0.46 in the non-viewing eye; in SM1, 0.96 ± 0.88 in the viewing eye and 2.62 ± 1.39 in the non-viewing eye; and in SM2, 1.86 ± 0.96 in the viewing eye and 3.22 ± 1.32 in the non-viewing eye. In SM3, the BCEA of viewing eye was 0.63 ± 0.39. The BCEA of non-viewing eye was not available since this monkey had only one eye coil. Table 2.2 shows BCEA (Mean ± SD) for viewing and non-viewing eye during monocular and binocular viewing conditions for each monkey. The fixation stability in SM1 and SM2 was poorer with left eye. This perhaps could be used as a marker for underlying amblyopia in the left eye in these animals.

-Insert Figure 2.6 and 2.7 near here-

-Insert Table 2.2 near here-

2.3.1 Influence of Target Parameters on Fixation Stability:

A principal goal of the study was to examine the influence of target parameters on gaze stability. Figures 2.8 - 2.10 shows the main effects of target shape, size and background on fixation stability of the viewing (Panel A in each figure) and covered (Panel B in each figure) eyes in each monkey.

A significant target shape effect was observed in viewing-eye BCEA in the NM, SM1 and SM2 but not SM3 (NM: $F(3, 288) = 6.18$, $p < 0.001$; SM1: $F(3, 288) = 2.89$, $p = 0.036$; SM2: $F(3, 288) = 6.92$, $p < 0.001$; SM3: $F(3,192) = 0.98$, $p = 0.4$) (Figure 2.8A). Post hoc testing revealed that solid circle resulted in higher BCEA when compared with 'X' in SM1, whereas with '%' in NM and SM2. A significant target-shape effect was also observed in the covered eye (Figure 2.8B) in NM, SM1 and SM2 (NM: $F(3,192) = 4.13$, $p = 0.007$, SM1: $F(3,288) = 4.57$, $p = 0.004$; SM2: $F(3,288) = 3.12$, $p = 0.026$). Post hoc testing indicated that solid circle produced higher BCEA in NM and SM1 as compared to '%' and 'X' respectively whereas combination stimulus produced higher BCEA in SM2 as compared to 'X'. Recall that covered eye BCEA was not available in SM3 due to a non-functional coil signal in the right eye.

The main effect of target size on viewing eye BCEA was significant in all monkeys (NM: $F(2,288) = 35.94$, $p < 0.001$; SM1: $F(2,288) = 7.26$, $p = 0.001$; SM2: $F(2,288) = 19.91$, $p < 0.001$; SM3: $F(2,192) = 6.38$, $p = 0.002$) with greatest instability resulting from the 2° stimulus (Figure 2.9A). Post hoc testing indicated that 2° target size produced higher BCEA as compared to both 0.5° and 1° in NM. In SM1 and SM2 significantly smaller BCEA was observed with 1° size. ANOVA followed by post-hoc testing for covered eye BCEA data showed that the 2° size stimulus resulted in significantly larger BCEA (Figure 2.9B) in NM and SM1 (NM: $F(2,192) = 15.33$, $p < 0.001$ and SM1: $F(2,288) = 6.13$, $p = 0.002$) but not SM2 ($F(2,288) = 1.28$, $p = 0.281$).

A significant background effect was observed in viewing eye BCEA of NM,SM1 and in non viewing eye BCEA of SM2. Post hoc test revealed that a black target on a white background produced significantly larger BCEA in the viewing eye in the NM ($F(1,288) = 7.07$, $p < 0.001$) and significantly smaller BCEA in SM1 ($F(1,288) = 18.62$, p

< 0.001) (Figure 2.10A) when compared to BCEA values measured when monkeys fixated a white target on a black background. In SM2, background did not produce significant changes in viewing-eye BCEA ($F(1,288) = 1.33$, $p = 0.250$); however the effect was significant for covered eye ($F(1,288) = 31.53$, $p < 0.001$). Non-significant background effects were observed in the covered eye in NM and SM1 (NM: $F(1,192) = 2.84$, $p = 0.093$; SM1: $F(1,288) = 1.32$, $p = 0.251$ (Figure 2.10B). In summary the background effects on the monkeys were rather idiosyncratic.

-Insert Figure 2.8 - 2.10 near here-

2.3.2 Statistical Examination of Interaction Effects between Target Parameters:

Our multi-factorial design also allowed us to examine interaction effects between the factors (shape, size, background and viewing conditions) and therefore determine if a particular combination of factors was influential in affecting fixation stability. No significant interaction effect was observed in the normal monkey; however certain interaction effects were statistically significant in each of the three strabismic monkeys. In the viewing eye, interaction between shape and size was significant in all strabismic monkeys (SM1: $F(6,288) = 6.59$, $p < 0.001$; SM2: $F(6,288) = 3.37$, $p = 0.003$ and SM3: $F(6,192) = 2.23$, $p = 0.042$). For example, combination of solid circle and 2° size produced higher BCEA values than combination of '%' and 2° size. There was also a significant interaction between size and background in SM1 and SM2 (SM1: $F(2,288) = 8.84$, $p < 0.001$; SM3: $F(2,192) = 6.75$, $p = 0.001$). Significant interaction was observed between background and viewing conditions in SM1 ($F(2,288) = 21.52$, $p < 0.001$) and between size and viewing conditions in SM2 ($F(4,288) = 3.78$, $p = 0.005$). In SM3, shape and background showed significant interaction, (SM3 $F(3,192) = 3.11$, $p = 0.028$).

In the covered eye, interaction between background and viewing condition was significant in SM1 and SM2 (SM1: $F(2,288) = 4.47$, $p = 0.012$ and SM2: $F(2,288) = 3.20$,

$p = 0.042$). Size and viewing condition interaction produced a significant result in SM2 ($F(4,288) = 4.16$, $p = 0.003$) whereas shape and size interaction produced a significant result in SM1 ($F(6,288) = 5.60$, $p < 0.001$). These interactions suggest that the effect of one target parameter on the BCEA is not the same at all levels of the other target parameters. Plots showing the interaction effects are shown in the Appendix in figures 4.1 - 4.7.

2.3.3 Fixation stability under monocular versus binocular conditions:

The main effect of viewing condition (monocular vs binocular viewing) on viewing eye BCEA was not significant in any monkey (NM: $F(1,238) = 2.90$, $p = 0.090$; SM1: $F(1,238) = 1.07$, $p = 0.302$; SM2: $F(1,238) = 0.76$, $p = 0.385$; SM3: $F(1,238) = 1.45$, $p = 0.229$). Also the main effect of viewing condition on covered eye BCEA was not significant in two monkeys, SM1, $F(1,238) = 0.02$, $p = 0.879$, SM2, $F(1,238) = 0.01$, $p = 0.934$. Mean ± standard deviations BCEA during monocular (right and left eye) and binocular viewing are included in table 2.2. For statistical testing, the eye that the animal used for fixation during binocular viewing was compared with the same eye BCEA during monocular viewing. For example, SM1 used right eye for fixation during binocular viewing and therefore BCEA was compared with monocular right eye viewing conditions.

2.3.4 Are changes in fixational stability due to target parameters proportional in the two eyes?

The next part of the analysis was to consider whether changes observed in the viewing and covered eyes due to target parameters were proportional. Proportional changes in both eyes would suggest that the influence of target parameters were via conjugate mechanisms. To perform this analysis, we first calculated the ratio of the Viewing eye (VE) and Non-viewing eye (NVE) BCEA value for each stimulus condition during monocular viewing and thereafter performed a multi-factorial ANOVA on the BCEA ratios to test for a significant influence of target parameters. An absence of a significant statistical effect would suggest that the particular factor produced proportional changes in the two eyes. In the NM, the VE/NVE BCEA ratio was close to 1 due to similar BCEA values in both eyes whereas it was less than 1 in SM1 and SM2 because NVE BCEAs were greater than VE BCEAs. BCEA ratio could not be calculated in SM3 because the NVE BCEA was not available.

ANOVA analysis on the BCEA ratios indicated that target shape was not a significant factor in any monkey (NM: $F(3,192) = 1.95$, p = 0.12; SM1: $F(3,192) = 0.48$, $p = 0.70$; SM2: $F(3,192) = 1.27$, $p = 0.28$) suggesting proportional changes in both eyes due to shape (Figure 2.11A). A main effect of size on BCEA ratios was not significant in NM and SM1 (NM: $F(2,192) = 0.94$, $p = 0.39$; SM1: $F(2,192) = 0.15$, $p = 0.86$) suggesting proportional changes in both eyes due to sizes (Figure 2.11B); however a significant effect was observed in SM2 (SM2: $F(2,192) = 8.32$, $p < 0.001$). The main effect of backgrounds on BCEA ratio was significant in NM, SM1, and SM2 (NM: $F(1,192) = 8.25$, $p = 0.005$; SM1: $F(1,192) = 17.46$, $p < 0.001$ and SM2: $F(1,192) = 11.05$, $p = 0.001$) (Figure 2.11C).

-Insert Figure 2.7 near here-

2.4 Discussion:

In this study, we evaluated fixation stability in normal and strabismic monkeys with the goal of examining whether target parameters and viewing conditions can influence the BCEA metric in strabismic monkeys. Our main finding is that stimulus factors that influence fixation stability in the normal animal are also effective in influencing fixation stability in strabismic monkeys. Moreover the changes due to these factors affected both the viewing and deviated eyes proportionally. Here we discuss our results with the goal of further understanding monocular and binocular influences on fixation stability in normals and strabismics.

Although we had only one normal monkey in our study, results from this animal agree with published data in the literature. For example, when this animal fixated a 0.5° target with his right eye, we found the mean right eye BCEA value was 0.24 degree^2 and the corresponding mean SDs of horizontal and vertical positions were 0.17 degrees and 0.19 degrees respectively. These values are similar to measurements estimated from figure 3 of a publication by Motter and colleagues where they examined fixation stability in monkeys (Motter and Poggio 1984). In another study, Skavenski and colleagues reported that viewing-eyes BCEAs in normal monkeys ranged from 150 to 1214 min arc^2 with horizontal standard deviations ranging from 4.2 arc minutes to 7.5 arc minutes and vertical standard deviations ranging from 4.2 arc minutes to 22.6 arc minutes. In this study, animals were trained to fixate on target of 2.5 arc minute and data were selected from 10 longest fixation records.

Overall fixation stability in strabismic monkeys was worse than in the normal monkey and fixation stability was worse in the deviated eye than in the fixating eye for the strabismic animals. These general findings are in accordance with the reports of Gonzalez and colleagues and Subramanian and colleagues on fixation stability in humans with strabismus and amblyopia (Gonzalez, Wong et al. 2012; Subramanian, Jost et al. 2013). Ciuffreda and associates (Ciuffreda, Kenyon et al. 1979; Ciuffreda, Kenyon et al. 1980) also reported abnormal fixational eye movements in strabismus and amblyopia that includes increased drift, saccadic intrusions, and latent nystagmus. We also observed all these abnormal eye movements in our strabismic monkeys which might account for the larger BCEAs values in the strabismic monkeys. Fixation stability in strabismic monkeys could also be degraded by factors such as reduced acuity and contrast sensitivity although in the present study only high-contrast targets were used. We were unable to measure visual acuity in our monkeys; however visual acuity and stereoacuity has been found to be correlated with BCEA in subjects with strabismus and anisometropic amblyopia (Subramanian, Jost et al. 2013) suggesting that our strabismic monkeys' visual acuity might be less than that of the normal animal.

2.4.1 Target Parameter Influences on VE BCEA in normal and strabismic monkeys

Previous studies on normal humans suggest that visual parameters such as contrast, luminance, blur and color exert little or no influence on fixation stability (Steinman 1965; Ukwade and Bedell 1993; McCamy, Najafian Jazi et al. 2013). However other studies have shown that target shape does indeed matter. Thus, Thaler and colleagues (Thaler, Schutz et al. 2013) reported, in normal subjects, that a target shaped as a 'solid circle' resulted in higher dispersion of eye movements and increased microsaccade rate as compared to a shape which looks like combination of bulls' eye and cross hair. These two shapes were replicated in our study and indeed the normal monkey showed higher BCEA values with the 'solid circle' shape when compared to other shapes. Bellman and colleagues (Bellmann, Feely et al. 2004) reported that fixation instability in normal subjects is significantly greater for peri-central fixation targets (large 4-point diamond) compared to targets that provide central stimulation (1° cross). We also observed smaller BCEA values with fixation targets that provided central stimulation such as the 'X' or '%' optotype. Finally, St. Cyr and Fender (St Cyr and Fender 1969) have reported that the spread of fixation points changes based on the target shape, which is in general agreement with our findings. Target size influences were also observed in the normal monkey data in our study. Thus a target size of 0.5 degree yielded greatest stability and a target size of 2 degree was least stable. The target size effect is in basic agreement with previous studies in normal humans and monkeys, all of which showed increased standard deviations of horizontal or vertical eye positions for larger targets (Steinman 1965; Sansbury, Skavenski et al. 1973; Brian J Murphy 1974; Motter and Poggio 1984; Zhang, Pansell et al. 2011; Thaler, Schutz et al. 2013). Sansbury and colleagues (Sansbury, Skavenski et al. 1973) and Zhang and associates (Zhang, Pansell et al. 2011) reported that fixation stability with the larger target was still better than no target (i.e., darkness condition). A study by McCamy and associates (McCamy, Najafian Jazi et al. 2013) found that microsaccade rate decreased linearly and microsaccade magnitude increased linearly with target size in normal human subjects and this could be the reason for increased BCEA value in normal subjects or monkeys.

We were surprised to find that the target shape and size influences were significant in the strabismic monkeys as well and that these effects were very similar to the effects in the normal. The strabismic animals showed the largest VE BCEA values for the disk target compared to the other targets (statistically significant in two of three animals). In strabismic monkeys, the smallest VE BCEA was observed with the 1.0 degree target and the BCEA increased for 0.5 and 2.0 degree targets. From our results it appears that abnormal drifts and nystagmus eye movements do not mask the influence of fixation target parameters. Note that, in the strabismic monkeys, the improvement in BCEA by the ‘best’ target shape or ‘best’ target size is quite small (see figures 4-7). In the present study we did find significant changes in the strabismic monkeys’ BCEA in spite of abnormal eye movements. This appears contrary to the finding of Bellman and colleagues (Bellmann, Feely et al. 2004) who did not find significant changes in BCEA due to different target shapes in AMD subjects.

2.4.2 Comparison of Target Influences in Viewing and Deviated Eyes

In the NM, we observed similar BCEA values in both eyes (VE/NVE BCEA ratio close to 1.0) whereas in SM1 and SM2 the NVE BCEAs were greater than the VE BCEA (VE/NVE BCEA ratio is less than 1). Interestingly, proportional changes in viewing and non-viewing eye BCEA were observed as a function of target shape and size in both normal and strabismic monkeys (in general BCEA ratios did not change significantly as a function of target parameters). This finding suggests that the mechanism that drives the target parameter influences is basically conjugate.

2.4.3 Influence of Binocular Viewing on Fixation Stability

Cyr and Fender (St Cyr and Fender 1969) reported that horizontal and vertical eye movement component patterns differ under binocular viewing conditions than under monocular fixation in normal subjects. However, in our study fixation stability of the viewing eye did not change significantly under monocular versus binocular viewing conditions in normal and strabismic monkeys. Similar results were observed for the non-viewing (deviated) eye in strabismic monkeys.

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Figure 2.1

Four shapes used as fixation target

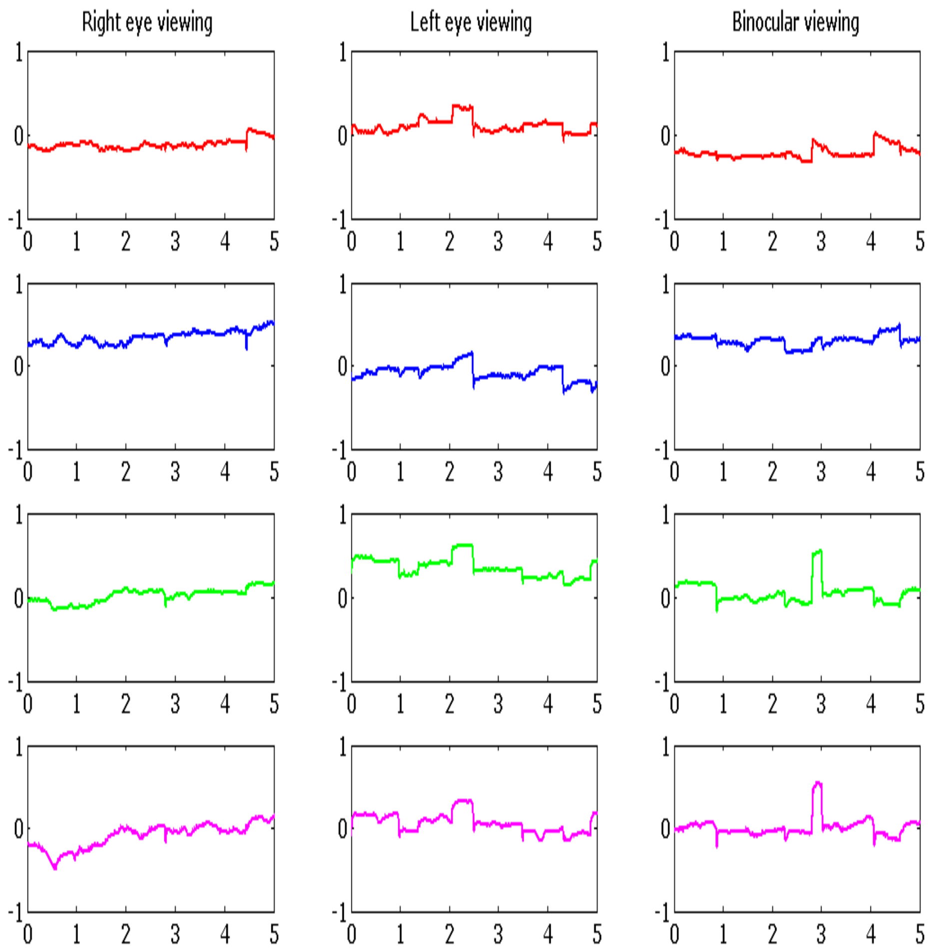


Figure 2.2: This plot indicates eye position traces during different viewing conditions in NM. The left panel shows right eye viewing, middle panel indicated left eye viewing and right panel indicates binocular viewing conditions. Red color shows right eye horizontal eye movement, Blue color shows left eye horizontal, Green color shows right eye vertical and Pink color indicates left eye vertical eye movements. Negative numbers indicate leftward or downward movement whereas positive numbers indicate rightward or upward movement. X axis indicated time in seconds and Y axis indicates amplitude in degrees.

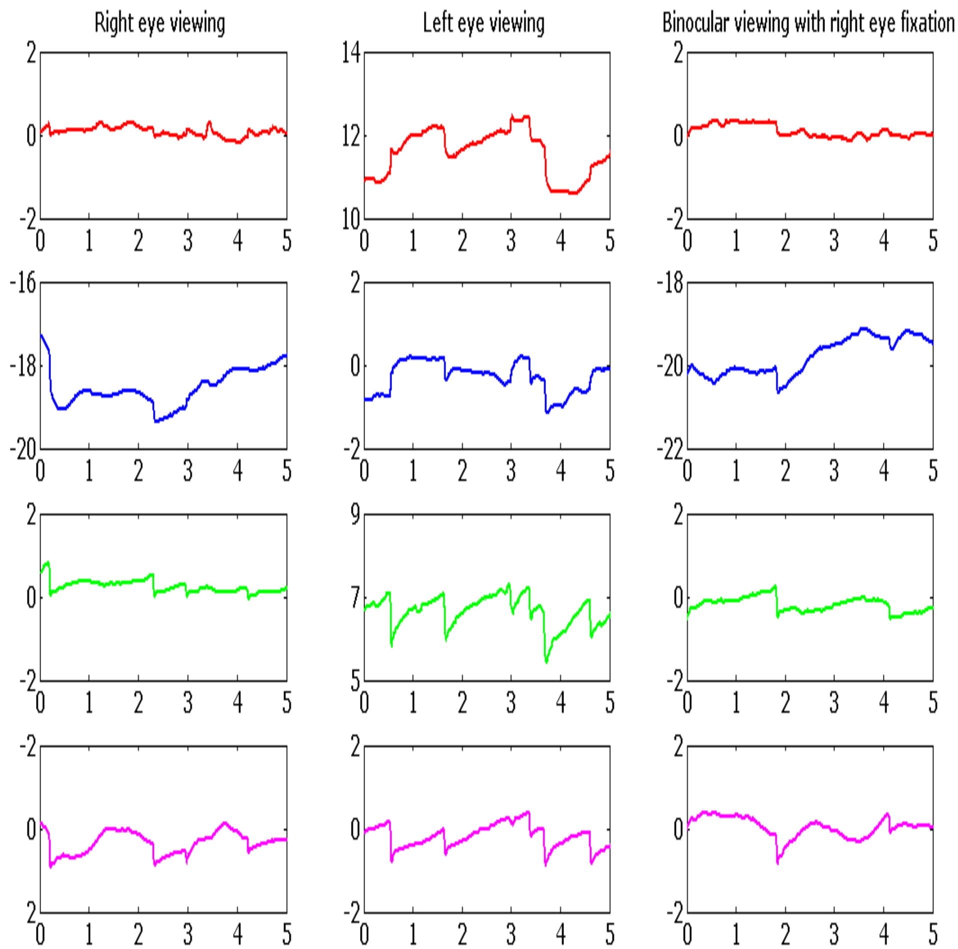


Figure 2.3: This plot indicates eye position traces during different viewing conditions in SM1. The axes, legends and conventions are same as above.

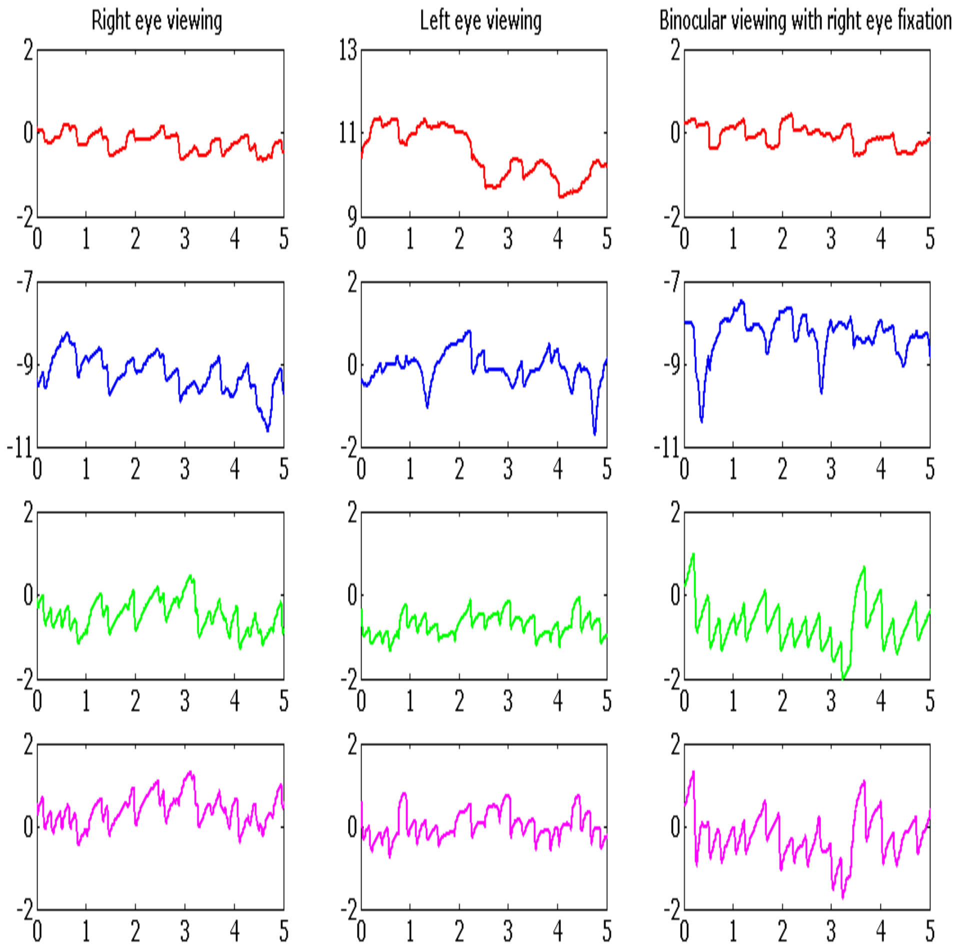


Figure 2.4: This plot indicates eye position traces during different viewing conditions in SM2. The axes, legends and conventions are same as above.

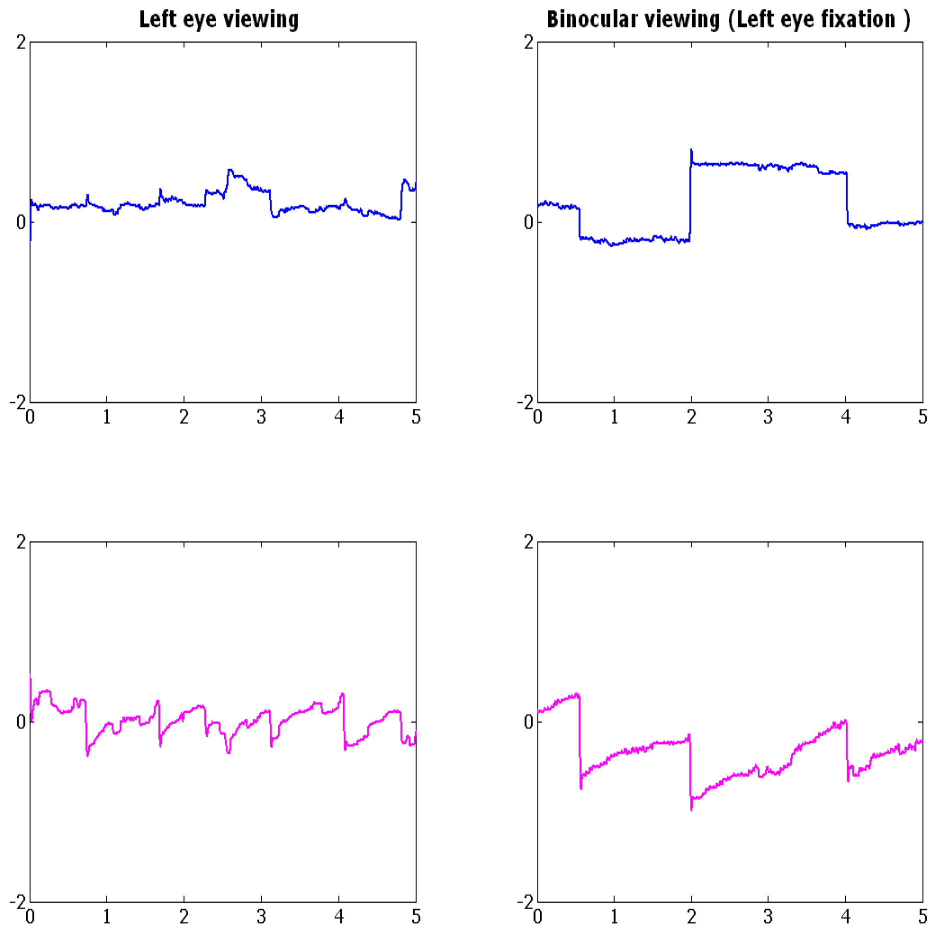


Figure 2.5: This plot indicates eye position traces during different viewing conditions in SM3. The axes, legends and conventions are same as above.

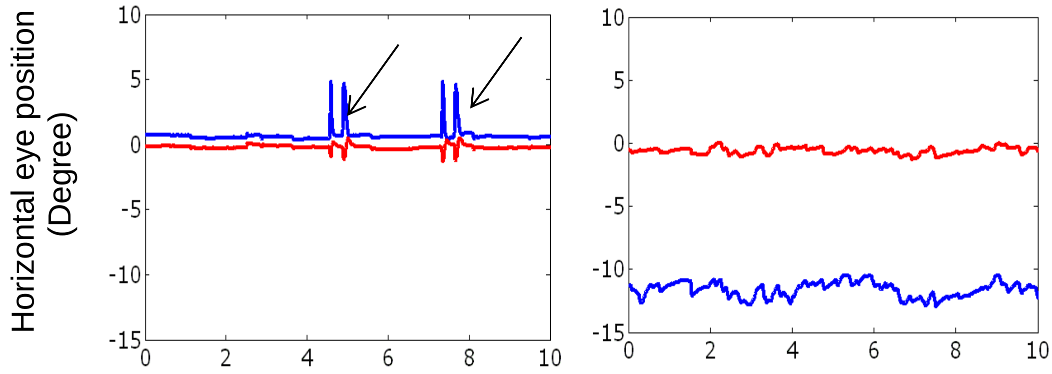


Figure 2.6A **Figure 2.6B**

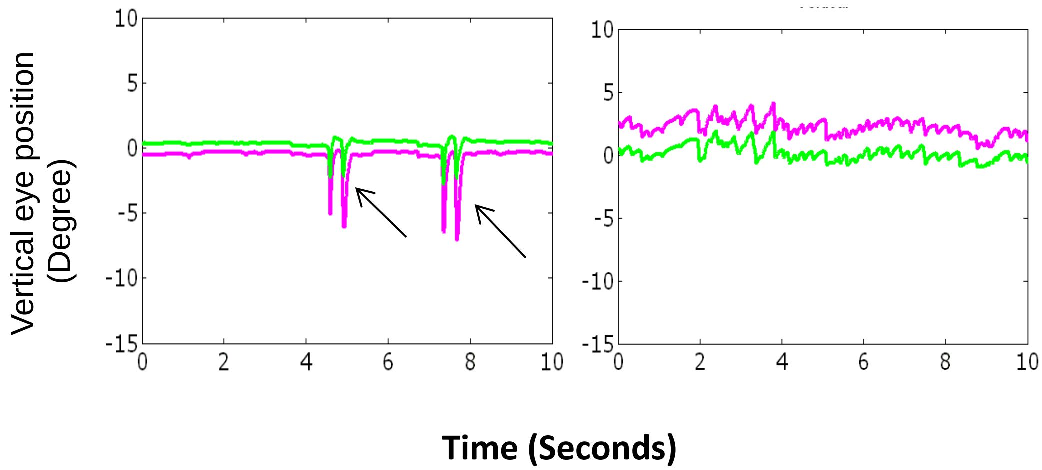


Figure 2.6C **Figure 2.6D**

Figure 2.6 shows raw horizontal (A,B) and vertical (C,D) eye position data during monocular right eye viewing of 1° solid circle shape on a black background in the normal (Panels A, C) and one of the strabismic monkeys (Panel B, D). Top panel shows horizontal eye movements and the bottom panel shows vertical eye movements. Legend: Red - Right eye horizontal; Blue - Left eye horizontal; Green Right eye vertical; Pink - Left eye vertical. Rightward and Upward eye positions and positive. Figure 2.2A shows some blinks (marked with arrows) during attempted fixation that were excluded from the data analysis.

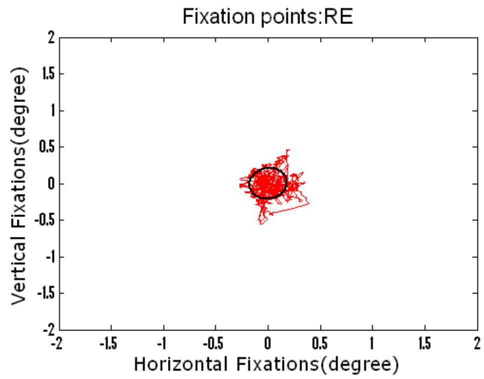


Figure 2.7A

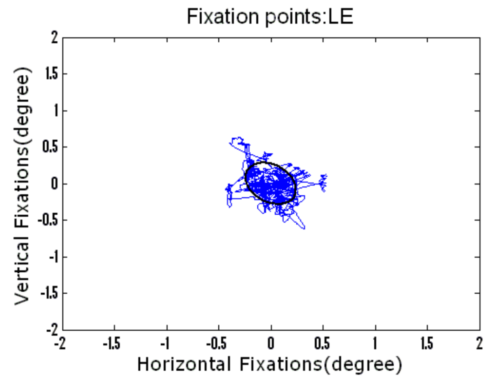


Figure 2.7B

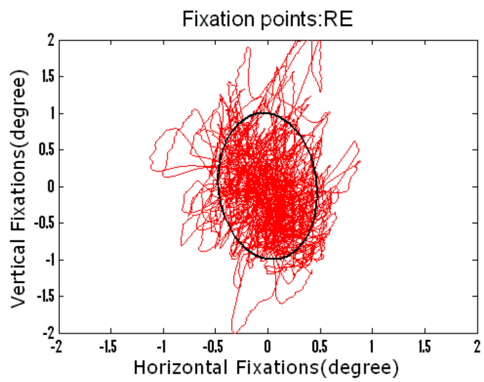


Figure 2.7C

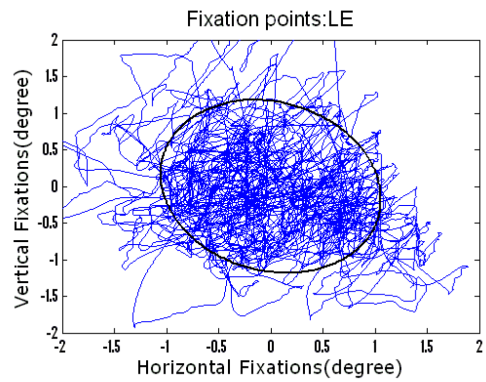


Figure 2.7D

Figure 2.7A-D shows 2- dimensional fixation points along with the fitted ellipse for the same stimulus as that shown in Fig 2.6. Panels A and B are from the normal monkey and Panels C and D are from the strabismic monkey. The left column (panels A, C) shows the viewing eye data and right column shows the non-viewing eye.

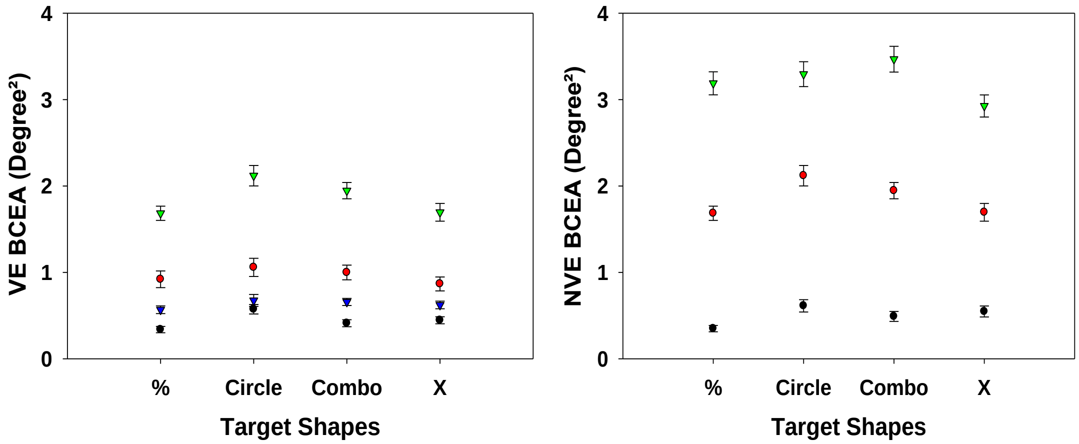


Figure 2.8A **Figure 2.8B**

Figure 2.8A and 2.8B shows the main effect of target shapes on viewing (Panel A) and non-viewing eye (Panel B) respectively. Legend: Black - normal monkey NM; Red - strabismic monkey SM1; Green - strabismic monkey SM2; Blue - strabismic monkey SM3; VE - viewing eye; NVE - non-viewing eye. Symbols and error bars indicate mean +/- 1 Standard error.

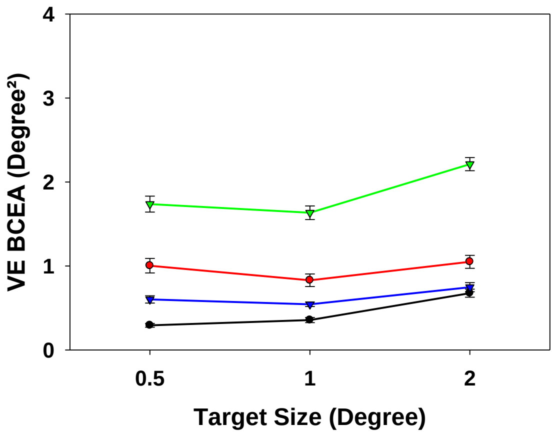


Figure 2.9A

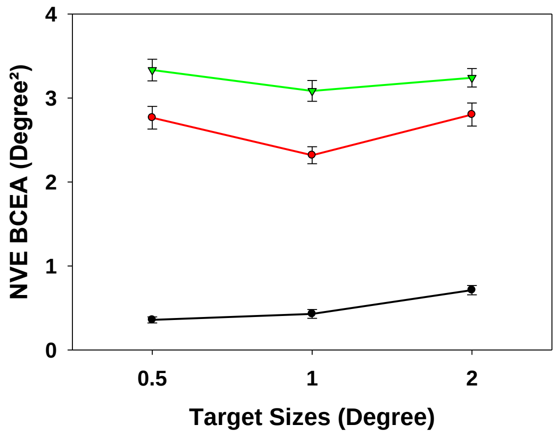
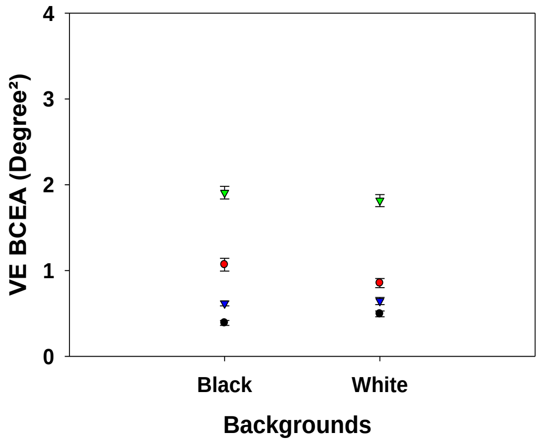


Figure 2.9B

Figure 2.9A and 2.9B shows the main effect of target size on viewing and non-viewing eye respectively. Legend same as in Figure 2.8.



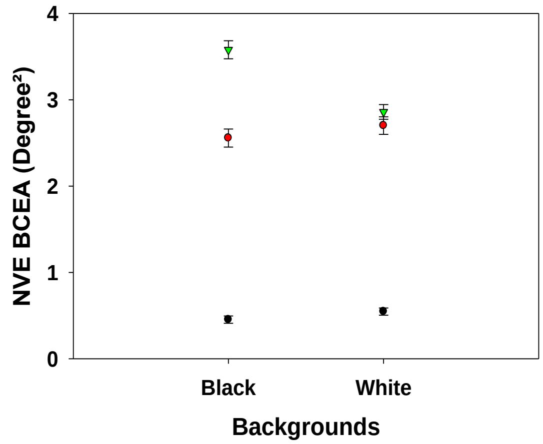


Figure 2.10A

Figure 2.10B

Figure 2.10A and 2.10B shows the main effect of backgrounds on viewing and non-viewing eye respectively. In NM, monocular viewing trials were used to calculate NVE BCEA. NVE BCEA was not calculated for SM3 because this monkey did not have a functional coil in the right eye.

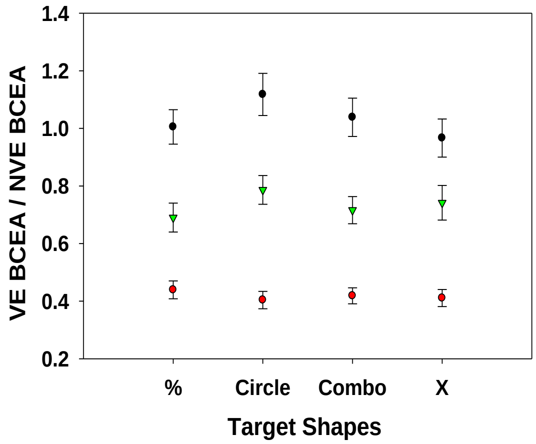


Figure 2.11A

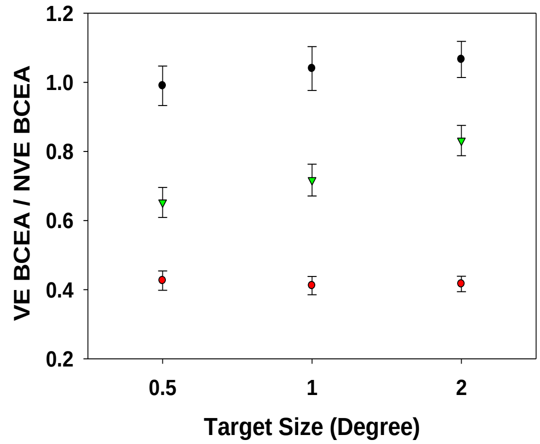


Figure 2.11B

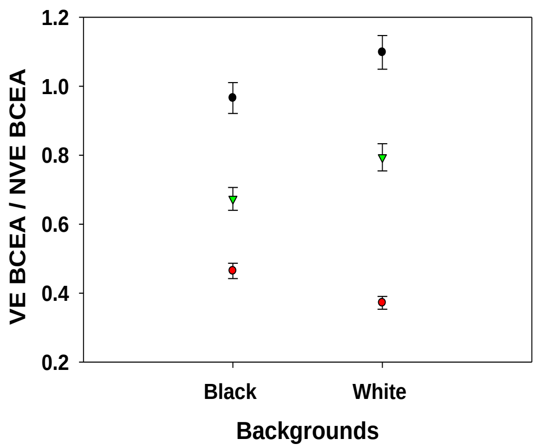


Figure 2.11C

Figure 2.7 shows the main effect of target shapes (A), sizes (B), and backgrounds (C) on BCEA ratio during monocular viewing conditions. Legend: Black - normal monkey NM; Red - strabismic monkey SM1; Green - strabismic monkey SM2; VE BCEA- viewing eye BCEA; NVE BCEA- non-viewing eye BCEA. Symbols and error bars indicate mean +/- 1 Standard error.

Monkeys	Age (Years)	Strabismus properties	Strabismic angle (°)	Fixation times(seconds) Range, Mean ± Std dev
NM	8	-	-	10.3 to 52.8, 31.4 ± 8.5
SM1	5	DVD, LN	OD: 20-25° Exo OS: 10° Exo, 7° Hyper	21.3 to 55.6, 44.7 ± 5.9
SM2	8	DVD, LN	OD: 10° Exo OS: 10° Exo, 2° Hyper	10.0 to 52.0, 32.2 ± 10.7
SM3	9	DVD, LN	OD: 15° Exo OS: 15° Exo	10.4 to 53.5, 38.1 ± 8.9

Table 2.1 describes age, strabismus properties, strabismus angle and fixation times for each monkey.

Monkeys	Viewing Conditions	VE BCEA (Mean ± SD)	NVE BCEA (Mean ± SD)
	Monocular RE	0.36 ± 0.27	0.60 ± 0.53
	Monocular LE	0.58 ± 0.58	0.40 ± 0.36
NM	Binocular (RE+LE)/2	0.39 ± 0.31	NA
	Monocular RE	0.52 ± 0.37	2.24 ± 1.44
	Monocular LE	1.89 ± 0.89	3.43 ± 1.26
SM1	Binocular (RE Fixation)	0.47 ± 0.30	2.21 ± 1.09
	Monocular RE	1.58 ± 0.77	3.46 ± 1.25
	Monocular LE	2.52 ± 1.02	2.75 ± 1.24
SM2	Binocular (RE Fixation)	1.49 ± 0.71	3.45 ± 1.36
	Monocular RE	NA	NA
	Monocular LE	0.67 ± 0.47	NA
SM3	Binocular (LE Fixation)	0.60 ± 0.38	NA

Table 2.2 shows mean ± standard deviation BCEA during monocular (right and left eye) and binocular viewing for normal and strabismus monkeys.

Chapter 3: General Discussion

In this chapter, I provide additional discussion about my research project focusing on topics that were not covered in chapter two. The discussion is organized around the following major topics – 1) Methodological Considerations 2) Additional discussion of results and 3) Future directions.

3.1. Methodological Considerations

3.1.1 Rearing paradigms:

There are different ways by which strabismus can be induced in monkeys. Surgical approaches include recession of one muscle and resection of its antagonist muscle. This can result in incomitant strabismus with large angle of deviation. Also the cut muscles frequently reattach and the strabismus that is produced tends to be quite variable. (Kiorpes, Walton et al. 1996; Crawford and Harwerth 2004; Economides, Adams et al. 2007). Lid suturing can induce strabismus in monkeys (Tusa, Mustari et al. 2002); however there is risk of the monkey becoming severely amblyopic due to form deprivation. Strabismus induced by *Botulinum A* neurotoxin injections can exhibit a change in the direction of deviation with respect to time and therefore is not considered a reliable method (Kiorpes, Walton et al. 1996). We used alternate monocular occlusion (Tusa, Mustari et al. 2002) and an optical prism viewing paradigm (Crawford and von Noorden 1980) which are well established sensory methods for the induction of strabismus (description of these methods is in Chapter 2).

3.1.2 Refractive error measurement

Refractive errors were measured in all monkeys using an auto-refractometer (Retinomax, Nikon) and were confirmed with retinoscopy by two independent optometrists. Measurements were performed in awake monkeys under 1% tropicamide as it is an effective cycloplegic agent and controls accommodation during refractive error measurement (Manny, Hussein et al. 2001). Although measurements from the auto refractometer and retinoscopy correlate well (Smith and Hung 1999), reports suggest that the auto refractometer gives more myopic values than retinoscopy values. Table 3.1 describes the refractive error determined by retinoscopy in all monkeys. In our study, refractive error was not corrected while acquiring fixation data. However Ukwade and Bedell (Ukwade and Bedell 1993) have reported that blur induces little effect on fixational eye movements.

Monkeys	Age (Years)	Refractive error (Diopters)
NM	8	RE: 0.75DSph
		LE: 1.25DSph
SM1	5	RE: PL/-0.25 X 135
		LE: -1.50DSph
SM2	8	RE: +2.75Dsph
		LE: +4.75/-0.50X140
SM3	9	RE: +8.00 DSph
		LE: +4.25 DSph

Table 3.1 Age and refractive error of monkeys measured with retinoscopy.

3.1.3 Instrument considerations:

We used the magnetic scleral search coil technique to acquire eye movement data. This method measures horizontal and vertical eye positions unlike limbal eyetrackers which reliably measures only the horizontal eye position during fixation (Ciuffreda, Kenyon et al. 1979). The search coil also does not limit the field of view and can measure eye position during binocular viewing in strabismic monkeys. Video-based eyetrackers (Eyelink 1000 for example) (Gonzalez, Wong et al. 2012) have a limited range within which they can record eye positions, which makes it difficult to record strabismic eye position. Houben and associates (Houben, Goumans et al.

2006) reported erroneous eye positions due to noise in one video eyetracker (Chronos) during fixation at an eccentric gaze position, when compared to the search coil in humans. van der Geest and Frens (van der Geest and Frens 2002) have also mentioned that another video eye tracker (EyeLink) is not a reliable method for eye movement measurement, particularly during fixation experiments, due to the higher noise level in the system.

3.2 Additional Discussion on Results

3.2.1 Horizontal versus vertical eye position in viewing and non-viewing eye:

Kosnik and colleagues (Kosnik, Fikre et al. 1986) reported that the horizontal standard deviation of eye fixations is significantly greater than the vertical standard deviation in older subjects with normal visual acuity, when subjects viewed a 6 min-arc white square target (Table 3.2). On the other hand, Skavenski et al (Skavenski, Robinson et al. 1975) found greater eye positions SDs in the vertical than horizontal meridian in 3 out of 4 monkeys (Table 3.3).

	BA (Minarc^2)	*H (Minarc)*	*V (Minarc)*
Old	198	8.28	3.96
	(90.4)	(3.48)	(0.84)
Young	165	6.24	4.80
	(90.2)	(1.86)	(2.58)

Table 3.2 shows mean bivariate contour ellipse areas and mean standard deviations along horizontal and vertical meridian. (Printed from: Kosnik, Fikre et al. 1986*)*

Subject:	Henry	Buddah	Alvin	Albert
Fixation Interval (sec)	11.5	17.0	15.0	17.0
S D H (min arc)	5.4	4.6	4.2	7.5
S D V (min arc)	4.2	6.4	5.8	22.6
BCEA (68%)	150	213	171	1214
Intersaccadic Interval (sec)	0.8	1.2	7.4	2.0
Saccade Amplitude (min arc)	9.9	16.4	18.6	40.3

Table 3.3 represents 68%BCEA, SDs of horizontal and vertical eye position.

(Printed from: Skavenski, Robinson et al. 1975)

In our study, the SDs of horizontal and vertical eye positions in the normal monkey are similar to previously published data (Table 3.4). Further, a significant difference in the mean SDs for horizontal and vertical eye positions of viewing eye was observed in NM, SM1, and SM2 (paired t test, $p< 0.05$). In NM and SM2 the variability was higher in vertical meridian whereas in SM1 it was higher in horizontal meridian. Also in SM1 and SM2, the SDs of non-viewing eye horizontal eye positions were significantly greater than viewing eye horizontal eye positions. A similar effect was observed with the SDs in the vertical meridian (Table 3.4). Note that the SDs in the strabismic monkeys are likely to reflects the direction of any underlying nystagmus. For example, monkey SM2 had a vertical nystagmus while viewing with his right eye and the mean SD in the vertical direction (mean SD: 0.58 degree) is correspondingly higher than in the horizontal direction (mean SD: 0.37 degree).

Monkey	Viewing eye		Non-viewing eye	
	H	V	H	V
NM	0.24 ± 0.11	0.26 ± 0.15	0.26 ± 0.11	0.26 ± 0.15
SM1	0.39 ± 0.20	0.31 ± 0.15	1.14 ± 0.41	0.37 ± 0.16
SM2	0.47 ± 0.20	0.55 ± 0.14	0.82 ± 0.28	0.58 ± 0.14
SM3	0.31 ± 0.12	0.31 ± 0.10	NA	NA

Table 3.4 shows mean ± standard deviation for standard deviation of eye position (in deg) along the horizontal and vertical meridian for the viewing and non-viewing eye across three (Right eye, left eye, binocular) viewing conditions in normal and strabismic monkeys. For NM only monocular viewing trials are used. Recall SM3 has a functional coil signal from only one eye.

3.2.2 Influence of Target Parameters on horizontal and vertical standard deviations:

Figures 3.1 and 3.2 shows horizontal and vertical standard deviation as a function of target shape and size respectively. A general observation was that standard deviation of horizontal and vertical eye movements in strabismic monkeys were greater than the normal monkey. Another observation was that the standard deviation of horizontal eye movements in the non-viewing eye was greater than in the viewing eye. However vertical standard deviations were similar in both viewing and non-viewing eyes.

ANOVA followed by post-hoc testing showed that the solid circle' resulted in significantly higher viewing-eye horizontal standard deviation (HSD) values than 'X' and '%' shape in the NM and SM1 but not in SM2 and SM3 (NM: $F(3, 288) = 11.91$, $p < 0.001$; SM1: $F(3, 288) = 6.71$, $p < 0.001$; SM2: $F(3, 288) = 2.11$, $p = 0.09$; SM3: $F(3,192) = 1.16$, $p = 0.32$) (Figure 3.1A). A significant target-shape effect was also observed in the covered eye in all monkeys (NM: $F(3,192) = 5.37$, $p = 0.001$, SM1: $F(3,288) = 6.94$, $p < 0.001$, SM2: $F(3,288) = 4.44$, $p = 0.005$) (Figure 3.1B). Post hoc revealed that solid circle produced significantly higher deviations than '%' in NM and other three shapes in SM1 whereas combination stimulus produced higher deviation as compared to 'solid circle in SM2.

The target shapes did not significantly affect viewing-eye vertical standard deviation (VSD) in NM, SM1 and SM3 (NM: $F(3, 288) = 2.29$, $p = 0.078$; SM1: $F(3, 288) = 0.54$, $p = 0.65$; SM3: $F(3,192) = 0.47$, $p = 0.7$); however in SM2, the solid circle resulted in significantly higher vertical standard deviation a compared to other three shapes (SM2: $F(3,192) = 9.95$, $p < 0.001$; post-hoc test) (Figure 3.1C). In the covered eye, a significant target shape effect was observed in NM and SM2 (NM: $F(3, 192) = 2.58$, $p = 0.05$; SM2: $F(3, 288) = 8.81$, $p < 0.001$) but not in SM1 (SM1: $F(3,288) = 0.21$, $p = 0.89$) (Figure 3.1D). Post hoc indicated that solid circle produced higher vertical deviation than '%' in NM and other three shapes in SM1. Our findings suggest that target shapes affect horizontal deviations more than vertical deviations.

The main effect of target size on viewing-eye HSD was significant in all monkeys (NM: $F(2,288) = 47.14$, $p < 0.001$; SM1: $F(2,288) = 15.19$, $p < 0.001$; SM2: $F(2,288) = 28.12$, $p < 0.001$; SM3: $F(2,192) = 7.19$, $p = 0.001$) with greatest deviation resulting from the 2° stimulus as compared to 0.5° and 1° (post-hoc testing; Figure 3.2A). The 2° size stimulus resulted in significantly larger horizontal standard deviation in the covered eye in the NM (NM: $F(2,192) = 21.87$, $p < 0.001$; post-hoc test) but not in SM1 and SM2 (SM1: $F(2,288) = 1.62$, $p = 0.2$; SM2: $F(2,288) = 1.33$, $p = 0.32$) (Figure 3.2B). Similar target size effect was observed on viewing-eye VSD in all monkeys except SM3 (NM: $F(2,288) = 18.87$, $p < 0.001$; SM1: $F(2,288) = 7.75$, $p = 0.001$; SM2: $F(2,288) = 3.92$, $p < 0.021$; SM3: $F(2,192) = 1.06$, $p = 0.35$) (Figure 3.2C). Post hoc indicated that 2° size produced significantly larger deviations than both 0.5° and 1° in NM and 1° in SM1 and SM2. Similar Post hoc results were observed for covered eye. In the covered eye target shape produced significant effect on VSD in NM, SM1 and SM2 (NM: $F(2,192) = 9.28$, p

< 0.001; SM1: $F(2,288) = 9.28$, $p < 0.001$; SM2: $F(2,288) = 4.11$, $p = 0.017$) (Figure 3.2D). Our findings suggest that target size affects fixational eye movements in both meridians.

Background produced significant effect on viewing-eye HSD in all monkeys. (NM: $F(2,288) = 19.91$, $p < 0.001$; SM1: $F(2,288) = 8.44$, $p = 0.004$; SM2: $F(2,288) = 5.61$, $p = 0.019$; SM3: $F(2,192) = 11.57$, $p = 0.001$). Similar effect was observed in covered eye (NM: $F(2,192) = 7.13$, $p = 0.008$; SM1: $F(2,288) = 30.88$, $p < 0.001$; SM2: $F(2,288) = 10.51$, $p = 0.001$). The main effect of Background on viewing-eye VSD was observed in all monkeys except NM (NM: $F(2,288) = 3.61$, $p = 0.059$; SM1: $F(2,288) = 59.82$, $p < 0.001$; SM2: $F(2,288) = 29.75$, $p < 0.001$; SM3: $F(2,192) = 20.0$, $p < 0.001$). Similar effect was observed in covered eye except in NM (NM: $F(2,192) = 1.54$, $p = 0.216$; SM1: $F(2,288) = 27.37$, $p < 0.001$; SM2: $F(2,288) = 44.58$, $p < 0.001$). Post hoc analysis indicated that in white background produced significantly higher viewing-eye horizontal standard deviation in NM, SM1and SM2 and lower in SM3 where as black background produced significantly higher viewing-eye vertical standard deviation in SM1 and SM2 but lower in SM3. In the covered eye white background produced higher horizontal standard deviation in NM and SM1 and lower in SM2 whereas black background produced higher vertical standard deviation in SM1 and lower in SM2. Therefore the idiosyncratic background effects observed in the BCEA analysis was replicated in the analysis of HSD and VSD.

3.2.3 Statistical Examination of Interaction Effects between Target Parameters:

We also examined the interaction effects on HSD and VSD. Shape-size interaction was observed on viewing-eye HSD in NM, SM1 (NM: $F(6,288) = 3.51$, $p = 0.002$; SM1: $F(6,288) = 7.87$, $p < 0.001$ and vertical standard deviation in SM1, SM2 and SM3 (SM1: $F(6,288) = 2.40$, $p = 0.028$; SM2: $F(6,288) = 3.12$, $p = 0.006$; SM3: $F(6,192)$

= 3.17, $p = 0.006$). Shape-size interaction on horizontal deviation was also observed in covered eye of NM, SM1 (NM: $F(6,192) = 2.89$, $p = 0.01$; SM1: $F(6,288) = 5.82$, $p < 0.001$) and on vertical deviation in SM1, SM2 (SM1: $F(6,288) = 2.87$, $p = 0.01$; SM2: $F(2,288) = 3.12$, $p <= 0.006$). Size-background interaction were observed in viewing-eye vertical standard deviation in all three strabismic monkeys (SM1: $F(2,288) = 8.85$, $p < 0.001$; SM2: $F(2,288) = 4.41$, $p < 0.013$; SM3: $F(2,192) = 10.14$, $p < 0.001$) and in covered-eye vertical deviation in SM1 and SM2 (SM1: $F(2,288) = 12.67$, $p < 0.001$; SM2: $F(2,288) = 4.98$, $p = 0.007$. Also there was size-viewing condition effect on viewing-eye vertical standard deviation in all three strabismic monkey SM1: $F(4,288) = 5.41$, $p < 0.001$; SM2: $F(4,288) = 2.57$, $p = 0.038$; SM3: $F(2,192) = 5.64$, $p = 0.004$).

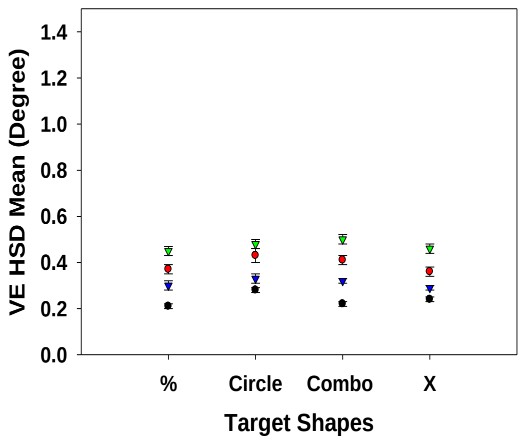


Figure 3.1A.

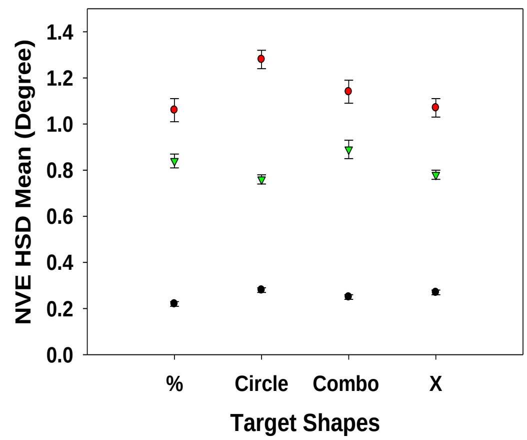


Figure 3.1B.

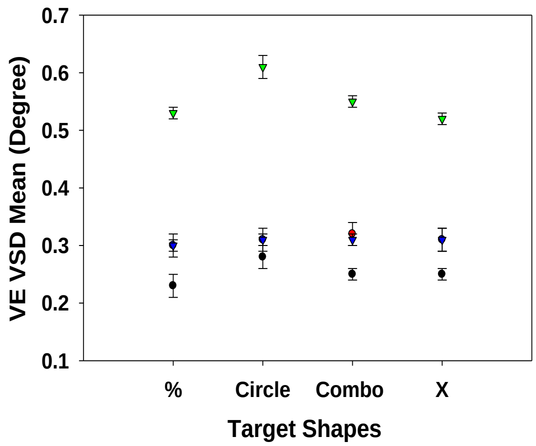


Figure 3.1C.

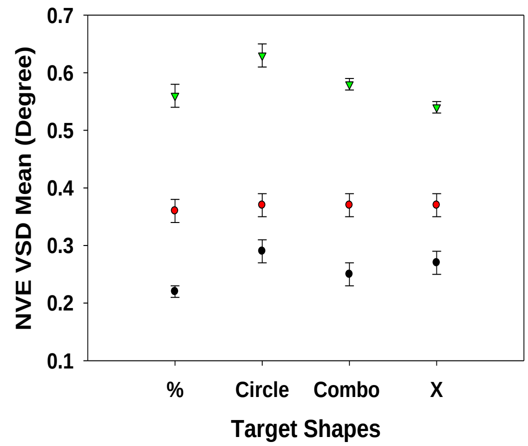


Figure 3.1D.

Figure 3.1 A-D shows the main effect of target shapes on horizontal and vertical standard deviation of viewing (A, C) and non-viewing eye (B, D) respectively. Legend: Black - normal monkey NM; Red - strabismic monkey SM1; Green - strabismic monkey SM2; Blue - strabismic monkey SM3; VE - viewing eye; NVE - non-viewing eye. Symbols and error bars indicate mean +/- 1 Standard error.

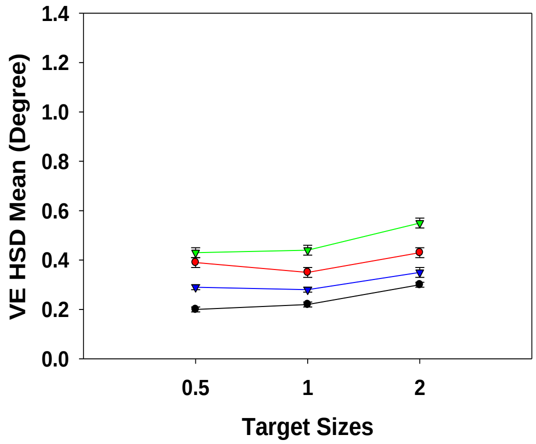


Figure 3.2A.

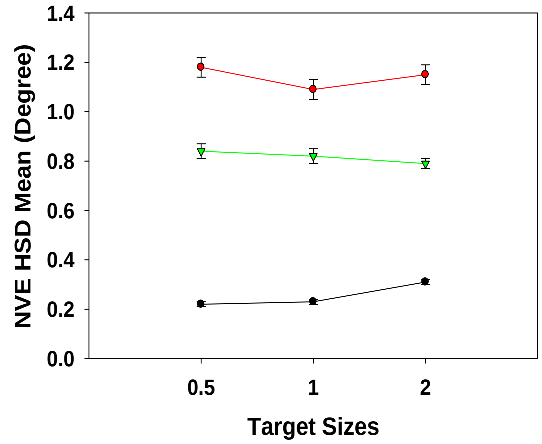


Figure 3.2B.

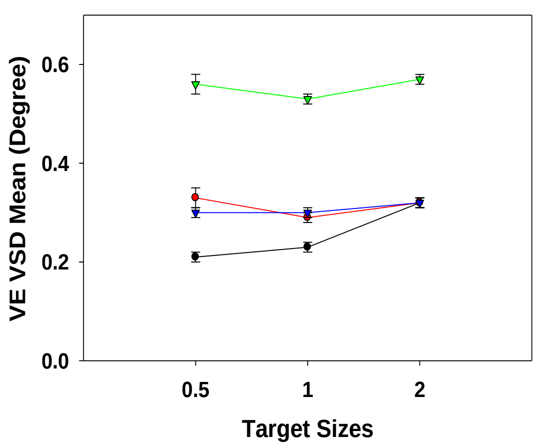


Figure 3.2C

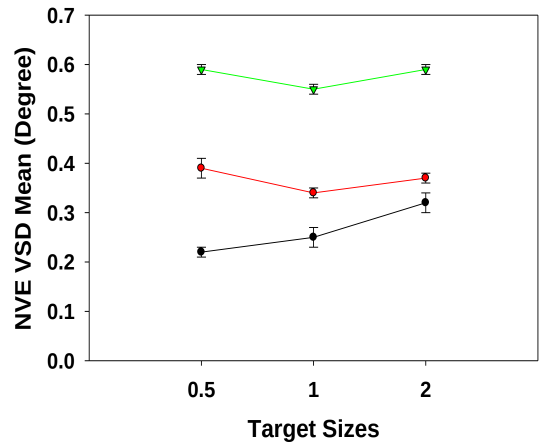


Figure 3.2D.

Figure 3.2 A-D shows the main effect of target sizes on horizontal and vertical standard deviation of viewing (A, C) and non-viewing eye (B, D) respectively. Legend is same as figure 3.1.

3.2.5 Target structure and its influence on ellipse parameters:

St Cyr and Fender (St Cyr and Fender 1969) reported that target structure influences fixation patterns and thereby bivariate contour ellipse parameters in normal subjects. They also mentioned that during dichoptic viewing, the ellipse corresponding to the viewing eye conforms more closely to the target structure than the ellipse corresponding to the occluded eye. We were interested in investigating whether ellipse parameters follow target shape and/or nystagmus eye movement patterns in the strabismic monkeys. We looked at the length of the major axis, ratio of the major and minor axis of the ellipse for viewing and non-viewing eye and orientation of the ellipse. The length of the major axis will tell us about instability. The ratio will describe the shape of the ellipse, for example if the fixation points are distributed equally along horizontal and vertical meridian, the major and minor axis will have the similar length and the resultant ellipse will be approximately circular. On the other hand, if the distribution of fixation points is more along one meridian than the other, then the length of the major axis will be greater than minor axis and the resultant ellipse will be of smaller width and less circular. The orientation of the ellipse will describe the distribution of fixation points including nystagmus eye movement.

3.2.5.1 Length of major axis of the ellipse as a function of target parameters in strabismic monkeys

In the viewing eye, the length of major axis was significantly changed as a function of target shape in SM1 ($F(3,351) = 3.78$, $p = 0.011$) and SM2 ($F(3,351) = 3.94$, $p < 0.009$) with greatest for the solid circle target. The main effect of target size on major axes of the ellipse was observed in SM1 ($F(2,351) = 9.90$, $p < 0.001$) and SM2 ($F(2,351) = 5.13$, $p < 0.006$) with greatest value for the 2° target size and least for 1°. None of the target parameters showed significant influence on major-axis length for SM3 (Shape: $F(3,232) = 0.66$, $p = 0.57$; Size: $F(2,232) = 3.18$, $p = 0.043$). In the non-viewing eye, target shape produced a significant change in the length of the major axis in SM1 and SM2. In SM1, major axis length was significantly greater with a solid circle *(F*(3,351) = 6.01, p = 0.001) whereas in SM2, it was significantly greater with the combo target *(F*(3,351) = 3.34, p = 0.019). The length of the major axis was greater with 0.5° target size; however the effect was non-significant in SM1: $F(2,351) = 1.69$, $p = 0.186$ and SM2 : $F(2,351) = 2.25$, $p = 0.10$. Length of the major axis basically describes fixational stability, mirroring the BCEA metric. The more the length, the more the instability.

Does ratio of major and minor axis change as a function of target shape and size in viewing and non-viewing eyes?

In SM1 and SM3, the ratio of the major and minor axes of the ellipse corresponding to the viewing eye did not show significant change as a function of target shape and size whereas in SM2, the ratio was significantly lower with 2° targets ($F(2,351) = 13.92$, $p < 0.001$). The ratio of the ellipse axes corresponding to the non-viewing eye showed significant changes as a function of target shape and size. In SM1 the ‘X’ shape: $F(3,351) = 2.58$, $p = 0.05$ and 2° target size: F(2,351 = 4.09, $p = 0.017$) produced significantly lower ratios whereas in SM2 the solid circle shape: $F(3,351) = 4.06$, $p = 0.007$; 2° size: $F(2,351) = 5.44$, $p = 0.005$) produced a significantly lower ratio changes. Target shape and size were deciding factors for ellipse shape. A 2° target produced lower ratio indicating similar lengths of the major and minor axis. The same was true for solid circle and ‘X’.

3.2.5.2 Orientation of the ellipse

Figures 3.3 and 3.4 show examples of ellipses fit to fixation data. Figure 3.3 is from monkey SM1 and Figure 3.4 is from SM2. Target parameters and viewing conditions for each dataset are provided in the legend to the figure. Since each stimulus condition was repeated 5 times, each subplot has 5 ellipses. In addition to the lengths of major and minor axes discussed before, we also examined the orientation of the ellipse with respect to the x-axis. The goal was to ascertain whether different stimuli might induce different ellipse orientations. Tables 3.5 and 3.6 shows the axis lengths and ellipse orientations associated with each trial for a single fixation stimulus as shown in Figures 3.3 and 3.4. The overall observation from Figures 3.3 and 3.4 is that ellipse size and orientation is more or less consistent from trial to trial and also from one stimulus to another (compare Figure 3.3A and Figure 3.3B; compare Figure 3.4A and Figure 3.4B). However ellipse orientation between the viewing and non-viewing eye can differ significantly (compare the left and right panels in Figure 3.3 for example).

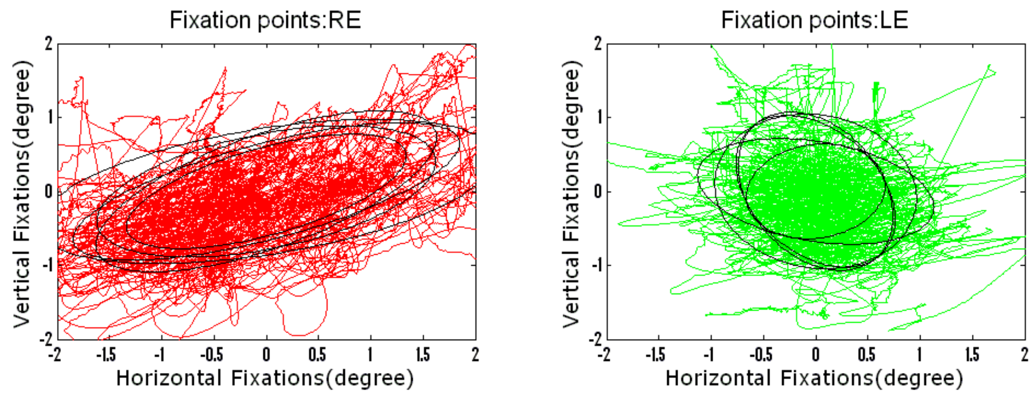


Figure 3.3A

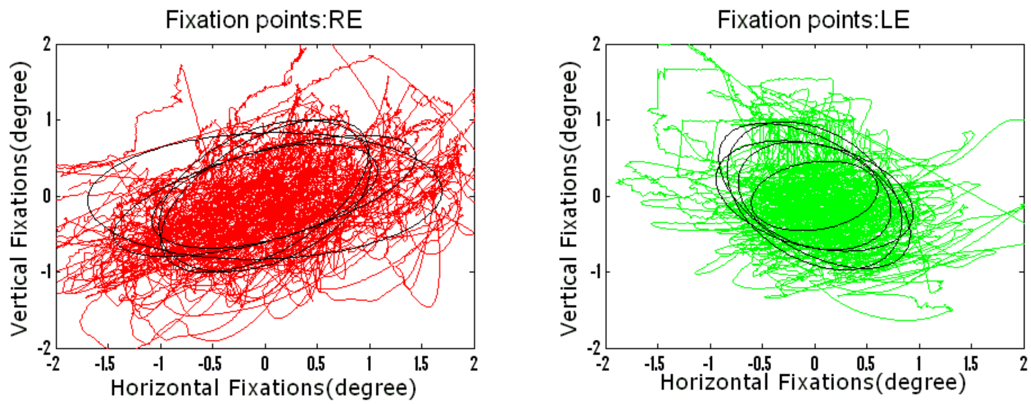


Figure 3.3B

Figure 3.3A (top panels) and 3.3B (bottom panels) shows fitted ellipses for SM1 during left eye viewing the 1 degree combo stimulus against a black background (3.3A) and left eye viewing the 1 degree 'X' stimulus against a black background (3.3B). Ellipses fit to all 5 trials are shown. Left panels show data from the non-viewing eye (RE) and right panels show data from the viewing eye (left eye). Legend: Red: RE fixation data, Green: LE fixation data.

Right eye			Left eye		
Major Axis	Minor Axis	Orientation	Major Axis	Minor Axis	Orientation
2.88	1.13	-23.50	1.38	1.22	-36.69
3.64	1.45	-29.01	2.18	1.33	64.62
3.94	1.25	-21.57	2.22	1.34	69.70
4.49	1.68	-13.80	2.13	1.89	65.61
3.37	1.52	-17.75	2.32	1.32	16.16

Table 3.5A

Right eye			Left eye		
Major Axis	Minor Axis	Orientation	Major Axis	Minor Axis	Orientation
2.37	1.57	-43.83	2.31	1.28	47.25
3.39	1.66	-2.85	1.99	1.31	24.87
2.30	1.13	-22.88	1.24	0.88	-17.21
2.50	1.31	-45.54	2.12	1.44	56.94
3.10	1.13	-22.76	1.61	1.25	41.63

Table 3.5B

Table 3.5A and 3.5B shows parameters corresponding to the ellipses fit to data in Figures 3.3A and 3.3B respectively. Clockwise tilted ellipses are positive (from positive X axis) whereas anticlockwise ellipses are negative. The lengths of the major and minor axes (degrees) and ellipse orientations (degrees) are tabulated.

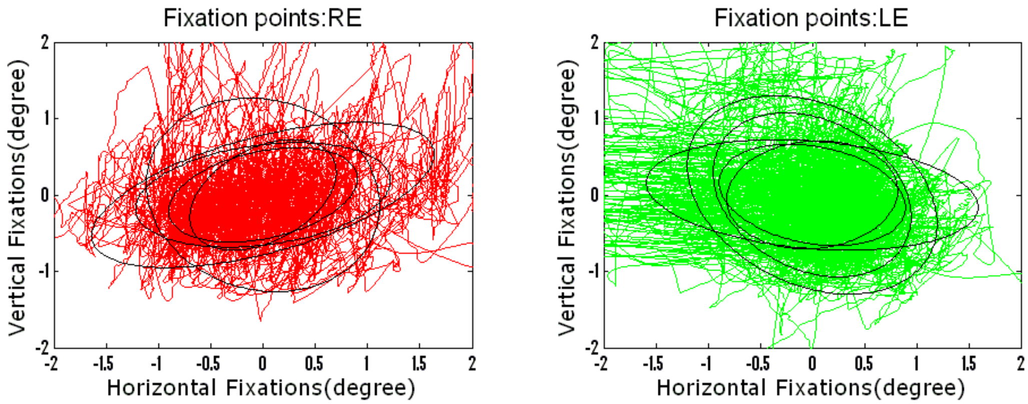


Figure 3.4A

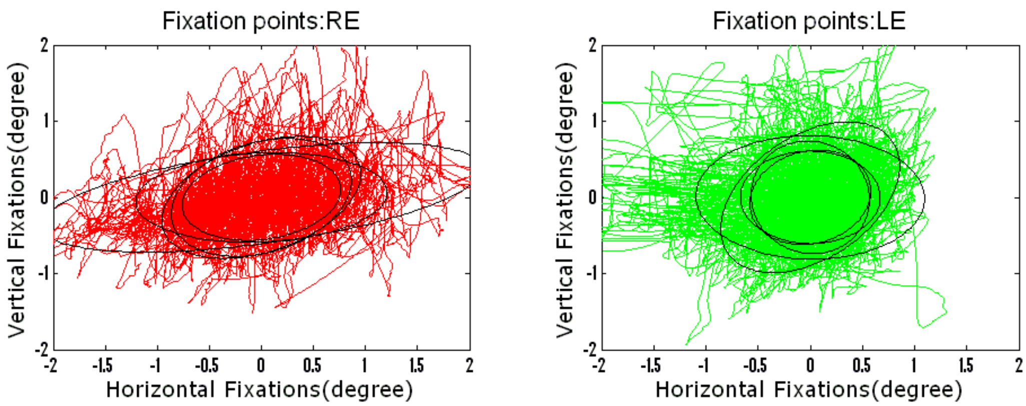


Figure 3.4B

Figure 3.4A (top panels) and 3.4B (bottom panels) shows ellipse fits for data from SM2 during left eye viewing the 1 degree combo stimulus against a black background (3.4A) and left eye viewing the 1 degree 'X' stimulus against a black background (3.4B). Legend and conventions same as figure 3.3.

Right eye			Left eye		
Major Axis	Minor Axis	Orientation	Major Axis	Minor Axis	Orientation
3.49	1.47	-22.37	2.30	1.70	57.40
2.48	1.30	-11.90	3.20	1.40	7.10
1.69	1.10	42.72	1.65	1.40	-14.08
1.89	1.11	-20.90	1.84	1.25	19.60
2.58	2.18	69.28	2.85	2.08	52.67

Table 3.6A

Right eye			Left eye		
Major Axis	Minor Axis	Orientation	Major Axis	Minor Axis	Orientation
2.42	1.17	-3.73	1.34	1.22	0.79
4.31	1.20	-10.90	2.22	1.39	-54.59
1.87	1.37	-34.17	1.48	1.13	85.39
2.10	1.35	-32.51	2.19	1.62	1.90
1.55	1.09	-15.33	1.29	1.11	-59.32

Table 3.6B

Table 3.6A and 3.6B shows ellipse fit parameters corresponding to ellipses fit to data in figure 3.2A and 3.2B respectively. . The lengths of major and minor axes (degrees) and ellipse orientations (degrees) are tabulated.

The plots shown in Figures 3.5-3.7 shows the ellipse orientation (in deg) for all 120 trials collected during right eye viewing (Panel A), left eye viewing (Panel B) and binocular viewing (Panel C) conditions for each strabismic monkey.

In SM1 (Figure 3.5), during right eye viewing ellipse orientation for viewing eye seems to be scattered widely whereas for the non-viewing eye it is mostly along horizontal axis (Figure 3.5A). During left eye viewing, ellipse orientation for the viewing and non-viewing eye is close to horizontal on most trials (Figure 3.5B). During binocular viewing, this monkey habitually fixated with his right eye on the straight ahead target. Therefore the orientation plot in Figure 3.5C most closely reflects the right eye viewing condition shown in Figure 3.5A. Overall in SM1, we observed scattered ellipse orientations for the viewing eye and horizontal orientations for the non-viewing eye during all the three viewing conditions and across all stimuli.

In SM2, during right eye viewing (Figure 3.6A) ellipse orientation for the viewing eye is almost vertical (note that an orientation of +90 and -90 are the same ellipse) whereas for the non-viewing eye it is horizontal to oblique. During left eye viewing, the ellipse orientation for the viewing and non-viewing eyes are both scattered (Figure 3.6B). During binocular viewing (Figure 3.6C), ellipse orientation was similar to that observed during right eye monocular viewing (Figure 3.6A). The binocular viewing data for SM1 and SM2 suggest that uncovering the fellow eye during binocular viewing does not change the fixation pattern.

In SM3, monocular and binocular (both with left eye fixating the target), ellipses are oriented in an oblique direction with substantial scatter (Figure 3.7A and 3.7B).

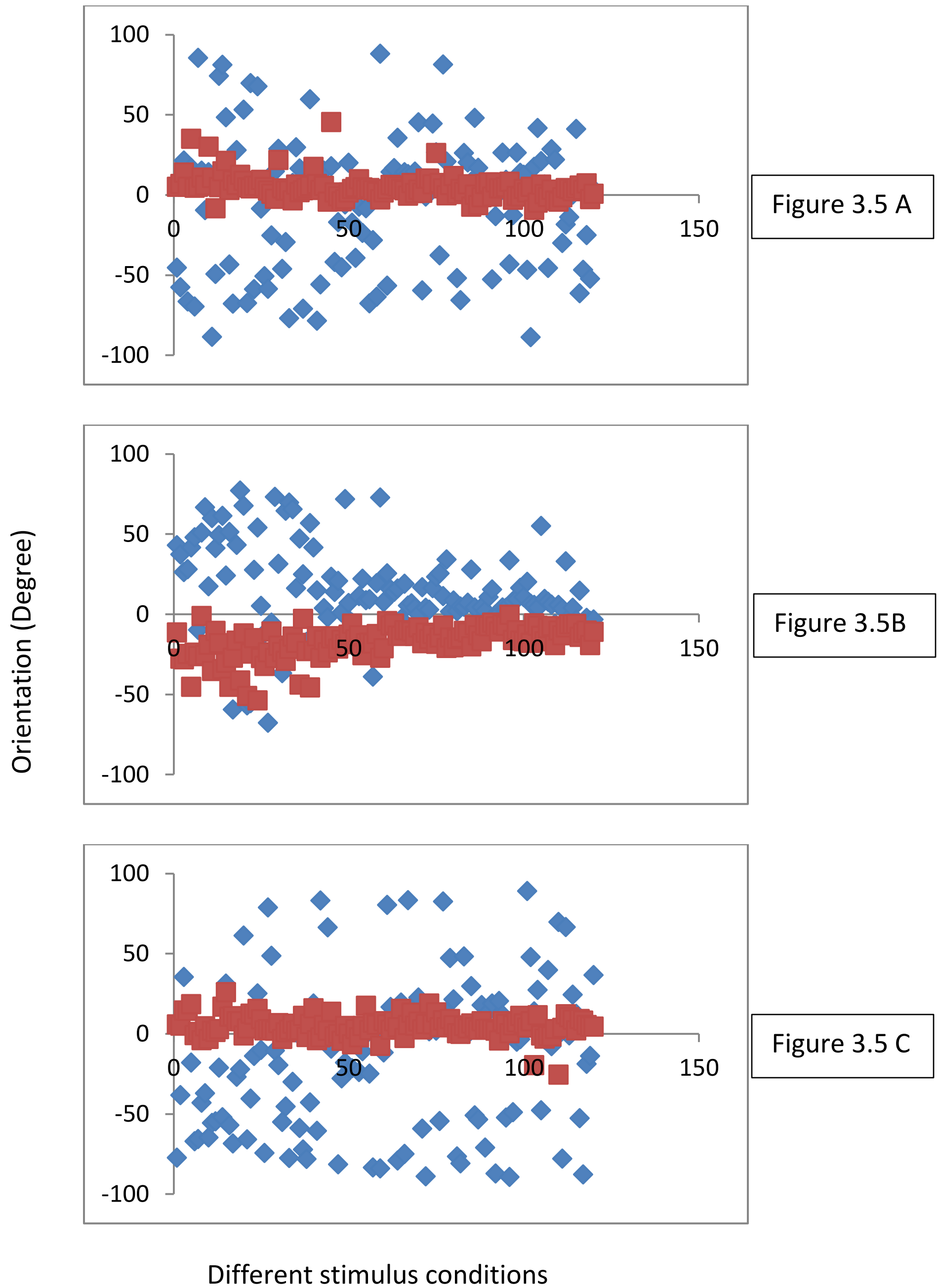


Figure 3.5A-C: Ellipse orientation (in deg) for SM1 during right eye viewing (A), left eye viewing (B) and binocular viewing (Right eye fixation) (C). Legend: Blue: viewing eye; Red: Non-viewing eye; X-axis: numbered according to the different stimulus conditions, Y-axis: orientation of ellipse (degrees).

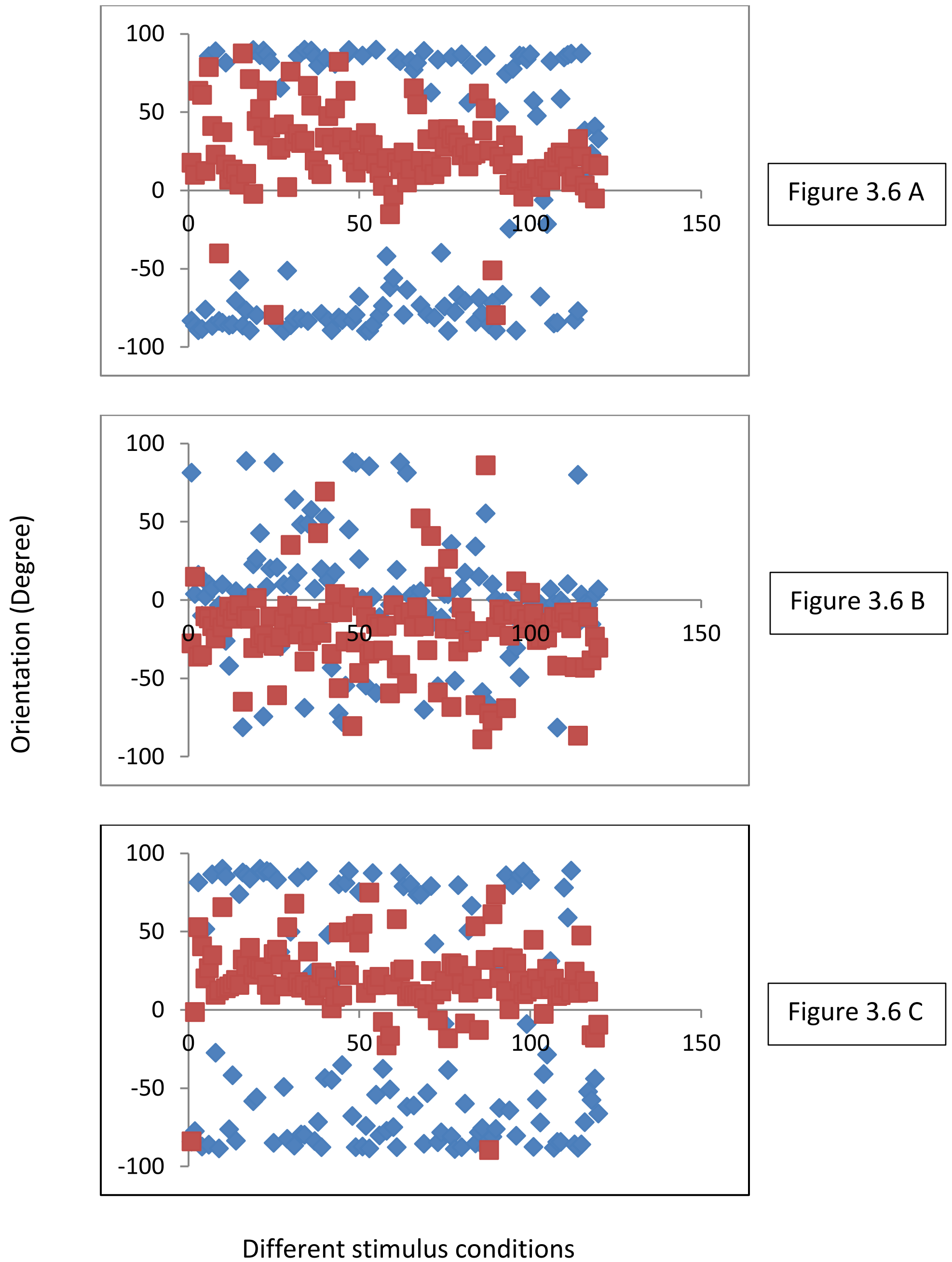


Figure 3.6A-C. Orientations of the ellipses for the viewing and non-viewing eye for SM2 during right-eye viewing (A), left-eye viewing (B) and binocular viewing (right eye fixation)(C). Legend is the same as figure 3.5.

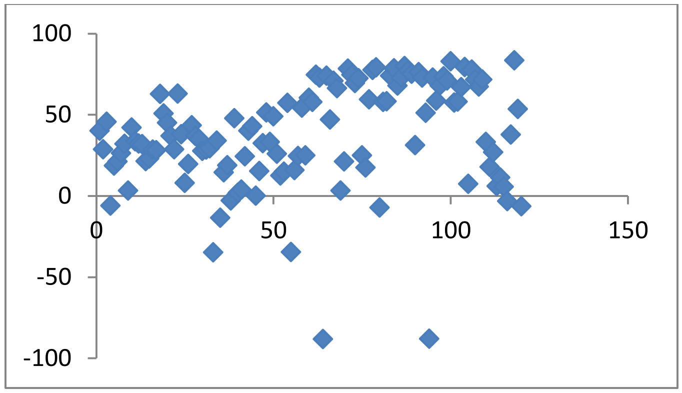


Figure 3.7A

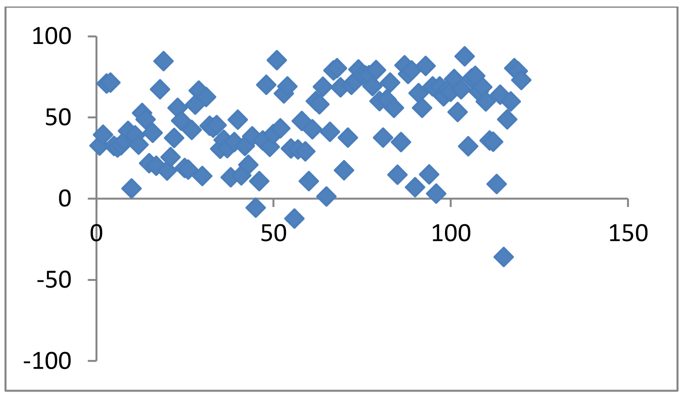


Figure 3.7B

Different stimulus conditions

Figure 3.7A-B shows the orientations of the ellipses for SM1 during left eye viewing (A) and binocular viewing with left eye fixation (B). Legend is the same as figure 3.5. Ellipse orientations for the fellow non-viewing (right) eye could not be calculated due to a non-functional coil.

3.2.5.3 Weighted orientation:

The scatter plots in Figures 3.5-3.7 showed that ellipse orientation was more or less consistent across the different fixation stimuli in the non-viewing eye but was more scattered in the viewing eye. We interpret this to be due to the direction of the nystagmus that is usually more apparent in the covered eye. When we consider the scatter in the viewing eye orientations, it is apparent that examining orientation alone, i.e., without consideration of other ellipse parameters could lead to erroneous conclusions. For example, when the major and minor axis lengths are similar, the shape of the ellipse approaches a circle and thus consideration of the ellipse orientation is not very important. On the other hand, ellipse orientations are quite significant when the major and minor axes are very different (i.e., less circular ellipses). Therefore we decided that ellipse orientations must be scaled, or weighted according to the shape of the ellipse (i.e., more elongated ellipse orientations are more important than more circular ellipse orientations).

To develop the weighting factor, we first took a ratio of the major to the minor axis. A more or less circular ellipse would have a ratio close to 1.0 while more elongated ellipses would have ratio much greater than 1.0. This ratio was then normalized by the maximum ratio value to give us the required weighting factor. The weighting factor was therefore 1.0 for the most elongated ellipse and was small for more circular ellipses. In other words, a smaller weighting factor indicates a circular shape whereas a bigger weighting factor indicates an elliptical shape. Finally, the orientation of the ellipse as measured in degrees (Figures 3.5-3.7) was multiplied by the weighting factor to obtain the weighted orientation of each ellipse. By using this method we were able to give more significance to the ellipses that are less circular.

Figures 3.8-3.10 shows the weighted ellipse orientations in the strabismic monkeys. The main observation was that the scatter observed in the orientation data in

Figures 3.5-3.7 was significantly reduced. Therefore the aforementioned scatter in the orientations of the viewing eye ellipses is partly due to more or less equal dispersion in the horizontal and vertical planes (especially in the viewing eye) resulting in more or less circular shaped fits. *Our overall finding with regard to orientation is that target parameters do not much affect the orientation. Viewing condition (i.e., right eye or left eye viewing) can indeed influence orientation most likely to differing directions of nystagmus.*

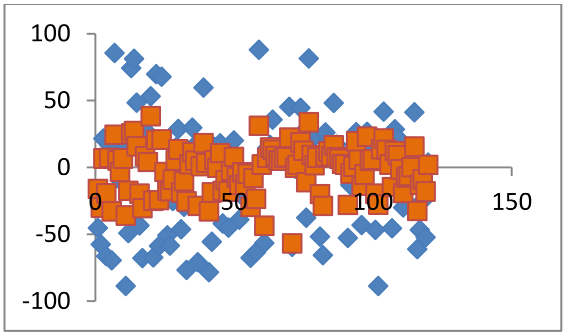


Figure 3.8A

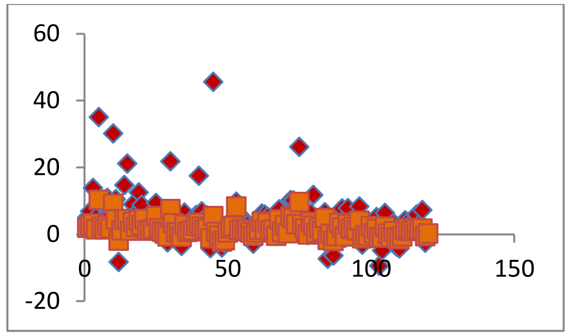


Figure 3.8B

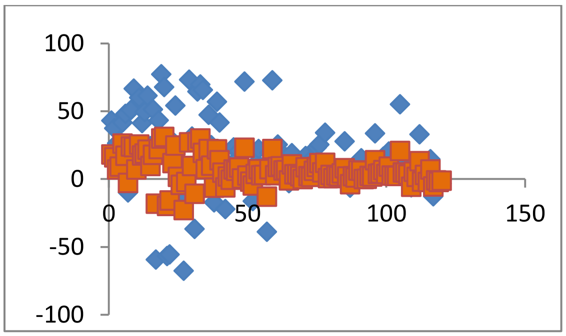


Figure 3.8C

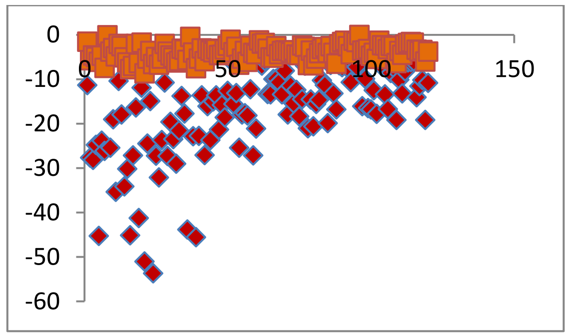


Figure 3.8D

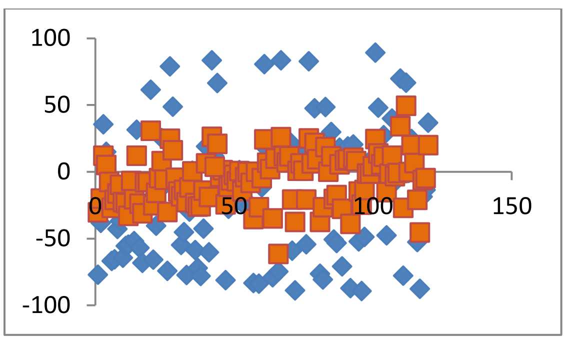


Figure 3.8E

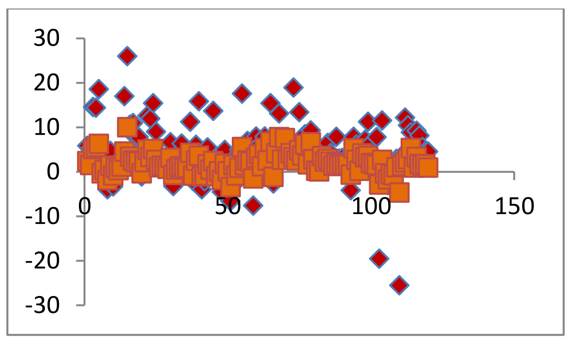
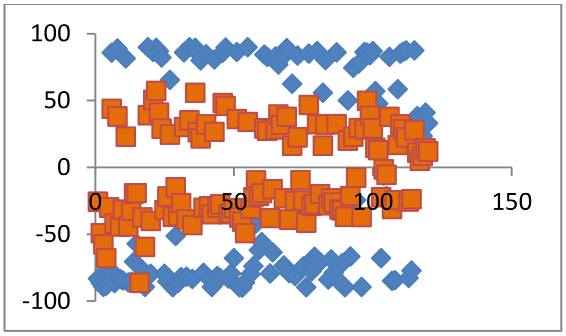


Figure 3.8F

Figure 3.8A-F shows the weighted orientations for fixation ellipses in the viewing and non-viewing eye for SM1 during right-eye viewing (3.8A-B), left-eye viewing (3.8C-D) and binocular viewing (right eye fixation)(3.8E-F). Right panel: (B, D, F) non-viewing eye, left panel: (A, C, E) viewing eye. Legend: Blue: viewing eye ellipse orientation (deg); Red: non-viewing eye ellipse orientation (deg); Orange: weighted ellipse orientation;



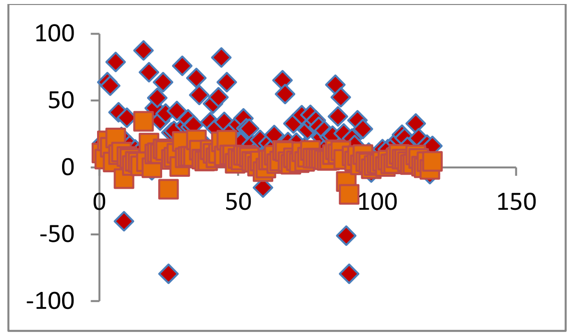
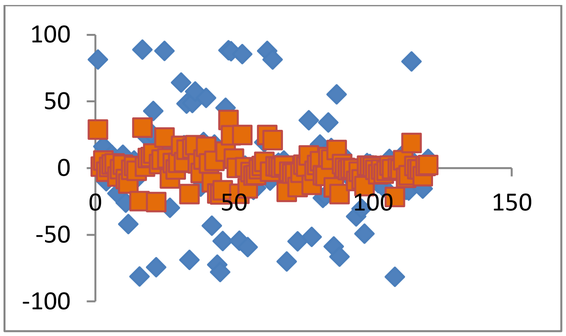


Figure 3.9A **Figure 3.9B**



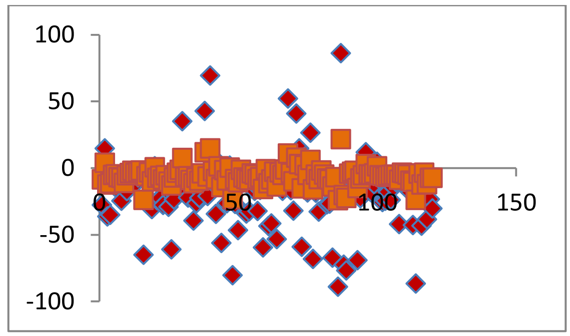
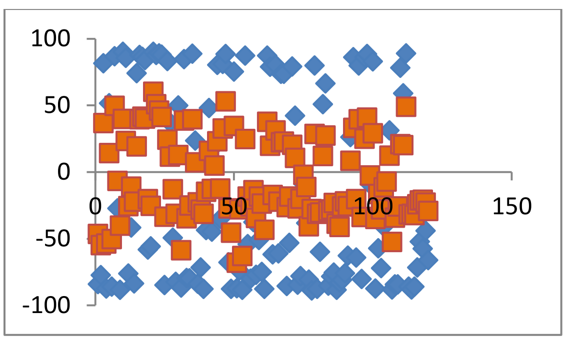


Figure 3.9C **Figure 3.9D**



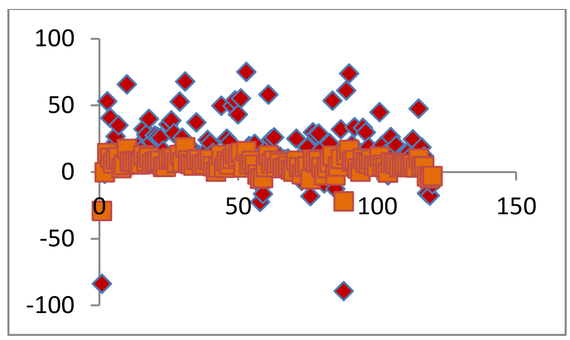


Figure 3.9E **Figure 3.9F**

Figure 3.9A-F: shows the weighted orientations of the ellipses for data from viewing and non-viewing eye for SM2. All plot conventions are the same as in Fig 3.8.

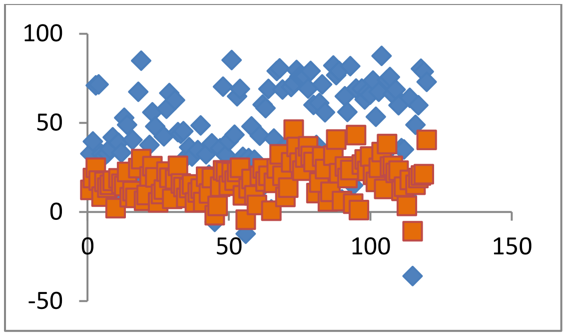


Figure 3.10A

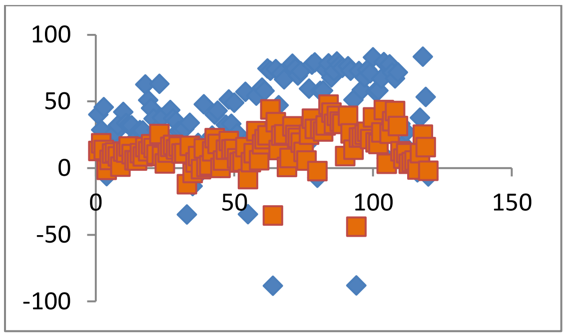


Figure 3.10B

Figure 3.10A-B shows the weighted orientations in the viewing eye for SM3 during left eye monocular (3.10A) and binocular viewing (left eye fixation) (3.10B). Legend is the same as figures 3.8 & 3.9.

3.3 Future directions:

Correlating Fixation Stability with Visual acuity

With our current experimental setup we were unable to measure the visual acuity in the monkeys and therefore we do not know the depth of amblyopia in our strabismic monkeys, if any. Visual acuity and stereoacuity were found to be correlated with BCEA in subjects with strabismic and anisometropic amblyopic eyes (Subramanian, Jost et al. 2013). Poor visual acuity from ocular abnormalities such as amblyopia and macular disease resulted in greater fixation instability and larger BCEA (Tarita-Nistor, Gonzalez et al. 2008; Subramanian, Jost et al. 2013).

The measurement of visual acuity will not change the data collected from the monkeys, however it will allow an assessment of correlation between fixation stability and visual acuity similar to Subramanian and Tarita-Nistor (Tarita-Nistor, Gonzalez et al. 2008; Subramanian, Jost et al. 2013). In the future, visual acuity could be measured monocularly by the Forced Choice Preferential Looking technique (Teller 1979). In this procedure, monkeys will be presented with two separate patches, one blank and the second one with a spatial frequency grating at a pre-defined location similar to Teller visual acuity card presentations. The trials will be repeated multiple times for each spatial frequency. If the stimulus is visible, the monkey will fixate on the stimulus and with the help of eye coil we can easily assess the location of the eye fixation. Finally, a psychometric function will be plotted to obtain a threshold with the percentage of correct fixations on the Y axis and spatial frequencies on the X axis. The measurement of visual acuity will allow us to determine the amount of amblyopia and a better correlation with fixation stability.

Possible Neural Substrates for Fixation Instability

Research could be conducted to investigate the neurological structures responsible for fixational instability in strabismic monkeys. Abnormal fixation suggests that the mechanism responsible for steady fixation is affected. In the present study we sometimes observed downbeat nystagmus in our strabismic monkeys, which is usually associated with lesions of the cerebellar flocculus (Abadi 2002). In addition to this, neural structures that are responsible for holding the eyes steady in eccentric positions should also be investigated. This includes the nucleus prepositus hypoglossi, medial vestibular nuclei, and interstitial nucleus of cajal. Also, previous studies have proposed that fixational saccades and saccadic intrusions are generated by the same neural circuits (Rolfs, Laubrock et al. 2006). A future study could be conducted to investigate activation of the rostral superior colliculus (Hafed, Goffart et al. 2009), burst neurons and pause cell (Brien DC 2009) in the brainstem during microsaccades and saccadic intrusions in strabismic monkeys. To be specific, activity in right and left superior colliculus could be investigated with respect to direction of microsaccdes in strabismic monkeys.

Fixation Instability in different Gaze Positions

In the present study we measured fixation stability in the primary gaze position. A further study could be considered to assess fixation in different positions of gaze. According to Alexander's law, nystagmus intensity increases when the eye turns in the direction of the fast phase (Bockisch and Hegemann 2008; Thurtell and Leigh 2011). For example, nystagmus intensity will be greatest on down gaze for downbeat nystagmus. Also it has been noted that nystagmus amplitude increases when subjects fixate in lateral gaze as compared to centerally. Assessing fixation in different positions of gaze will allow us to confirm Alexander's law in strabismic monkeys.

Fixation Instability after Strabismus Correction Surgery

Measurement of fixation stability pre- and post-strabismus surgery in monkeys will allow us to measure the outcome of surgery in terms of improvement in oculomotor functions. To be specific, the study would determine whether fixation stability in the monkey improves post strabismic surgery due to reduction in angle of deviation. Secondly, the study could investigate neural firing from structures responsible for fixational eye movement’s pre- and post- strabismus surgery.

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Chapter 4: Appendix

4.1 Extended Abstract and Summary of Study

Purpose: Fixation instability has been reported in ocular abnormalities such as strabismus, amblyopia and age-related macular degeneration. Several factors influence fixation stability in normal humans and monkeys including some parameters of the visual stimulus such as size and shape of the target. However it is unclear if these visual stimulus parameters would exert a significant influence in disease conditions such as strabismus. One possibility is that the influence of fixation target parameters on fixation stability in strabismus is significant and perhaps similar to that observed in normal. Alternatively, in the strabismic subject, the ongoing drifts and nystagmus eye movements could mask any potential influence of fixation target parameters. Our first aim was to assess whether manipulating fixation target shape, size and background affects fixation stability of the viewing and deviated eye in strabismic monkeys and compare these effects to the effects observed in a normal monkey. Second, we compared fixation stability of the viewing and deviated eyes under monocular and binocular viewing conditions in the strabismic monkeys. Third, we assessed whether fixation stability changes due to target parameters are proportional in the viewing and deviated eyes of the normal and strabismic monkeys.

Methods: One normal (NM) and three exotropic monkeys (SM1,SM2, SM3) were presented with four different fixation targets (symbol 'X', solid circle, Optotype '%', a combination of bulls eye and cross hair), each in three sizes (0.5°, 1° and 2°) against two backgrounds (white target on black background or black target on white background). Each stimulus condition was repeated 5 times yielding a total of 360 presentations per animal. Each stimulus, selected in random order, was presented at the center of a tangent screen, 114 cm away from the monkey. Eye movements were

recorded for 60s using implanted scleral search coils during monocular and binocular viewing. Eye position data were processed with anti-aliasing filters at 400Hz prior to sampling at a frequency of 2.79 KHz with 12-bit precision. Fixation stability was quantified by calculating the Bivariate Contour Ellipse Area (BCEA). Greater fixation instability results in a larger value of the BCEA metric. Multi-factorial ANOVA was performed to analyze the effects of target shape, size, background and viewing condition on BCEA. Paired t-tests were used to compare BCEA in the fixating and covered eyes.

Results: Two fundamental observations were that BCEA was greater in the three strabismic monkeys compared to the normal and that BCEA of the deviated eye of the strabismic monkeys was significantly greater than BCEA in the fixating eye (paired t-test $p<0.001$). Under monocular viewing conditions, the normal monkey did not show any significant difference in fixation stability between the viewing and covered eyes (paired t-test; $p = 0.162$). Statistical testing revealed that target shape and size significantly affected fixation stability in both normal and strabismic monkeys. Among the four shapes, the 'solid circle' resulted in significantly higher BCEAs in the viewing eyes of the NM and two of the three strabismic monkeys ($p < 0.001$). A significant target-shape effect was also observed in the covered eye in these monkeys ($p < 0.03$). With respect to target size, the greatest instability was associated with the largest target size (2°). Best fixation was elicited with a 0.5° target in the normal monkey and a 1.0° target in the strabismic monkeys ($p<0.005$). Once again, similar effects were observed in the covered eye in these monkeys. Background effects tended to be idiosyncratic. For example, a black target on a white background produced significantly larger BCEA in NM the and a significantly smaller BCEA in SM1 ($p < 0.001$). Background effects were not statistically significant in the covered eye. There was no statistical significance observed when comparing fixation stability under monocular and binocular viewing conditions in either the fixating or the covered eyes in all monkeys. In order to assess whether target effects were proportional in the two eyes, we performed a multifactorial ANOVA on the ratio of the BCEA values measured in the viewing and non-viewing eyes. Analysis of the BCEA ratios indicated that target shape was not a significant factor in any monkey, suggesting proportional changes in both eyes due to shape. Only SM2 appeared to show a disproportionate target size effect. Again, background effects were idiosyncratic.

Conclusions: The overall difference in fixation stability between normal and strabismic monkeys is likely be due to underlying amblyopia, abnormal drifts and nystagmus eye movements. Target parameters (shape and size) that influence fixation stability in a normal animal also affect fixation stability in strabismus. Further, these target parameter influences appear to function via conjugate mechanisms since proportional effects were observed in both the viewing and covered eyes in both normal and strabismic monkeys. One example of a potential conjugate mechanism would be the accommodation system.

4.2 Fixation stability of the viewing eye:

Table 4.1 Normal monkey:

General Linear Model: VE BCEA (Degree^2) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	3	BINO, LE, RE

Analysis of Variance for VE BCEA (Degree^2), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	2.6131	2.6131	0.8710	6.18
Sizes	2	10.1378	10.1378	5.0689	35.94
Backgrounds	1	0.9977	0.9977	0.9977	7.07
Viewing Conditions	2	3.3488	3.3488	1.6744	11.87
Shapes*Sizes	6	1.6912	1.6912	0.2819	2.00
Shapes*Backgrounds	3	0.1250	0.1250	0.0417	0.30
Shapes*Viewing Conditions	6	0.1610	0.1610	0.0268	0.19
Sizes*Backgrounds	2	0.0037	0.0037	0.0019	0.01
Sizes*Viewing Conditions	4	0.8379	0.8379	0.2095	1.49
Backgrounds*Viewing Conditions	2	0.8053	0.8053	0.4027	2.85
Shapes*Sizes*Backgrounds	6	0.0965	0.0965	0.0161	0.11
Shapes*Sizes*Viewing Conditions	12	0.5406	0.5406	0.0450	0.32
Shapes*Backgrounds* Viewing Conditions	6	0.2399	0.2399	0.0400	0.28
Sizes*Backgrounds*Viewing Conditions	4	0.9302	0.9302	0.2325	1.65
Shapes*Sizes*Backgrounds* Viewing Conditions	12	1.0051	1.0051	0.0838	0.59
Error	288	40.6198	40.6198	0.1410	
Total	359	64.1536			

Source	P
Shapes	0.000
Sizes	0.000
Backgrounds	0.008
Viewing Conditions	0.000
Shapes*Sizes	0.066
Shapes*Backgrounds	0.829
Shapes*Viewing Conditions	0.979
Sizes*Backgrounds	0.987
Sizes*Viewing Conditions	0.207
Backgrounds*Viewing Conditions	0.059
Shapes*Sizes*Backgrounds	0.995
Shapes*Sizes*Viewing Conditions	0.986
Shapes*Backgrounds* Viewing Conditions	0.945
Sizes*Backgrounds*Viewing Conditions	0.162
Shapes*Sizes*Backgrounds* Viewing Conditions	0.847
Error	
Total	

S = 0.375554   R-Sq = 36.68%   R-Sq(adj) = 21.07%

Table 4.2 Strabismus monkey (SM1):

General Linear Model: VE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	3	'Binocular', 'Left eye', 'Right eye'

Analysis of Variance for VE BCEA(Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	1.9300	1.9300	0.6433	2.89
Sizes	2	3.2300	3.2300	1.6150	7.26
Backgrounds	1	4.1407	4.1407	4.1407	18.62
Viewing Conditions	2	155.0748	155.0748	77.5374	348.59
Shapes*Sizes	6	8.7934	8.7934	1.4656	6.59
Shapes*Backgrounds	3	0.6848	0.6848	0.2283	1.03
Shapes*Viewing Conditions	6	1.9873	1.9873	0.3312	1.49
Sizes*Backgrounds	2	3.9334	3.9334	1.9667	8.84
Sizes*Viewing Conditions	4	1.1296	1.1296	0.2824	1.27
Backgrounds*Viewing Conditions	2	9.5727	9.5727	4.7863	21.52
Shapes*Sizes*Backgrounds	6	1.0708	1.0708	0.1785	0.80
Shapes*Sizes*Viewing Conditions	12	8.8188	8.8188	0.7349	3.30
Shapes*Backgrounds* Viewing Conditions	6	1.1288	1.1288	0.1881	0.85
Sizes*Backgrounds*Viewing Conditions	4	7.4127	7.4127	1.8532	8.33
Shapes*Sizes*Backgrounds* Viewing Conditions	12	2.5828	2.5828	0.2152	0.97
Error	288	64.0605	64.0605	0.2224	
Total	359	275.5509			

Source	P
Shapes	0.036
Sizes	0.001
Backgrounds	0.000
Viewing Conditions	0.000
Shapes*Sizes	0.000
Shapes*Backgrounds	0.381
Shapes*Viewing Conditions	0.182
Sizes*Backgrounds	0.000
Sizes*Viewing Conditions	0.282
Backgrounds*Viewing Conditions	0.000
Shapes*Sizes*Backgrounds	0.569
Shapes*Sizes*Viewing Conditions	0.000
Shapes*Backgrounds* Viewing Conditions	0.535
Sizes*Backgrounds*Viewing Conditions	0.000
Shapes*Sizes*Backgrounds* Viewing Conditions	0.480
Error	
Total	

S = 0.471627   R-Sq = 76.75%   R-Sq(adj) = 71.02%

Table 4.3 Strabismus monkey (SM2):

General Linear Model: VE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	3	'Binocular', 'Left eye', 'Right eye'

Analysis of Variance for VE BCEA (Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	11.9135	11.9135	3.9712	6.92
Sizes	2	22.8529	22.8529	11.4265	19.91
Backgrounds	1	0.7625	0.7625	0.7625	1.33
Viewing Conditions	2	77.8986	77.8986	38.9493	67.87
Shapes*Sizes	6	11.5886	11.5886	1.9314	3.37
Shapes*Backgrounds	3	1.7989	1.7989	0.5996	1.04
Shapes*Viewing Conditions	6	3.4100	3.4100	0.5683	0.99
Sizes*Backgrounds	2	0.9034	0.9034	0.4517	0.79
Sizes*Viewing Conditions	4	8.6770	8.6770	2.1692	3.78
Backgrounds*Viewing Conditions	2	0.7197	0.7197	0.3598	0.63
Shapes*Sizes*Backgrounds	6	9.5507	9.5507	1.5918	2.77
Shapes*Sizes*Viewing Conditions	12	6.5726	6.5726	0.5477	0.95
Shapes*Backgrounds* Viewing Conditions	6	4.3622	4.3622	0.7270	1.27
Sizes*Backgrounds*Viewing Conditions	4	3.7592	3.7592	0.9398	1.64
Shapes*Sizes*Backgrounds* Viewing Conditions	12	3.2759	3.2759	0.2730	0.48
Error	288	165.2732	165.2732	0.5739	
Total	359	333.3187			

Source	P
Shapes	0.000
Sizes	0.000
Backgrounds	0.250
Viewing Conditions	0.000
Shapes*Sizes	0.003
Shapes*Backgrounds	0.373
Shapes*Viewing Conditions	0.432
Sizes*Backgrounds	0.456
Sizes*Viewing Conditions	0.005
Backgrounds*Viewing Conditions	0.535
Shapes*Sizes*Backgrounds	0.012
Shapes*Sizes*Viewing Conditions	0.493
Shapes*Backgrounds* Viewing Conditions	0.273
Sizes*Backgrounds*Viewing Conditions	0.165
Shapes*Sizes*Backgrounds* Viewing Conditions	0.928
Error	
Total	

S = 0.757539   R-Sq = 50.42%   R-Sq(adj) = 38.19%

Table 4.4 Strabismus monkey (SM3):

General Linear Model: VE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	2	'Binocular', 'Left eye'

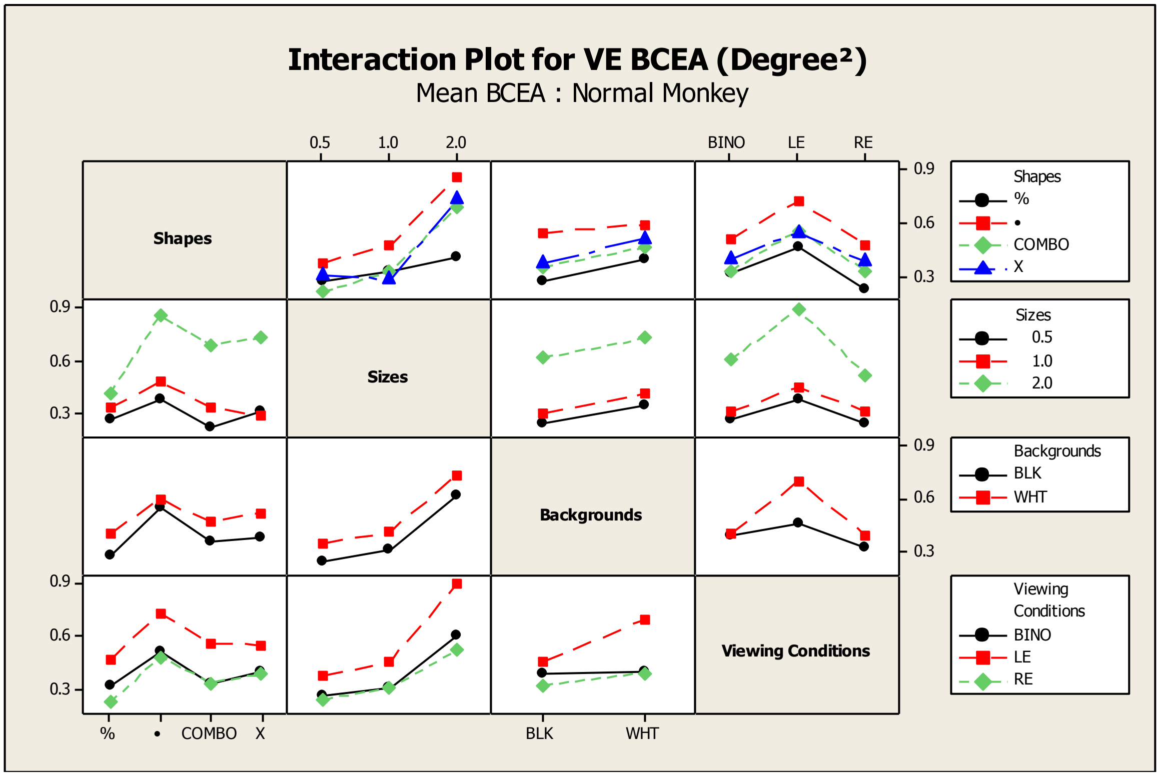
Analysis of Variance for VE BCEA(Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	0.4088	0.4088	0.1363	0.98
Sizes	2	1.7645	1.7645	0.8822	6.38
Backgrounds	1	0.0479	0.0479	0.0479	0.35
Viewing Conditions	1	0.2240	0.2240	0.2240	1.62
Shapes*Sizes	6	1.8486	1.8486	0.3081	2.23
Shapes*Backgrounds	3	1.2899	1.2899	0.4300	3.11
Shapes*Viewing Conditions	3	0.2885	0.2885	0.0962	0.70
Sizes*Backgrounds	2	1.8664	1.8664	0.9332	6.75
Sizes*Viewing Conditions	2	0.5925	0.5925	0.2962	2.14
Backgrounds*Viewing Conditions	1	0.3465	0.3465	0.3465	2.50
Shapes*Sizes*Backgrounds	6	0.5979	0.5979	0.0996	0.72
Shapes*Sizes*Viewing Conditions	6	0.2113	0.2113	0.0352	0.25
Shapes*Backgrounds* Viewing Conditions	3	0.2787	0.2787	0.0929	0.67
Sizes*Backgrounds*Viewing Conditions	2	0.0818	0.0818	0.0409	0.30
Shapes*Sizes*Backgrounds* Viewing Conditions	6	0.5184	0.5184	0.0864	0.62
Error	192	26.5623	26.5623	0.1383	
Total	239	36.9277			

Source	P
Shapes	0.401
Sizes	0.002
Backgrounds	0.557
Viewing Conditions	0.205
Shapes*Sizes	0.042
Shapes*Backgrounds	0.028
Shapes*Viewing Conditions	0.556
Sizes*Backgrounds	0.001
Sizes*Viewing Conditions	0.120
Backgrounds*Viewing Conditions	0.115
Shapes*Sizes*Backgrounds	0.634
Shapes*Sizes*Viewing Conditions	0.957
Shapes*Backgrounds* Viewing Conditions	0.571
Sizes*Backgrounds*Viewing Conditions	0.744
Shapes*Sizes*Backgrounds* Viewing Conditions	0.711
Error	
Total	

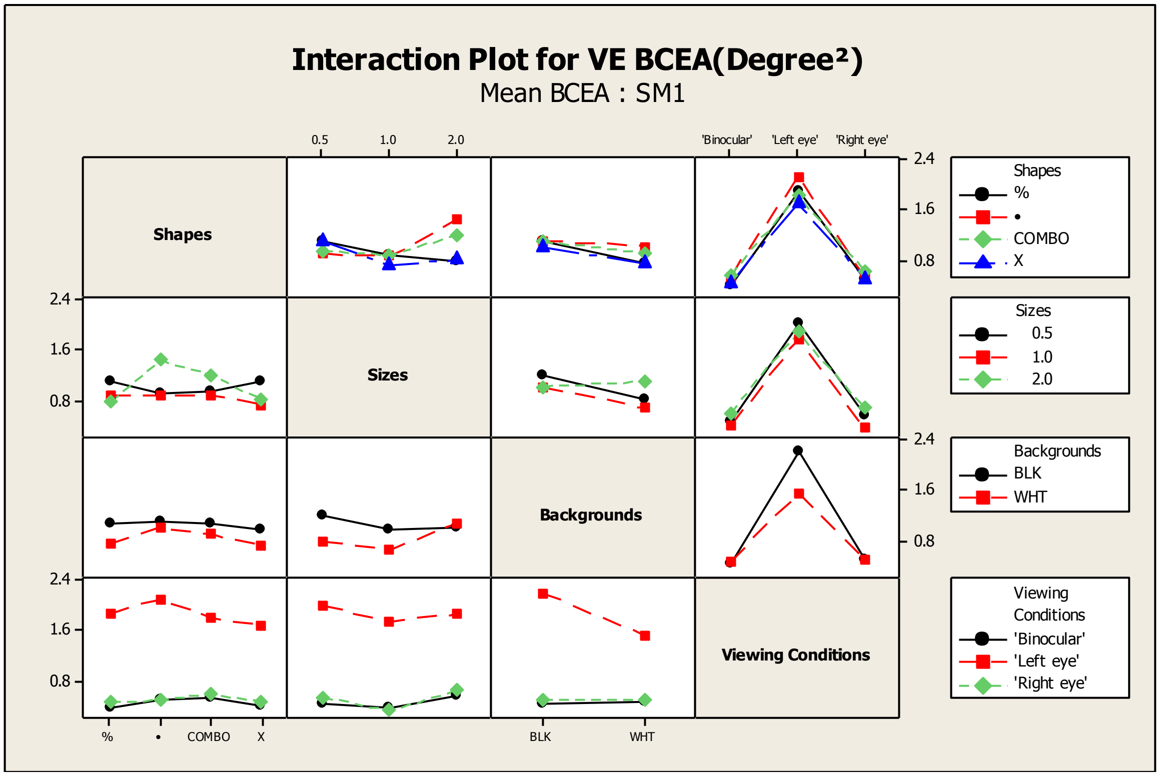
S = 0.371948   R-Sq = 28.07%   R-Sq(adj) = 10.46%

Figure 4.1 Interaction plot for VE BCEA: Normal monkey



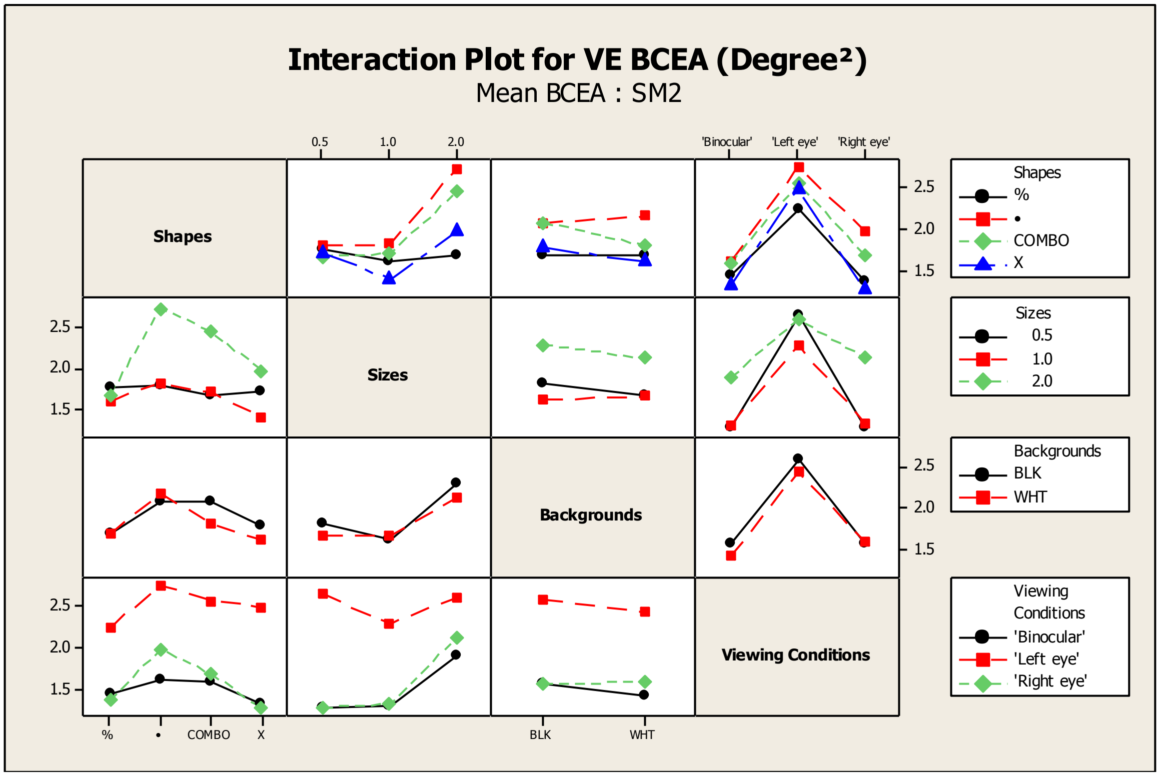
Post hoc indicated that solid circle produced higher BCEA as compared to Combo and '%' stimulus. 2° size resulted in higher BCEA as compared to 0.5° and 1°. Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that there are non-significant interactions between all factors.

Figure 4.2 Interaction plot for VE BCEA: Strabismic monkey (SM1)



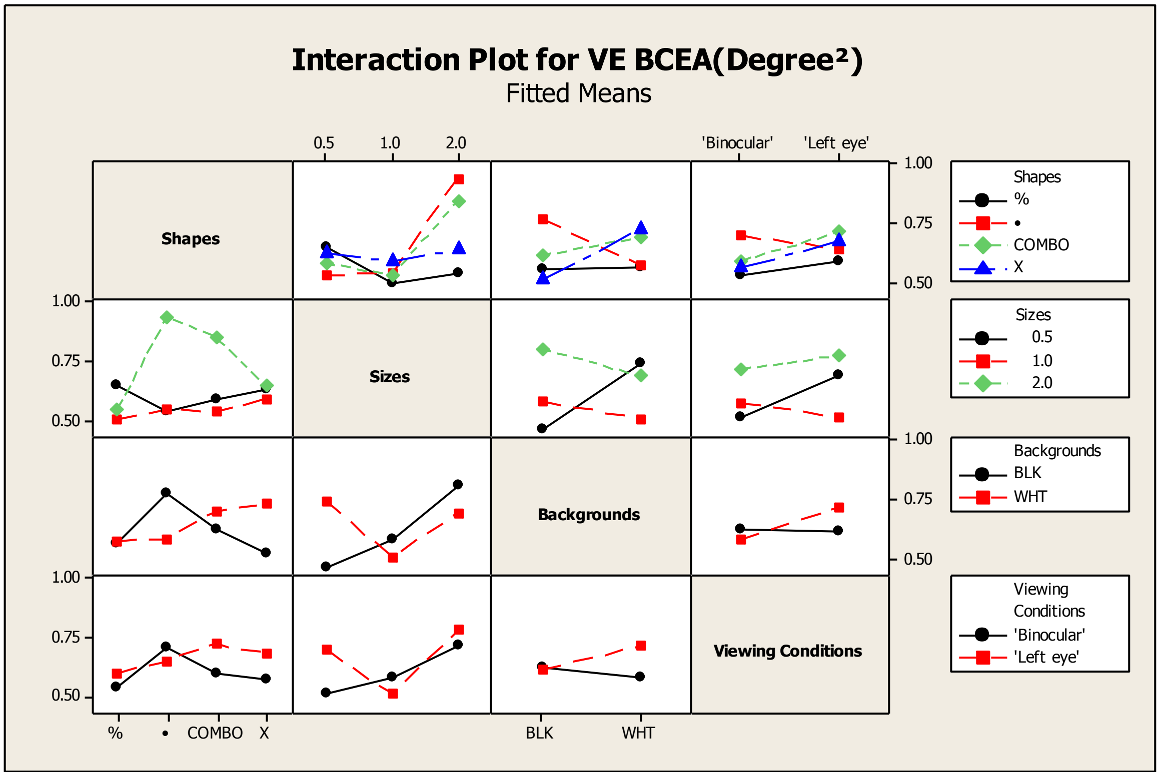
Solid circle and 2° size produced higher BCEA as compared to 'X' and 1° size respectively. Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that in SM1 there is significant interaction between target size and background, $F\ (2{,}288) = 8.84$, $p < 0.001$; background and viewing conditions, $F\ (2{,}288) = 21.52$, $p < 0.001$ and between target shape and size, $F\ (6{,}288) = 6.59$, $p < 0.001$.

Figure 4.3 Interaction plot for VE BCEA: Strabismic monkey (SM2)



Solid circle and 2° size produced higher BCEA as compared to '%' and 1° size respectively. Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that in SM2, there is significant interaction between target shape and size, F (6,288) = 3.37, p = 0.003; size and viewing condition, F (4,288) = 3.78, p = 0.005.

Figure 4.4 Interaction plot for VE BCEA: Strabismic monkey (SM3)



2° size produced higher BCEA as compared to 1° size. Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that in SM3, there is significant interaction between target shape and size F (6,192) = 2.23, p = 0.042; size and background, F (2,192) = 6.75, p = 0.001 and between shape and background, F (3,192) = 3.11, p = 0.028.

4.3 Fixation stability in non-viewing eye:

Table 4.5 Normal monkey (NM):

General Linear Model: NVE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	2	LE, RE

Analysis of Variance for NVE BCEA (Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	2.2687	2.2687	0.7562	4.13
Sizes	2	5.6093	5.6093	2.8046	15.33
Backgrounds	1	0.5202	0.5202	0.5202	2.84
Viewing Conditions	1	2.2989	2.2989	2.2989	12.57
Shapes*Sizes	6	1.6177	1.6177	0.2696	1.47
Shapes*Backgrounds	3	0.1106	0.1106	0.0369	0.20
Shapes*Viewing Conditions	3	0.8426	0.8426	0.2809	1.54
Sizes*Backgrounds	2	0.0175	0.0175	0.0087	0.05
Sizes*Viewing Conditions	2	0.0831	0.0831	0.0415	0.23
Backgrounds*Viewing Conditions	1	0.1346	0.1346	0.1346	0.74
Shapes*Sizes*Backgrounds	6	0.3505	0.3505	0.0584	0.32
Shapes*Sizes*Viewing Conditions	6	0.9414	0.9414	0.1569	0.86
Shapes*Backgrounds* Viewing Conditions	3	0.1114	0.1114	0.0371	0.20
Sizes*Backgrounds*Viewing Conditions	2	0.1659	0.1659	0.0830	0.45
Shapes*Sizes*Backgrounds* Viewing Conditions	6	0.9646	0.9646	0.1608	0.88
Error	192	35.1161	35.1161	0.1829	
Total	239	51.1530			

Source	P
Shapes	0.007
Sizes	0.000
Backgrounds	0.093
Viewing Conditions	0.000
Shapes*Sizes	0.189
Shapes*Backgrounds	0.895
Shapes*Viewing Conditions	0.207
Sizes*Backgrounds	0.953
Sizes*Viewing Conditions	0.797
Backgrounds*Viewing Conditions	0.392
Shapes*Sizes*Backgrounds	0.926
Shapes*Sizes*Viewing Conditions	0.527
Shapes*Backgrounds* Viewing Conditions	0.894
Sizes*Backgrounds*Viewing Conditions	0.636
Shapes*Sizes*Backgrounds* Viewing Conditions	0.511
Error	
Total	

S = 0.427664   R-Sq = 31.35%   R-Sq(adj) = 14.55%

Table 4.6 Strabismus monkey (SM1):

General Linear Model: NVE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	3	'Binocular', 'Left eye', 'Right eye'

Analysis of Variance for NVE BCEA (Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	19.481	19.481	6.494	4.57
Sizes	2	17.395	17.395	8.697	6.13
Backgrounds	1	1.876	1.876	1.876	1.32
Viewing Conditions	2	116.276	116.276	58.138	40.95
Shapes*Sizes	6	47.656	47.656	7.943	5.60
Shapes*Backgrounds	3	2.760	2.760	0.920	0.65
Shapes*Viewing Conditions	6	4.467	4.467	0.744	0.52
Sizes*Backgrounds	2	6.487	6.487	3.243	2.28
Sizes*Viewing Conditions	4	5.578	5.578	1.394	0.98
Backgrounds*Viewing Conditions	2	12.680	12.680	6.340	4.47
Shapes*Sizes*Backgrounds	6	3.664	3.664	0.611	0.43
Shapes*Sizes*Viewing Conditions	12	21.257	21.257	1.771	1.25
Shapes*Backgrounds* Viewing Conditions	6	4.332	4.332	0.722	0.51
Sizes*Backgrounds*Viewing Conditions	4	8.909	8.909	2.227	1.57
Shapes*Sizes*Backgrounds* Viewing Conditions	12	10.704	10.704	0.892	0.63
Error	288	408.835	408.835	1.420	
Total	359	692.357			

Source	P
Shapes	0.004
Sizes	0.002
Backgrounds	0.251
Viewing Conditions	0.000
Shapes*Sizes	0.000
Shapes*Backgrounds	0.585
Shapes*Viewing Conditions	0.790
Sizes*Backgrounds	0.104
Sizes*Viewing Conditions	0.417
Backgrounds*Viewing Conditions	0.012
Shapes*Sizes*Backgrounds	0.859
Shapes*Sizes*Viewing Conditions	0.250
Shapes*Backgrounds* Viewing Conditions	0.802
Sizes*Backgrounds*Viewing Conditions	0.183
Shapes*Sizes*Backgrounds* Viewing Conditions	0.818
Error	
Total	

S = 1.19146   R-Sq = 40.95%   R-Sq(adj) = 26.39%

Table 4.7 Strabismus monkey (SM2):

General Linear Model: NVE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	3	'Binocular', 'Left eye', 'Right eye'

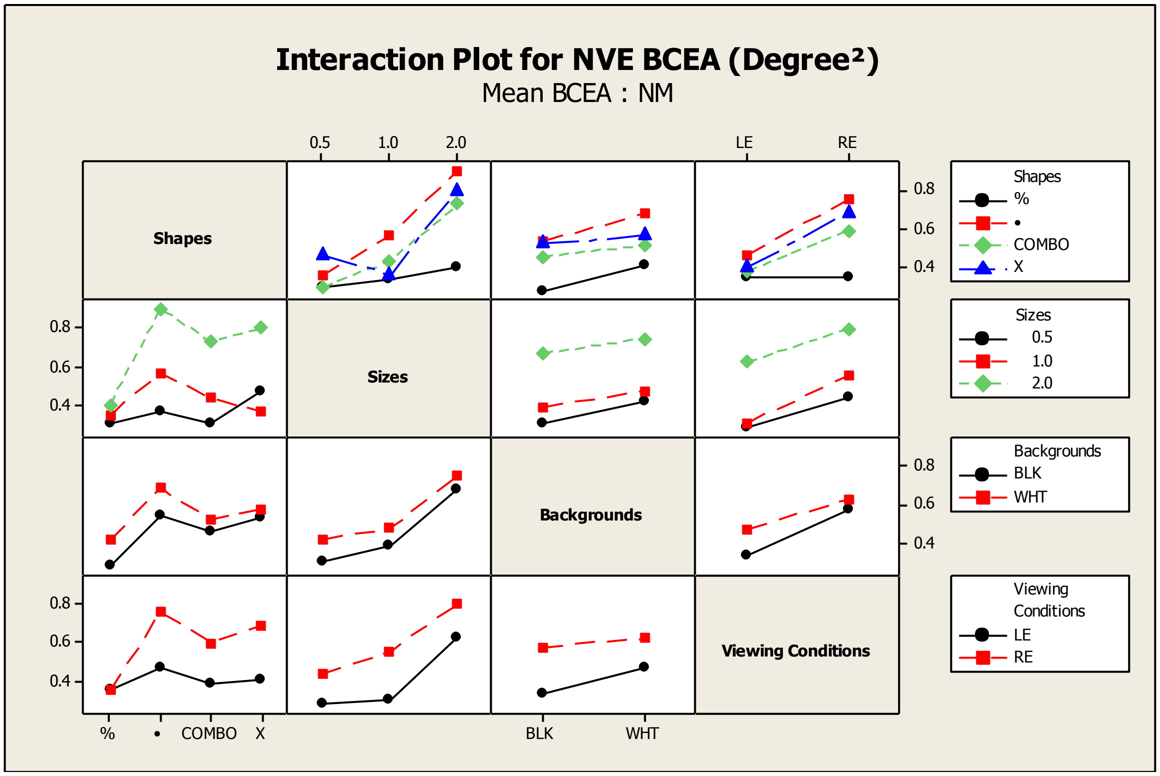
Analysis of Variance for NVE BCEA (Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	13.859	13.859	4.620	3.12
Sizes	2	3.775	3.775	1.888	1.28
Backgrounds	1	46.657	46.657	46.657	31.53
Viewing Conditions	2	39.791	39.791	19.896	13.45
Shapes*Sizes	6	8.956	8.956	1.493	1.01
Shapes*Backgrounds	3	1.890	1.890	0.630	0.43
Shapes*Viewing Conditions	6	3.501	3.501	0.583	0.39
Sizes*Backgrounds	2	0.127	0.127	0.064	0.04
Sizes*Viewing Conditions	4	24.595	24.595	6.149	4.16
Backgrounds*Viewing Conditions	2	9.456	9.456	4.728	3.20
Shapes*Sizes*Backgrounds	6	7.934	7.934	1.322	0.89
Shapes*Sizes*Viewing Conditions	12	18.495	18.495	1.541	1.04
Shapes*Backgrounds* Viewing Conditions	6	6.765	6.765	1.128	0.76
Sizes*Backgrounds*Viewing Conditions	4	6.367	6.367	1.592	1.08
Shapes*Sizes*Backgrounds* Viewing Conditions	12	13.611	13.611	1.134	0.77
Error	288	426.164	426.164	1.480	
Total	359	631.945			

Source	P
Shapes	0.026
Sizes	0.281
Backgrounds	0.000
Viewing Conditions	0.000
Shapes*Sizes	0.420
Shapes*Backgrounds	0.735
Shapes*Viewing Conditions	0.882
Sizes*Backgrounds	0.958
Sizes*Viewing Conditions	0.003
Backgrounds*Viewing Conditions	0.042
Shapes*Sizes*Backgrounds	0.500
Shapes*Sizes*Viewing Conditions	0.411
Shapes*Backgrounds* Viewing Conditions	0.600
Sizes*Backgrounds*Viewing Conditions	0.369
Shapes*Sizes*Backgrounds* Viewing Conditions	0.685
Error	
Total	

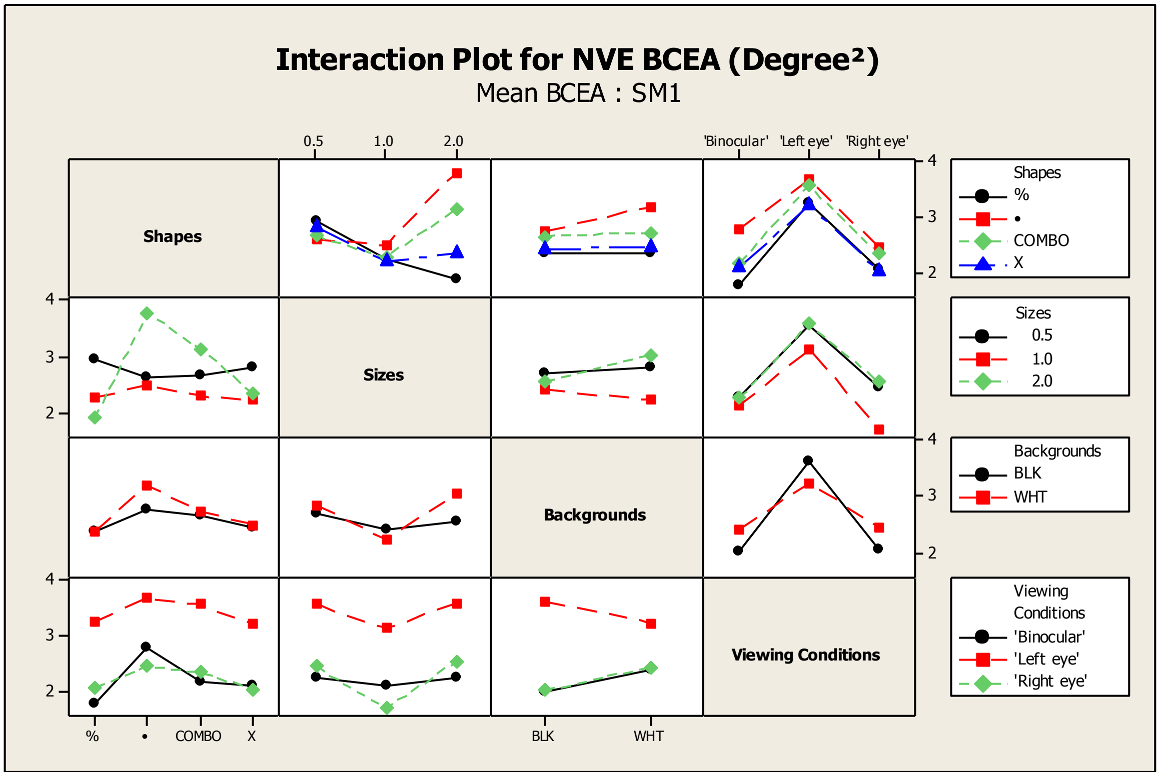
S = 1.21644   R-Sq = 32.56%   R-Sq(adj) = 15.94%

Figure 4.5 Interaction plot for NVE BCEA: Normal monkey (NM)



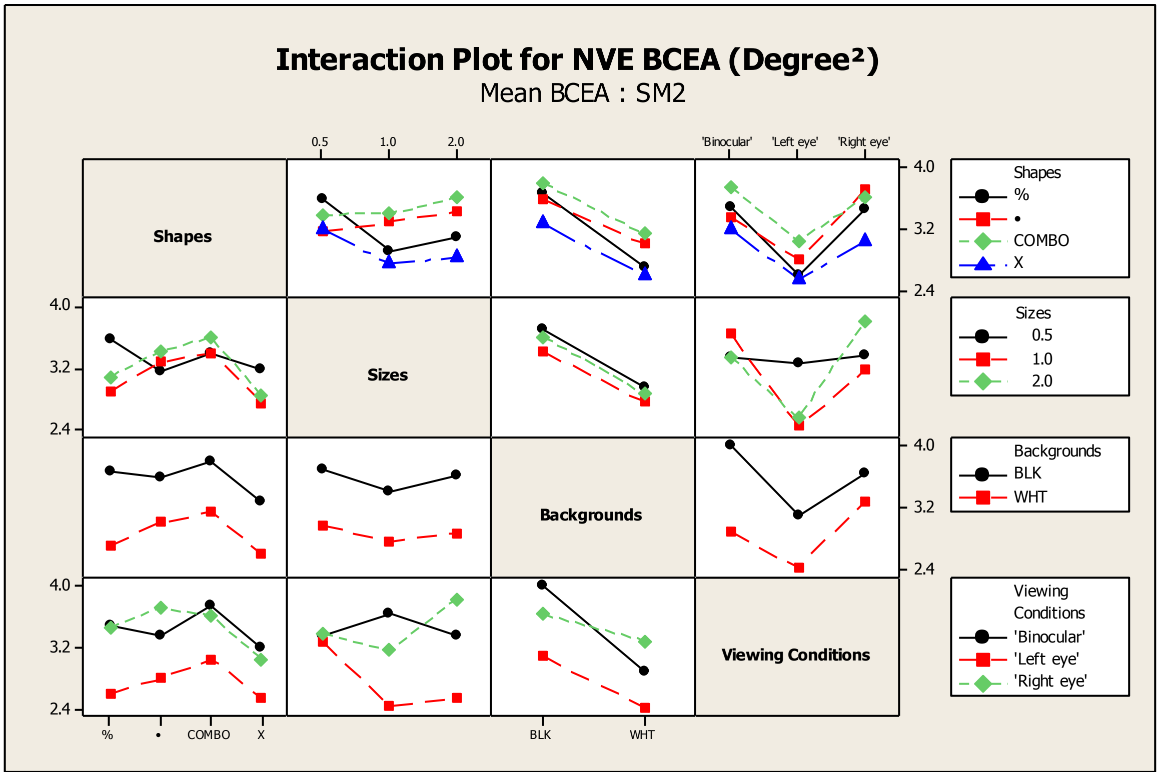
Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that there are non-significant interactions between all factors.

Figure 4.6 Interaction plot for NVE BCEA: Strabismic monkey (SM1)



Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that in SM1, there is significant interaction between target background and viewing conditions was significant, F (2,288) = 4.47, p =0 .012 and shape and size, F (6,288) = 5.60, p < 0.001.

Figure 4.7 Interaction plot for NVE BCEA: Strabismic monkey (SM2)



Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that in SM2, interactions between background and viewing condition F (2,288) = 3.20, p = 0.042 and target size and viewing condition was significant in SM2, F (4,288) = 4.16, p = 0.003

4.4 Proportional changes in BCEA:

Table 4.8 Normal monkey (NM):

General Linear Model: VE/NVE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	2	LE, RE

Analysis of Variance for VE/NVE BCEA (Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	0.7460	0.7460	0.2487	1.95
Sizes	2	0.2403	0.2403	0.1202	0.94
Backgrounds	1	1.0528	1.0528	1.0528	8.25
Viewing Conditions	1	30.9392	30.9392	30.9392	242.39
Shapes*Sizes	6	1.1450	1.1450	0.1908	1.50
Shapes*Backgrounds	3	0.8878	0.8878	0.2959	2.32
Shapes*Viewing Conditions	3	0.2309	0.2309	0.0770	0.60
Sizes*Backgrounds	2	0.0719	0.0719	0.0360	0.28
Sizes*Viewing Conditions	2	0.0064	0.0064	0.0032	0.02
Backgrounds*Viewing Conditions	1	0.1047	0.1047	0.1047	0.82
Shapes*Sizes*Backgrounds	6	0.7913	0.7913	0.1319	1.03
Shapes*Sizes*Viewing Conditions	6	0.8698	0.8698	0.1450	1.14
Shapes*Backgrounds* Viewing Conditions	3	0.8519	0.8519	0.2840	2.22
Sizes*Backgrounds*Viewing Conditions	2	0.5983	0.5983	0.2991	2.34
Shapes*Sizes*Backgrounds* Viewing Conditions	6	0.6002	0.6002	0.1000	0.78
Error	192	24.5076	24.5076	0.1276	
Total	239	63.6439			

Source	P
Shapes	0.123
Sizes	0.392
Backgrounds	0.005
Viewing Conditions	0.000
Shapes*Sizes	0.182
Shapes*Backgrounds	0.077
Shapes*Viewing Conditions	0.614
Sizes*Backgrounds	0.755
Sizes*Viewing Conditions	0.975
Backgrounds*Viewing Conditions	0.366
Shapes*Sizes*Backgrounds	0.405
Shapes*Sizes*Viewing Conditions	0.343
Shapes*Backgrounds* Viewing Conditions	0.087
Sizes*Backgrounds*Viewing Conditions	0.099
Shapes*Sizes*Backgrounds* Viewing Conditions	0.584
Error	
Total	

S = 0.357272   R-Sq = 61.49%   R-Sq(adj) = 52.07%

Table 4.9 Strabismus monkey (SM1):

General Linear Model: VE/NVE BCEA (Degree²) versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	2	'Left eye', 'Right eye'

Analysis of Variance for VE/NVE BCEA (Degree²), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	0.04253	0.04253	0.01418	0.48
Sizes	2	0.00864	0.00864	0.00432	0.15
Backgrounds	1	0.51415	0.51415	0.51415	17.46
Viewing Conditions	1	4.68417	4.68417	4.68417	159.06
Shapes*Sizes	6	0.11643	0.11643	0.01940	0.66
Shapes*Backgrounds	3	0.03199	0.03199	0.01066	0.36
Shapes*Viewing Conditions	3	0.13640	0.13640	0.04547	1.54
Sizes*Backgrounds	2	0.09680	0.09680	0.04840	1.64
Sizes*Viewing Conditions	2	0.14356	0.14356	0.07178	2.44
Backgrounds*Viewing Conditions	1	0.10408	0.10408	0.10408	3.53
Shapes*Sizes*Backgrounds	6	0.16694	0.16694	0.02782	0.94
Shapes*Sizes*Viewing Conditions	6	0.45208	0.45208	0.07535	2.56
Shapes*Backgrounds* Viewing Conditions	3	0.10314	0.10314	0.03438	1.17
Sizes*Backgrounds*Viewing Conditions	2	0.08337	0.08337	0.04169	1.42
Shapes*Sizes*Backgrounds* Viewing Conditions	6	0.18542	0.18542	0.03090	1.05
Error	192	5.65438	5.65438	0.02945	
Total	239	12.52408			

Source	P
Shapes	0.696
Sizes	0.864
Backgrounds	0.000
Viewing Conditions	0.000
Shapes*Sizes	0.683
Shapes*Backgrounds	0.780
Shapes*Viewing Conditions	0.205
Sizes*Backgrounds	0.196
Sizes*Viewing Conditions	0.090
Backgrounds*Viewing Conditions	0.062
Shapes*Sizes*Backgrounds	0.464
Shapes*Sizes*Viewing Conditions	0.021
Shapes*Backgrounds* Viewing Conditions	0.323
Sizes*Backgrounds*Viewing Conditions	0.245
Shapes*Sizes*Backgrounds* Viewing Conditions	0.395
Error	
Total	

S = 0.171610   R-Sq = 54.85%   R-Sq(adj) = 43.80%

Table 4.10 Strabismus monkey (SM2):

General Linear Model: VE/NVE BCEA versus Shapes, Sizes, ...

Factor	Type	Levels	Values
Shapes	fixed	4	%, •, COMBO, X
Sizes	fixed	3	0.5, 1.0, 2.0
Backgrounds	fixed	2	BLK, WHT
Viewing Conditions	fixed	2	'Left eye', 'Right eye'

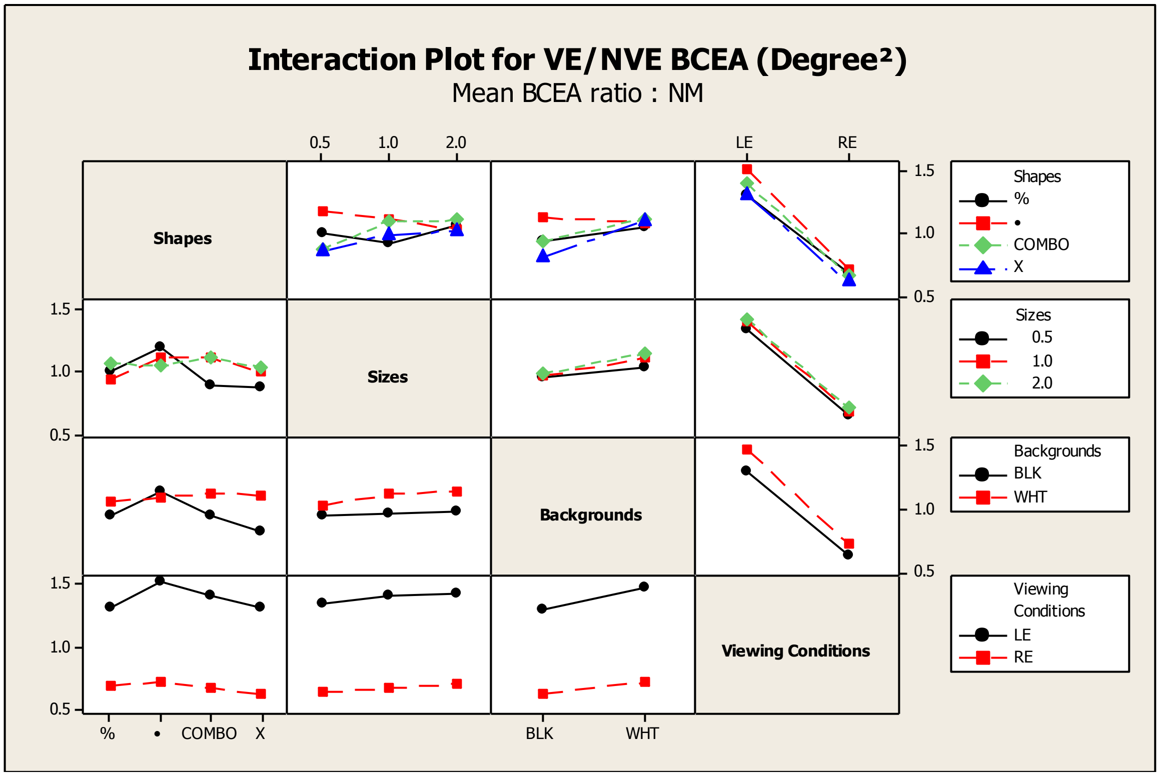
Analysis of Variance for VE/NVE BCEA, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F
Shapes	3	0.30226	0.30226	0.10075	1.27
Sizes	2	1.31508	1.31508	0.65754	8.32
Backgrounds	1	0.87334	0.87334	0.87334	11.05
Viewing Conditions	1	17.64495	17.64495	17.64495	223.16
Shapes*Sizes	6	0.47141	0.47141	0.07857	0.99
Shapes*Backgrounds	3	0.22321	0.22321	0.07440	0.94
Shapes*Viewing Conditions	3	0.23791	0.23791	0.07930	1.00
Sizes*Backgrounds	2	0.06651	0.06651	0.03325	0.42
Sizes*Viewing Conditions	2	0.02147	0.02147	0.01073	0.14
Backgrounds*Viewing Conditions	1	0.34606	0.34606	0.34606	4.38
Shapes*Sizes*Backgrounds	6	0.41948	0.41948	0.06991	0.88
Shapes*Sizes*Viewing Conditions	6	1.18298	1.18298	0.19716	2.49
Shapes*Backgrounds* Viewing Conditions	3	0.14210	0.14210	0.04737	0.60
Sizes*Backgrounds*Viewing Conditions	2	0.02749	0.02749	0.01374	0.17
Shapes*Sizes*Backgrounds* Viewing Conditions	6	0.24897	0.24897	0.04149	0.52
Error	192	15.18150	15.18150	0.07907	
Total	239	38.70470			

Source	P
Shapes	0.284
Sizes	0.000
Backgrounds	0.001
Viewing Conditions	0.000
Shapes*Sizes	0.431
Shapes*Backgrounds	0.422
Shapes*Viewing Conditions	0.393
Sizes*Backgrounds	0.657
Sizes*Viewing Conditions	0.873
Backgrounds*Viewing Conditions	0.038
Shapes*Sizes*Backgrounds	0.508
Shapes*Sizes*Viewing Conditions	0.024
Shapes*Backgrounds* Viewing Conditions	0.616
Sizes*Backgrounds*Viewing Conditions	0.841
Shapes*Sizes*Backgrounds* Viewing Conditions	0.789
Error	
Total	

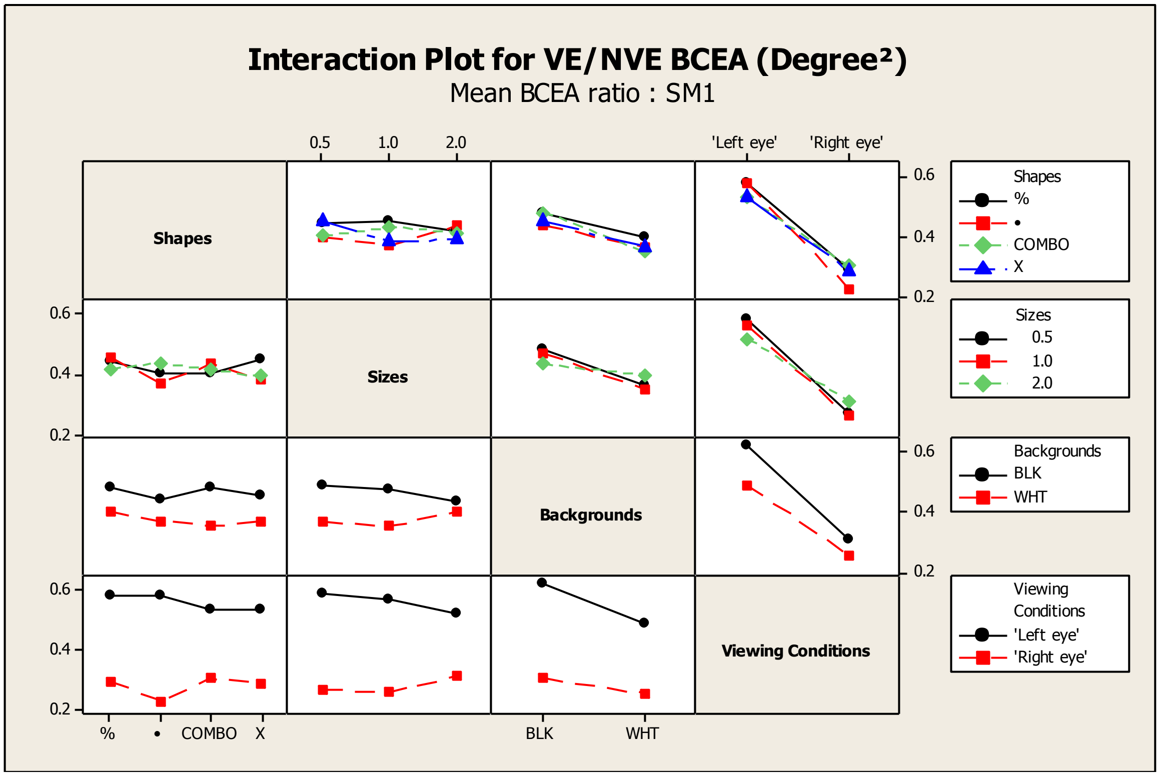
S = 0.281194   R-Sq = 60.78%   R-Sq(adj) = 51.17%

Figure 4.8 Interaction plot for BCEA ratio: Normal monkey (NM)



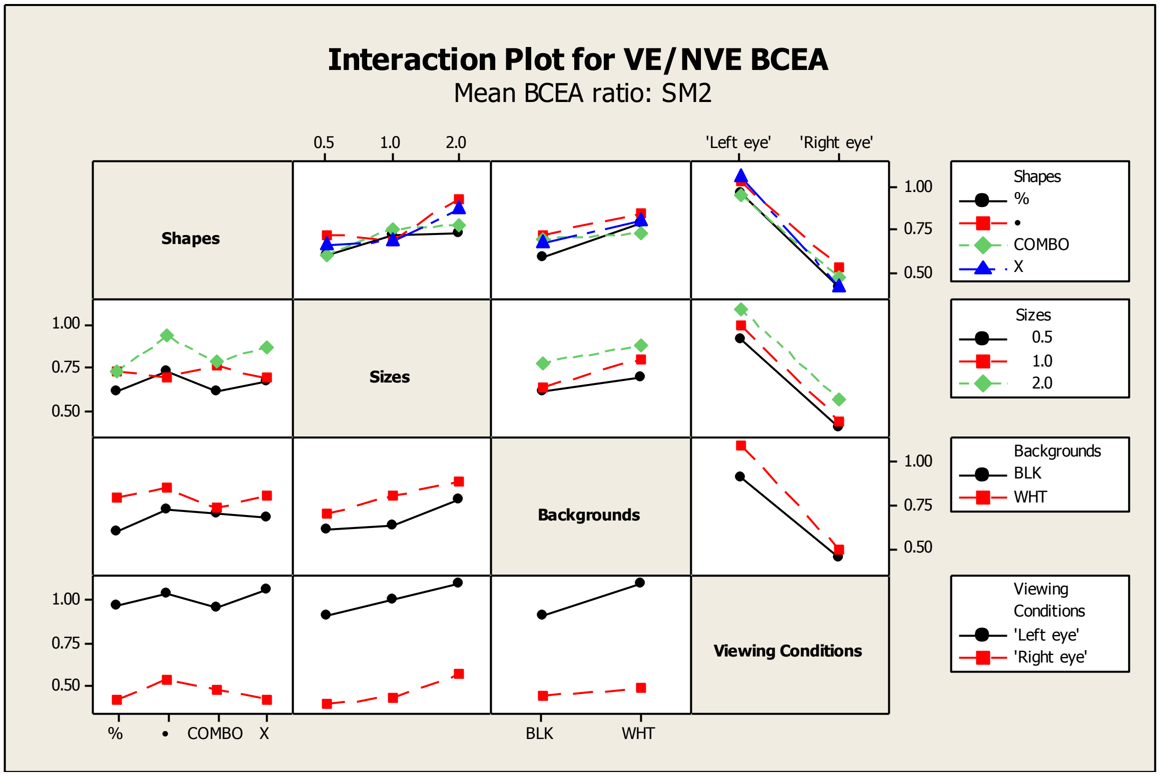
Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that there is non-significant interaction between all factors.

Figure 4.9 Interaction plot for BCEA ratio: Strabismic monkey (SM1)



Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that there is non-significant interaction between all factors.

Figure 4.10 Interaction plot for BCEA ratio: Strabismic monkey (SM2)



Parallel lines indicate no interaction whereas less parallel lines indicate more likely significant interactions. The above plot indicates that there is significant interaction between background and viewing conditions F (1,192) = 4.38, p = 0.038.

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