INVESTIGATING THE ROLE OF THE SUPERIOR COLLICULUS IN STRABISMUS

By

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DISSERTATION

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Dedication

This work is dedicated to my parents Guru Dutta Upadhyaya, Prabha Kumari Upadhyaya and my brother Sunil Upadhyaya (MD).

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Few people were behind the curtain during my academic journey in Houston but without their support and guidance I would not have even started my Ph.D. and would not have been writing this dissertation. Dr. Frishman, thank you for believing in me. I would also like to thank Dr. Bedell who taught to see through the numbers in the article and correlate it with the conclusion. I am very grateful to meet both of you.

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Abstract

Purpose: Strabismus is a global problem with prevalence of 3-5% in infants around the world. Besides eye misalignment, which leads to difficulty in perception of depth, other associated problems include fixation instability, unequal saccades in two eyes and nystagmus. Traditionally, extraocular muscles were thought to be responsible for ocular misalignment. However, recent data suggests that disrupted vergence circuits in the brain contributes towards strabismus. Before improving treatment modalities, a better understanding of basic neural mechanism and structures involved in strabismus is necessary. Superior Colliculus (SC) is an important visual-oculomotor structure that has been implicated in control of vergence in cats, monkeys, and humans. The goal of this dissertation was to understand the role of the SC in strabismus.

Methods: Prism-reared adult strabismic monkeys (n=6) were used. Scleral search coils were used to measure eye movements. Behavioral-study: Fixational data from five strabismic and one normal monkeys were collected to determine the relationship between fixational saccade amplitude and fixational stability. Electrical stimulation-study: Electrical stimulation (10-40µamp, 400Hz, 500msec) was applied to the SC of three strabismic monkeys to investigate the effect of SC activation on strabismus angle. Neurophysiology-study: Single-cell recording within the rostral SC of two strabismic monkeys was used to localize cells related to eye misalignment. Firing properties of these cells were also studied during 5° and 15° ipsilateral and contralateral saccades and during brief periods of target extinction (300-400ms). Muscimol inactivation-study: Muscimol, a GABA-a agonist inhibitory neurotransmitter, was used to pharmacologically inactivate

the SC in two strabismic monkeys to determine a causal role between SC activity and eye misalignment as well as fixation instability of strabismic animals.

Results: Behavioral-study: Amplitude of fixational saccades was larger in strabismic monkeys than that of normal monkeys ($p<0.001$; one-way-ANOVA). There was a nonlinear relationship between amplitude of fixational saccades and fixation instability such that fixational saccade amplitude saturates for larger fixation instability. Vergence BCEA (instability in depth) was poor in strabismic monkeys. Electrical stimulation-study: Electrical stimulation of SC produced significant changes in the horizontal misalignment that could be either convergent or divergent. Amplitude of electrically-evoked saccades was similar in the two eyes ($p>0.05$; paired t-test), but directions were different. Approximately 50% of the change in strabismic angle was due to saccade disconjugacy and the other 50% was due to disconjugate post-saccadic drift. Neurophysiological-study: Cells related to eye misalignment were found within the rSC. Some cells showed increased responses for small angles of exotropia (Convergence or near-response cells - NRC) while others showed increased response for larger angles of exotropia (Divergence or far-response cells - FRC). Neural sensitivity of these cells was similar to that of normal monkeys for both NRC (M1=3.2±1.6 spks/sec/°; M2= 3.0±2.6 spks/sec/°) and FRC (M1= -3.3±1.8 spks/sec/°; M2= -2.2±1.2 spks/sec/°), but firing thresholds were significantly shifted towards the habitual strabismus angle. These cells decrease their discharge during saccades and were not affected by the presence of a visual stimulus. A subset of cells also encoded the quick phases of nystagmus. Muscimol inactivation-study: We performed a total of 13 injections (11 muscimol; 2 saline control) within the right (n=6) or left (n=7) SC of two strabismic monkeys. There was significant

convergent (1.7°±1.3°) or divergent (-4.7°±3.2°) change due to inactivation of SC ($p<0.01$). The change in fixation instability was not significant.

Conclusion: This series of experiment shows that the SC is part of a disrupted vergence circuit that contributes to the development and maintenance of strabismus. Anatomical connections from the SC to the abducens nucleus and the lateral rectus muscle or from the SC to the supraoculomotor area and then to the oculomotor nucleus and medial rectus muscle are potential circuits by which the SC might influence strabismus angle.

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Chapter 1 - General Introduction

The overall goal of my dissertation is to examine the role of the Superior Colliculus in strabismus. This introductory chapter will provide background information on critical elements of this dissertation including binocular vision, strabismus, and its physiological properties and neural aspects, animal models of strabismus, fixational eye movements and neural mechanisms, and the SC. This chapter will end with an overall rationale for the project and introduce dissertation chapters.

1.1 Binocular vision and Strabismus

Binocular single vision (BSV) is the ability to fuse the images falling on the retina of the two eyes to see single objects. Binocular vision helps us to extract detail, something sometimes impossible for one eye alone. For example, in random dot stereograms, an embedded figure is invisible during monocular viewing (Blakemore and Julesz 1971). In primates who have fovea and frontally located eyes, eye movements are binocularly coordinated. The visual angle over which images can be separated and still be perceived as one is called panum's fusional area. Humans and non-human primates have highly specialized retina capable of perceiving a single mental image of two similar images formed on corresponding retinal points two eyes. In some cases when similar images in two eyes do not fall on corresponding retinal points, it can cause diplopia. If the two images are different but fall on the corresponding retinal points of two eyes, it causes visual confusion. Further, coordinated eye movements are necessary for maintaining precise ocular alignment to support and maintain fixation. In addition, binocular single

vision is obtained by coordinated effort of both eyes to place object or regard on or near fovea of each eye (Leigh and Zee 2015). Together sensory and motor processes help to attain this binocular single vision, including central foveal fixation in both eyes, accurate and steady oculomotor control, and sensory fusion. Humans have an obvious advantage by having two eyes; there is a larger binocular field of view, masking of optical and neural defects in one eye image (Mills 1998, Nelson-Quigg et al. 2000, Crabb and Viswanathan 2005), and normal binocular vision—all of which improves functional vision by binocular summation and stereopsis (Campbell and Green 1965). Stereopsis is the perception of relative distance, or the depth separation, between objects that occurs as a result of neural processing of the relative horizontal disparities. Horizontal disparities are present because the lateral separation of the eyes in the head provides each eye with a slightly disparate view of a given object. These horizontal retinal disparities produce stereoscopic depth.

Strabismus is a binocular vision disorder primarily characterized by misalignment of two eyes. In most cases, developmental strabismus is inward, followed by outward horizontal deviations, and sometimes combined with vertical deviation (hyper or hypo). It is one of the most common developmental disorders, affecting 2–5% of all human infants (Govindan et al. 2005, Mohney 2007). Accommodative and binocular vision disorders are more prevalent than other ocular diseases in children six months to 5 years of age (Scheiman 1996, Scheiman et al. 1996). In the United States alone, about 700,000 children are affected by strabismus (Friedman et al. 2009).

The primary characteristic of strabismus is that two eyes are not pointed in the same direction. Humans with strabismus may also be present with dissociated deviation, in addition to the primary horizontal deviation (Guyton 2000, Brodsky 2007). In dissociated vertical deviation (DVD), the non-fixating eye is deviated upward compared to the viewing eye (Guyton et al. 1998). Similarly, when horizontal deviation varies depending on viewing eye, it is called dissociated horizontal deviation (DHD). Latent nystagmus or fusion maldevelopment nystagmus is usually associated with developmental strabismus. This type of nystagmus is conjugate, jerky in nature, with quick phase directed towards viewing eye during monocular occlusion. Normally, when viewing with monocularly or binocularly, leftward and rightward image motions of an object can drive the smooth eye movement response symmetrically. Nasotemporal asymmetry during monocular tracking of a smoothly moving target is frequently seen in infantile strabismus. The optokinetic response motion in the temporal-to-nasal direction is robust (high gain), but nasal-to-temporal motion yields low gain OKN (i.e., is more saccadic) (Bedell et al. 1990). Stable visual fixation is necessary for normal foveal vision. Inability to fixate steadily on a fixed target is termed as fixation instability. Fixation instability in strabismus is due to not only large amplitude of fixational saccades and nystagmus but also increased drifts (Ciuffreda et al. 1979, Upadhyaya et al. 2017). Disconjugate eye movements are also seen in strabismic humans and monkeys (Kapoula et al. 1997, Ghasia et al. 2015). Strabismic people take advantage of position of eyes to switch eyes when fixating eccentric targets - this is called fixation switch behavior. Fixation-switch behavior follows the partial suppression of temporal retina and in esotropes follows nasal retinal suppression (Economides et al. 2012).

1.2 Animal models of strabismus

Research involving nonhuman primates (NHPs) has being crucial in many health-related issues. As humans and NHPs share many anatomical and physiological similarities, basic science research in NHPs is applicable to the human clinical population. Moreover, our ability to address diseases via applied research comes entirely from efforts in basic science research. In my opinion, translational research would be difficult without testing in NHPs. Monkeys also have specifically been a critical element in neuroscience research. NHP models are important for neuroscience research due to their resemblance of central and peripheral nervous system with humans. Examining healthy and diseased monkey models will help us to understand what has gone wrong in human disease conditions. In other words, monkeys are the bridge to the clinic.

In order to understand the neural aspect of strabismus, the best model we have available is NHPs. An animal model of strabismus can be created either by altering the extraocular muscles or by altering the sensory input. Surgical and pharmacological approaches are directed mostly to extraocular muscles. A particular muscle or a group of extraocular muscles are altered by resection or recession (both muscle surgical technique) or by injecting weakening agents, such as botulinum toxins. This changes the muscles normal torque to pull the eye ball. These methods may also change the neuromuscular junctions, which hampers the ability of the neural signals to communicate effectively with the muscles. Due to an imbalance between the push and pull forces in two eyes, binocular balance is disturbed. This disruption of binocular balance eventually disrupts the sensory input, which leads to strabismus. This method has been successfully used in kittens and

monkeys to understand abnormal brain areas (Hubel and Wiesel 1965, Crawford and von Noorden 1979, Kiorpes et al. 1996, Horton et al. 1999, Candy 2000, Economides et al. 2007).

Another method of inducing strabismus is by disrupting sensory fusion during the critical period of development. There are various ways to disrupt fusion, such as monocular or binocular lid suturing, daily alternating monocular occlusion (daily AMO), and an optical prism-viewing paradigm (Hubel and Wiesel 1965, Crawford et al. 1975, Smith et al. 1979, Crawford and von Noorden 1980, Mustari et al. 2001, Tusa et al. 2001, Tusa et al. 2002). Optical prism-rearing method closely mimics the development of sensory strabismus of humans without tampering with the oculomotor plant. In our lab, we use a lightweight helmet with a twenty-prism diopter base-in Fresnel prism placed in front of the right eye and a twenty-prism diopter base-down Fresnel prism in front of the left eye. The infant monkey wears this helmet 24hr from 1-2 days post-birth to 4 months of age as shown in Figure 1.2.1.

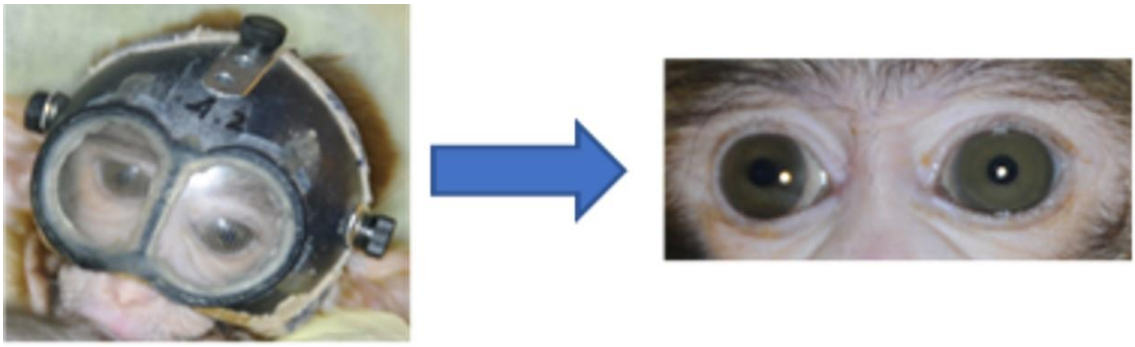

Figure 1.2.1 Monkey with light weight helmet and Fresnel prisms

Figure 1.2.1 It shows optical Prism reared infant monkey. Left photograph shows infant monkey wearing light helmet and viewing through Fresnel prism. Right photograph shows the same monkey with ~ 15-20° right eye exotropia after ~6 months of age.

Combination of vertical and horizontal Fresnel prism avoids any possibility of fusion. In this method, visual information (although de-correlated) is concurrently accessible via each eye, which reduces the possibility of an amblyogenic factor. Due to having prisms constantly in front of the eyes, the two foveas never get a chance to have similar retinal images, which prevents fusion during a critical period of stereopsis development. This binocular decorrelation strengthens suppressive signals, leading to disruption in the development of binocular vision—which ultimately leads to strabismus (Boothe et al. 1985, Crawford et al. 1996).

Monkey model of strabismus replicates many eye alignment and eye movement properties seen in strabismic humans. One of the biggest features of animal models is that, while looking at the primary gaze, these animals show a significant amount of ocular misalignment, similar to strabismic humans (Economides et al. 2007). Animal models created by primarily sensory insult could show DVD, DHD, or both (Das et al. 2005). Nasotemporal asymmetry has been observed in strabismic monkeys as well (Mustari et al. 2001, Tusa et al. 2002, Richards et al. 2008, Tychsen et al. 2008, Joshi et al. 2017). In addition, disconjugate eye movements are prevalent in animal models (Fu et al. 2007, Walton et al. 2014). This strabismic model has also simulated A or V pattern deviation, fixation instability in viewing as well as non-viewing eyes, and alternating fixation or fixation-switch behavior similar to that of many strabismic humans (Das and Mustari 2007, Economides et al. 2007, Das 2009, Agaoglu et al. 2014).

1.3 Neural mechanism of strabismus

In order to precisely align two eyes on to the target, the brain should send exact and synchronized neural signals to extraocular muscles. As horizontal relative disparity is behind the perception of stereopsis, this disparity also drives vergence. Vergence could also be driven by blur stimulus. Improper vergence command sent to extraocular muscles make normal ocular alignment impossible (Kenyon et al. 1980, Kenyon et al. 1981, Tychsen 2007).

We have recently accumulated evidence that neural structures involved in eye movements show different activity in normal and strabismic monkeys, implicating the brain in strabismus. Although no existing longitudinal studies show how the functional properties of oculomotor structures changed as the strabismus was induced, studies from adult strabismus monkeys (that resembles the adult stage of strabismus) have provided convincing evidence of the role of the brain.

Comparing the neural activity recorded from oculomotor and abducens nuclei of normal and strabismic monkeys revealed the first evidence indicating a neural correlate to strabismus (Das and Mustari 2007, Joshi and Das 2011, Walton et al. 2015). The next evidence came from the supra oculomotor area (SOA) or horizontal vergence center (Das 2011). It is known that there is a monosynaptic connection between medial rectus motor neurons and the supraoculomotor area (Zhang et al. 1992). When comparing the activity of majority convergence neurons and a few divergence neurons found in the SOA of normal monkeys, with similar near response cells and far response cells found in the SOA of strabismic monkeys, population neuronal sensitivity was significantly lower in

strabismic monkeys. Similarly, the threshold to firing for vergence was also shifted toward the strabismic state (Mays 1984, Judge and Cumming 1986, Das 2012). The third line of evidence comes from the cerebellum - anatomical connection between the cerebellar vermis to contralateral SOA and feedback connection from SOA to cerebellar nuclei—provides the basis to investigate cerebellum in strabismus (Noda et al. 1990, May et al. 1992). Joshi and Das showed that inactivation of the caudal fastigial nucleus (cFN) in strabismic monkeys produced divergent change in strabismus angle, whereas in normal monkeys, it is responsible for convergence, showing evidence that cFN is also a part of disrupted vergence circuit in strabismus (Gamlin et al. 1996, Joshi and Das 2013). Similarly, inactivation of posterior interposed nucleus (PIN) in strabismic monkeys showed convergent change in misalignment state (Zhang and Gamlin 1998, Joshi and Das 2013). Incidence of latent and manifest deviation has also been seen in humans with cerebellar degenerations (Versino et al. 1996, Kheradmand and Zee 2011, Ghasia et al. 2015). The fourth line of evidence comes from the brainstem. When the paramedian pontine reticular formation (PPRF - critical for producing horizontal saccades in normal monkeys) was electrically stimulated in strabismic monkeys, it evoked disconjugate saccades in various directions, (Fuchs et al. 1985, Walton et al. 2013). Neurons collected from excitatory burst neurons (EBN) in strabismic monkeys were also not purely horizontal, in fact out of 60 neurons collected, 12 or 20% preferred vertical saccades (Walton and Mustari 2015).

Higher cortical areas are also compromised in strabismus. Substantial loss of binocular cells in visual cortex (V1 and V2) of strabismic monkeys has been shown (Crawford and von Noorden 1979, Crawford et al. 1984). There was an increase in disparity tuning

width and interocular suppression in V1 and V2 (Smith et al. 1997, Mori et al. 2002, Zhang et al. 2005, Bi et al. 2011). Moreover, suppression of local metabolic activity has been seen in the striate cortex when measured using cytochrome oxidase in strabismic monkeys (Horton et al. 1999). Middle temporal (MT) and medial superior temporal (MST) showed reduced binocular response in strabismic monkeys (Kiorpes et al. 1996).

1.4 Fixational eye movements

Fixational eye movements, specifically microsaccades or fixational saccades, in strabismus constitute another portion of my dissertation and this section provides background information on this type of eye movements. Fixational eye movements are critical to normal vision. Without fixational eye movements, the visual scene will fade away due to neural adaptation to stimuli. On the other hand, exaggerated eye movements lead to unstable and blurred vision (Martinez-Conde 2006). Foveate vertebrate eyes are never still even when fixating at a stationary target (Martinez-Conde et al. 2008, Martinez-Conde and Macknik 2008). Fixational eye movements include microsaccades or fixational saccades, drifts, and tremors. Tremors are very small amplitude and high frequency eye movement usually in the range of the recording system's noise. Drift are usually slow and once presumed to be randomly moving eye movement. Microsaccades seem the most important fixational eye movement in foveate species, whereas they are scarce or absent in afoveate species (i.e., rabbits, frogs, and goldfish). Broadly microsaccades serves as a corrective movement to bring the drifting image near to the fovea. (Cornsweet 1956). These miniature movements also help in enhancing spatial resolution (Engbert and Kliegl 2004, Engbert 2006, Engbert and Mergenthaler 2006).

Fixational saccades or microsaccades are the fastest and largest of the three types of fixational eye movements. Fixational saccades can carry the retinal image across several photoreceptors. Microsaccade velocities are related to microsaccade amplitudes, following the "main sequence" in both macaques and humans (Zuber et al. 1965, Martinez-Conde et al. 2002, Engbert and Mergenthaler 2006, Martinez-Conde 2006, Martinez-Conde et al. 2006). Microsaccades are generally involuntary and conjugate (Ditchburn and Ginsborg 1953, Krauskopf et al. 1960). Visual perception thresholds are elevated during microsaccades (Herrington et al. 2009). Microsaccade rates can be reduced intentionally during specific tasks. Theoretically, voluntary saccades can be as small in amplitude as microsaccades. Therefore it is difficult to differentiate between voluntary small amplitude saccades and microsaccades (Otero-Millan et al. 2008). Intuitively, the term "fixational saccade" makes more sense than "microsaccade" because irrespective of amplitude, these are saccades occurring during fixation. These fixational saccades change the retinal neural responses by displacing the visual stimulus across many receptive fields (Bair and O'Keefe 1998, Kagan et al. 2008). Bursting of neurons in the lateral geniculate nucleus (LGN), and primary visual cortex (V1) immediately after the microsaccade has being found in awake monkeys suggesting enhancement of visual perception by microsaccades (Martinez-Conde et al. 2000, Martinez-Conde et al. 2002). Microsaccades rates can be modulated by attention (Hafed and Clark 2002). There is a transient decrease in the microsaccade rate ~100–200 ms after cue onset, followed by momentary enhancement ~300–400 ms after cue onset (Laubrock et al. 2005, Valsecchi and Turatto 2007, Valsecchi and Turatto 2009).

Van Gisbergen and colleagues found motor neurons in the primate abducens nucleus and burst neurons in the nearby PPRF which were active during saccades and microsaccades (Van Gisbergen et al. 1981). Recently, Hafed and colleagues have found individual neurons in rSC of normal monkey which has preferred small amplitude and direction vectors (Hafed et al. 2009). Furthermore, the data showed a continuous representation of saccade amplitude from large to small throughout the SC, suggesting a microsaccade-saccade continuum (Zuber et al. 1965, Otero-Millan et al. 2008). Inactivation of the rSC reduces microsaccade rates, backing a causal role of rSC in the generation of microsaccade (Hafed et al. 2009). All these results suggest that neural circuitry for saccades and microsaccades is the same (Van Gisbergen et al. 1981, Otero-Millan et al. 2008).

1.5 Superior colliculus

The SC is an evolutionary preserved structure located on the roof of the vertebrate midbrain. SC is a vital structure in oculomotor neural circuitry which helps in shifting of gaze (Goldberg and Wurtz 1972). The SC is a laminar structure delineated by alternating strata of fibers. Broadly, the SC consists of seven layers: stratum zonale, stratum griseum superficiale(SGS), stratum opticum, stratum griseum intermediate (lower sublamina and upper sublamina), stratum album intermediale, and stratum griseum profundum from dorsal to ventral (May 2006). In primates, SC is broadly divided into superficial, intermediate, and deep layers. The superficial layers receive direct projections from both retinal ganglion cells and the striate cortex and have neurons that exhibit various

responses to salient visual stimuli (Pollack and Hickey 1979). Each SC represents contralateral visual field with larger representation for foveal and parafoveal area than far peripheral area (Hafed and Chen 2016). Intermediate and deep layers receive input from other areas. The diverse anatomical connections of these layers, make neurons respond during planning and execution of orienting movements (Gandhi and Katnani 2011). SC provides interconnection between many sensory, motor, and cognitive circuits. SC acts as a relay station where it receives sensory information from cortex and directs brainstem activity (Krauzlis et al. 2013). Its primary function is to direct the sensory structures of the head toward objects of interest. The SC has evolved to provide the brain with the location of targets and threats in the peripheral world. As noted, to fulfill this requirement, the colliculus receives retinal input and a variety of input from the subcortical and cortical sensory structure. Visual information is concentrated in the SGS, which acts as part of the extrageniculate sensory system. The SGS also supplies visual target information to deeper layers, where it correlates with inputs from other modalities. In addition, the deep layers are provided with crucial information needed for target selection by motor systems, such as the basal ganglia and the cerebellum. Finally, other regions of the brain that also redirect the gaze, such as the frontal eye field, supply the deep SC with inputs. To process this information, the colliculus has many more cell types than most nuclei and a complex system of intralaminar, interlaminar, and intertectal connections. The main targets for collicular information are the brainstem structures in which the premotor circuitry for gaze control resides. However, this collicular information is of value to many regions of the brain for other functions, such as attention.

1.6 Rationale for project

The basic oculomotor mechanism underlying disruption in binocular alignment and binocular synchronization of eye movements in strabismus is not clear. Disruption of binocular vision during the critical period of visual development leads to a cascade of events resulting in eye misalignment in humans and monkey models. Still, little is known about structures, circuitry, and properties of neurons in visual and oculomotor areas in strabismic monkeys. Therefore, basic science research on the understanding of neural structures and mechanisms involved in strabismus is necessary to gain a better understanding of the disorder. Further, discovering the neural basis of strabismus is critical to developing better treatments in the future. There is now growing evidence that suggests that disruption within a vergence circuit could lead to strabismus in monkeys – described earlier in this chapter (Joshi and Das 2011, Das 2012, Walton et al. 2013, Walton and Mustari 2015, Walton et al. 2015, Economides et al. 2016, Fleuriet et al. 2016, Economides et al. 2017, Pallus et al. 2017, Upadhyaya et al. 2017, Walton et al. 2017).

The maintenance of both static and dynamic ocular alignment is under long-term adaptive control to ensure that, with every change of gaze, the lines of sight of both eyes are promptly brought to the fixation target and kept there. Fast vergence movement brings the two eyes near to the target of interest, but to tune fine binocular alignment a slow vergence circuit may be necessary. Disruption in slow vergence circuit has been suggested as a neural basis for eye misalignment. Different neurophysiological studies, electrical stimulation studies, and pharmacological inactivation studies have clearly

shown that motor neurons, cerebellum, brainstem, and SOA are involved in strabismus. It has been suggested that vergence circuits involving projection from cerebellar nuclei (fastigial nucleus and posterior interposed nucleus) to the midbrain SOA, and thereafter to the oculomotor nucleus (OMN) and medial rectus (MR) muscle, are partially responsible for maintaining horizontal eye deviation in strabismus (Walton and Mays 2003, Joshi and Das 2011, Das 2012, Joshi and Das 2013, Walton and Mustari 2015, Walton et al. 2015). We now know that motor neuron signals are altered in strabismic animals, but motor neurons simply execute the command—they do not compute these signals. Rather, these signals are transmitted to them by higher brain structures.

In order to investigate the neural substrate for misalignment, we took an approach to investigate midbrain structures, particularly SC, in light of the following evidence suggesting the role of SC in vergence. Bohlen et al. have shown anatomical projections from the SC to the abducens nucleus via the central mesencephalic reticular formation (cMRF) (Bohlen et al. 2016). SC also anatomically connects with OMN through the cMRF and SOA in monkeys. Early studies in the cat (Jiang H 1996) and recent studies in normal monkeys have found convergent and divergent neurons in the rSC, suggesting its role in vergence eye movement (Van Horn et al. 2013). Moreover, a case report of a 30-year-old human with bilateral superior colliculus lesions showed accommodation and convergence palsy (Jiang H 1996, Ohtsuka et al. 2002). Suzuki and colleagues have shown changes in accommodation and vergence due to electrical stimulation and pharmacological inactivation of the rSC of cat (Suzuki et al. 2004, Suzuki 2007). In regard to monkeys, some studies were unable to elicit vergence response by electrical stimulation in the SC (Billitz 1997). On the other hand, it has been shown that electrical

stimulation in the rostral part of the SC of normal monkeys caused disruption during vergence or combined saccade-vergence tasks (Chaturvedi and van Gisbergen 1999, Chaturvedi and Van Gisbergen 2000). In another study involving ablation of rSC in monkeys, problems with both disparity processing and eye alignment were observed (Lawler and Cowey 1986). Few other studies found a population of vergence-related neurons located 4–5 mm dorsal and 2–3mm lateral to the OMN and suggested that these neurons could be within the rSC (Judge and Cumming 1986), although the location of this area was not verified.

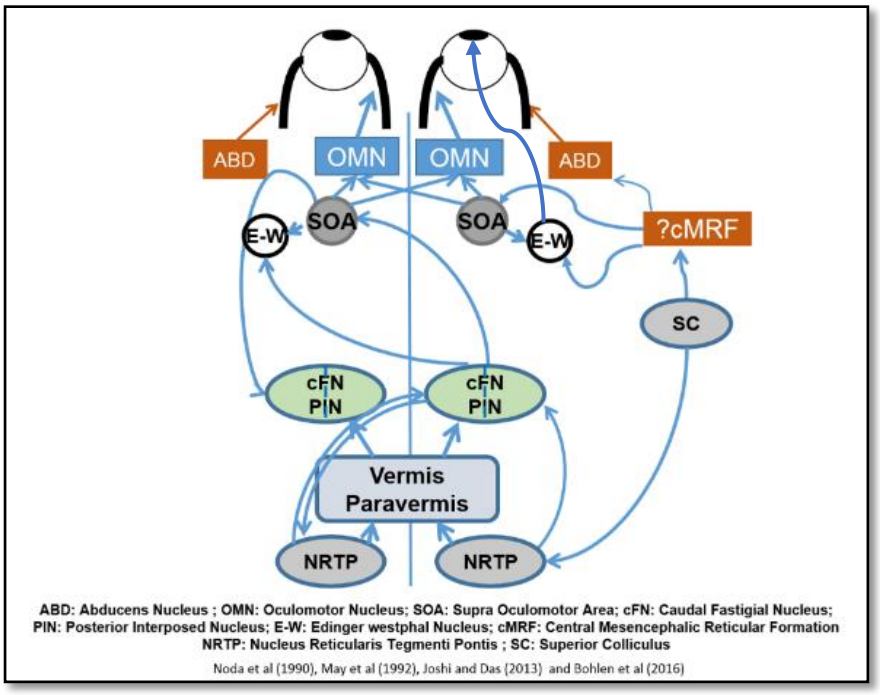

Figure 1.6.1 Hypothetical vergence pathway

Figure 1.6.1 This hypothetical vergence circuit shows all the known brain structures found to be involved in strabismus and vergence eye movements

Moreover, anatomically, the SC also receives depth information from the lateral intraparietal cortex (LIP) and frontal eye field (FEF) (Mays et al. 1986, May et al. 1990). It is also connected with the cerebellum (cFN and PIN). A major efferent target of the SC is cMRF. Paul May and colleagues have shown that cMRF is indeed connected with abducens nucleus, SOA, and E-W (May et al. 2015, Bohlen et al. 2016). Since the abducens nucleus projects to lateral rectus and medial rectus motor neuron projects to medial rectus, the SC signals could hypothetically innervate (via separate pathways) both medial and lateral rectus muscles. It is figuratively shown in figure 2. Therefore, it is worthwhile to study SC in relation to its role in horizontal strabismus.

The other motivation to study the superior colliculus in strabismus is, the causal role of the rSC in generation of microsaccades or fixational saccades (Hafed et al. 2009). Fixation instability is one of the problems in strabismus partly due to large amplitude of fixational saccades or microsaccades. Thus, it is possible that the fixational saccades and quick and/or slow phases of nystagmus observed in strabismic monkeys could be encoded within the rSC.

Figure 3 shows the flow of experiments. Specifically, this project will answer the following specific questions 1) How does electrical microstimulation of the SC in strabismic monkeys influence strabismus angle? 2) Is increased fixation instability in

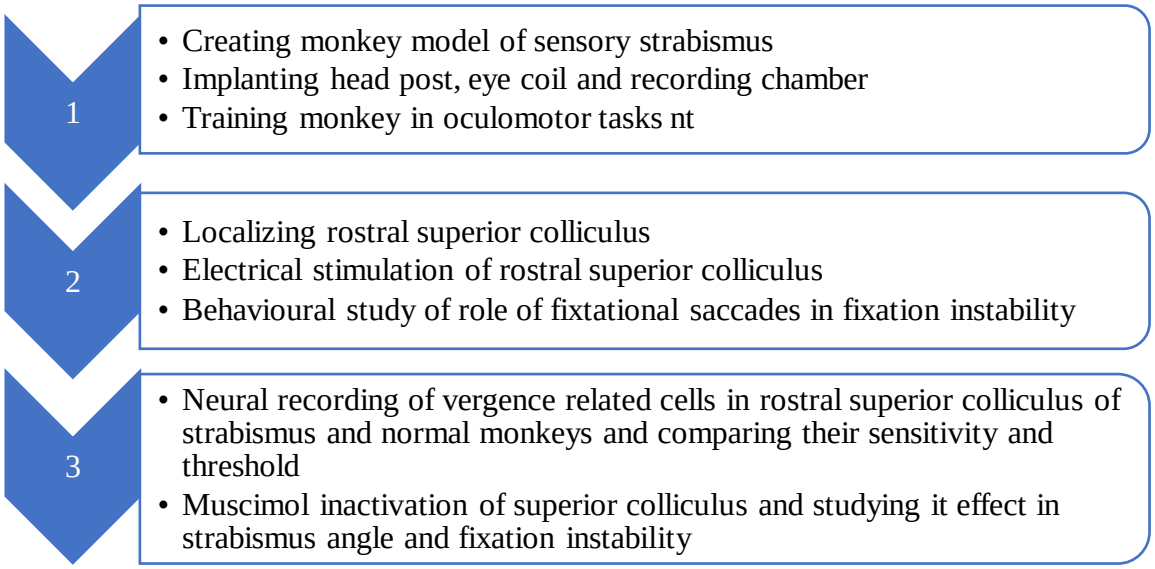

Figure 1.6.2 Experimental flow chart

Figure 1.6.2 This flow chart shows different steps of the complete project

strabismic monkeys due to increased amplitude or frequency of fixational saccades that are generated in the SC? 3) Are there neurons related to eye misalignment in the SC similar to those observed within the SOA in strabismic monkeys, and does the sensitivity and threshold of this cell population in strabismic monkeys differ from population sensitivity of convergent and divergent cells in the SC of normal monkeys? 4) Does inactivation of the SC affect strabismus angle and fixation stability in strabismic monkeys?

The subsequent chapters provide the details of each of the question proposed above.

Chapter 2: Fixational saccades and their relationship to fixation instability in strabismic monkeys.

Fixation stability in strabismic monkeys and humans is poor. A quantitative metric to measure fixation stability is the bivariate contour ellipse area (BCEA). It has been shown that BCEA is influenced by target parameters such as size, shape, and viewing condition. This chapter evaluates the contribution of fixational saccades toward fixation instability in strabismic monkeys as they are generated in the SC. It also examines the effect of target parameters in amplitude and frequency of fixational saccades because fixation instability is shown to be affected by target parameters.

Chapter 3: Electrical stimulation of superior colliculus in strabismic monkeys.

It has been previously shown that a vergence eye movement circuit may be involved in maintaining horizontal eye deviation in strabismus. Recent studies in normal monkeys have shown convergence and divergence neurons in the superior colliculus. Anatomical

studies have also shown a connection between SC and the supraoculomotor area, cerebellum, Edinger Westphal nucleus, and abducens nucleus, directly or indirectly. This study will use electrical stimulation methods (10 μA–40 μA, 400 Hz, 500 ms) to determine whether superior colliculus may also be involved in eye misalignment in a monkey model of strabismus. If SC is not involved in strabismus, then we would expect no change in strabismus angle during electrical stimulation of the SC.

Chapter 4: Properties of cells associated with strabismus angle in rostral superior colliculus of strabismic monkey.

Studies in normal monkeys and cats have suggested a role for the superior colliculus in vergence. Humans with bilateral superior colliculus ablation have shown convergence insufficiency. We have used single unit recording methods to study the properties of cells associated with eye misalignment in the SC of strabismic animals. The fourth chapter comprehensively describes response properties including population sensitivity and threshold of near response cells and far response cells in strabismic monkeys.

Chapter 5: Effect of muscimol inactivation of superior colliculus on strabismus angle and fixation instability in a monkey model of strabismus.

Studies in strabismic and amblyopic humans and monkeys have identified increased fixation instability that is dominated by drifting eye movement. Our previous study showed that small fixation instability is positively correlated with the amplitude of fixational saccades. Current literature suggests that optimal fixation location and fixational saccades behavior are governed by balance activity across the right and left superior colliculi. This study investigates the role of the SC and downstream neural

circuits in fixation instability and misalignment by using muscimol to inactivate the SC reversibly.

Chapter 6: Discussion

This chapter discuss all the experiments in the context of what we have learned from studying SC in strabismic monkeys. I also discuss limitations of above studies and propose ideas for future studies.

Chapter 2 - Fixational saccades and their relation to fixation instability in strabismic monkeys

2.1 Introduction

Our eyes are continuously moving even when consciously fixating a stationary object (Ditchburn and Ginsborg 1953, Martinez-Conde 2006, Rucci and Poletti 2015). Such fixational eye movements can be broadly categorized into two components - quick, rapid flicks of the eye that are termed microsaccades or fixational saccades and a much slower drift component that occurs in between fixational saccades (Martinez-Conde et al. 2009, Poletti et al. 2010, Pansell et al. 2011, Rucci and Victor 2015). A third kind of fixational eye movement, tremor, is too small to be physiologically relevant (Martinez-Conde et al. 2004). Fixational eye movements are necessary to prevent retinal fading and also to help gather information about features of the object of regard (Ditchburn et al. 1959, Ko et al. 2010, Rucci and Victor 2015). On the other hand, excessive fixational eye movements lead to fixation instability and are detrimental to visual function, e.g., reading (Amore et al. 2013). Among fixational eye movements, we know the most about fixational saccades (Rolfs 2009, Poletti and Rucci 2016, Martinez-Conde and Macknik 2017). These saccadic movements are also called microsaccades but we prefer the term fixational saccades since the term microsaccades implies an arbitrary amplitude limit. These movements are basically tiny saccades that are generated by the same neural circuitry as that of the large saccade (Hafed et al. 2009, Hafed and Krauzlis 2012). Fixational saccades follow the same main-sequence relationships as larger saccades and may be

used to reorient gaze between closely spaced objects just like a large saccade is used to reorient gaze between widely spaced objects (Hafed et al. 2009, Ko et al. 2010). Although the object of regard is already on the fovea, fixational saccades are subject to adaptive control in the face of consistent retinal error, similar to large saccades (Havermann et al. 2014).

Instability in fixation, i.e., larger than normal fixational eye movements, is often a hallmark of many forms of visual system pathology, such as in strabismus and amblyopia, macular degeneration, etc (Ciuffreda et al. 1979, Bellmann et al. 2004, Gonzalez et al. 2012, Kumar and Chung 2014). One of the many quantitative measures used to quantify fixation instability is the Bivariate Contour Ellipse Area (BCEA), which is a metric that measures area over which eye position is dispersed during fixation(Timberlake et al. 2005). Since this is a measure of dispersion of eye position, it takes into account both fixational saccade and drift components. Using the BCEA metric, we have recently shown that strabismic monkeys have significantly greater fixation instability compared to normal monkeys. We also showed that fixation instability was influenced by target parameters such as size and shape of the target, in both normal and strabismic monkeys (Pirdankar and Das 2016). The goal of the current study was to further investigate the components that contribute to larger fixation instability in strabismus. Specifically, we were interested in investigating the contribution of fixational saccades towards fixation instability in strabismic monkeys, while also investigating the influence of target parameters such as target shape and size on fixational saccades. Some of these data have appeared before in abstract form and in our previous publication on

fixation instability in strabismic monkeys (Upadhyaya et al. ARVO 2017 e-abstract 3411; Pullela et al ARVO 2017 e-abstract 751) (Pirdankar and Das 2016).

2.2 Methods

2.2.1 Subjects, Rearing paradigms and Surgical Procedures

We examined fixational instability and fixational saccades in seven adult rhesus macaque (macaca mulatta) monkeys of which two had normal ocular alignment (NM, PM) and the rest exhibited ocular misalignment as a consequence of either optical prism rearing (SM1, SM4, SM5) or daily alternating monocular occlusion (AMO – SM2, SM3). In SM1-5, strabismus was induced in infancy by disrupting binocular vision starting from day 1 after birth for the first four months of life. In the optical prism paradigm, the infant monkeys wore lightweight helmets fitted with either a base-in or base-out prism in front of one eye and a base-up or base-down in front of the other eye till they were four months of age after which they were allowed unrestricted viewing (Smith et al. 1979, Crawford and von Noorden 1980). The AMO rearing consisted of the infant animals wearing an opaque contact lens in front of one eye while the fellow eye was unrestricted. The opaque lens was alternated between each eye every day till the age of four months (Das 2009). Both rearing paradigms disrupt binocular vision during the critical period for visual development thus resulting in strabismus (Tusa et al. 2002). One animal (PM) was also specially reared using optical prism methods but did not develop ocular misalignment. However, this animal developed a consistent nystagmus and therefore could not be

categorized as either normal or strabismic. Data from this animal are presented as a separate entity.

When the animals were ~4 years of age, they underwent a surgical procedure carried out under aseptic conditions with isoflurane anesthesia (1.25%-2.5%) to implant a head stabilization post (Adams et al. 2007). Later in a second surgery, a scleral search coil was implanted in one eye using the technique of Judge and colleagues and in a third surgery, a scleral search coil was implanted in the fellow eye (Judge et al. 1980). All procedures were performed according to National Institute of Health 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 (IACUC) at the University of Houston.

NM and SM1-3 were previously part of a published study from our lab that examined influence of target parameters on fixation instability in normal and strabismic monkeys (Pirdankar and Das 2016). In that study, we examined fixation instability using the BCEA metric. A subset of the fixation data that were collected for the previous study from NM and SM1-3 are now analyzed to quantify fixational saccades. In addition, we have collected and analyzed new data from SM4, SM5 and PM.

2.2.2 Experimental Paradigms, Data acquisition and analysis

Movements of both eyes were recorded as the monkeys, trained previously on a variety of oculomotor tasks, fixated a stationary target back-projected onto a tangent screen at a

distance of 114cm using a DepthQ LCD projector (Lightspeed Design Inc., Bellevue WA, USA) running at 120-Hz frame rate. Eye movements were calibrated as the monkey monocularly viewed targets at ±15° horizontally and vertically. During experiments, each fixation trial was developed from one of 12 different conditions – two target shapes (optotype – '%' sign or disk), two target sizes (0.5° or 2°) and three viewing conditions (monocular right eye or left eye viewing and binocular viewing). Our target choices for this study were motivated from our recently published study that showed these target parameters produced maximal differences in fixation instability in both normal and strabismic monkeys (Thaler et al. 2013, Pirdankar and Das 2016). An additional motivation for using the '%' optotype was that this target is typically used in our lab for training the animal on oculomotor tasks. All the fixation targets were white (luminance 470 cd/m^2) on a black background (luminance 0.5 cd/m^2). Monocular and binocular viewing was facilitated by occluding an eye using liquid crystal shutter goggles (Citizen Fine devices, Nagano, Japan) under computer control. Five fixation trials (60s duration each) were obtained for each combination of target shape, size and viewing conditions. Fixation trails were inter-leaved with saccade and smooth pursuit tasks so as to avoid after-images and adaptation across target parameters.

Eye movements of both eyes of all animals were recorded using the magnetic search coil technique (Judge et al. 1980), except in SM3 who had a functional scleral coil in only the left eye. Advantages of using the scleral search coil technique include high resolution and precision and the ability to measure movements of both eyes equally well during both monocular and binocular viewing. Eye movement data were processed with anti-aliasing filters at 400Hz before sampling at 2.79kHz with 12-bit precision (AlphaLab SNR

system; Alpha-Omega Engineering, Nazareth, Israel). All eye movement data were additionally calibrated offline and filtered using a software finite impulse response (FIR) low-pass filter with a pass-band of 0-80Hz. Epochs of fixation were selected by visual inspection of data. Saccades, blinks and any sections of data that the monkey was not looking at the target (readily apparent on visual inspection of data) were not a part of selected fixation periods. Nystagmus (e.g., latent nystagmus), drifts and fixational saccades would not be removed by our selection method.

We used an unsupervised clustering algorithm published by Otero-Millan and colleagues (Otero-Millan et al. 2014) to detect fixational saccades (MATLAB from Mathworks, Natick, MA, USA). Briefly, this method detected saccade-like events using a velocity and acceleration threshold of 8°/s and 100°/s^2 respectively and organized these events into clusters by principal component analysis. In a minor deviation from the published method, any detected event smaller than 0.05° was removed and considered to be noise. Also saccade events from the viewing and non-viewing eyes of all animals were independently detected by the clustering program during both monocular and binocular viewing conditions in the strabismic monkeys (SM1-5) and during monocular viewing in the normally aligned monkeys (NM, PM). In conditions when the normally aligned monkeys viewed binocularly, the clustering program for detection of saccade events used an average of right and left eye data. Once fixational saccades were identified, radial amplitude, direction and peak velocity of the saccade was calculated. Frequency of fixational saccades in each trial was calculated as the number of fixational saccades per second over the length of the trial. BCEA, a metric that quantifies the area over which eye positions are dispersed during attempted fixation was also calculated as an overall

measure of fixation instability. A smaller value of BCEA indicates lower fixation instability. The BCEA encompassing 68.2% of fixation points was calculated using the following equation

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

σx = Standard deviation of horizontal eye position,

σy = standard deviation of vertical eye position,

2.291 is the chi-squared value (2df) corresponding to a probability of 0.68,

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

MATLAB (Mathworks, Natick, MA, USA) and Sigmaplot 12.0 (Systat, Inc; San Jose, USA) were used for statistical analysis. A Kruskal-Wallis ANOVA on ranks test was used to test differences in amplitude and frequency of fixational saccades between the strabismic and normal monkeys and a three-way ANOVA followed by Holm-Sidak post-hoc testing was used to test the main effect of target shape, target size and viewing condition on fixational saccades.

2.3 Results

2.3.1 Properties of Strabismus

Table 2.3.1 summarizes the alignment properties of the animals used in this study. Along with ocular misalignment the strabismic monkeys also showed nystagmus and dissociated deviations as indicated in the table. PM showed no misalignment but a robust downbeat

nystagmus was present. Table 2.3.1 also shows high frequency thresholds obtained during monocular contrast sensitivity testing using a psychophysical method, that we have described previously(Agaoglu et al. 2015), in three monkeys and the mean fixation duration within each 60sec trial in each monkey.

In Figure 2.3.1, we show eye position traces of the left eye over a 10s duration while the normal monkey and one of the strabismic monkeys (NM and SM5) viewed a 2° disk-shaped target with their left eye (right eye occluded). Stable fixation was observed in the viewing eye of the normal monkey with fixational saccades on the order of approximately 0.5° in amplitude and small amplitude of drifts. The strabismic monkeys showed significant instability due to large fixational saccades, nystagmus and increased drifts. In each plot, fixational saccades as identified by the automated clustering method are marked.

Table 2.3 1 Properties of strabismus in experimental animals

Monkeys	Age (Years)	Strabismus Angle (deg) RE View	LE View	Refractive Error (Diopter) RE	LE	Strabismus Properties	High Frequency Cutoff in Contrast Sensitivity Function (cyc/deg) RE	LE	Fixation Times(s) (Mean ± SD)
NM	8	-	-	+0.75	+1.25	-	15	12	31.4±8.5
PM	6.5	-	-	+2	+1.75	N	-	-	34.2±10.2
SM1	5	20°-25° XT	10° XT	Plano	-1.50	DVD, DHD, N	17	10	44.7±5.9
SM2	8	10° XT	10° XT	+2.75	+4.75	DVD, N	-	-	32.2±10.7
SM3	9	15° XT	15° XT	+8.00	+4.25	DVD, N	-	-	38.1±8.9
SM4	7	5° ET-15°XT	1°ET-12°XT	+4.50	+0.75	DHD, N	8	15	45.3±7.2
SM5	6	25° XT	25° XT	Plano	Plano	DVD, N	16	11	42.8±7.0

Legend: XT – exotropia; ET – esotropia; RE – Right eye; LE – Left eye; DVD – Dissociated Vertical Deviation; DHD – Dissociated Horizontal Deviation; N – nystagmus. Contrast sensitivity testing was only performed in animals NM, SM1, SM4 and SM5.

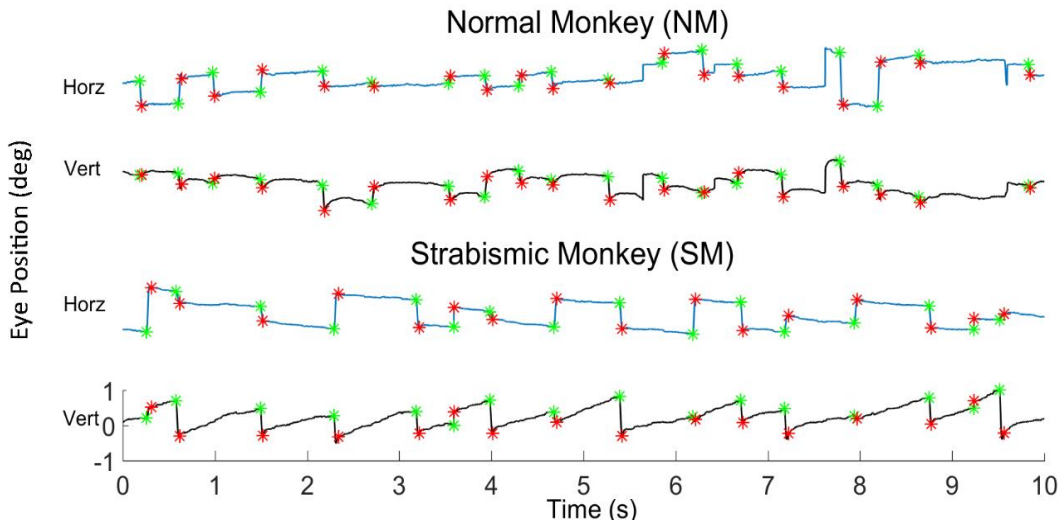

Figure2.3.1 Plot showing position of eyes

Figure 2.3.1 Raw data showing horizontal (blue) and vertical (black) left eye position of a normal monkey and a strabismic monkey (SM5) while fixating a 2° disk target with their left eye. Green and red asterisks denote start and end of saccade events identified by the automated clustering algorithm that include quick phases of nystagmus and other fixational saccades. Positive values indicate rightward and upward eye positions.

2.3.2 Fixational Saccade Amplitude and Frequency

In all, we analyzed ~6000 fixational saccades from NM, ~47000 fixational saccades from SM1-5 and ~11000 fixational saccades from PM. Fig 2.3.1 shows the distribution of fixational saccade radial amplitudes in the normal and one of the strabismic monkeys (SM2). Overall, the pattern of amplitude distribution was similar in normal and strabismic monkeys. The median amplitude of fixational saccades in the normal monkey was 0.33° and is similar to that previously published (Hafed et al. 2009). The median amplitude of fixational saccades in the strabismic monkey was 0.74° (dotted line) which is significantly greater compared to that of the normal monkey. Figs 2.3.3A and 2.3.3B shows a box-plot summary of fixational saccade amplitude across all targets and monocular/binocular viewing conditions in the viewing and non-viewing eye of each animal. Note that for this and other plots there was no data included for the non-viewing eye during binocular viewing in normally aligned animals NM and PM. In all strabismic animals, the viewing eye during binocular viewing is the eye that is fixating the target and the non-viewing eye is the deviated eye. Median amplitudes of fixational saccades from the viewing eye of three strabismic monkeys were larger than that of the normal monkey (Kruskal-Wallis Analysis of variance on ranks $H(6) = 9624.03$ $p<0.001$; Dunn's method for post-hoc testing $p<0.05$). Fixational saccades in the non-viewing eye of all the strabismic monkeys were larger than that of the normal monkey (Kruskal-Wallis Analysis of variance on ranks $H(5) = 4510.70$ $p<0.001$; Dunn's method for post-hoc testing $p<0.05$). Although PM did not show eye misalignment, fixational saccade amplitude in this animal was also significantly greater than that of NM.

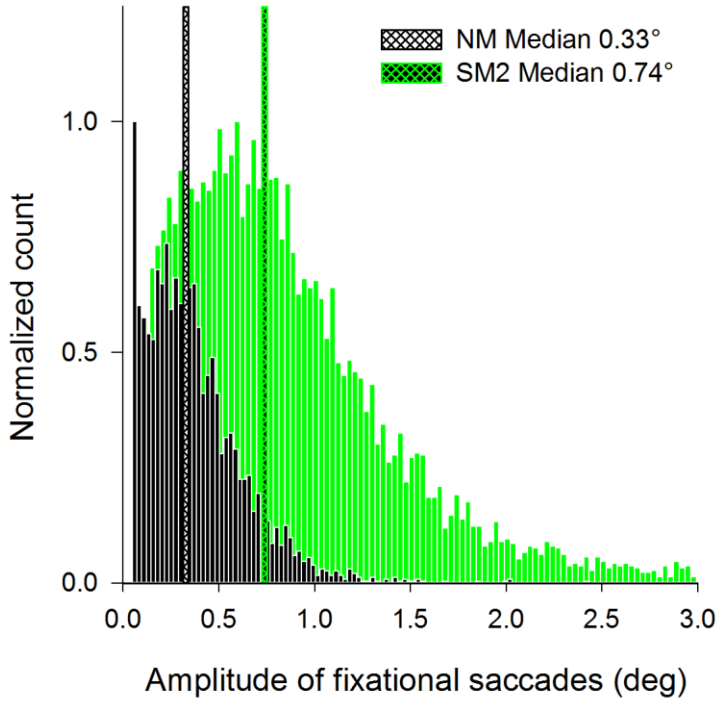

Figure 2.3.2 Amplitude histogram

Figure 2.3.2: Histogram showing amplitudes of all fixational saccades in viewing eye of Normal monkey (NM – black), and a strabismic monkey (SM2 – green)

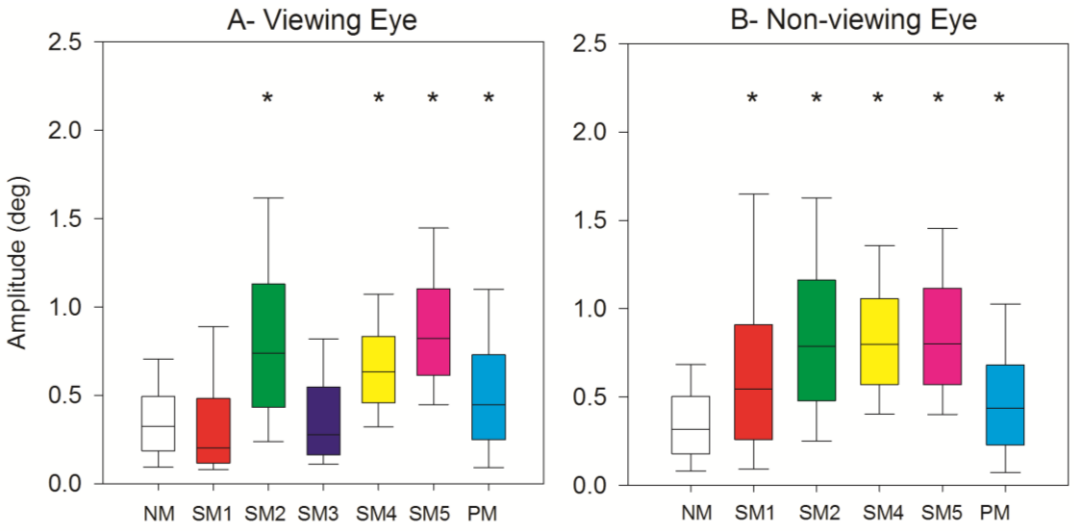

Figure 2.3.3 Box plot showing amplitude of fixational saccades

Figure 2.3.3: Box plots of amplitude of fixational saccades in viewing eye (Panel A) and non-viewing eye (Panel B) of each monkey in the study pooled across all experimental conditions. Asterisks indicate significant difference from the normal monkey (NM) as determined by one-way ANOVA on ranks followed by post-hoc testing using Dunn's method. SM3 had only one functional eye coil and therefore no data from the non-viewing eye was acquired.

We also calculated the frequency of fixational saccades in the viewing and non-viewing eyes of the animals and these data are summarized in Fig 2.3.4. Frequency of fixational saccades was increased in 3/5 strabismic monkeys in comparison to the normal monkey in the viewing eye (Kruskal-Wallis ANOVA on ranks $H(6)=196.69$ $p<0.001$; Dunn's test $p<0.05$) and 1/5 monkey in the non-viewing eye (Kruskal-Wallis ANOVA on ranks $H(5)=201.82$ $p<0.001$; Dunn's test $p<0.05$). Fixational saccade frequency in PM was significantly higher than NM and in many cases even higher than in the strabismic monkeys.

2.3.3 Influence of nystagmus on estimates of fixational saccade amplitude and frequency

As seen in Figure 2.3.1, strabismic monkeys usually show significant nystagmus during fixation. We wondered whether the nystagmus quick-phase components could be driving the increased amplitude and/or frequency of fixational saccades in SM1-SM5 and PM. In our sample of animals, we observed that although nystagmus quick-phases tended to be oriented in a specific direction that was different for an individual monkey, they all showed a downward component (see SM5 data in Fig 2.3.1 for example). Therefore to compare fixational saccade amplitude and frequency in normal and strabismic monkeys without the influence of nystagmus, we simply compared fixational saccades with an upward component in the animals. Upward fixational saccade amplitude data from the viewing and non-viewing eye is shown in Fig 2.3.5A, B and frequency data is shown in Fig 2.3.5C, D.

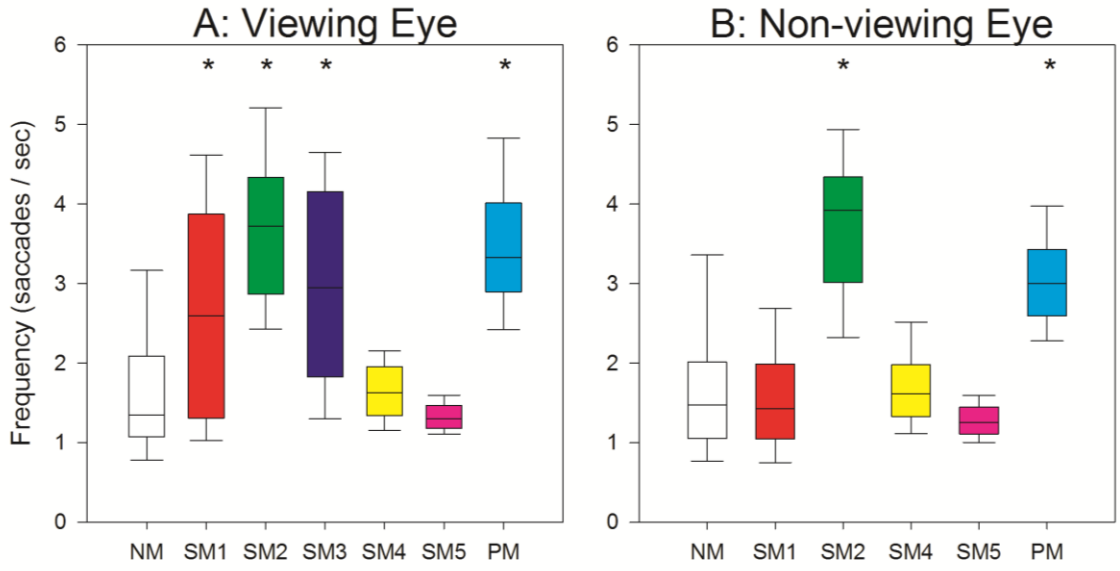

Figure 2.3.4 Box plot of frequency of fixational saccades

Figure 2.3.4: Box plots of frequency of fixational saccades in viewing eye (Panel A) and non-viewing eye (Panel B) of each monkey in the study. Asterisks indicate significant difference from the normal monkey (NM).

Median amplitudes following the removal of nystagmus quick-phases were smaller than when the nystagmus was included in all the strabismic monkeys in both the viewing and non-viewing eyes (figs 2.3.5A, B). Despite the reduction, saccade amplitudes in the strabismic monkeys were still higher than in the normal monkey in 3/5 strabismic monkeys in the viewing eye and in all the strabismic monkeys in the non-viewing eye (Kruskal-Wallis Analysis of variance on ranks viewing eye $H(6)=2777.93$ $p<0.001$; non-viewing-eye $H(5)=1210.921$ $p<0.001$; Dunn's method for post-hoc testing $p<0.05$). The two animals whose fixational saccade amplitudes following removal of nystagmus were not significantly different from the normal were the same two animals that showed small amplitude fixational saccades to begin with (Fig 2.3.3A, B; SM1, SM3). Our findings suggest that even after accounting for nystagmus, larger fixational saccades could drive larger fixation instability in strabismic monkeys. Amplitude of fixational saccades in PM also did not significantly change after removing quick phase of nystagmus and was still greater than that of the normal monkey.

Only considering fixational saccades with an upward component in the normal and strabismic monkeys significantly impacted frequency estimates (Fig 2.3.5C, D). Percentage reductions in median frequency estimates in the viewing eye were ~63% for NM, ~60% for SM1, ~78% for SM2, ~70% for SM3, ~74% for SM4 and ~91% for SM5. Note that a reduction of greater than 50% suggests that downward components (including nystagmus quick phases) biased the estimates of frequency when all fixational saccade events are included. Further, frequency of fixational saccades in the strabismic monkeys was either similar to or less than in the normal monkey for 4/5 strabismic monkeys in the viewing eye and all strabismic monkeys in the non-viewing eye (Kruskal-Wallis Analysis

of variance on ranks viewing eye $H(6)=179.79$ $p<0.001$; non-viewing-eye $H(5)=167.21$ $p<0.001$; Dunn's method for post-hoc testing $p<0.05$). There was significant reduction in the frequency estimate in PM (~68%), although still higher than that of NM in both the viewing and non-viewing eye.

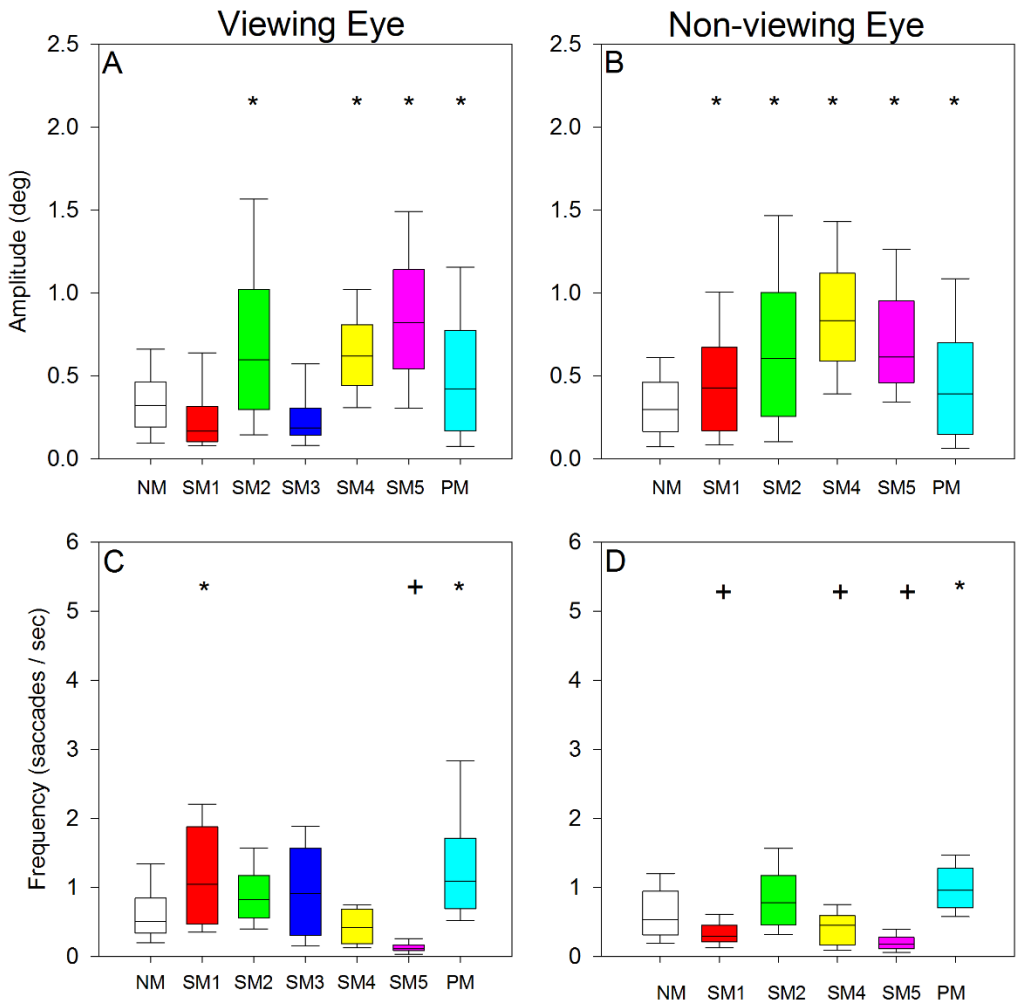

Figure 2.3.5Box plot comparing fixational saccades amplitude and frequency with and without nystagmus

Figure 2.3.5: Box plots of amplitude (panels A, B) and frequency (Panels C, D) of only upward directed fixational saccades in the viewing eye (Panels A, C) and non-viewing eye (Panels B, D) of each monkey in the study. Asterisks indicate significant difference from the normal monkey (NM). '*' indicates significantly higher values than NM and '+' indicates significantly lower values than NM.

2.3.4 Relationship between fixational saccade amplitude and BCEA

Fixation instability in the viewing and non-viewing eyes of all five strabismic monkeys and PM, as quantified using the BCEA metric, was increased (larger BCEA) compared to fixation instability in the normal monkey. In order to further understand how fixational saccades might affect stability of fixation, we sought to determine a relationship between fixational saccade amplitude and the BCEA metric. We plotted median amplitude of fixational saccades from the viewing eye along with the corresponding BCEA for each of the 60 experimental trials and found that an exponential rise to maximum model provided the best fit to the data for each monkey (Figure 2.3.6). Note that for each animal, we also attempted linear regression between fixational saccade amplitude and BCEA and found that the exponential rise to maximum fit yielded better coefficients of determination in all monkeys except SM3. The plateauing of fixational saccade amplitude for larger BCEA suggests that factors other than fixational saccades (likely drift) contribute significantly towards increased BCEA conditions in the monkeys.

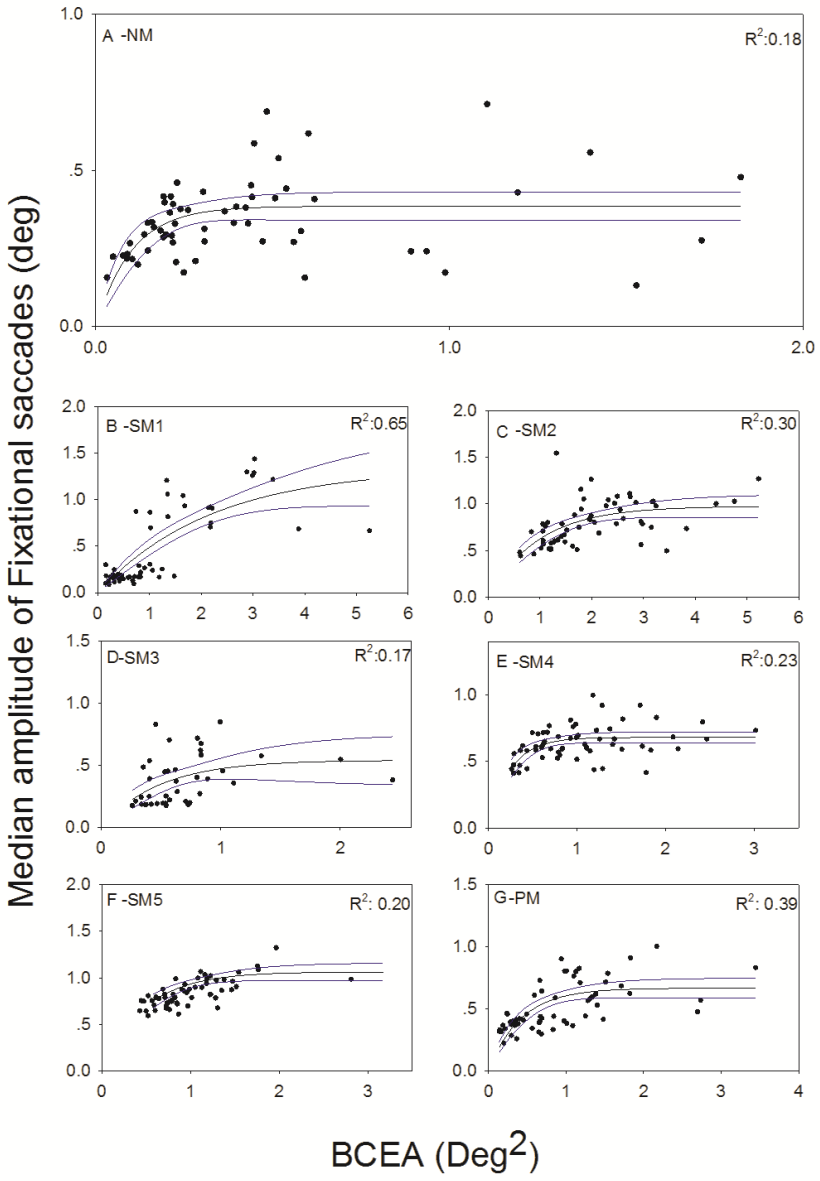

Figure 2.3.6 Scatter plot of BCEA vs mean amplitude of fixational saccades

Figure 2.3.6: Relationship between median amplitude of fixational saccades and overall fixation instability as measured by the BCEA metric in each monkey. Each data point represents the calculated BCEA and median amplitude measures from the viewing eye data during a single experimental trial. The continuous lines in each panel are the exponential rise to maximum fit and 95% confidence intervals.

2.3.5 Fixational stability in depth

Although, historically, fixation instability has been investigated for each eye individually, it is also reasonable to consider fixation instability in depth (vergence fixation instability) in strabismic patient populations since their binocular vision capabilities are compromised. To interpret our data from a vergence standpoint, we calculated a vergence (left eye position – right eye position) BCEA for our study cohort (Fig 2.3.7A). Vergence BCEA for the normal monkey was small (median = 0.15 deg^2) and less variable as opposed to the strabismic monkeys (range of medians: 0.63 deg^2- 2.15 deg^2). These findings suggest that fixation in depth is stable in the normal animal but is not in the strabismic population (Kruskal-Wallis ANOVA on ranks $H(5) = 265.47$ $p<0.001$). Interestingly, monkey PM who showed significant fixation instability due to nystagmus was relatively stable in depth (median = 0.42 deg^2). We also calculated a versional (left eye position/2 + right eye position/2) BCEA and these data are plotted in Fig 2.3.7B. Our data show that versional fixation stability is also disrupted in the strabismic animals (Kruskal-Wallis ANOVA on ranks $H(5) = 196.56$ $p<0.001$).

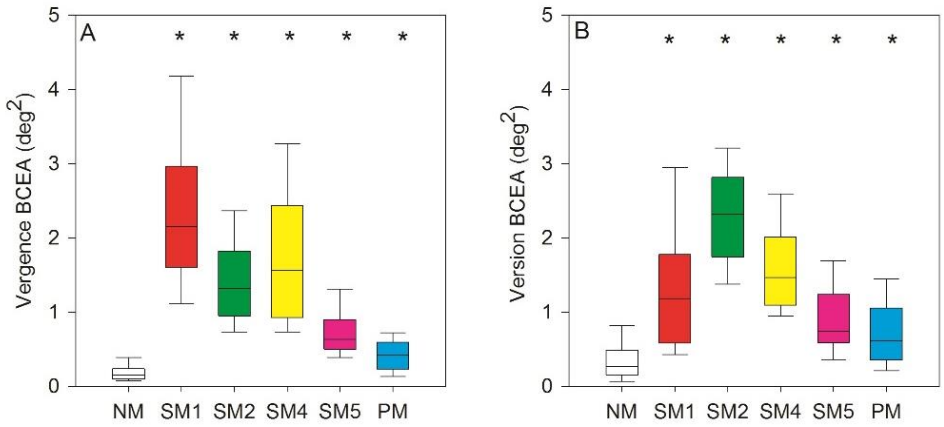

Figure 2.3.7 Box plot showing vergence and version BCEA

Figure 2.3.7: Box plots of vergence (left eye position – right eye position) BCEA (Panel A) and version (left eye position/2 + right eye position/2) BCEA (Panel B) of each monkey in the study. Asterisks indicate significant difference from the normal monkey (NM).

2.3.6 Influence of target parameters and viewing conditions on amplitude of fixational saccades

In a previous study, we have shown that target shape and size parameters influenced fixation instability as measured by the BCEA in normal and strabismic monkeys. Changes in BCEA were generally small in magnitude. One of the objectives of this study was to examine the influence of target parameters (those deemed significant in the previous study) on amplitude of fixational saccades. Three-way ANOVA followed by Holm-Sidak post-hoc testing was used to assess the main effects of target size, target shape and viewing condition in the viewing eye (Fig 2.3.8A,C,E) and non-viewing eye (Fig 2.3.8B,D,F) of the animals. Table 2.3.2 summarizes the main effects outcomes of the 3-way ANOVA and post-hoc testing for the viewing and non-viewing eyes.

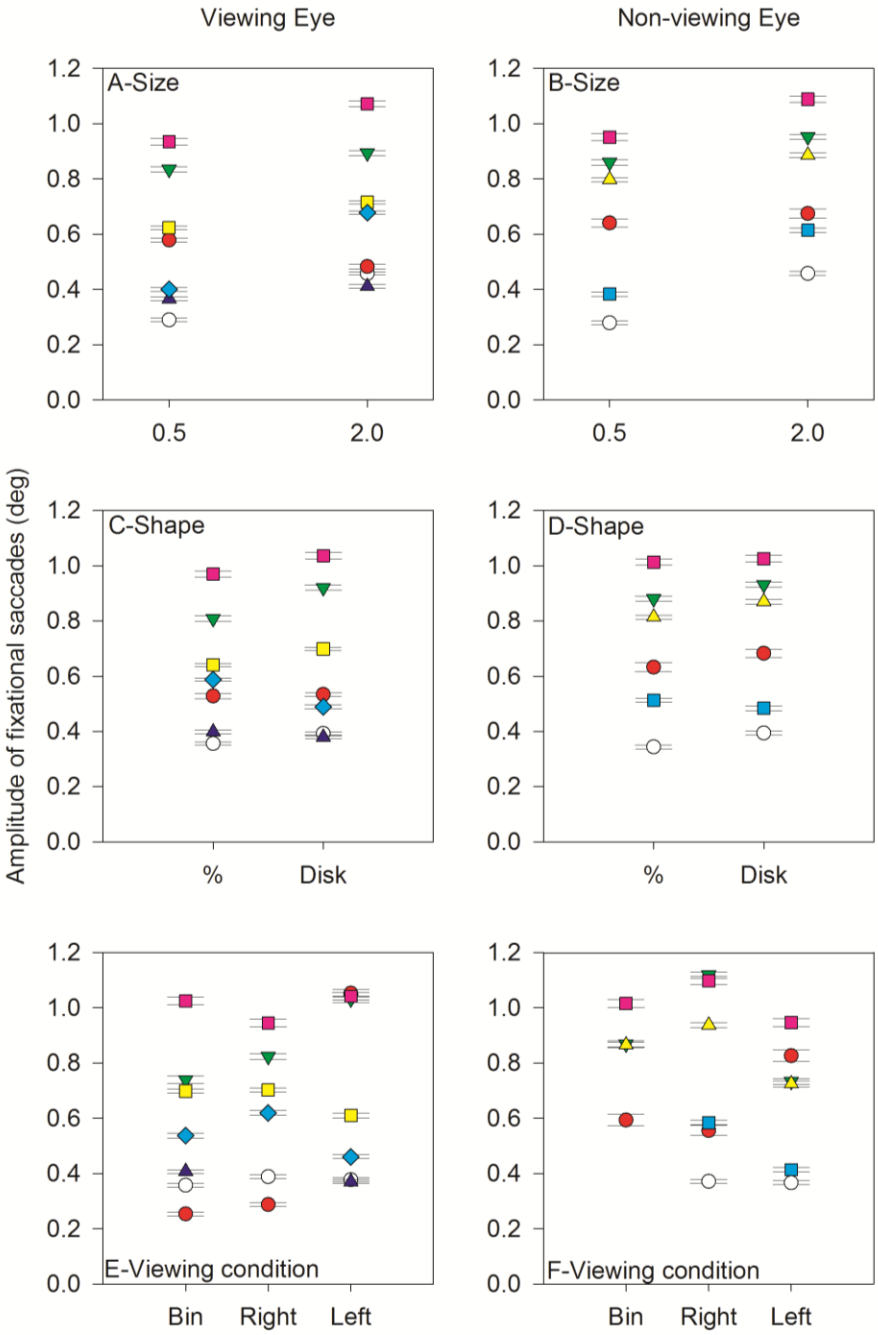

Figure 2.3.8 Plot showing main effect of different conditions on amplitude of fixational saccades

Figure 2.3.8: Main effect plots of amplitude of fixational saccades in viewing eye (Panels A, C, E) and non-viewing eye (Panels B, D, F) of all the monkeys as a function of target size (Panels A, B), target shape (Panels C, D) and viewing condition (Panels E, F). Panels A-D include data from both monocular and binocular viewing conditions. Colors represent monkeys and are same as in other plots. Legend: Right – right eye viewing; Left – left eye viewing, Bin = binocular viewing.

Table 2.3 2 Table showing 3-way ANOVA and post hoc results of multiple factors on the amplitude of fixational saccades

Factors	Monkey	Viewing Eye		Non-Viewing Eye	
		ANOVA Results ([df effect, df error] = *F* value, *P* value)	Post-hoc tests (larger amplitude)	ANOVA Results ([df effect, df error] = *F* value, *P* value)	Post-hoc tests (larger amplitude)
Shape	NM	F(1,3495)=14.16, <0.001	Disk	F(1,2411)=22.66, <0.001	Disk
	SM1	F(1, 7066) = 0.24, NS	-	F(1, 3014)=2.38, NS	-
	SM2	F(1,7827)=65.87, <0.001	Disk	F(1,7800)=14.36, <0.001	Disk
	SM3	F(1, 4978)=4.41, <0.05	%	-	-
	SM4	F(1,4529)=42.69, <0.001	Disk	F(1,4501)=24.42, <0.001	Disk
	SM5	F(1,3165)=15.98, <0.001	Disk	F(1,3117)=0.12, NS	-
	PM	F(1,7249)=116.94, <0.001	%	F(1,4027)=7.04, <0.008	%
Size	NM	F(1,3495)=440.81, <0.001	2	F(1,2411)=360.40, <0.001	2
	SM1	F(1, 7066) = 63.54, <0.001	0.5	F(1, 3014) = 1.30, NS	-
	SM2	F(1,7827)=18.61, <0.001	2	F(1,7800) = 49.67, <0.001	2
	SM3	F(1,4978)=25.41, <0.001	2	-	-
	SM4	F(1,4529)=108.09, <0.001	2	F(1,4501)=58.62, <0.001	2
	SM5	F(1,3165)=70.19, <0.001	2	F(1,3117)=14.49, <0.001	2
	PM	F(1,7249)=948.3, <0.001	2	F(1,4027)=445.04, <0.001	2
Viewing condition	NM	F(2,3495)=4.23, <0.05	Right eye viewing	F(1,2411)=0.786, NS	
	SM1	F(2, 7066) = 1378, <0.001	Left eye viewing	F(2, 3014) = 232.71, <0.001	Left eye viewing
	SM2	F(2,7827)= 155.95, <0.001	Left eye viewing	F(2,7800) = 301.53, <0.001	Right eye viewing
	SM3	F(1,4978)= 16.58, <0.001	Binocular viewing	-	-
	SM4	F(2,4529)=42.38, <0.001	Right eye viewing	F(2,4501)=117.04, <0.001	Right eye viewing
	SM5	F(2,3165)=13.18, <0.001	Left eye viewing	F(2,3117)=27.43, <0.001	Right eye viewing
	PM	F(1,7249)=108.4, <0.001	Right eye viewing	F(1,4027)=243.5, <0.001	Left eye viewing

Amplitude of fixational saccades was significantly larger for the disk shaped target than for the optotype in the normal monkey and 3/5 strabismic monkeys in the viewing eye and 2/4 strabismic monkeys in the non-viewing eye. Fixational saccade amplitudes were also significantly larger for larger targets (2° vs 0.5°) in the normally aligned monkeys (NM and PM) as well as in all strabismic monkeys (except SM1) in the viewing and non-viewing eyes. Amplitude of fixational saccades was larger for one of the monocular viewing conditions for 5/6 monkeys including the normally aligned monkey. Binocular viewing was idiosyncratic but generally the same as the 'better' monocular viewing condition. Although statistically significant, the magnitudes of the changes in fixational saccade amplitudes are generally small and not likely to be functionally significant. Consequently, analysis of interaction effects among the ANOVA variables was not pursued. Note that these findings are largely in line with our earlier observations of influence of target parameters on the BCEA.

2.4 Discussion

In this study we examined fixational saccades and their contribution towards fixation instability in strabismic monkeys and also considered how target parameters and viewing conditions affect fixational saccades. The main findings of our study were 1) Amplitude and frequency of fixational saccades were larger than those in the normal monkeys; nystagmus quick phases significantly influenced fixational saccade frequency but only slightly influenced fixation saccade amplitude 2) Relationship between overall fixation instability and fixational saccade amplitude was nonlinear and showed a saturation of fixational saccade amplitudes. 3) Strabismic monkeys show significantly larger fixation

instability in depth (vergence fixation instability) compared to the normal animal. 4) Target shape, size and viewing conditions affects amplitude of fixational saccades in both normal and strabismic monkeys. Below we discuss the implication of each of these findings in detail.

2.4.1 Fixational saccade metrics in normal and strabismic monkeys

Strabismic monkeys tended to exhibit larger and more frequent fixational saccades when compared to normal animals. Nystagmus quick phases affected estimates of fixational saccade amplitude only marginally but significantly reduced estimates of frequency towards normal levels. A previous study by Gonzalez and colleagues(Gonzalez et al. 2012) also did not find differences in microsaccade amplitudes between amblyopic and fellow eyes in a cohort of strabismic and anisometropic amblyopes. One way to interpret our data is that fixation instability in strabismic monkeys is at least partially due to larger fixational saccades and when nystagmus is present, more frequent fixational saccade type events. Studies that have examined the frequency of fixational saccades in disease conditions have yielded inconsistent results. Thus Shaikh and colleagues(Shaikh et al. 2016) and Ghasia and colleagues(Ghasia et al. 2017) suggested that the frequency of fixational saccades was decreased in strabismic and anisometropic amblyopes, while Gonzalez and colleagues found that frequency of fixational saccades was similar to controls(Gonzalez et al. 2012) and Ciuffreda and colleagues(Ciuffreda et al. 1979) and Chung and colleagues(Chung et al. 2015) suggested that frequency of fixational saccades was increased in strabismic amblyopes. In AMD patients, it appears that the frequency of fixational saccades is similar to controls (Kumar and Chung 2014). From our data

analysis, we suggest that the inconsistency could be due to the presence or absence of nystagmus. The presence of nystagmus quick phases could present as an increase in frequency, when in fact the frequency of fixational saccades after accounting for nystagmus quick phases is actually same as controls or even decreased. As shown by the data from PM, the presence of nystagmus, even in the absence of strabismus, can significantly influence estimates of fixational saccade frequency.

An issue for debate is the relative role of drift in fixation instability and whether larger fixational saccades can completely account for increased instability in strabismic monkeys. A previous study on fixational eye movements in human strabismic and anisometropic amblyopes suggested that fixational saccades were the limiting factor that determined fixation instability (Chung et al. 2015). We took advantage of the different viewing conditions and multiple trials in our experimental design to investigate this issue. Our analysis showed that fixation instability (BCEA value) and amplitude of fixational saccades could be related via an exponential rise to maximum model fit (Fig 2.3.6). The implication of this relationship is that amplitude of fixational saccades is a primary driver for BCEA when stability is relatively high (low BCEA); however there is a saturation effect of fixational saccade amplitudes and therefore other factors (most likely drift) must drive fixation instability at higher BCEA.

2.4.2 Fixation Instability in Depth

The analysis of fixation instability and fixational eye movements has generally been uniocular, for example investigating right eye stability and left eye stability or instability of the viewing and non-viewing eye in the case of strabismus, or in the case of

amblyopia, instability of the amblyopic eye and that of the fellow eye (Ciuffreda et al. 1979, Gonzalez et al. 2012, Kumar and Chung 2014). These analyses have been partially driven by considerations of relationship between instability on the fovea and visual acuity. However fixation must also be relatively stable in depth in order to maintain the image within Panum's fusional area and therefore maintain clear and single vision. For a human with inter-pupillary distance (IPD) of 6cm, the size of Panum's fusional area for a straight-ahead object is ~20 min of arc horizontally and ~8min of arc vertically. A recent study has shown that fixation in depth in normal adults and children is quite stable and remains within these limits (Shaikh and Ghasia 2017).

In our data, we found that indeed the vergence BCEA of the normal monkey was quite stable and comparable to that of normal human subjects (Fig 2.3.7). In other words, the stability of fixation in depth for the normal monkey is adequate to maintain a clear image. We found significantly greater vergence BCEA values in the strabismic monkeys when compared to the normal. The absence of disparity information in the strabismic monkeys could lead to poorer vergence control and vergence instability indicated by the larger vergence BCEA. Alternatively, developmental disruption of binocular vision also leads to disruption of accommodation control; increased accommodation instability in these strabismic animals leads to the increased vergence instability due to cross links between the vergence and accommodation systems. We did not measure accommodation in our studies and therefore cannot directly comment on instability of accommodation. However other studies have suggested that accommodation is unstable (increased accommodative microfluctuations) in strabismus populations (Quick et al. 1994) (Joshi AC, ARVO 2016 e-abstract 4577). It has been shown that office-based accommodative/vergence therapy

improves control of accommodation and it would be interesting to investigate whether these therapies have a direct effect on improving accommodation instability and vergence fixation instability in strabismic patients (Ma et al. 2016). Note that, in the strabismic monkeys, fixation stability is not rooted only in the vergence system. The increased versional BCEA (Fig 2.3.7B) shows that there is instability driven via conjugate oculomotor pathways as well.

2.4.3 Influence of target parameters on fixational saccades

In a previous study, we found that fixation instability, as measured by the BCEA, was significantly influenced by target shape and size(Pirdankar and Das 2016). A disk shaped target resulted in greater instability compared to the optotype and larger targets resulted in greater instability compared to the smaller target. In this study, we found that fixational saccade amplitude also changed, similar to the BCEA, depending on the target parameters. A larger sized target and a disk shaped target resulted in larger fixational saccades in both the strabismic group and normal. Since the target parameter mediated changes in BCEA (previous study) and fixational saccade amplitude (this study) are small in magnitude and occur in the presence of large ongoing instability, we suggest that there is little functional utility to the target-mediated effects that are observed. The influence of monocular vs. binocular viewing on fixational saccade amplitudes were also similar to that found previously on the BCEA, viz., binocular viewing did not result in better stability than the “better” monocular viewing condition.

In summary, larger and more frequent fixational saccades and inter-saccadic drifts contribute detrimentally to fixation in strabismic monkeys. Fixational saccade parameters

are influenced by target parameters similar to target parameters effects on overall measures of fixational stability. These studies provide a framework for future neurophysiological investigation into the neural substrate for fixation instability and for quick phases of nystagmus in monkey models for strabismus and nystagmus.

Chapter 3 - Electrical stimulation of Superior Colliculus affects strabismus angle in monkey models for strabismus

3.1 Introduction

Strabismus is a misalignment of the visual axes of the two eyes with a worldwide prevalence in childhood of approximately 2-5% (Lorenz 2002, Govindan et al. 2005, Greenberg et al. 2007, Mohney 2007). A disruption in binocular vision during the critical period for visual and oculomotor development leads to developmental strabismus and this is the basis for the induction of strabismus in animal models (Boothe et al. 1985, Harwerth et al. 1986, Kiorpes 2015). Recent studies have shown that neural activity within oculomotor and abducens motor nuclei innervating the extraocular muscle (EOM) drives the state of eye misalignment in strabismic monkeys (Das and Mustari 2007, Joshi and Das 2011, Walton et al. 2015). In addition, neural recording of neurons in the midbrain supraoculomotor area (SOA) in strabismic monkeys has shown that activity in these cells is correlated with strabismus angle (Das 2012). Also, muscimol inactivation of the caudal fastigial nucleus (cFN) and posterior interposed nucleus (PIN) of the cerebellum in strabismic monkeys induces changes in eye misalignment (Joshi and Das 2013). These findings in different brain areas have led to a framework wherein disruption in oculomotor neural circuits related to vergence eye movements leads to eye misalignment.

The superior colliculus (SC) is a laminated midbrain structure known to be critical for saccadic eye movements (Gandhi and Katnani 2011). Superficial layers of the SC encode

a retinotopic map of visual space, while the intermediate and deeper layers of the SC encode a motor map, i.e, encode a specific saccade vector from initial position (Wurtz and Goldberg 1971, Cynader and Berman 1972, Goldberg and Wurtz 1972, Schiller and Stryker 1972, Wurtz and Goldberg 1972, Gandhi and Katnani 2011). The SC map is topographically organized such that saccadic amplitude increases from rostral to caudal and saccade direction changes from upward to downward along the mediolateral direction. The SC, especially the rostral part of the SC, is also involved in smooth-pursuit (SP), although its role in SP appears not to be related to motor commands for smooth-pursuit (SP eye movements are not elicited by rSC electrical stimulation during fixation), but rather to aspects of target selection/movement initiation (specifying an eye-movement goal) and perhaps providing a position signal to the smooth-pursuit system during ongoing pursuit (Basso et al. 2000, Krauzlis and Dill 2002, Krauzlis 2004, Gandhi and Katnani 2011).

Although the role of the SC in saccade and smooth-pursuit eye movements has been the focus of the majority of published studies, there is a fair bit of evidence supporting the role of the SC in vergence eye movements, an aspect that could be of specific interest for strabismus. Convergence related neurons have been identified in the cat rostral superior colliculus (rSC) (Jiang et al. 1996). Also, electrical stimulation or pharmacological inactivation of the cat rSC produced changes in both accommodation and vergence (Sawa and Ohtsuka 1994, Ohtsuka and Sato 1996, Suzuki et al. 2004). Monkey studies also suggest a role of the SC in vergence, though perhaps interpretation is more complex. Billitz and Mays were not able to elicit vergence by electrical stimulation in the SC during far viewing but electrical stimulation during near viewing caused a relaxation of

vergence (Billitz and Mays 1997). In another study, electrical stimulation in SC interfered with vergence only if applied just before or during a vergence only movement or a combined saccade-vergence movement (Chaturvedi and van Gisbergen 1999, Chaturvedi and Van Gisbergen 2000). One hypothesis for the apparent lack of vergence changes due to electrical stimulation in normal monkeys is that a net 'zero vergence' command is initiated because both convergence and divergence related neurons are activated. Lawler and Cowey performed ablations of the rSC in monkeys and suggested that there were problems with both disparity processing and eye alignment although eye movements were not explicitly recorded in this study (Lawler and Cowey 1986). Neurons in the SC have been shown to receive monosynaptic projections from neurons in lateral intraparietal cortex (LIP) that also encoded depth information (Gnadt and Beyer 1998). Walton and Mays showed that saccade related neurons in the caudal colliculus showed a weak relationship to vergence in that many burst neurons showed a reduction in saccade velocity sensitivity when looking at near targets compared to when looking at far targets (Walton and Mays 2003). However, they were unable to identify any systematic 3-D tuning of neurons. Interestingly, early studies investigating SOA vergence neurons also reported another population of vergence neurons located 4-5mm dorsal and 2-3mm lateral to the oculomotor nucleus (OMN) that they did not unequivocally localize using histological methods, but could be in the rSC (Mays 1984, Judge and Cumming 1986, Mays et al. 1986). A recent study by Van Horn and colleagues indeed identified convergence and divergence neurons in the rSC that were modulated during slow vergence but not conjugate or fast vergence eye movements thereby postulating that the

rSC only contributes to slow vergence (Van Horn et al. 2013). Also, electrical stimulation in this area produced vergence angle changes when looking at near targets.

Considering the evidence for SC involvement in vergence and the potential for disruption in vergence circuits as a neural substrate for strabismus, we decided to examine the role of the SC in maintaining the state of misalignment in strabismus monkey models. We used the strategy of electrical stimulation of the SC as it has commonly been used in this structure to elicit saccades and more recently also in the rSC to elicit vergence. Thus, the specific goal of the study was to determine whether stimulation of the superior colliculus could influence the state of eye misalignment in juvenile strabismic monkeys. Some of these data have appeared before in abstract form (Upadhyaya et al. 2016).

3.2 Methods

3.2.1 Subjects and Surgical Procedures

Three adult strabismic monkeys (Macaca Mulatta) were used in this study (Monkeys E, L and H; ages 6-7 years; weights 5-10kg). These monkeys were previously reared using an optical prism-viewing paradigm. In this paradigm, infant monkeys viewed through a 20 prism-diopter Fresnel prism oriented base-in and placed in front of one eye and another 20 prism-diopter Fresnel prism oriented base-up and placed in front of the other eye. These horizontal and vertical Fresnel prisms were fitted in a lightweight helmet-like device that the animal wore for the first four months of life starting from 1-2 days after birth. After the initial four months of prism rearing, monkeys were reared for several years, in a normal visual environment at the University of Houston College of

Optometry, before experiments were begun. Disruption of binocular vision due to prism-viewing during this initial period leads to strabismus as it is the critical period for development of eye alignment, stereopsis and binocular sensitivity.

Prior to experimentation, each juvenile monkey went through three sterile surgical procedures carried out under aseptic conditions under isoflurane anesthesia (1.25%-2.5%). In the first surgery, a head stabilization post was implanted (Adams et al. 2007). In the second surgery, we stereotaxically implanted a 21mm diameter titanium neural recording chamber along with a scleral search coil in one eye and in the third surgery we implanted a scleral search coil in the other eye (Judge et al. 1980). In monkeys L and H, the chamber was implanted at a stereotaxic location centered 3-mm anterior and 1-mm lateral to stereotaxic zero and tilted 20° dorsolateral to ventromedial in the coronal plane. In monkey E, the chamber was implanted in the mid-sagittal plane and centered at Anterior-Posterior 0-mm and tilted posteriorly by 38°. All surgical and experimental procedures were performed in strict compliance with National Institute of Health and The Association for Research in Vision and Ophthalmology (ARVO) guidelines and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Houston. After recovery from surgery and additional behavioral acclimatization to the laboratory environment, training in standard oculomotor tasks such as saccades, smooth-pursuit and fixation was undertaken.

3.2.2 Eye Movement Measurements and Experimental Procedures

Binocular eye position was measured using the magnetic search coil method (Primelec Industries, Regensdorf, Switzerland). Eye coils were calibrated at the beginning of each

experiment by rewarding the animals with small amounts of juice as they looked at a series of targets along the horizontal or vertical meridian projected onto a tangent screen at 57cm. Calibration of each eye was performed independently during monocular viewing forced by occluding one or the other eye using liquid crystal shutter goggles (Citizen Fine Devices, Nagano, Japan) that were under computer control. Visual stimuli were generated using the BITS# visual stimulus generator (Cambridge Research Systems, Cambridge, UK) and Psychtoolbox 3(Brainard 1997) operated under computer control and presented using a DepthQ projector running at a 120Hz frame rate (Lightspeed Design Inc, Bellevue, WA).

We identified the superior colliculus using a combination of neural activity recording and electrical stimulation methods. During neural recording (epoxy-coated tungsten electrodes; 1-5 Mohm Frederik Haer, Brunswick, ME), as the electrode was advanced into the area of SC, we first encountered visual (superficial layers) and then saccade related (intermediate and deeper layers) activity. SC locations were confirmed by electrical microstimulation methods wherein a train of low current cathodal pulses (10-40μA, 400Hz, 500ms) delivered via the recording electrode elicited a staircase of saccades in a specific direction. After determining that a particular site yielded consistent staircase saccades at low currents, electrical stimulation of 500ms duration was repeated multiple times with each eye viewing and these data were used for later analysis. Binocular eye position signals were processed with anti-aliasing filters (Krohn-Hite; Krohn-Hite Corporation, Brockton, MA) at 400 Hz before digitization at 2.75 kHz with 12-bit precision (Alpha-Lab System; Alpha-Omega Engineering, Nazareth, Israel).

3.2.3 Data Analysis

The fundamental goal of the analysis was to examine changes in vertical and horizontal eye alignment during electrical stimulation and correlate them with amplitude and direction of saccades elicited due to stimulation. Binocular eye position data from electrical stimulation sites that resulted in staircase saccades at low threshold currents were analyzed using custom MATLAB programs. Eye velocity was calculated from eye position data using a central difference algorithm. Eye position and velocity data were further filtered using an 80-point finite impulse response digital filter with a pass band of 0 to 80 Hz prior to analysis.

Each electrical stimulation trial was initially reviewed by the investigator to determine whether the stimulation evoked staircase saccades, an indication to accept the trial. Trials were rejected if the animal broke fixation just before the stimulation or if no saccades were initiated within 100ms of stimulation. Once the trial was accepted, strabismus angle (difference in position of left and right eyes) was calculated just before stimulation and at the end of stimulation (i.e, after 500ms) and a mean change in strabismus angle was calculated as the mean difference in strabismus angle before and after stimulation over the multiple stimulation trials at each site.

For analysis of electrically evoked saccades, saccade onset and offset were identified using a combination of velocity criterion of 30°/sec and acceleration criterion of 3000°/sec^2. Although the staircase saccades following electrical stimulation were evident visually in the data records from all stimulation sites, the saccade detection criteria needed to be adjusted to reliably detect saccade onset and offset at a minority of

stimulation sites (15/51). The adjustments to the criteria were relatively small and consisted of changing the velocity criteria between 20-60°/sec and changing the acceleration criteria between 1000-4000°/sec^2. At one site we used a velocity criterion of 100°/sec. Generally, the higher threshold values were necessary to reliably detect saccades in the presence of significant post-saccadic movement and lower threshold values were necessary to identify smaller saccade amplitudes. All saccade onset and offset locations were visually verified prior to acceptance. Radial amplitude and direction of each saccade in each eye were calculated and a mean radial amplitude and mean direction for a single electrically elicited saccade at a particular SC site were calculated (mean of all electrically evoked saccades at a specific site). Saccades were grouped according to viewing eye (right eye viewing or left eye viewing) and also site of stimulation. Statistical analysis (SigmaPlot V12.5) focused on evaluating disconjugacy in radial saccade amplitude, disconjugacy in polar direction and correlating change in misalignment with saccade parameters.

3.3 Results

Monkey E presented with an exotropia (XT) with either eye viewing of ~25° while the other two animals had strabismus that was variable and also changed depending on viewing eye (evidence for a Dissociated Horizontal Deviation). Strabismus in Monkey H ranged 5° esotropia (ET) -15° XT (median: 8° XT) during right eye viewing and 1° ET - 12° XT (median: 7° XT) during left eye viewing. Strabismus in Monkey L ranged 5° ET - 18° XT (median: 13° XT) during left eye viewing and 5° ET - 25° XT (median: 12° XT) during right eye viewing. The large variability in the strabismus angle was not on a

moment-to-moment basis, i.e., was not related to fixation instability. Rather we observed variations over the several hours of a recording session and sometimes less exotropia during fixation compared to smooth-pursuit in the same recording session. These relatively slow changes in strabismus angle may be the result of slow variations in the tonic accommodative state of the monkey. There were also variations that occurred over the several months during which data for this study were acquired which is sometimes observed in human strabismus also. Note however, that the current study focuses on changes in strabismus angle over a very short time period of electrical stimulation (500ms) and therefore innate variability in strabismus angle is not a factor. Monkeys also showed a vertical misalignment that was determined to be a Dissociated Vertical Deviation (DVD) in monkeys E and L of ~0° – ~6° and a pure vertical strabismus in monkey H of ~0° - 2°. Alignment properties are also evident from the Hess screen chart representation of eye positions shown in Figure 3.3.1, obtained as the animals' performed smooth-pursuit tracking of a target moving along the horizontal or vertical meridian. Although each of the monkeys showed some evidence for pattern strabismus (variation of horizontal misalignment with vertical gaze position) and also variation of vertical misalignment with horizontal gaze position, these gaze-position dependent variations in strabismus angle were small (Figure 3.3.1), as is typical of many humans with strabismus.

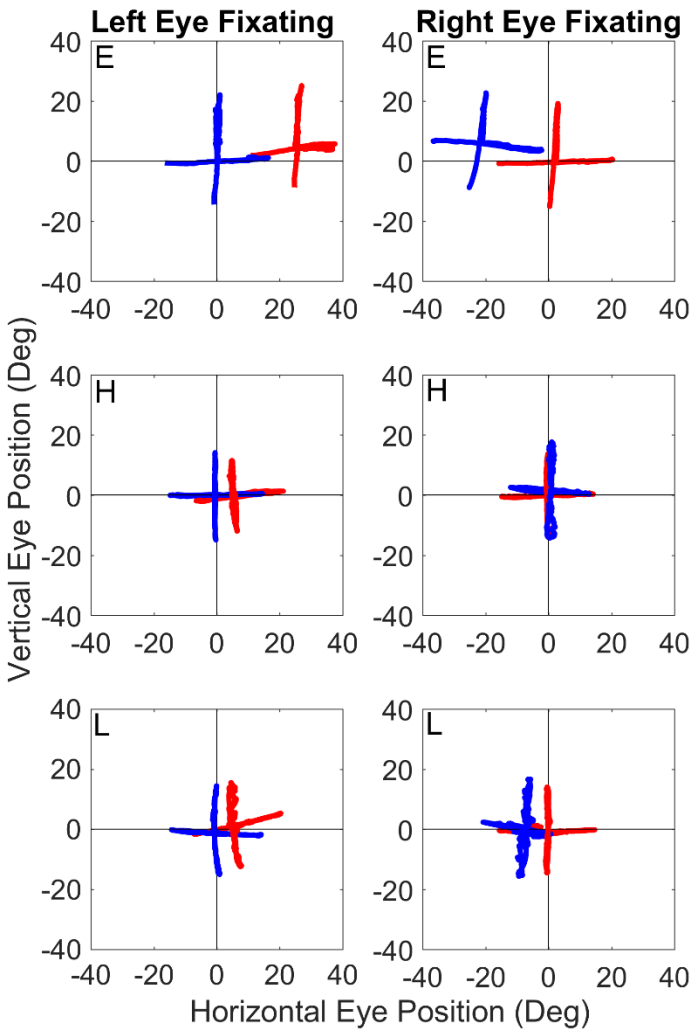

Figure 3.3.1 Plots showing pattern strabismus in three monkeys

Figure 3.3.1: Plot showing horizontal and vertical misalignment between right eye (red trace) and left eye (blue trace) as observed during monocular smooth-pursuit tracking (0.3Hz, ±15°) along the horizontal or vertical meridian in Monkeys E, H and L. Left column shows data acquired during left eye viewing and right column shows data acquired during right eye viewing. Positive values indicate rightward or upward positions and negative values indicate leftward or downward position. Traces are means developed

from several cycles of desaccaded eye positions during smooth-pursuit tracking. All monkeys presented with varying degrees of exotropia and small vertical misalignment and pattern strabismus.

3.3.1 Description of SC Stimulation sites

Electrical stimulation of SC was performed in 51 sites in the three animals of which 12 were in right superior colliculus and 39 in left superior colliculus. Of these 51 sites, 41 sites were tested under both right and left eye viewing conditions. 7 sites were tested under only left eye viewing and 3 other sites were tested under only right eye viewing. Stimulation at each of these 51 sites evoked staircase saccades of various amplitudes and directions. Figure 3.3.2 shows a polar plot of the evoked saccade vector in the viewing eye at each of these sites acquired during left eye viewing. Due to the previous finding of vergence neurons in rostral SC, our sample of stimulation sites was skewed towards rostral SC. During left eye viewing, 36/48 stimulation sites yielded saccades of radial amplitude <5° (mean radial amplitude = 1.79°; Range = 0.29° - 4.95°). Mean radial amplitude of electrically evoked saccades from the other 12 sites was 9.39° (Range = 5.35°-16.74°).

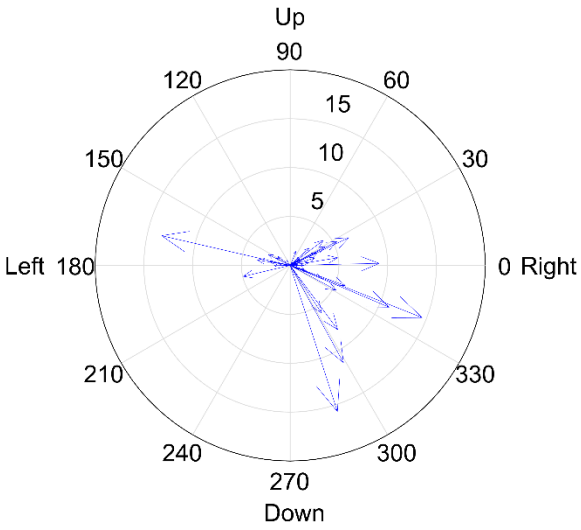

Figure 3.3.2 Polar plot showing direction and amplitude of saccade vectors

Figure 3.3.2: Polar plot showing amplitude and direction of left eye saccade vector in all animals evoked at each of the 48 electrical stimulation sites during left eye viewing.

3.3.2 Change in strabismus angle due to electrical stimulation of the superior colliculus

Electrical stimulation of the SC invariably resulted in a change in strabismus angle. Figure 3.3.3A-D shows raw horizontal and vertical eye position data (Panels A, C) and horizontal and vertical strabismus angle (Panels B, D; strabismus angle = left eye position minus right eye position) from seven superimposed trials following electrical stimulation of the left SC in monkey H. Eye movement traces are aligned on stimulation onset and the animal was fixating with her left eye (right eye under cover) prior to stimulation. As seen in Figure 3, electrical stimulation at this SC site results in a staircase of rightward and upward saccades with radial amplitude of ~2° (rostral site). Electrical stimulation for 500ms at this site also results in a significant divergent change in horizontal misalignment of ~6° (more exotropic) and little change in vertical misalignment (Figure 3.3.3B, D).

Panels E-H show data from another stimulation site in the left SC of Monkey E wherein electrical stimulation evoked a convergent change in misalignment of ~2-6° (Panel F - less exotropic) and also a significant change in vertical misalignment of ~2-4° (Panel H). Radial amplitude of the electrically evoked saccade at this stimulation site was ~1.5°.

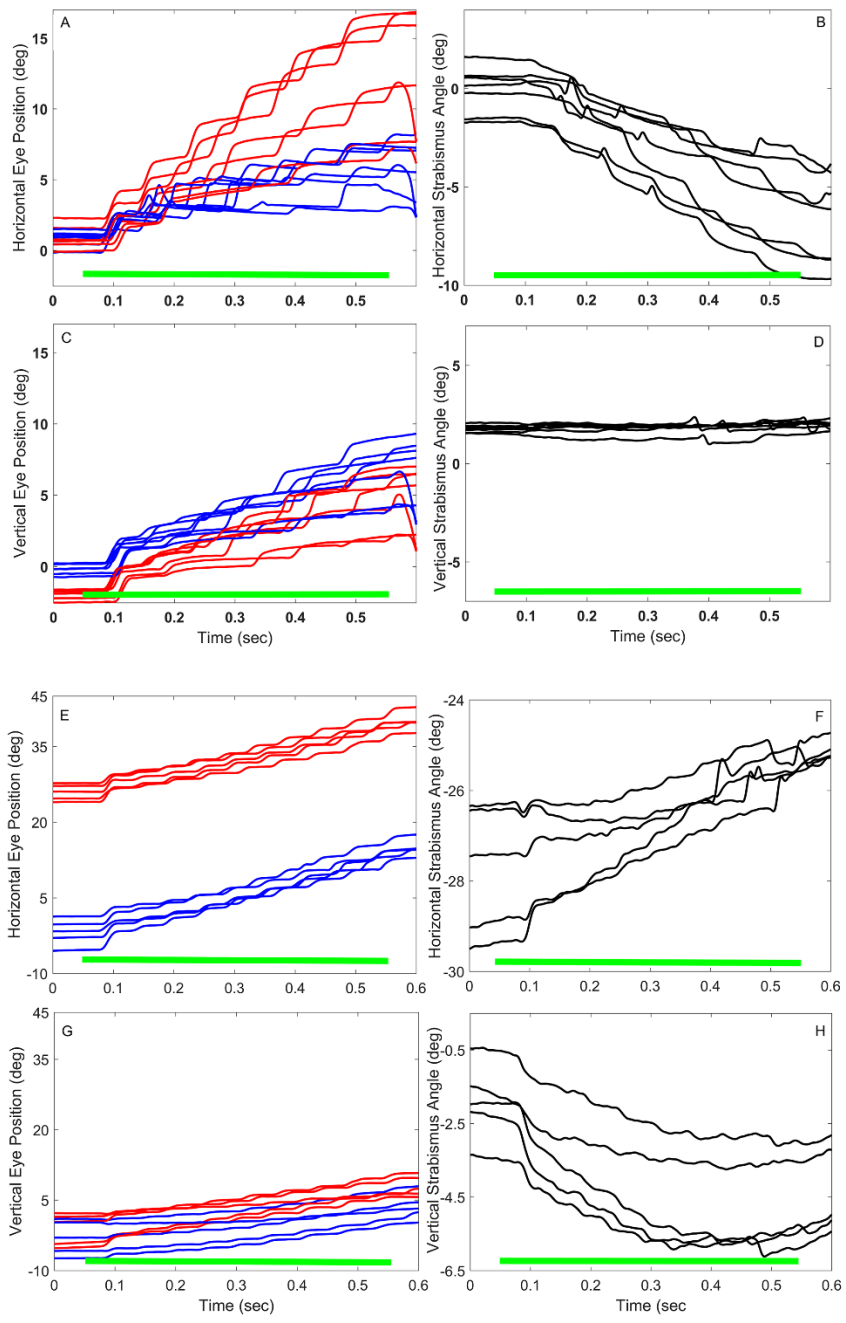

Figure 3.3.3 Position plot showing staircase saccades

Figure 3.3.3: Raw data showing eye movement responses following electrical stimulation in the left superior colliculus of monkey H (Panels A-D) and Monkey E (Panels E-H)

during monocular left eye viewing. Panel A, E: Multiple traces of horizontal eye position of the right (red) and left (blue) eyes. Data are aligned on start of stimulation. Contralateral staircase saccades are evoked during the period of electrical stimulation (shown by green bar). Panel C, G: Vertical eye position data of the right and left eyes during the same time period shows a staircase of upward saccades. Panel B, F: Traces of horizontal strabismus angle (Left eye position – right eye position) shows significant change in alignment (divergent change in Monkey H and convergent change in Monkey E) during stimulation. Panel D, H: There is little change in vertical misalignment due to stimulation in Monkey H and ~5° change in Monkey E. In all panels, +ve values indicate rightward or upward eye positions and -ve values indicates leftward or downward eye positions.

Stimulation at the different SC sites resulted in a varied amount of change in horizontal and vertical strabismus angle and these data are summarized in Figure 3.3.4. During left eye viewing (Figure 3.3.4A), 36/48 sites evoked a divergent change in strabismus angle (shift towards more exotropia or less esotropia; mean divergent change = 5.7°) and 12/48 sites evoked a convergent change in strabismus angle (shift towards less exotropia or more esotropia; mean convergent change =1.8°). Similarly during right eye viewing (Figure 3.3.4C), 27/44 sites evoked a divergent change and 17/44 evoked a convergent change (mean divergent change =6.2°; mean convergent change = 1.4°). The amount of change in vertical strabismus angle (Figures 3.3.4B, 3.3.4D) was generally small across all sites. Magnitude (absolute value) of change in vertical strabismus angle during left eye view was 2.3° and during right eye view was 4.3°. Although in general electrical stimulation evoked a consistent divergent or convergent change during both right and left eye viewing, at 10 sites stimulation evoked a divergent change while one eye was viewing and a convergent change while the other eye was viewing. However, the magnitude of the convergent/divergent changes at these sites was generally small (mean divergent change across 10 sites= 2.6°; mean convergent change=1.5°).

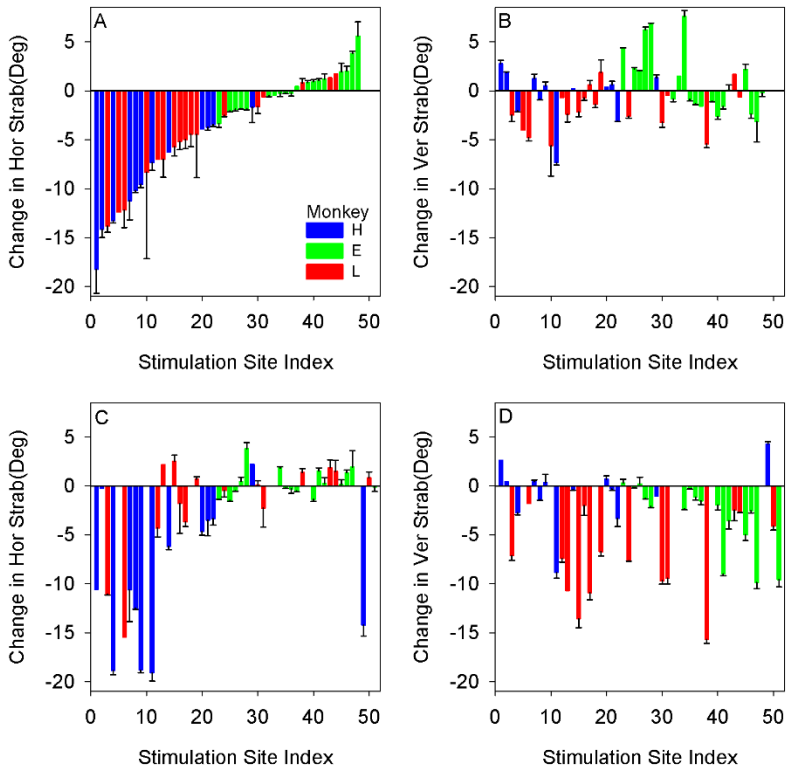

Figure 3.3.4 Bar plot showing change in strabismus angle

Figure 3.3.4: Change in horizontal and vertical strabismus angle (Panel A, B – left eye viewing; Panel C, D – right eye viewing) as a result of electrical stimulation. Data are arranged in increasing order of convergence obtained during left eye viewing and the same stimulation site index is used for all panels. Site index 1, 2, 3 are sites tested only under right eye viewing. Data obtained from each monkey is color coded.

3.3.3 Analysis of saccade amplitude and direction disconjugacy

In an attempt to further understand the effect of SC electrical stimulation on strabismus angle, we asked whether the changes in strabismus angle were due to disconjugate saccades (saccade amplitude disconjugacy or saccade direction disconjugacy). To analyze saccade amplitude disconjugacy, we calculated the radial amplitude of the electrically evoked saccade in the right and left eyes and these data are plotted in Figure 3.3.5. The slope of the regression line of mean radial amplitude in right vs left eyes during either eye viewing was 1.01 and statistical testing using a paired t-test showed radial amplitude of saccades was not significantly different in the two eyes ($p>0.05$).

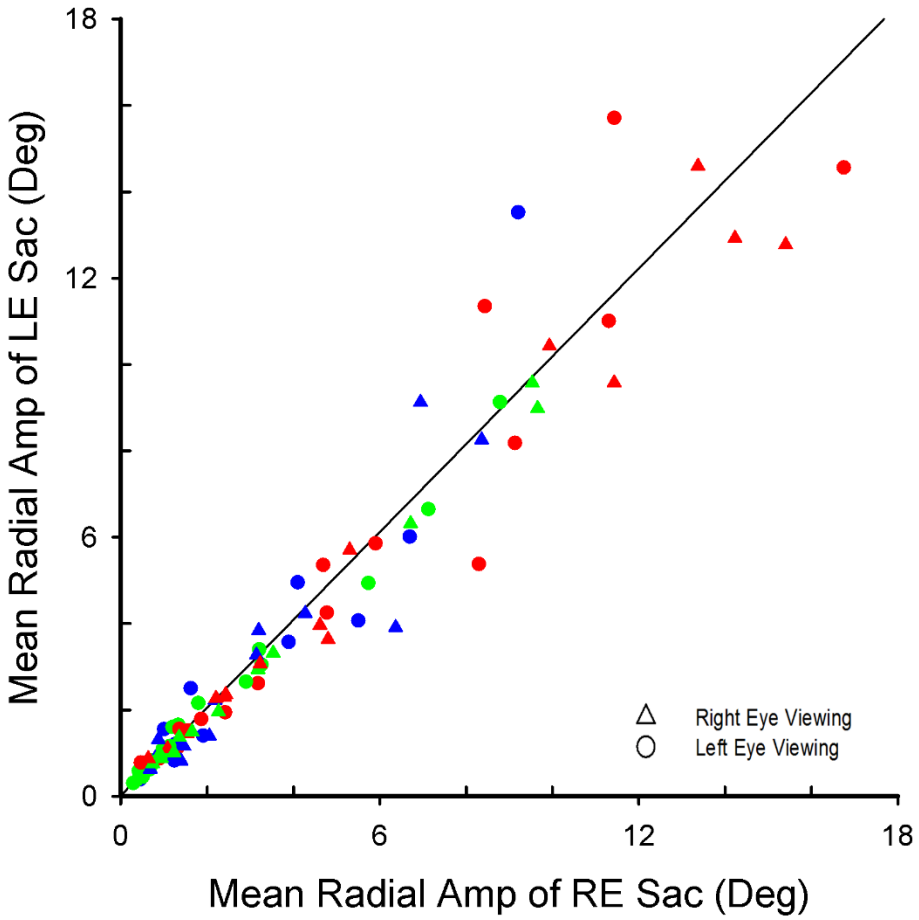

Figure 3.3.5 Scatter plot showing radial amplitude of electrically evoked saccades

Figure 3.3.5: Correlation between mean radial amplitude of saccade evoked in the right and left eyes during both right and left eye viewing conditions (regression line slope = 1.01; $r^2 = 0.90$). Data from individual animals are color-coded using the same scheme as in Figure 3.3.4.

Potentially, change in eye misalignment due to electrical stimulation could also be due to differing directions of left and right eye movements. Figure 3.3.6 is a polar plot showing the electrically evoked eye movements in the right and left eyes at two different SC sites. In each case, it is apparent that the eye movement directions in the two eyes are indeed different. Some of this eye movement direction difference could be due to a difference in the saccade direction. Saccade directional differences between right and left eyes were observed at almost all stimulation sites and these data are summarized in Figure 3.3.7. The data show that saccade direction differences could be either positive or negative and at most sites, the magnitude of the difference in saccade direction is <20°. Statistical testing showed that saccade direction differences were not correlated with saccade vector amplitude (Pearson correlation; $p>0.05$). In summary, analysis of saccade parameters in the two eyes showed that differences in saccade vector direction but not saccade vector amplitude could potentially contribute towards change in strabismus angle due to electrical stimulation.

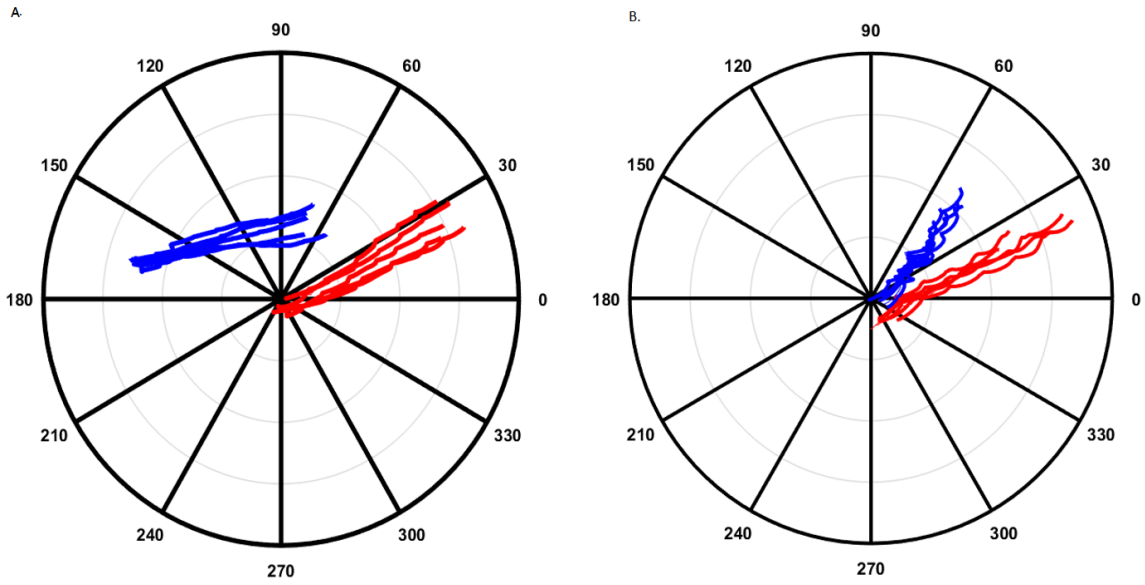

Figure 3.3.6 Polar plot showing direction of eye position during electrical stimulation

Figure 3.3.6: Polar plots showing electrically evoked saccades in right eye (red traces) and left eye (blue traces) at two different sites in Monkey E (Panel A) and Monkey H (Panel B). In Panel A, monkey is viewing with the right eye and in Panel B, monkey is viewing with the left eye. Plots show that the evoked movements are in different directions. Panel A: RE Direction ~25°, LE Direction ~ 9°; Panel B: RE Direction ~ 27°, LE Direction ~38°.

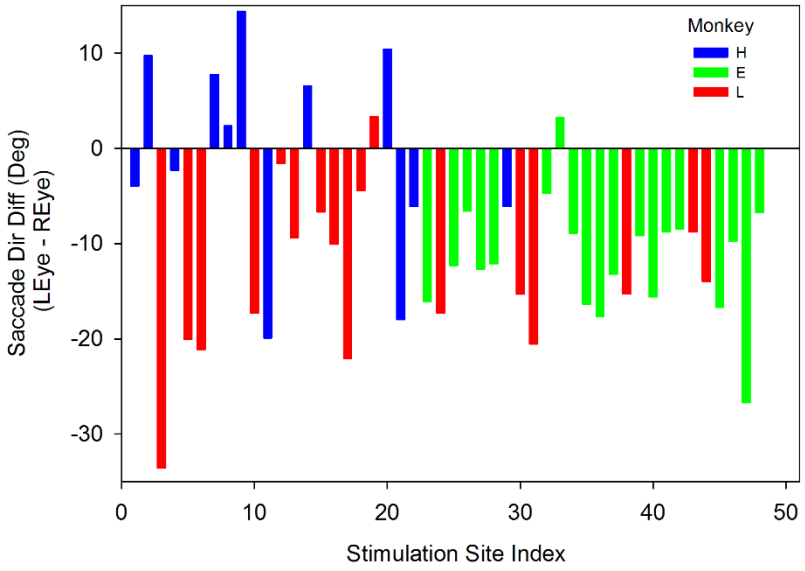

Figure 3.3.7 Bar plot showing difference in direction of saccades in two eyes

Figure 3.3.7: Summary of directional difference between left and right eyes electrically evoked saccades in the three monkeys obtained during left eye viewing conditions. Stimulation site index and color-coding scheme is the same as that used in Figure 4.

3.3.4 Disconjugate saccadic eye movements do not fully account for the change in horizontal and vertical strabismus angle due to electrical stimulation

We asked whether the observed change in horizontal and vertical strabismus angle was simply the cumulative consequence of the several disconjugate saccades (due to saccade direction difference) that were evoked during the 500ms of electrical stimulation. To perform this analysis, for each stimulation site, we calculated the contribution of horizontal (or vertical) saccade disconjugacy by multiplying the mean difference in horizontal (or vertical) saccade component of the two eyes due to a single electrically evoked saccade at that site with the number of saccades evoked in 500ms at the same site. These data are plotted in Figure 3.3.8A, B against the total change in misalignment at each stimulation site. The slope of the regression line in Figure 8A (horizontal misalignment) is 0.49, which means that only ~50% of the change in horizontal strabismus angle upon electrical stimulation is due to horizontal saccade disconjugacy. The regression line slope in Figure 8B (vertical misalignment) is 0.83 which means that most of the change in vertical strabismus angle is due to vertical component of saccade disconjugacy.

3.3.5 Disconjugacy in slow post-saccadic movements contributes significantly to change in misalignment during electrical stimulation

The change in misalignment due to electrical stimulation is fundamentally a sum of disconjugacy due to saccades and the disconjugacy due to slow post-saccadic movement. Examination of the raw data plots in Figure 3, indeed show significant post-saccadic

movement disconjugacy. In Panels 3.3.8C and 3.3.8D, we plot the predicted change in horizontal or vertical misalignment due to slow post-saccadic movement disconjugacy (derived as the difference between total change in misalignment and the contribution from saccade disconjugacy) against the total change in misalignment. These data highlight the significant contribution of slow post-saccadic movement disconjugacy towards the total change in misalignment. Fundamentally this analysis shows that during electrical stimulation, ~50% of change in horizontal misalignment is due to the slow post-saccadic movement disconjugacy. For vertical misalignment, there was no correlation between the change in vertical misalignment and the amount of post-saccadic movement disconjugacy (Figure 3.3.8D), which might be expected if saccadic disconjugacy (Figure 3.3.8B) can explain almost all of the change in vertical misalignment due to electrical stimulation. There was also no correlation between the amount of change in horizontal or vertical misalignment due to slow post-saccadic movements and the radial amplitude of the electrically evoked saccade ($p>0.05$).

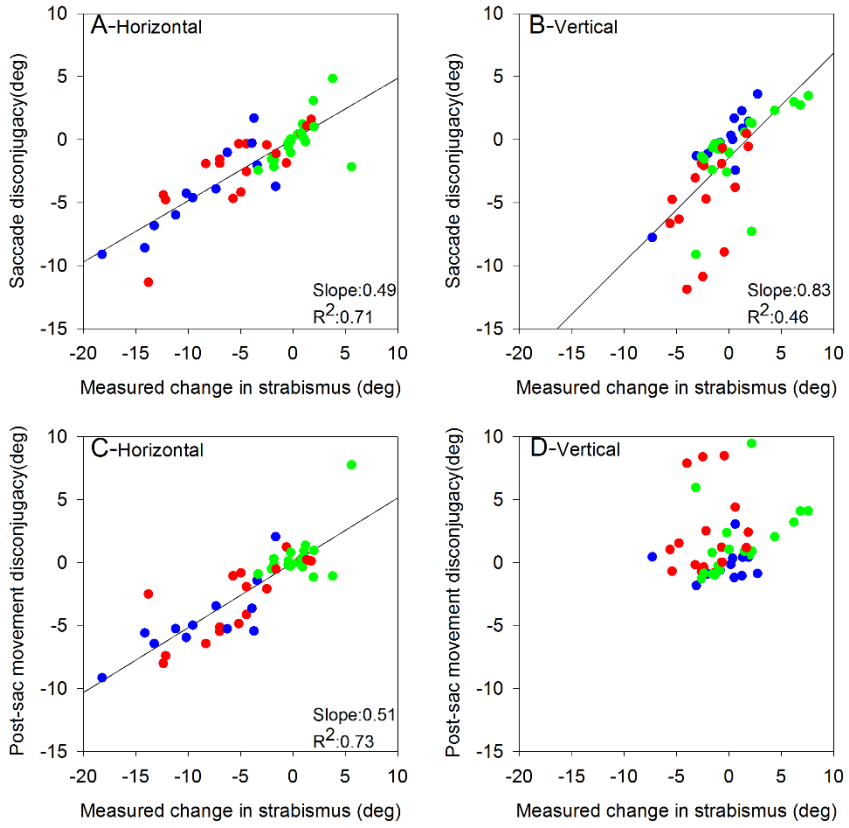

Figure 3.3.8 Scatter plot showing contribution of saccades and post saccadic drift in change of strabismus angle

Figure 3.3.8: Panels A and B show contribution of horizontal (Panel A) and vertical (Panel B) saccadic disconjugacy (estimated from cumulative sum of horizontal saccade disconjugacy) towards total change in horizontal or vertical strabismus angle over 500ms stimulation period at all stimulation sites during left eye viewing. Panels C and D show contribution of horizontal (Panel A) and vertical (Panel B) slow post-saccadic movement disconjugacy towards total change in horizontal or vertical strabismus angle over 500ms stimulation period These data show that horizontal saccade disconjugacy and slow post-

saccadic movement disconjugacy accounts for ~50% each of total change in horizontal strabismus angle and that vertical saccade disconjugacy accounts for ~85% of total change in vertical strabismus angle due to electrical stimulation. Color coding scheme is same as in Figure 3.3.4.

3.3.6 Rostral-caudal influence on saccade disconjugacy and post-saccadic movement disconjugacy

Since vergence neurons were previously found within the rostral part of SC, we wondered whether there might be different influence on eye misalignment upon stimulation at rostral vs caudal SC sites. To perform this analysis, we arbitrarily divided our data into stimulation sites that yielded saccades of radial amplitude <5° (36 rostral sites during left eye viewing) and sites that yielded saccades of radial amplitude >5° (12 caudal sites – sites 4, 6, 8, 9, 13, 14, 18, 20, 34, 41, 48, 50). From figure 3, it is apparent that stimulation at either rostral or caudal sites can induce significant changes in horizontal or vertical strabismus angle.

Data in figure 8 shows the contributions of saccade disconjugacy and post-saccadic movement disconjugacy towards change in misalignment at all sites. We also re-calculated the contribution of saccade disconjugacy and post-saccadic movement disconjugacy separately for rostral and caudal sites. Figure 3.3.9A, B shows the contribution of saccade disconjugacy towards change in horizontal and vertical misalignment and Figure 3.3.9C, D shows the contribution of post-saccadic movement disconjugacy towards change in misalignment. In each panel, black line fits are for rostral sites data and light grey line fits are for caudal sites data. Line fits are provided only when there is a correlation between x-axis and y-axis values, i.e., if the slope values of the regression are statistically significant. Slope values in Panels 3.3.9A suggest that contribution of saccade disconjugacy towards change in horizontal misalignment is slightly greater at caudal sites (~54%) than rostral sites (~43%). Similarly slope values in

Panels 3.3.9C suggest that contribution of post-saccadic movement disconjugacy is larger at rostral sites (~57%) than at caudal sites (~46%). However, a statistical comparison of slopes of rostral and caudal sites regression lines within each panel yielded no significant difference (t-test; p=0.21). A similar consideration of vertical misalignment and rostro-caudal influences showed that although there was a linear relationship between saccade disconjugacy or post-saccadic movement disconjugacy and change in vertical misalignment for rostral sites data, there was no such relationship for the caudal site data. For the rostral sites, saccade disconjugacy and post-saccadic movement disconjugacy contribute ~50% each of the total change in vertical misalignment.

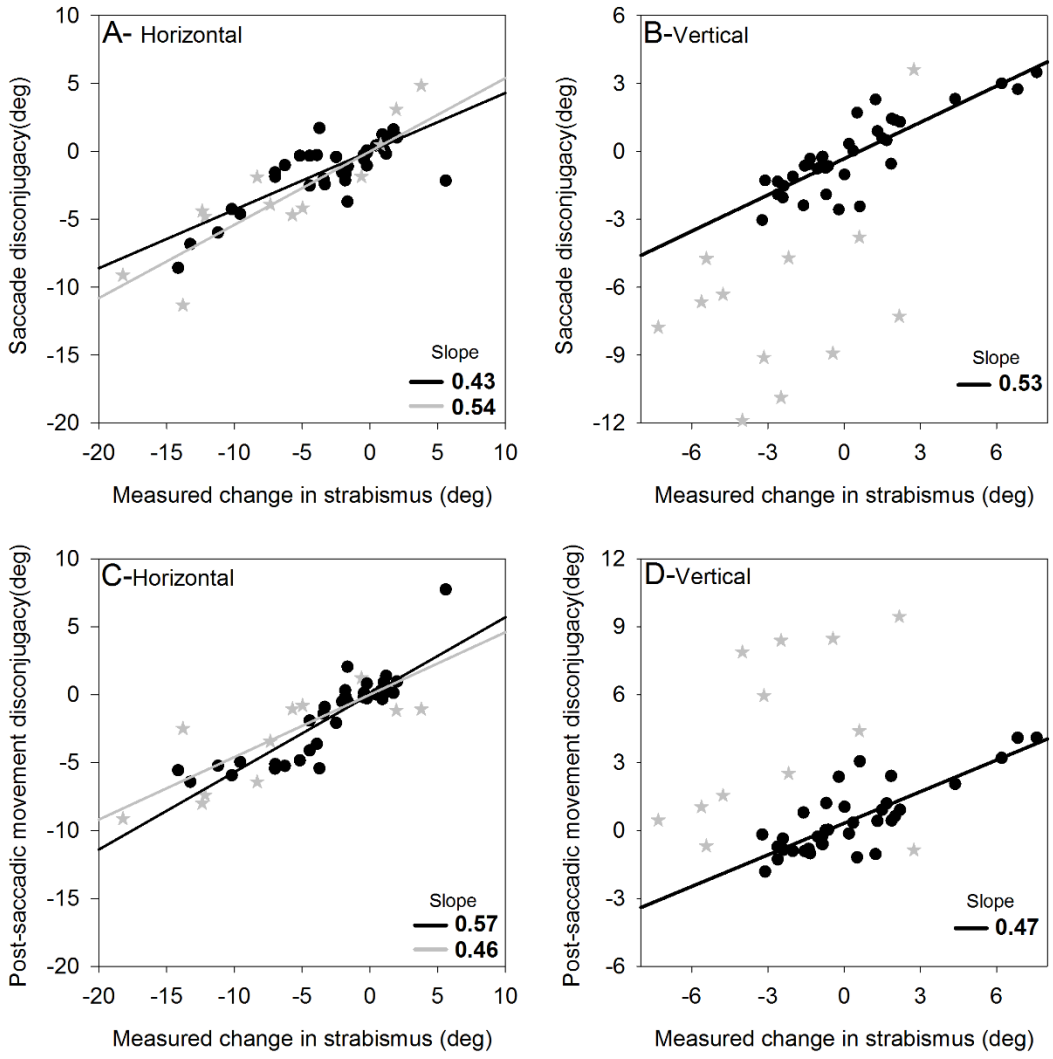

Figure 3.3.9 Plots showing contribution of rostral and caudal Superior Colliculus in change of strabismus angel

Figure 3.3.9: Contribution of saccade disconjugacy (Panels A, B) and post-saccadic movement disconjugacy (Panels C, D) towards change in horizontal (Panels A, C) and vertical misalignment (Panels B, D) at rostral (black circles; black regression line) and caudal (grey stars; grey regression line) sites. Slopes of regression lines indicate relative contribution of each quantity towards change in horizontal or vertical misalignment. At

caudal sites, there was no linear relationship between vertical saccade disconjugacy or vertical post-saccadic movement and vertical change in misalignment.

3.3.7 Could the differences in saccade direction and changes in eye misalignment be unrelated to electrical stimulation of the Colliculus?

Eye movement disconjugacies have been described before in strabismic monkeys (Das et al. 2005, Fu et al. 2007, Walton et al. 2014). It is possible that the saccade direction and eye misalignment changes that we observed are not specifically related to involvement of the colliculus and saccadic circuits, but rather a function of other mechanisms, for example disrupted downstream vergence circuits or possibly orbital factors that alter pulling directions of extraocular muscles. In order to assess the effect of these factors, we performed two control experiments. The first control experiment involved acquiring data during visually guided saccades that were matched in amplitude and direction with the previously collected electrically evoked saccades in the three monkeys. Although target movement was matched to the previously acquired electrically evoked saccade amplitude and direction, the actual amplitude and direction of the visually guided saccade was slightly different, perhaps due to small drift of the eye prior to saccade onset. Therefore, we paired visually guided saccades with electrically evoked saccades that were within 1° of amplitude and within 30° of saccade direction. This criterion resulted in matching of one visually guided saccade with more than one electrically evoked saccade site. Figure 10 summarizes the comparisons between electrically evoked saccades (ES) and visually guided saccades (VGS) of matched amplitude and direction. Panel 3.3.10A compares difference in saccade direction of the right and left eyes between ES and VGS and shows no significant difference between these two quantities (paired signed-rank test $p>0.05$). The difference in RE and LE radial amplitude (Panel 3.3.10B) was also not significantly

different between ES and VGS (paired signed rank test; $p>0.05$). In order to examine effects on misalignment (Panels 3.3.10C, D), we calculated a change in horizontal or vertical misalignment following a visually guided saccade (calculated at 200msec after saccade offset) and compared this value to change in misalignment after a single electrically evoked saccade (total change in misalignment due to 500ms stimulation divided by number of electrically evoked saccades in each staircase). Note that the change in misalignment in Figure 3.3.10C, D includes both the effect of saccade disconjugacy and also post-saccadic movement disconjugacy for both ES and VGS. Change in horizontal misalignment due to ES was significantly larger than VGS (Figure 3.3.10C; paired signed rank test $p<0.05$). Note in Figure 10C that although the data points are above the unity line, the magnitude of change in horizontal misalignment is actually larger for ES because it is more negative or divergent for ES than VGS. Change in vertical misalignment (Figure 3.3.10D) due to ES was not significantly different from that that during VGS (paired signed rank test; $p>0.05$). In summary, these control data suggest that saccade disconjugacy (saccade amplitude and saccade direction) is not significantly different between ES and VGS but change in horizontal misalignment is indeed larger during ES than VGS. Thus, electrical stimulation within the SC may indeed activate special circuits or cell populations, possibly vergence related, that causes changes in misalignment (especially horizontal misalignment) that are larger than during VGS.

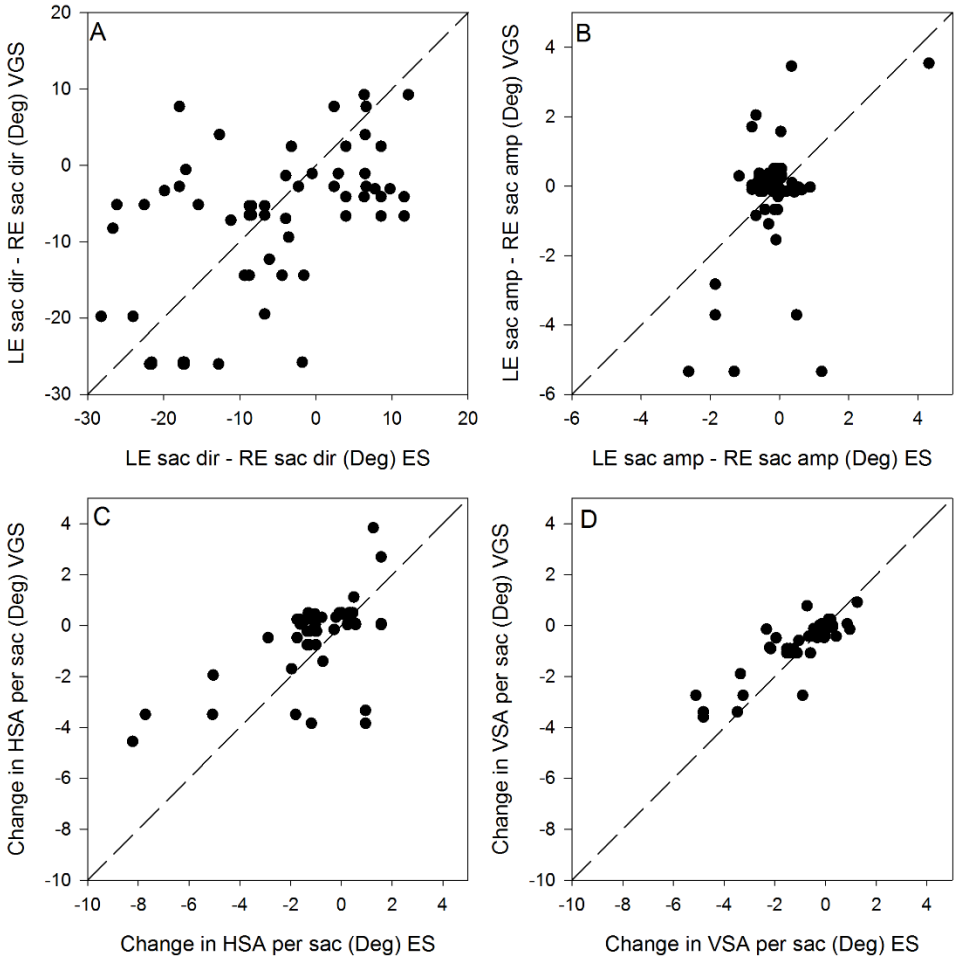

Figure 3.3.10 Comparison of change in strabismus angle between visually guided saccades and electrically evoked saccades

Figure 3.3.10: Comparison of electrically evoked saccades (ES) and visually guided saccades (VGS) of similar amplitude and direction in all animals. Panel A: Comparison of difference in saccade direction of right and left eyes during ES or VGS shows no significant differences (points distributed around the dotted unity line). Panel B: Difference in radial saccade amplitude of right and left eyes during ES or VGS showed no significant difference. Panel C: Comparison of the change in horizontal strabismus

angle due to a single ES or VGS shows larger (more negative; data points above the unity line) effects due to electrical stimulation than visually-guided movements. Panel D: Comparison of the change in vertical strabismus angle due to a single ES or VGS shows no significant difference between electrical stimulation and visually-guided movements.

In a second control, we analyzed eye movement direction differences between the two eyes during smooth-pursuit eye movements in the horizontal and vertical directions. Similar direction differences during smooth-pursuit and electrically evoked saccades would suggest that areas downstream of the colliculus, perhaps related to generation of pattern strabismus, are responsible for the stimulation effects observed in this study. Direction differences during smooth-pursuit were calculated from the desaccaded eye movements shown in Figure 1. In each monkey, there was indeed difference in smooth-pursuit direction between the two eyes but the difference was constant and relatively small (Right Eye Viewing: Monkey E = ~5°, Monkey H = ~4°, Monkey L = ~6°; Left Eye Viewing: Monkey E = ~8°, Monkey H = ~4°, Monkey L = ~5°). In contrast, saccade direction differences and misalignment change due to electrical stimulation of the SC are either positive or negative as shown in Figure 3.3.4 and 3.3.7 and are larger in magnitude. To illustrate these differences more clearly, Figure 3.3.11 plots the position of the right eye versus the left eye for both smooth-pursuit (after desaccading) and a single electrically evoked saccade staircase in Monkey H. The slope of the regression line is 0.94 for smooth-pursuit and 2.13 for the electrically evoked saccade train. Therefore, these second set of control data show that direction differences and misalignment changes due to SC electrical stimulation are significantly greater than might be predicted due to smooth-pursuit disconjugacy and is therefore likely a product of activation of specific circuits or cell types within the SC.

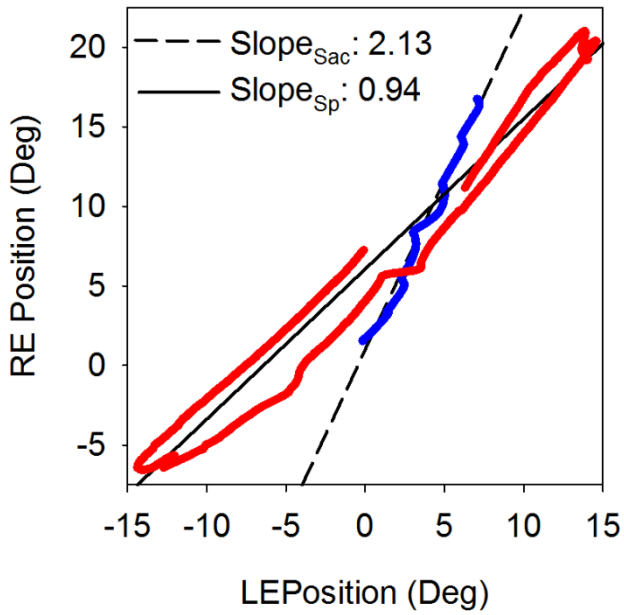

Figure 3.3.11 Line plot showing relation between change in eye position between smooth pursuits and electrical stimulation

Figure 3.3.11: Comparing horizontal component of right and left eye movement during horizontal visually guided smooth-pursuit (red trace; solid line regression fit) and electrically evoked saccades (blue trace; dotted line regression fit) in Monkey H. Disconjugacy and change in alignment is significantly larger during electrical stimulation (saccade slope =2.13; smooth-pursuit slope=0.94).

3.4 Discussion

In this study, we have used the strategy of electrical stimulation to study the role of the superior colliculus in driving eye misalignment. The main findings in our study were 1) electrical stimulation of the SC induced significant changes in eye misalignment that could be either divergent or convergent depending on stimulation site. 2) The electrically evoked saccades in the two eyes were of similar radial amplitudes but differed in saccade direction. 3) The change in misalignment evoked by electrical stimulation was due to both disconjugate saccades and disconjugate post-saccadic movement. These results advance our understanding of strabismus in primates in that it implicates the SC as being part of the circuit that serves as a neural substrate for steady-state ocular misalignment. Additionally, the mechanism by which the SC participates in strabismus is consistent with our previously proposed idea of a disrupted vergence circuit. Below we discuss the implications of these findings in the context of saccadic circuitry and maintenance of the state of misalignment in strabismus.

3.4.1 Disconjugate saccade behavior following electrical stimulation of the SC

Disconjugate saccade behavior was primarily due to saccade direction differences. The differences in saccade directions in the two eyes was quite varied but most frequently were under 20°. Differences in saccade direction in strabismic monkeys have been observed before. While examining oblique saccade disconjugacy, Walton and colleagues observed that for the most part saccade direction was fundamentally affected (Walton et al. 2014). They also showed that electrical stimulation of the PPRF, structure that

receives projections from the SC, often evoked saccades of varying directions (Walton et al. 2013) and that direction preference of PPRF was disrupted in comparison to the normal (Walton and Mustari 2015). In a recent study, Fleuriet and colleagues also reported horizontal and vertical saccade disconjugacies following SC stimulation in strabismic monkeys (Fleuriet et al. 2016) and proposed that that stimulation in strabismic monkeys activates different desired displacements for the two eyes. In other words, the saccade disconjugacy observed with SC stimulation is due to separate right eye and left eye maps within the colliculus. However, Economides and colleagues (Economides et al. 2016) found some amplitude and direction differences upon electrical stimulation of caudal colliculus in a cohort of surgically induced strabismic monkeys, but concluded that tectal maps were not different in the two eyes.

Our analysis showed that only part of the change in horizontal misalignment (~50%) is due to disconjugate horizontal saccades (Figures 3.3.6-3.3.8). It is unlikely that disconjugacy is driven by initial eye position differences between the two eyes because two of the animals in the study (Monkeys L and H) presented with small misalignment but still showed significant changes in misalignment following stimulation. In their study of strabismic monkeys, Fleuriet and colleagues also did not find a significant effect of eye position upon saccade disconjugacy due to SC stimulation (Fleuriet et al. 2016). Also, the small amount of pattern strabismus in our monkeys (Figure 3.3.1) was insufficient to account for horizontal or vertical disconjugacy during stimulation. However the fact that saccade disconjugacy during visually guided saccades was not significantly different from saccade disconjugacy during electrically evoked saccades (Figure 3.3.10, A, B) suggests that there was nothing special about electrical stimulation

vis-**à**-*vis* saccade disconjugacy. We suggest that it is likely that disruption in saccadic circuitry either within or downstream from the SC was responsible for saccadic disconjugacy.

3.4.2 Role of the SC in eye misalignment – post-saccadic movement disconjugacy

In our study, about 50% of the change in horizontal misalignment is due to differential post-saccadic movement in the two eyes, i.e., a slow vergence drift. It is unlikely that these post-saccadic movements are due to faulty neural integration (post-saccadic drift due to pulse-step mismatch) because one could reasonably expect that the neural integrator functions similarly during smooth-pursuit, visually guided saccades and electrically evoked saccades, but the changes in horizontal misalignment are greater during electrical stimulation than during either pursuit or visually guided saccades (Figure 3.3.10C, 3.3.11). Also almost none of the change in vertical misalignment evoked by electrical stimulation is due to vertical post-saccadic movement disconjugacy (Figure 8D). It should however be noted that vertical misalignment is usually significantly smaller than horizontal misalignment.

The afferent and efferent anatomical projections of the SC are diverse (May 2006); focusing on potential 'vergence' pathways, the SC receives extensive projections from cortical areas encoding disparity information including LIP and Frontal eye field (FEF). It also receives projections from the cFN and the PIN (May et al. 1990), areas that we showed are related to the strabismus state (Joshi and Das 2013). There are major reciprocal connections between the SC and the central mesencephalic reticular formation (cMRF) (lateral to OMN) and studies have identified connections between the cMRF and

the SOA (related to vergence), Edinger-Westphal nucleus (related to accommodation), oculomotor and abducens nuclei (Buttner-Ennever et al. 2001, Ugolini et al. 2006, May et al. 2015, Bohlen et al. 2016). This slow vergence circuit could be involved in static alignment and is potentially disrupted in strabismus. As part of the horizontal saccade circuitry, the SC and the cMRF connect to the Paramedian Pontine Reticular Formation (PPRF) and electrical stimulation and neural recording within the PPRF in strabismic monkeys has suggested that saccade disconjugacy in strabismus may be a consequence of disruption within this circuit (Walton et al. 2013, Walton and Mustari 2015). It is possible that the circuit that mediates saccade disconjugacy is separate from the afore-mentioned slow vergence circuit. The difference between electrically evoked saccades and similar amplitude and direction visually guided saccades (Figure 3.3.10) also suggests that specific vergence circuits or cell population within the SC that affect alignment are being recruited by stimulation.

3.4.3 Role of the SC in eye misalignment – rostral vs caudal influences

Ohtsuka and colleagues reported a patient with a focal lesion in the rostral SC, who showed a deficit in convergence and a static exotropia (Ohtsuka et al. 2002). In the normal monkey, Van Horn and colleagues found vergence cells while specifically targeting the rSC and also found that electrical stimulation induced divergence when looking at near targets (Van Horn et al. 2013). It was therefore possible that, in the strabismic monkey, the changes in misalignment due to stimulation might have been restricted to rostral areas in the SC. However, we found that stimulation in the caudal colliculus (defined arbitrarily as sites yielding radial saccade amplitude >5°) also induced

changes in misalignment (Figure 3.3.3, Figure 3.3.9). Although their studies were primarily focused on investigating saccadic tectal maps in strabismus, examination of Figure 3.3.1 from Fleuriet and colleagues (Fleuriet et al. 2016) and Figure 3.3.2 from Economides and colleagues (Economides et al. 2016) also suggest that stimulation at some caudal colliculus sites can result in change in misalignment due to disconjugate post-saccadic movements. Comparing relative contributions of saccade disconjugacy and slow post-saccadic movement disconjugacy towards total change in horizontal misalignment at rostral and caudal sites yielded no significant differences (Figure 3.3.9), although the trend was for increased contribution of slow post-saccadic movement in rostral SC and increased contribution of saccadic disconjugacy in caudal SC. In summary, evidence points to a continuum along the rostro-caudal axis for saccade amplitude representation and similarly horizontal vergence cells may also be spread throughout the SC. Relative contributions of vertical saccade disconjugacy and vertical post-saccadic movement disconjugacy to change in vertical misalignment were similar to each other and to relative contributions to change in horizontal misalignment (~50% each) at rostral sites, but not at caudal sites. However, this finding is difficult to interpret since nothing is known about the neural substrate for vertical vergence.

Chapter 4: Properties of cells associated with strabismus angle in rostral superior colliculus of strabismic monkeys

4.1 Introduction

About 5% of all infants in the world have some form of ocular misalignment or strabismus (Govindan et al. 2005, Mohney 2007). This developmental disorder is treated mostly at the level of muscles using surgical methods with a notion that over-action or under-action of specific extraocular muscles is causing strabismus. Recent data from animal models of strabismus acquired using neurophysiological methods such as electrical stimulation, muscimol inactivation and single cell recording within numerous brain areas including the motor nuclei, supraoculomotor area, fastigial and posterior interposed nuclei of the cerebellum, paramedian pontine reticular formation and the superior colliculus, have shown that various structures within a vergence neural circuit contributes towards maintenance of the state of strabismus (Das and Mustari 2007, Joshi and Das 2011, Joshi and Das 2013, Walton et al. 2015, Fleuriet et al. 2016, Upadhyaya et al. 2017, Walton et al. 2017).

The superior colliculus has been extensively studied for its involvement in saccadic eye movements (Gandhi and Katnani 2011), but this structure also appears to have a role in vergence. Van Horn and colleagues, in a study in normal monkeys, have shown that the rostral superior colliculus contains vergence related neurons (convergence and

divergence) which modulate with eye movements made to target motion in depth (Van Horn et al. 2013). It has also been shown that stimulation of the rostral superior colliculus during an asymmetric vergence task affects vergence eye movement in normal monkeys (Chaturvedi and van Gisbergen 1999, Chaturvedi and Van Gisbergen 2000). Vergence related neurons have also been recorded in rSC of cat (Jiang 1996). In humans, a case of bilateral superior colliculus lesion resulted in convergence and accommodation palsy (Ohtsuka et al. 2002). Afferent and efferent anatomical connections from cerebellar areas to the central mesencephalic reticular formation and supraoculomotor areas provide supporting evidence that the SC also lies within a vergence and accommodation circuit (May et al. 2015, Bohlen et al. 2016).

Motivated by the evidence in normal monkeys and humans, we recently investigated the SC in strabismic monkeys using electrical stimulation techniques and showed that low current electrical stimulation within mostly the rostral part of the superior colliculus of strabismic monkeys resulted in a change in strabismus angle (Upadhyaya et al. 2017). Depending on the site of stimulation, we elicited either convergent or divergent change of strabismus angle. Other studies in strabismic monkeys using SC electrical stimulation have also shown that there were influences on strabismic angle during electrical stimulation (Economides et al. 2016, Fleuriet et al. 2016, Economides et al. 2017). Our analysis of the stimulation data indicated that the strabismus angle changes were a consequence of disconjugate saccades and also disconjugate post-saccadic drift. We hypothesized that the disconjugate post-saccadic drift could be the consequence of stimulating a population of vergence (misalignment in the case of strabismic monkeys) cells within the SC. Therefore, the goal of the current study was to identify and examine

the firing properties of any misalignment related cells within the SC. Since the previous work by Van Horn and colleagues and also our own electrical stimulation study was primarily focused in the rostral regions of the SC, we focused the neural recording also in the rSC. Our data indicates that certain cells in the rostral superior colliculus (rSC) show responses that are related to strabismus angle. Some of these data have appeared before in abstract form (Upadhyaya and Das 2018).

4.2 Methods

4.2.1 Subjects, Rearing Paradigms and Surgical Procedures:

The subjects of this study were two adult exotropic (divergent strabismus; Monkey M1 and Monkey M2) monkeys whose strabismus was previously induced in infancy by disrupting binocular vision during the critical period of development using an optical prism-rearing method. In the optical prism rearing paradigm, the infant monkeys wore lightweight helmets fitted with either a base-in or base-out prism in front of one eye and a base-up or base-down in front of the other eye starting from day one after birth till they were four months of age after which they were allowed to grow under unrestricted viewing conditions (Smith et al. 1979, Crawford and von Noorden 1980). This paradigm decorrelates binocular vision during the critical period for visual development thus resulting in development of strabismus (Tusa et al. 2002).

When the animals were ~4 years of age, they underwent a surgical procedure carried out under aseptic conditions with isoflurane anesthesia (1.25%-2.5%) to implant a head stabilization post (Adams et al. 2007). Later in a second surgery, we stereotaxically implanted a 21mm diameter titanium recording chamber in each animal (M1 location: 3mm anterior and 1mm lateral with respect to ear-bar-zero and a 20° tilt angle with respect to the sagittal plane; M2 location: on the mid-sagittal plane and 15mm above ear-bar-zero and a 38° tilt angle to the coronal plane). In the same surgery we also implanted a scleral search coil in one eye using the technique of Judge and colleagues and in a third surgery, a scleral search coil was implanted in the fellow eye (Judge et al. 1980). All procedures were performed per National Institute of Health 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 (IACUC) at the University of Houston. Monkey M1 was used in our previously published study (Monkey H) that examined effect of electrical stimulation within the superior colliculus of strabismic monkeys (Upadhyaya et al. 2017).

4.2.2 Experimental Paradigms, Data acquisition and analysis

Monkeys were trained on a variety of oculomotor tasks prior to data collection for this study. Eye movements were calibrated as the monkey monocularly viewed target stimuli at ±15° horizontally and vertically. A 2° sized white optotype target (luminance 470 cd/m^2) on a black background (luminance 0.5 cd/m^2) was used in the study. Targets were back-projected onto a tangent screen at a distance of 57cm using a DepthQ LCD

projector (Lightspeed Design Inc., Bellevue WA, USA) running at 120-Hz frame rate. Liquid crystal shutter goggles (Citizen Fine devices, Nagano, Japan) under computer control were used to facilitate monocular viewing. Changing the viewing eye resulted in a change in strabismus angle that we were able to leverage to identify cells related to eye misalignment.

The SC was identified by visual responses from cells in the superficial layer followed by saccade related bursting as we descended into the intermediate and deep layers. Electrical stimulation, resulting in staircase saccades, was also used to map the area and the area that evoked a radial saccadic amplitude of <5° was defined as our target zone (rostral SC) for neural recording. Once a cell in the rostral SC was isolated and then identified as being related to eye misalignment, the following four tasks were performed –

- Monocular fixation with either eye for 4-7 sec each. We were able to leverage the change in strabismus angle depending on fixating eye to identify cells whose response correlated with eye misalignment.
- 10°-15° amplitude ipsilateral and contralateral saccades during monocular viewing to examine whether the misalignment cells paused during large saccades, similar to previously described fixation cells (Munoz and Wurtz 1993).
- A target blink paradigm (in which animal monocularly fixated a straight-ahead target for 1-3 sec during which the target was randomly blanked for 300-400ms) to determine if the cell showed visual sensitivity.

- Fixation at vertical +/- 10° to determine whether changes in horizontal strabismus angle at different vertical gaze positions (A/V pattern strabismus) was correlated with any changes in firing rate of the misalignment related cells.

Eye movement data were processed with anti-aliasing filters at 400Hz before sampling at 2.79 kHz with 12-bit precision (Alpha Lab SNR system; Alpha-Omega Engineering, Nazareth, Israel). All eye movement data were additionally calibrated offline and filtered using a software finite impulse response (FIR) low-pass filter with a pass-band of 0-80Hz. Single cell recording was performed using epoxy coated tungsten electrode with ~1Mohm resistance (Frederik Haer, Brunswick, ME). Raw spike data were acquired at a sampling rate of 44 kHz. Spike sorting was performed offline using a template matching algorithm (Spike 2 Software; Cambridge Electronics Design, England). Unit response was represented as a spike density function that was generated by convolving action potential time stamps with a 15ms gaussian. Data analysis was performed with custom software routines developed in MATLAB (Mathworks, Natick, MA, USA) and SigmaPlot 12.0 (Systat, Inc; San Jose, USA) was used for statistical analysis. The overall goal of the data analysis was to establish whether changes in neuronal firing rates observed in the rSC cells corresponded to the changes in angle of misalignment.

4.3 Results

4.3.1 Properties of Strabismus

Animal M1 had an exotropia of ~5°-10° during right eye viewing and ~10°-15° during left eye viewing and monkey M2 had an exotropia of ~20°-27° during right eye viewing and ~15°-20° during left eye viewing. Monkey M1 did not show any prominent pattern deviation (i.e., no change in strabismus angle with up or down viewing), whereas monkey M2 showed A-pattern strabismus (i.e., reduction in angle of exotropia during up-gaze compared to down-gaze). Note that animal M1 is the same animal that we used previously for an SC electrical stimulation study (Monkey H in that study). Table 4.3.1 summarizes properties of strabismus in both of the monkeys.

Table 4.3.1 Properties of animals used in single cell recording

Monkeys	**Age(yrs)**	**Strabismus Angle(°)**		**Refractive Error(D)**		**Strabismus Properties**
		RE View	LE View	RE	LE	
M1	7	3°-18°XT	10°-20°XT	+4.50	+0.75	DHD, N
M2	9	15°-22°XT	20°-28°XT	-4.50	-1.50	DHD, N

Legend: DHD= Dissociated Horizontal Deviation, N =Nystagmus, XT= Exotropia, D=Diopters

4.3.2 SC Recording Locations

Cells related to eye misalignment, which were the target of this study, were generally recorded ~1.5-2mm deeper than the initial visual background response that was characteristic of penetrating the superficial layers of the SC. Once an individual cell was isolated and neuronal data collected within the various paradigms described earlier, we delivered small amplitude electrical stimulation (10-40micramp, 400HZ, 500ms) to examine the amplitude of the evoked staircase saccades and thereby get an estimate of our recording location within the SC. The mean radial amplitude of saccades evoked in the viewing eye at the recording sites were 2.1°±1.3°(M1) and 0.90°±0.41°(M2) with range of 0.6°-4.9°(M1) and 0.2°-2.1°(M2). Six cells were recorded from the right superior colliculus and 43 cells were recorded from the left superior colliculus. The polar plot in Figure 4.3.1 shows amplitude and direction of the first electrically evoked saccade from the two monkeys. By design, all of the recording sites were within the rostral SC as shown by electrically evoked radial saccade amplitude being less than 5° (~88% of recording sites evoked saccades less than 2°).

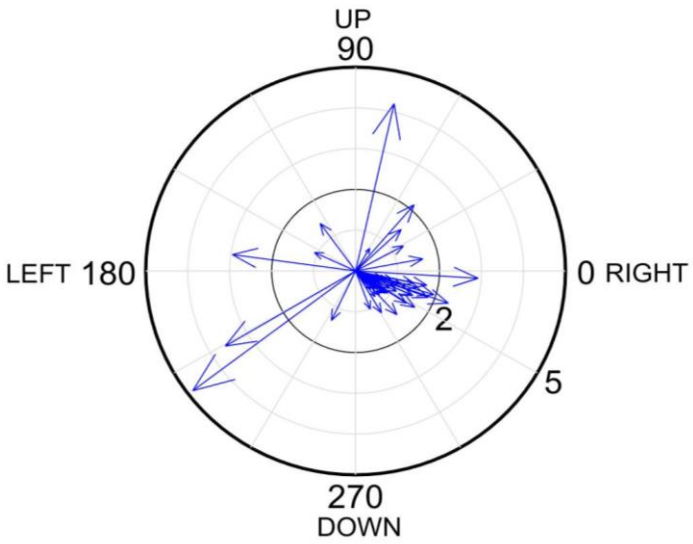

Figure 4.3.1 Polar plot showing amplitude and direction of the electrically evoked first saccade vector in the viewing eye at sites where misalignment cells were recorded

4.3.3 SC Misalignment Cell Response during Change in Strabismus Angle

A total of 49 cells which were modulated by a change in eye misalignment were recorded in the two animals (M1- 28 cells, M2- 21 cells). Two different types of strabismus-related cells were encountered. The more commonly encountered cells (M1 - 17/28 cells, 61% and M2 - 16/21 cells, 76%) were near-response cells that showed an increased firing rate for a smaller angle of exotropia (e.g. brought about by changing the eye of fixation from left eye to right eye in monkey M1). The other type of cell were far-response cells (M1 - 11/28 cells, 39% and M2 - 5/21 cells, 24%), as they showed an increased firing rate for a larger angle of exotropia. Note that since exotropia is a divergent strabismus, an increase in exotropia is an increase in divergence and a decrease in exotropia is a decrease in divergence (or equivalently an increase in convergence).

Figure 4.3.2 shows an example of a far-response cell (panel A – left column) and a near-response cell (panel B – right column) in the rSC of monkey M1. The far-response cell was recorded from left rSC and the near-response cell was from the right rSC. Also shown in the figure (Fig 4.3.2A-i and B-i) are the staircase saccades evoked due to electrical stimulation at these sites. Electrical stimulation not only evoked staircase saccades but also produced a divergent change in strabismus angle as we have shown in our previously published study.

In these data, a change in the eye of fixation brought about a change in strabismus angle (evidence for Dissociated Horizontal Deviation) and this property was leveraged to investigate the correlation between strabismus angle and neural response rate within the sub-population of rSC cells. Mean change in strabismus angle (calculated as difference

between left and right eye position; shown in Fig 4.3.2A-iv and B-iv) was ~8° in M1 and ~10.0° in M2. Average neural responses shows that in the cell shown in the left column (Fig 4.3.2A-vi), there was increased firing rate when angle of exotropia was greater (far-response cell) and in example cell in the right column (Fig 4.3.2B-vi), there was increased firing rate when the angle of exotropia was small (near-response cell).

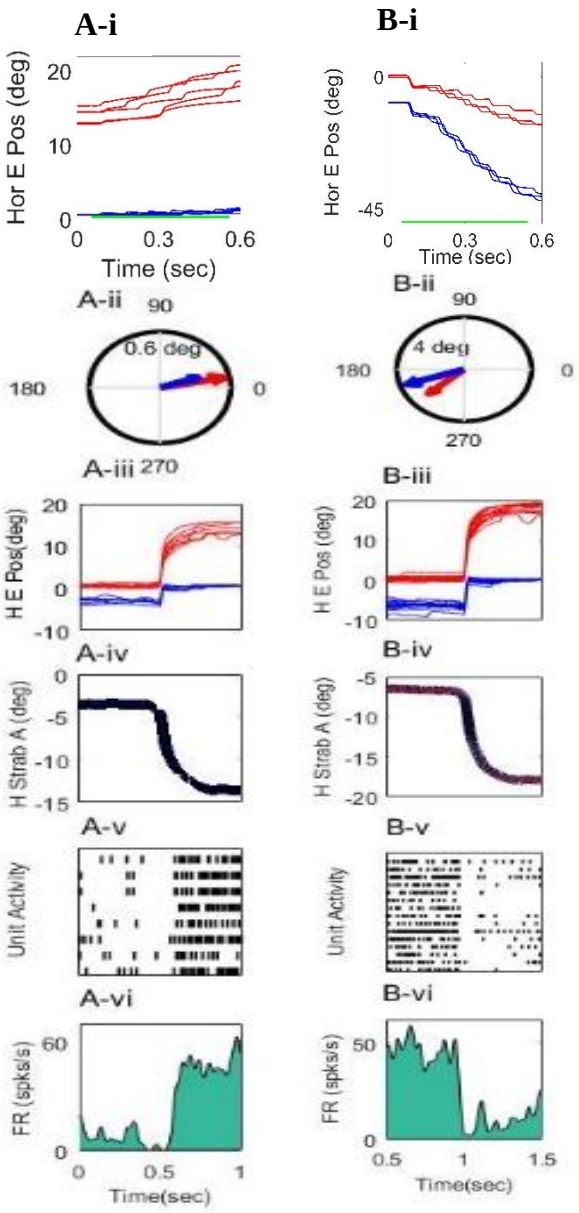

Figure 4.3.2 Raw position and neural data from a near response cell and far response cell collected from rostral superior colliculus of strabismic monkey M1

Figure 4.3.2: Raw eye and neural data from a far response cell (Panel A) and near response cell (Panel B) in monkey M1. A-i and B-i show staircase eye movement responses following electrical stimulation. Multiple trials are aligned on the start of stimulation shown by the green bar at the bottom of the plot. A-ii and B-ii are polar plots showing amplitude and direction of the first saccade vector elicited by electrical stimulation at this site. A-iii to A-vi and B-iii to B-vi are data associated with the neural response. A-iii and B-iii show multiple trials of an alternate cover test where the monkey was first viewing a straight-ahead target with his right eye (red) and then his left eye (blue). Positive numbers denote rightward eye positions. A-iv and B-iv shows horizontal strabismus angle (left eye position minus right eye position calculated from A-iii and B-iii) and illustrates the change in strabismus angle due to fixation switch. A-v and B-v are raster plots showing timestamps of neural spiking during each trial shown in A-iii and B-iii. A-vi and B-vi shows average firing rate of the far response cell and near response cell. Data in A-iii to A-vi and B-iii to B-vi are aligned with change in fixation from right eye to left eye. In all plots, right eye is denoted in red and left eye is denoted in blue.

4.3.4 Quantification of Eye Misalignment Sensitivities of rSC cells

For each cell, data obtained during several trials of change in fixation with each eye viewing were averaged and a linear regression was performed between the average firing rate and the corresponding mean strabismus angle. The slope of the regression line for each cell was a measure of the neuronal sensitivity (spikes/s/° of strabismus angle) of the cell and the threshold was a measure of the angle of misalignment at which the cell commenced firing. For the representative far-response cell shown in figure 4.3.2A, mean sensitivity was -3.1 spks/sec/deg of strabismus angle and mean threshold was about 0.4° and for the representative near-response cell shown in figure 4.3.2B, mean sensitivity was 3.7 spks/sec/deg of strabismus angle and mean threshold was about -21.6°. Note that negative angles imply exotropia or divergence and positive angles imply esotropia or convergence.

Figure 4.3.3 shows the relationship between firing rate and strabismus angle for the population of near and far-response cells (grey lines) in monkeys M1 and M2. Table 4.3.2 shows sensitivity, threshold and coefficient of determination for each of the cells in our sample. Also shown in Figure 4.3.3 are the population average of the strabismic animals (shown as red lines) and for comparison the population average of normal animals (NM), redrawn from the data published by Van Horn and colleagues, shown in green lines ((Van Horn et al. 2013); personal communication with Dr. K. E. Cullen). Comparing the strabismic and normal animals yields two observations. First, the population thresholds for near and far-response cells in the strabismic animals were substantially shifted towards exotropia (near-response cells: M1 18.2° XT; M2: 26.7°

XT; NM ~2.0° divergence & far-response cells: M1 3.8° ET; M2 8.8° XT; NM ~15.0° convergence). Second, the population sensitivity of near-response cells and far-response cells in rSC of strabismic animals and convergence and divergence cells found in rSC of normal animals were similar (near-response cells: M1 3.2±1.6 spks/sec/deg; M2 3.0±2.6 spks/sec/deg; NM 3.3±2.3 spks/sec/deg; one-way ANOVA df2, F=0.04, p=0.96 & far-response cells: M1 - 3.1±1.8 spks/sec/deg; M2 -2.1±1.2 spks/sec/deg; NM 2.6±1.0 spks/sec/deg; one-way ANOVA df2, F=0.86, p=0.44).

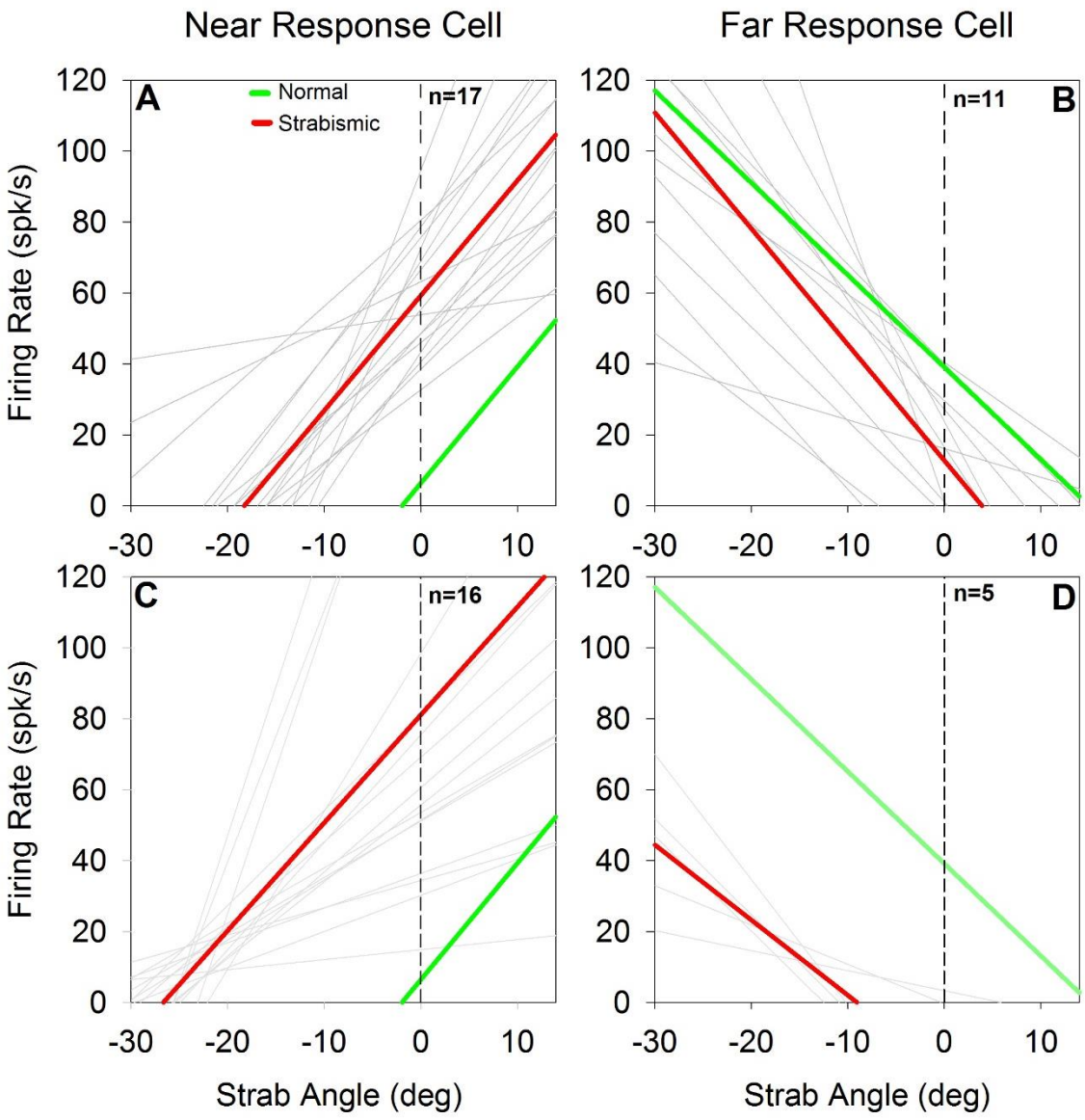

Figure 4.3.3 Population sensitivities and threshold of normal and strabismic monkeys

Figure 4.3.3 : Summary plot showing response properties of rSC near response cells (Panel A, C) and far-response cells (Panels B, D) in animals M1 (Panels A, B) and M2 (Panels C, D). Each grey line represents the rate-misalignment curve for a single misalignment cell found in rSC. The red line

is the population average in the strabismic monkey. The green line is the population average drived from data of Van Horn and colleagues in normal monkeys. The x-intercept is the threshold or angle of misalignment at which the cell commences firing and the slope is a measure of the cell's sensitivity to angle of misalignment.

Table 4.3.2 Summary of mean sensitivity, mean threshold, and R2 value of all the misalignment cells.

Coefficient of determination				Coefficient of determination			
Cell No	Sensitivity	Threshold		Cell No	Sensitivity	Threshold	
M1_01	4.07	-10.65	0.98	M2-01	-2.68	-12.49	0.88
M1_02	2.18	-21.11	0.71	M2-02	7.10	-25.59	0.94
M1_03	2.51	-19.29	0.82	M2-03	-0.56	5.87	0.54
M1_04	2.67	-14.42	0.97	M2-04	-3.53	-10.19	0.85
M1_05	0.42	-129.11	0.38	M2-05	0.97	-37.27	0.73
M1_06	6.28	-11.56	0.95	M2-06	1.76	-29.95	0.80
M1_07	3.36	-22.50	0.99	M2-07	1.59	-32.19	0.86
M1_08	7.11	-13.30	0.91	M2-08	1.02	-29.68	0.63
M1_09	4.33	-15.94	0.83	M2-09	-2.71	-10.88	0.96
M1_10	2.05	-16.00	0.85	M2-10	2.37	-25.50	0.88
M1_11	3.03	-16.05	0.89	M2-11	8.38	-25.63	0.94
M1_12	3.44	-19.33	0.99	M2-12	8.14	-23.10	0.98
M1_13	3.27	-16.92	0.99	M2-13	2.16	-25.78	0.65
M1_14	3.65	-21.56	0.99	M2-14	2.38	-29.03	0.91
M1_15	2.42	-33.25	0.91	M2-15	0.28	-52.79	0.39
M1_16	1.32	-47.84	0.95	M2-16	3.03	-24.92	0.97
M1_17	3.05	-13.32	0.95	M2-17	-1.11	-0.32	0.73
M1_18	-0.81	20.01	0.87	M2-18	2.68	-30.18	0.90
M1_19	-2.10	-6.79	0.82	M2-19	4.45	-22.12	0.96
M1_20	-2.65	-0.95	0.57	M2-20	0.77	-44.69	0.45
M1_21	-7.89	0.18	0.78	M2-21	1.56	-34.33	0.76
M1_22	-3.06	0.36	0.96				
M1_23	-1.92	21.13	0.86				
M1_24	-4.14	4.01	0.90				
M1_25	-3.27	8.33	0.92				
M1_26	-2.50	11.85	0.93				
M1_27	-5.09	4.67	0.89				
M1_28	-3.02	-8.44	0.92				

4.3.5 SC Misalignment Cells Response during Contralateral and Ipsilateral Saccades

Certain cells within the rostral superior colliculus pause during large saccades in any direction (Munoz and Wurtz 1993). Previously called “fixation cells”, these cells have been shown to be sensitive to microsaccades (Hafed et al. 2009) . We wondered if the misalignment cells in strabismic monkeys also show these properties. Figure 4.3.4 shows the saccade related response of one of the misalignment cells (near response cells) from the left rSC of monkey M1 that showed decreased discharge rate during saccades. We examined saccade related responses of 24 misalignment cells in M1 and 18 in M2 by calculating average firing rate during ipsilateral and contralateral saccades and comparing to average firing rate during a 50ms period, 100ms prior to saccade onset (Figure 4.3.5). The solid black diagonal line in Fig 4.3.5 represents the equality line and the dotted lines denote increase or decrease in firing rate of 5 spks/s around the equality line(Munoz and Wurtz 1993). The data in Figure 4.3.5 shows that 33/42 cells reduced discharge rate during saccades, among which 27 reduced discharged for both ipsilateral and contralateral saccades, and 6 reduced discharged only for ipsilateral. Among these 6 cells 4 cells burst and 2 cells remained unchanged for contralateral saccades. Furthermore 6/42 cells remained unchanged during saccades, and 3 /42 cells burst during both ipsilateral and contralateral saccades.

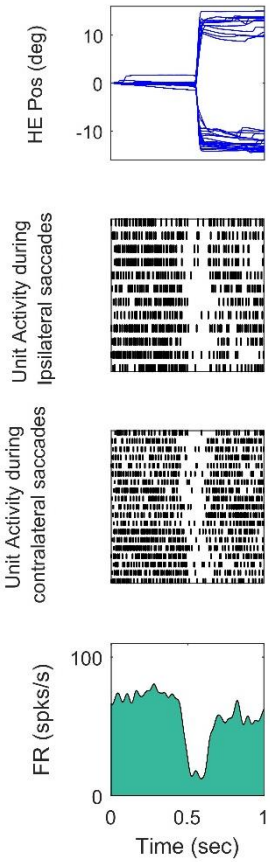

Figure 4.3.4 A near response cell showing decrease in neural activity during saccades

Figure 4.3.4: Firing properties of a misalignment cell during saccadic eye movements. Top panel shows raw traces of multiple saccades in rightward and leftward directions of different amplitudes aligned on saccade onset. Middle and bottom panel shows average firing rate of the cell and raster information during saccades. There is a reduction in firing associated with the saccade.

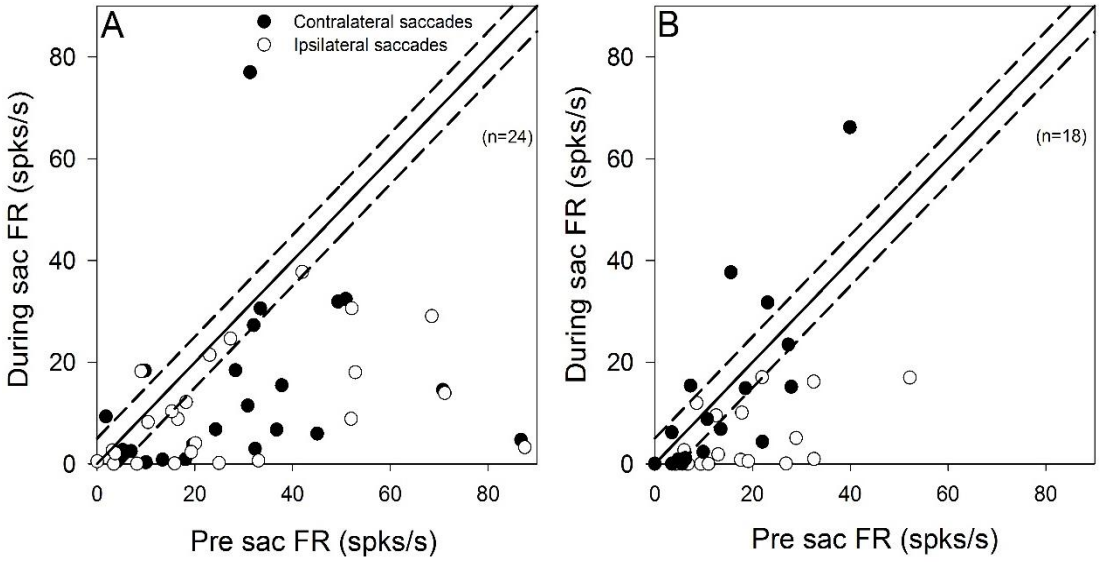

Figure 4.3.5 Scatter plot showing average neural response during saccades in both the animals

Figure 4.3.5: Scatter plot showing firing rate of misalignment cells during saccades (both ipsilateral and contralateral) and over a 50ms period 100ms before the saccade. Solid line is 1:1 line and dash line shows change of 5 spks/s over the equality line. Panel A is from monkey M1 and panel B is from monkey M2.

4.3.6 Visual response of SC misalignment related cells

Firing activity of the so-called fixation cells persists during momentary blanking of the fixation target, i.e., their responses are not visually driven. We employed a similar fixation-blank paradigm to test visual responsiveness of 18 misalignment related cells in M1 and 18 cells in M2. In this paradigm, the animal fixated the straight-ahead target for a period of 1000-1500ms during which time, the target was randomly blanked for a period of 300-400ms. Figure 4.3.6 shows raw data during the blank paradigm from a near-response cell shown during left eye viewing conditions. There was no visible difference in firing pattern due to blanking of target. Figure 4.3.7 summarizes the results of the target blank paradigm for all 36 cells. Again, the solid black diagonal line represents the equality condition and the dotted lines represents an increase or decrease of 5 spks/s over the equality condition. The firing rate of the cells are close to equality suggesting that there was little effect of target blanking, i.e., little effect of loss of visual information.

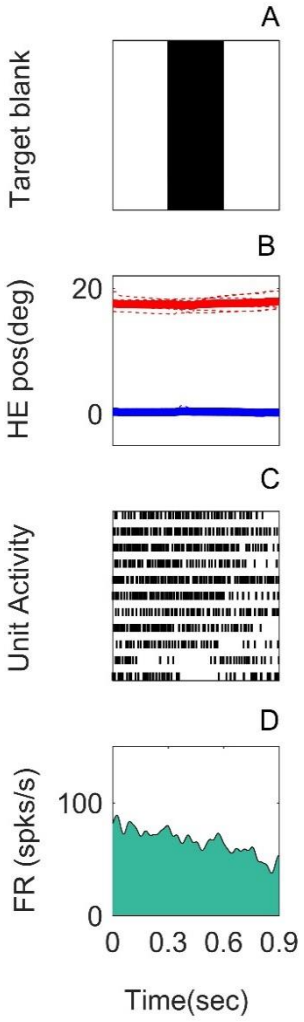

Figure 4.3.6 Neural response of cells during blank paradigm

Figure 4.3.6: Data acquired during target-blank paradigm in which visual target was turned off for 300ms. Panel B shows mean eye position (right eye – red; left eye – blue) during the paradigm, Panel C shows raster plot of each trial, and Panel D shows the cell's average firing rate.

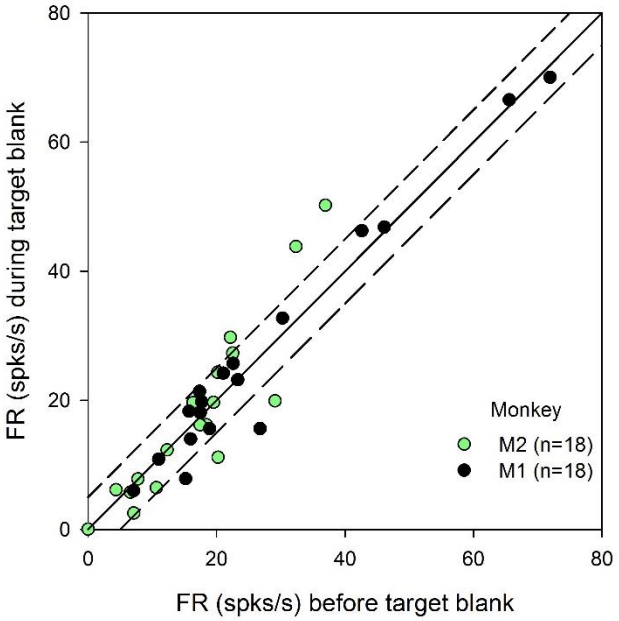

Figure 4.3.7 Summary data showing neural response during blank paradigm

Figure 4.3.7: Comparison of firing rate of misalignment cells (n=18) before target-blank and during the blank period. Solid black line is the equality line and dashed lines indicate increase or decrease in discharge of 5 spks/s during the blank period.

4.3.7 Misalignment cells show correlation with quick phase of nystagmus

Previous studies have shown that collicular cells respond during quick phases of physiological nystagmus such as optokinetic or vestibular nystagmus (Schiller and Stryker 1972) .To our knowledge, collicular responses during pathological nystagmus has not been reported. Since the strabismic monkeys showed significant downbeat nystagmus, we had an opportunity to investigate whether the SC cells responses correlated to the quick phase of nystagmus. Nystagmus in both the animals was down and right. Since some of our penetrations were in similar topographical location of amplitude and direction of nystagmus in these strabismic monkeys, we were able identify nystagmus related cells in both animals.

We found 10 cells (M1=2 and M2=8) in the rSC of these strabismic monkeys which showed bursts that were correlated with the nystagmus quick phase. Figure 4.3.8 shows eye position data (top four traces in red and blue) along with corresponding time stamps of a sample near-response cell that showed responses correlated with the rightward and downward quick phases of nystagmus. The same site in monkey M2 also yielded electrically evoked saccade directional responses that were in the same direction as the direction of nystagmus quick phases and of similar small amplitude. The mean amplitude and direction of right and left eye vector obtained by electrical stimulation at this site is also shown in the inset.

Figure 4.3.9 shows the average neural response to multiple quick phases of nystagmus during both right eye (Panel A) and left eye (Panel B) viewing conditions. It is clear from the mean data that this near response cell shows burst that are correlated with the quick

phase of nystagmus. This same cell was also previously determined to be a near-response cell because the baseline firing rate during right eye viewing (larger angle of exotropia) was less than during left eye viewing (smaller angle of exotropia). Another interesting observation was the apparent build-up in firing rate just before the burst which possibly could be related to the slow phase. Unfortunately, we did not have enough of a sample of cells to make any further conclusions on properties of the nystagmus related response. This small sample of cells serve as a proof of existence of SC responses related to quick phases of pathological nystagmus.

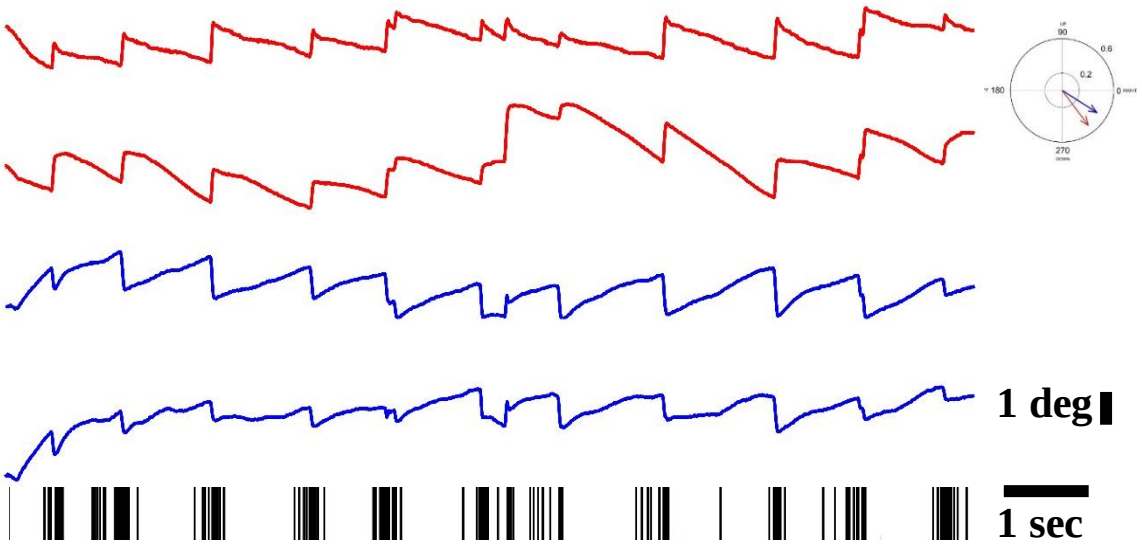

Figure 4.3.8: Raw data showing nystagmus related activity in a misalignment related cell

Figure 4.3.8: Example of cell that shows burst activity associated with nystagmus quick phases. First two rows in red shows horizontal eye position of right and left eyes respectively. Third and fourth rows in blue show vertical eye position of right and left eyes respectively. The bottom traces in black shows time stamp of neural spikes. The inset polar plot shows the amplitude and direction of the electrically evoked saccadic vector of the right (red) and left (blue) eyes.

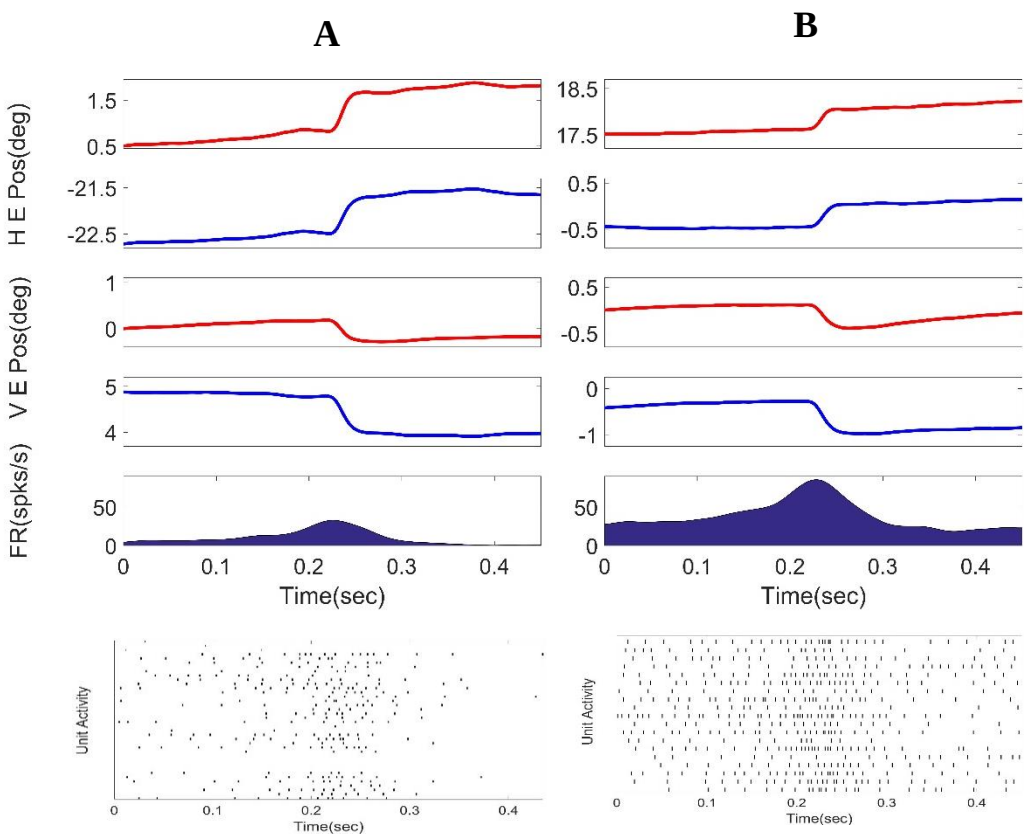

Figure 4.3.9: Average activity of a nystagmus related near response cell

Figure 4.3.9: Eye position and neural data from a cell encoding quick-phases of nystagmus during right eye (Panel A) and left eye viewing (Panel B) of a stationary straight-ahead target. Red trace denotes right eye and blue trace denotes left eye. Data are aligned on quick-phase onset and eye movement plots show the mean eye position over multiple quick-phases. Note the burst associated with the quick-phase. Also note that the baseline firing rate is less during right eye viewing (larger exotropia) than during left eye viewing (smaller exotropia) indicating that is cell also encoded eye misalignment and was a near-response cell.

4.3.8 SC Misalignment cell response in A-pattern deviation.

Many monkey models of strabismus have reported presence of A-V pattern deviation (Tusa, Mustari et al. 2002, Das, Fu et al. 2005), which is also common in human strabismic patients (Guyton 2000, Brodsky 2007). Studies in non-human primates have shown that activity at the motor neuron level (Das and Mustari 2007) and within the PPRF (Walton and Mustari 2015) in strabismic monkeys is correlated to the cross-axis movements that leads to the appearance of A/V patterns. However, near response cells in the supraoculomotor area of strabismic monkeys do not show the same correlation. One of our strabismic monkeys, animal M2, had an A-pattern deviation (reduction in exotropia in up-gaze compared to down-gaze) and so we were able to test whether changes in misalignment due to pattern deviation was reflected in SC activity. In order to determine whether firing rate of misalignment related cells changes during up and down gaze, we collected data from 11 misalignment cells in monkey M2 while this animal fixated at targets 10° up and 10° down.

Figure 4.4.10 shows average strabismus angle and average neural response of a near response cell from left rSC of monkey M2 at different vertical gaze positions. Although strabismus angle was higher at down-gaze compared to up-gaze, the firing rate of the cell was relatively similar. A similar analysis was performed for 11 additional cells in M2 where we acquired data during up and down gaze. Figure 4.4.11 plots summary data of all collected misalignment cell during both right eye viewing and left eye viewing conditions while the monkey viewed 10° up and 10° down targets. There was no significant difference (Wilcoxon signed-rank test df=21 t=0.452, p=0.66) in firing rate

during up gaze and down gaze implying that these misalignment related cells do not encode changes in misalignment due to A-pattern deviation.

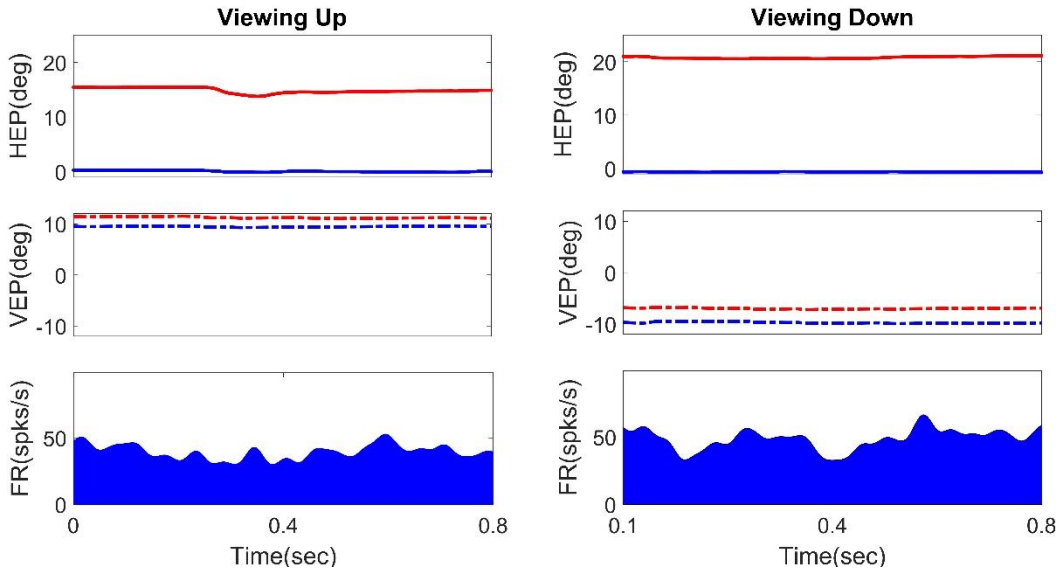

Figure 4.3.10 Average neural activity of near response cell during up and down gaze

Figure 4.3.10: Firing rate of near-response cell at different vertical gaze positions. First row shows horizontal eye position (red traces –right eye; blue traces-left eye), second row shows vertical eye position and third row shows corresponding firing rate of the near response cell from monkey M2 while monkey was fixating at a 10 deg up target (first column) and 10 deg down target (second column). Although the horizontal strabismus angle varies at different vertical gaze positions (indicative of A-Pattern), there is no change in firing response of the cell.

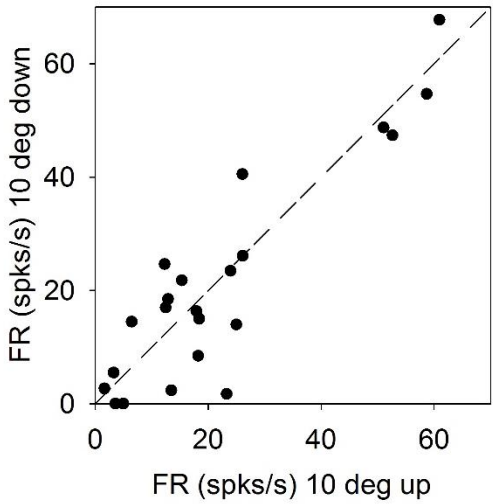

Figure 4.3.11: Summary plot showing average firing pattern during up and own gaze

Figure 4.3.11: Plot comparing firing rate of misalignment cells in monkey M2 during 10 deg gaze up and 10 gaze down. Diagonal dashed line is the equality line.

4.4 Discussion

In this study, neurons that appear to carry a signal related to the horizontal eye misalignment have been identified for the first time within the rostral superior colliculus of strabismic monkeys. These results, therefore, provide new insight into the role of the SC in the neural circuits that leads to the appearance of problems in binocular eye alignment and binocular coordination.

4.4.1 Role of rSC in strabismus

The anatomical location and physiological properties of misalignment related cells recorded in this study suggest that these cells are likely the same as those that have been reported to encode vergence angle in normal animals (Van Horn et al. 2013). The anatomical locations of vergence cells in normal monkey SC overlaps the location of misalignment cells in strabismic monkeys since electrical stimulation within this area in our sample animals also produced contralateral staircase saccades of <5° radial amplitude. Our search area was limited to the rostral part because of the report of convergence and divergence cells in this area by Van Horn and colleagues (Van Horn et al. 2013) and so searching within caudal colliculus may or may not have yielded identification of misalignment related cells. Comparison of the population response properties of misalignment (vergence) cells in strabismic and normal animals yielded significant differences in threshold but not neuronal sensitivity. The threshold of both the near- and far-response cells in normal animals is close to 0° (straight ahead) but were

significantly shifted towards exotropia in the strabismic monkey. The shift in threshold suggests that these cells are still active despite the significant exotropia and therefore this area and downstream areas receiving projections from the rSC cells still influence the state of alignment on a moment-to moment basis. On the other hand, neuronal sensitivity of near and far-response cells were not significantly different between normal and strabismic monkeys. So it is unlikely that these cells alone are providing the reduced vergence tone responsible for the strabismus.

Previously we have shown that the supraoculomotor area (SOA) that normally contains convergence and divergence cells shows responses related to strabismus angle (Das 2012). Analysis of these SOA cells showed that both threshold and sensitivity was reduced in animals with exotropia leading to the hypothesis that the SOA connection to medial rectus motoneurons was resulting in reduced vergence tone and therefore contributing to exotropia. Recently Pallus and colleagues (Pallus et al. 2017) also recorded from SOA of strabismic monkeys and showed similar results in their exotropic monkeys. The SOA connects monosynaptically to the medial rectus motoneurons (Zhang et al. 1991, Zhang et al. 1992) and therefore changes in SOA sensitivity and threshold are likely to directly impact the state of strabismus via changes in medial rectus contractility. On the other hand, the SC projects to the cMRF which in turn projects to SOA, the oculomotor nucleus and the abducens nucleus (Bohlen et al. 2016). Our finding of SC misalignment related cells places the SC squarely within a vergence and accommodation circuit that is potentially disrupted in strabismus. Interestingly, Pallus and colleagues found that the esotropic animals they tested also showed reduced SOA sensitivity but not altered thresholds compared to normal animals. In order to account for the reduced SOA

sensitivity (i.e., reduced vergence tone to medial rectus muscle) but still result in a convergent strabismus (esotropia), vergence input to the lateral rectus must be reduced even further. It is possible that the SC input to the abducens via the cMRF can account for the reduced vergence input to the lateral rectus in an esotrope. Recording studies must be performed in esotropic animals to test this hypothesis. In this scenario, the misalignment related cells in SOA play a stronger role in maintenance of exotropia, while the misalignment related cells in SC play a stronger role in maintaining esotropia.

4.4.2 Are the vergence and misalignment cells distinct from other cell types found in rSC of normal monkeys?

Previous studies in cats and monkeys have described the so-called "fixation cells" in rostral SC that are tonically active when an animal fixates straight ahead and pause during saccades. These cells are not influenced by the presence or absence of a visual target (Munoz and Guitton 1989, Peck 1989, Munoz and Wurtz 1993). Later, these cells have been shown to burst during microsaccades (Hafed et al. 2009). Other than the property of being sensitive to vergence and eye misalignment, the cells that we have described in this study share a lot in common with the previously described population. The anatomical location of these cells is within the rostral superior colliculus. Misalignment related far response and near response cells found in strabismic monkeys show sustained discharge before and during blanking of a visual target. Most of the misalignment cells decrease their firing rate during ipsilateral and contralateral large saccades. We therefore suggest that misalignment cells are the same population of

fixation cells or perhaps constitute a subset that also carries information about strabismus angle (vergence).

4.4.3 Misalignment cells also encode nystagmus quick-phases in strabismic monkeys

Nystagmus is observed in most strabismus humans and is replicated in monkey models of strabismus. Jerk nystagmus has both a quick phase during which the eye moves quickly with saccade-like amplitude and velocity (similar to microsaccades) and a slow phase drift. There is considerable evidence that microsaccades are generated within rSC in normal monkeys and we have shown in our study (Fig 4.3.8) that neurons in the superior colliculus also encode the quick phases of nystagmus. These neurons are located topographically in the same location of the motor map of SC as saccades of similar amplitude and direction, as we found that electrical stimulation resulted in saccades of similar amplitude and direction as the nystagmus quick phases. The period immediately before and after the quick phase is the slow phase of nystagmus. Examination of SC misalignment cell activity during the peri-quick phase period shows some indication that slow phases may also be encoded in these same cells. The low sample size of the nystagmus cells in our study precludes further conclusions on the role of SC in generating nystagmus, but it appears that quick phases of nystagmus are processed similarly as microsaccades of similar amplitude and direction.

4.4.4 Influence of Ocular Accommodation

SC has been shown to be involved in accommodation in cats (Billitz and Mays 1997, Billitz 1997, Billitz 1997, Ohtsuka and Nagasaka 1999). Further, an anatomical connection between EW nucleus and SC through cMRF has been found in monkeys (May et al. 2015). Thus, it is possible that these misalignment cells also encode ocular accommodation. The relatively low firing rates of these cells also suggest that accommodation could be encoded within these cells. We tried to minimize accommodative cues as much as possible by using a white optotype shaped target against a dark background and a fixed target distance of 57 cm (1.75D accommodative demand) making it unlikely that changes in firing rate (due to change in fixation for example) are solely due to changes in accommodation. Future experiments that include measurement of accommodation as a variable could be useful in characterizing these cells more thoroughly.

Chapter 5: Effect of muscimol inactivation of superior colliculus on strabismus angle and fixation stability in monkey model of strabismus

5.1 Introduction

Disruption of sensory binocular signals could lead to improper development of neural oculomotor circuits leading to eye misalignment (strabismus). The worldwide prevalence of strabismus in infants is about 2-5% (Lorenz 2002, Govindan et al. 2005, Greenberg et al. 2007, Mohney 2007). The disruption of binocular vision during development has also been used as a tool to develop animal models of strabismus (Boothe et al. 1985, Harwerth et al. 1986, Tusa et al. 2002, Kiorpes 2015). Various brain structures involved in normal oculomotor control have been shown to contribute to the state of misalignment or to disruptions in eye movements in monkey models of strabismus (Joshi and Das 2011, Das 2012, Joshi and Das 2013, Walton and Mustari 2015, Walton et al. 2015).

The superior colliculus (SC) is a visual and oculomotor structure that has been studied widely with regard to saccadic eye movements (Gandhi and Katnani 2011). The SC has a retinotopic map of visual space in superficial layers, while its intermediate and deeper layers encode a motor map, i.e. saccades of specific amplitude and direction (Wurtz and Goldberg 1971, Cynader and Berman 1972, Goldberg and Wurtz 1972, Schiller and Stryker 1972, Wurtz and Goldberg 1972, Gandhi and Katnani 2011). The SC motor map is organized such that the saccadic amplitude increases from the rostral to caudal, providing the same neural circuitry for large voluntary saccades and small microsaccades

(Hafed et al. 2009). The SC could be an important neural structure in strabismus also because of its potential role in vergence and in pathological nystagmus associated with strabismus because it encodes fixational saccades or microsaccades.

Neurons related to convergence have been found in the rostral superior colliculus of cat (Jiang et al. 1996) and a recent study by Van Horn et al. identified convergence and divergence neurons in the rSC of normal monkeys that were modulated during slow vergence (Van Horn et al. 2013). In humans, the bilateral ablation of SC has been associated with accommodation and convergence palsy (Ohtsuka et al. 2002). This suggests that the SC could be part of a disrupted vergence circuit that leads to strabismus. Fixation stability, the process of fixating steadily on a stationary object, is poor in strabismic humans and monkeys. Several studies have shown that increased drifts, nystagmus and saccadic intrusion are responsible for increased fixation instability in strabismic humans and monkeys (Ciuffreda et al. 1979, Gonzalez et al. 2012, Subramanian et al. 2013, Pirdankar and Das 2016, Ghasia et al. 2017, Upadhyaya et al. 2017, Kelly et al. 2018). Hafed et al. recently found neurons related to microsaccades in rostral SC (Hafed et al. 2009) and the hypothesis is that a small imbalance of activity across the two SC results in a microsaccade in the contralateral direction. We have recently shown that fixational microsaccades are larger in strabismic monkeys (Upadhyaya et al. 2017). Given the role of the SC in maintaining stable fixation and the generation of microsaccades, it is possible that this structure can also play a role in fixation instability in strabismus and nystagmus.

In our previous work on role of the SC in strabismus, we have electrically stimulated the rSC of strabismic monkeys and that stimulation can lead to convergent and divergent changes in strabismus angle depending on location of stimulation (Upadhyaya et al. 2017). We followed up the electrical stimulation study by conducting a study recording neural activity of cells in the rostral colliculus and were able to identify the presence of strabismus related activity in a population of cells in the rSC (Upadhyaya and Das 2018). In the present study, we use the approach of muscimol inactivation to gain additional insight into the potential role of SC in eye misalignment and fixation instability.

5.2 Methods

5.2.1 Subjects and Surgical Procedures

Two adult strabismic monkeys (Macaca Mulatta) were used in this study (Monkeys M1 and M2; ages ~7 and 9 years; weights ~5-8kg). These monkeys were previously reared using an optical prism-viewing paradigm. In this paradigm, the infant monkeys viewed through a 20D Fresnel prism oriented base-in and placed in front of one eye and another 20D Fresnel prism-oriented base-down and placed in front of the other eye. These horizontal and vertical Fresnel prisms were fitted in a lightweight helmet-like device that the animal wore for the first four months of life starting from 1-2 days after birth. After the initial four months of prism rearing, the monkeys were reared normally for several years in an unrestricted visual environment. Due to the prism-viewing during the critical period for development, there is disruption of binocular vision which leads to strabismus.

Prior to enrollment in the muscimol study, each adult monkey went through three sterile surgical procedures carried out under aseptic conditions using isoflurane anesthesia (1.25%-2.5%). In the first surgery, a head stabilization post was implanted (Adams et al. 2007). In the second surgery, we stereotaxically implanted a 21 mm diameter titanium neural recording chamber along with a scleral search coil in one eye, and in the third surgery we implanted a scleral search coil in the other eye (Judge et al. 1980). In monkey M1, the chamber was implanted at a stereotaxic location centered 3-mm anterior and 1-mm lateral to stereotaxic zero and tilted 20° dorsolateral to ventromedial in the coronal plane. In monkey M2, the chamber was implanted in the mid-sagittal plane, centered at 15mm above anterior-posterior 0, and tilted posteriorly by 38°. After recovery from surgery and additional behavioral training in oculomotor tasks such as saccades, and fixation, these animals were enrolled in this muscimol study. All surgical and experimental procedures were performed in compliance with National Institute of Health and The Association for Research in Vision and Ophthalmology (ARVO) guidelines and the Institutional Animal Care and Use Committee (IACUC) of the University of Houston. Monkey M1 is the same as that was used for our electrical stimulation study (Monkey H) and for the neural recording study (M1). M2 is also the monkey used in neurophysiology study (also denoted as M2).

5.2.2 Eye Movement Measurement, Experimental Paradigm

Binocular eye position was measured using the magnetic search coil method (Primelec Industries, Regensdorf, Switzerland). The eye coils were calibrated at the beginning of each experiment by rewarding the animal with small amounts of juice as they looked at a

series of targets along the horizontal or vertical meridian back projected onto a tangent screen at 57 cm. The calibration of each eye was performed independently during monocular viewing forced by occlusion of an eye using liquid crystal shutter goggles (Citizen Fine Devices, Japan) under computer control. Visual stimuli were generated using the BITS# visual stimulus generator (Cambridge Research Systems, Cambridge, UK) and Psychtoolbox 3 (Brainard 1997) run under computer control and presented using a DepthQ projector running at a 120Hz frame rate (Lightspeed Design Inc, Bellevue, WA).

Binocular eye position data were collected when the animal performed a fixation task, and a visually guided saccade task. In the fixation task, a 2°sized disk-shaped bright target was presented in a dark room at a distance of 57 cm, and the animal had to fixate the target monocularly for 60 sec. Any break in fixation or voluntary saccades away from the target were removed before processing the fixation data, which was then used to calculate fixation instability using a bivariate contour ellipse area (BCEA) metric.

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

σx = Standard deviation of horizontal eye position,

σy = standard deviation of vertical eye position,

2.291 is the chi-squared value (2df) corresponding to a probability of 0.68,

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

The same fixation data was also used to detect the amplitude and frequency of fixational saccades using a modified unsupervised clustering method (Otero-Millan et al. 2014). In the visually guided saccadic tasks, the target was randomly stepped to right and left locations with an amplitude of 5° or 15° from center. Saccade onset and offset were detected using a velocity criterion of 30 deg/sec. These data were used to calculate saccadic latency, saccadic duration, and peak velocity. Strabismus angle (left eye position – right eye position) was calculated from the same data when the monkey was fixating on the center target prior to making centrifugal saccades. Pre-injection data were collected immediately before the injection and post-injection data were collected at timepoints 10 min, 30 min, 70 min, and 120 min after injection. The fundamental goal of the analysis was examining changes in strabismus angle and fixation instability (BCEA) before and after injection of muscimol.

5.2.3 Muscimol Injections

Before starting the muscimol injection experiments in an animal, we identified and mapped the superior colliculus using a combination of neural recording and electrical stimulation methods. During neural recording (epoxy-coated tungsten electrodes; 1-5 Mohm Frederik Haer, Brunswick, ME), we first encountered visual (superficial layers) and then saccade-related (intermediate and deeper layers) activity as the electrode advanced into the SC area. SC locations were confirmed via electrical microstimulation methods wherein a train of cathodal pulses (40-90μA, 400Hz, 500ms) was delivered via the recording electrode and elicited a staircase of saccades in a contralateral direction.

During the actual muscimol injection experiments, we used an injectrode, which is a combination of an injection pipette and microelectrode. The availability of the microelectrode allows localization of the SC immediately prior to injection (Alpha Omega Co. USA). The injection pipette part of the injectrode consists of a 27-gauge or 28-gauge stainless steel hypodermic metallic tube with a beveled tip to deliver the muscimol. In addition, a tungsten electrode is inserted into the thin gauge metallic tube and glued on one end with just the tip of the electrode (~200-500um length) projecting out of the 27-gauge tubing allowing neural recording and electrical stimulation prior to injection. A polyethylene tube is connected at the other end of the pipette/microelectrode combination to complete the injectrode assembly. At the time of the experiment, the injectrode is protected within a 21-gauge stainless steel hypodermic guide tube with a beveled tip when penetrating the dura. In our experiments, a small volume of muscimol (conc 2mg/ml; Sigma-Aldrich) was delivered at the desired depth with a picoliter pump (WPI-PV830) connected to the micropipette to provide timed air pressure pulses (5-15 mm Hg; 10msec -200msec) allowing for a gradual delivery of muscimol over several (5-10) minutes (refer to table 5.3.1 for details). Pre- and post-inactivation eye movement data were collected for each injection experiment, and the injection experiments were separated by at least 1 week to allow the animal to fully recover prior to the next injection. Binocular eye position signals were processed with anti-aliasing filters (Krohn-Hite; Krohn-Hite Corporation, Brockton, MA) at 400 Hz before digitization at 2.75 kHz with 12-bit precision (Alpha-Lab System; Alpha Omega Engineering, Nazareth, Israel).

5.3 Results

Properties of strabismus of the two monkeys used in the study are provided in Table 5.3.1. Both of these animals were previously used for single cell recording studies in SC. Briefly, monkey M1 had exotropia of 3°-18° during right eye viewing and 10°-20° during left eye viewing. Similarly, monkey M2 has exotropia of 15°-22° during right eye viewing and 20°-28°during left eye viewing. A jerk-nystagmus with a significant downward component was present in both monkeys. In addition, monkey M2 showed prominent A-pattern deviation, i.e variation of horizontal deviation in up and down gaze.

Table 5.3.1 Properties of strabismic animals used in muscimol inactivation of SC

Monkeys	**Age(yrs)**	**Strabismus Angle(°)**		**Refractive Error(D)**		**Strabismus Properties**
		RE View	LE View	RE	LE	
M1	7	3°-18°XT	10°-20°XT	+4.50	+0.75	DHD, N
M2	9	15°-22°XT	20°-28°XT	-4.50	-1.50	DHD, N

Legend: XT= Exotropia, DHD= Dissociated horizontal deviation, N= nystagmus, and D= Diopters.

In total, 13 injections were delivered including two control (saline) injections in the two monkeys (M1: 8 muscimol and 1 control; M2: 3 muscimol and 1 control). Out of 11 muscimol injections, 6 were in the right superior colliculus (M1=6) and 5 were in left superior colliculus (M1=2; M2=3). Detailed injection characteristics are in Table 5.3.2 including muscimol concentration and volumes.

Table 5.3.2 Summary of all the injection in both the strabismic monkeys

Injection	Monkey	Injection Type	~Volume µl	Location	First staircase saccades
Inj 1	M1	Muscimol	2	R-SC	A: 10.7° ;D: 171°
Inj 2	M1	Muscimol	1	R-SC	A: 9.2° ;D: 170°
Inj 3	M1	Muscimol	3	R-SC	A: 11.4° ;D: 120°
Inj 4	M1	Muscimol	1	R-SC	A: 8.7° ;D: 124°
Inj 5	M1	Muscimol	0.5	L-SC	A: 0.9° ;D: 41°
Inj 6	M1	Muscimol	0.5	L-SC	A: 0.3° ;D: 5°
Inj 7	M1	Control	1	R-SC	A: 2.7° ;D: 173°
Inj 8	M2	Muscimol	0.7	L-SC	A: 3.1° ;D: 300°
Inj 9	M2	Control	0.6	L-SC	A:0.8° ;D: 221°
Inj 10	M1	Muscimol	0.8	L-SC	A: 1.0° ;D: 51°
Inj 11	M2	Muscimol	0.6	L-SC	A: 6.9° ;D: 280°
Inj 12	M1	Muscimol	1	R-SC	A: 3.8° ;D: 176°
Inj 13	M2	Muscimol	0.7	L-SC	A: 3.8° ;D: 0°

Legend: R-SC = right superior colliculus, L-SC = left superior colliculus, A= amplitude, and D= direction. Injection order in table and all subsequent figures is based on largest amplitude of divergent change to largest amplitude of most convergent that was obtained following muscimol injection.

Immediately prior to injecting muscimol, electrical stimulation was used to verify the topographic location of the injection within the SC. Figure 5.3.1 shows example staircase saccades evoked during electrical stimulation before injection. Mean radial amplitude of staircase saccades in viewing eye of panel A (M1 - Inj5) was ~0.9° and direction ~41° and in panel B (M1 - Inj12) was ~3.8° in amplitude and ~176° in direction.

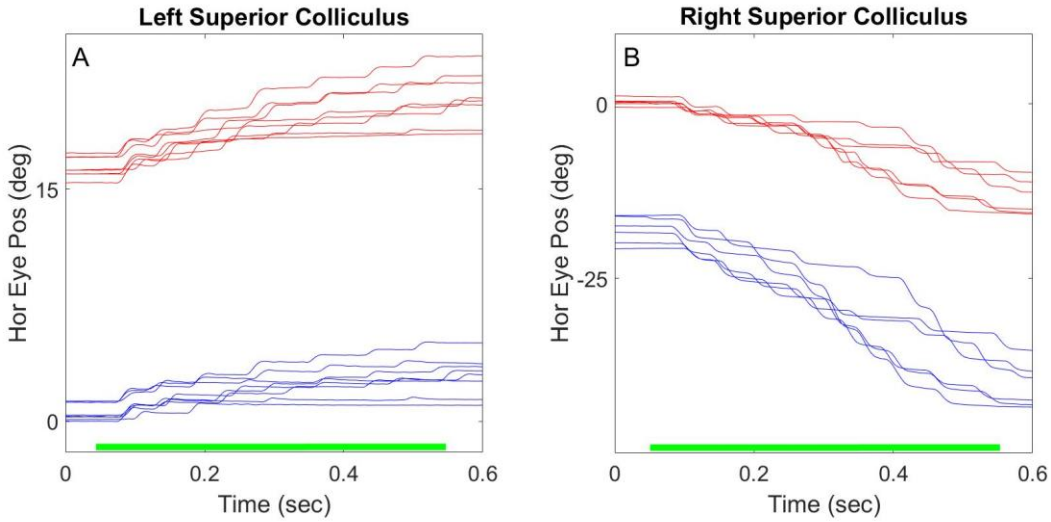

Figure 5.3.1 Effect of electrical stimulation at the inactivation site in two colliculi

Figure 5.3.1 Position plot showing horizontal eye position of right eye (red traces) and left eye (blue traces) during electrical stimulation. Green bar at the bottom indicates the duration of electrical stimulation. Panel A shows electrical stimulation effect in Left superior colliculus during left eye viewing (Inj 5) and panel B shows electrical stimulation in right superior colliculus during right eye viewing (Inj 12). Note that staircase saccades were evoked in contralateral direction. Mean amplitude of staircase saccades in panel A was ~0.9° and in panel B was ~3.8° . Positive numbers on the y-axis indicate rightward and negative numbers indicate leftward eye positions.

All of the injections were within 0.3°-11.4° range of radial amplitude with mean of 4.9°±4.0° (8/13 injections were within 4°). Rostral locations within the SC were targeted since our previous neural recording study indicated the presence of cells related to eye misalignment in rostral SC. However, data from caudal injections were also accepted for analysis since the spread of muscimol is likely to occur into rostral areas also. Control injections were performed by injecting saline within the SC in both monkeys.

5.3.1 Change in strabismus angle due to muscimol injection in the superior colliculus

The main aim of this study was to measure change in strabismus angle due to muscimol inactivation of SC. Effect of muscimol inactivation of SC on eye alignment was observed after 10mins in some experiments. In a few injections around 30mins after injection, robust horizontal nystagmus with quick phase towards the inactivated superior colliculus was observed. This is likely due to spread of muscimol to the nearby nucleus of optic tract as it has been shown that unilateral inactivation of NOT results in horizontal nystagmus (Hoffmann and Fischer 2001). Data from those time points where muscimol spread to the NOT were not used for further analysis.

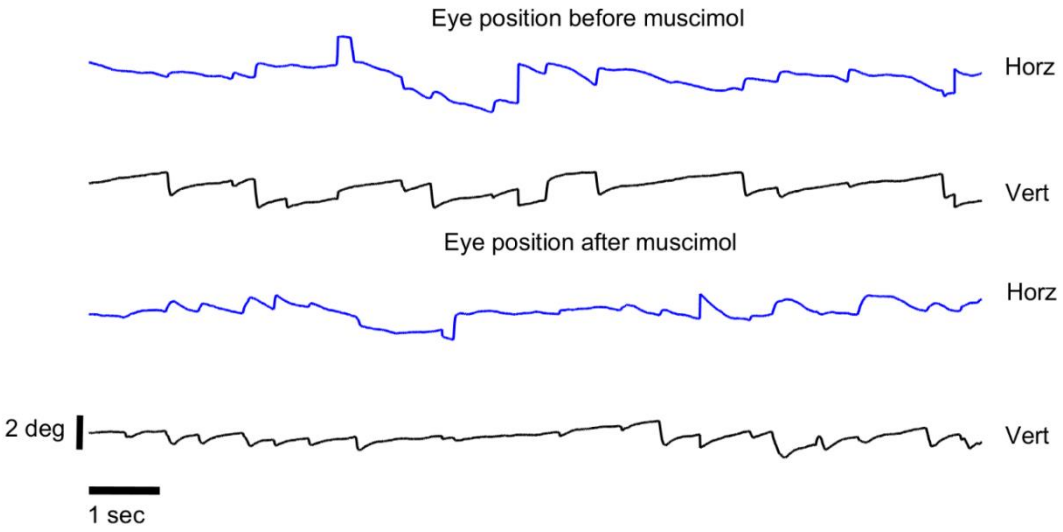

Figure 5.3.2. Eye position plot before and after muscimol injection

Figure 5.3.2. Horizontal (blue traces) and vertical (black traces) eye positions of viewing eye before and after muscimol injection (Inj 4) in right superior colliculus of monkey M1.

Figure 5.3.2 shows raw eye position traces of the fixating eye in monkey M1 while the monkey was viewing a straight-ahead target before and after muscimol injection. There was no significant visible change in eye traces before and after muscimol injection, although later analysis indicated changes in alignment. An ipsilesional positional offset after inactivation of superior colliculus has been shown before in normal monkeys (Hafed et al. 2008) and we observed this phenomenon during most injections. Figure 5.3.3 summarizes the positional offset (calculated during periods of central fixation) before and after muscimol injection in both monkeys. Panels A and B in figure 5.3.3 shows positional offset of the viewing eye in the animals during left superior colliculus (LSC) injections and the Panel C during right superior colliculus (RSC) injection. During LSC injection and right eye viewing (red symbols), 6/6 injections showed significant ipsilesional offset (M1: 3/3 Kruskal-Wallis One way ANOVA on ranks H [3] = 139.6, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$; M2: 3/3 Kruskal-Wallis One way ANOVA on ranks H [3] = 100.9, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$) and during left eye viewing (blue symbols) 4/6 injections showed significant ipsilesional offset (M1: 3/3 Kruskal-Wallis One way ANOVA on ranks H [3] = 170.5, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$; M2: 1/3 Kruskal-Wallis One way ANOVA on ranks H [3] = 15.7, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$). During RSC injection and right eye viewing, 3/5 injections showed significant offset (M1: 3/5 Kruskal-Wallis One way ANOVA on ranks H [5] = 126.6, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$;) and during left eye viewing also, 3/5 injections showed significant offset (M1: 3/5 Kruskal-Wallis One way ANOVA

on ranks H [5] = 278.9, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$). During RSC injection, 2/3 were a contralesional offset and 1/3 was an ipsilesional offset.

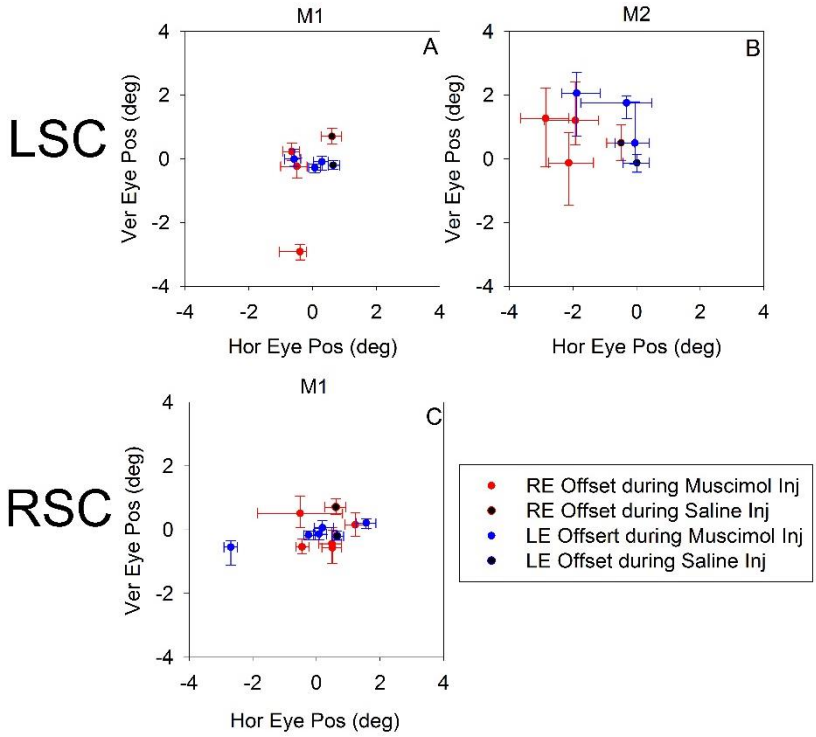

Figure 5.3.3. Positional offset seen after muscimol and saline injections

Figure 5.3.3. Scatter plot showing vertical and horizontal offset of the viewing eye before and after muscimol and control injections. Filled circles represent viewing eye offset after injections and unfilled circles represent viewing eye position before injection. Blue and red colors denote right and left eye viewing conditions and black color denotes control (saline) injection in each panel. Panel A, B shows effect from left superior colliculus injections from both animals and panel C shows effect from right superior colliculus injection in both animals.

There was a significant change in strabismus angle (left eye position – right eye position) in 8/11 injections during right eye viewing and 7/11 injections in left eye viewing. Some injections showed a gradual change in strabismus angle over the different time points at which we collected data. However, since this was not always apparent, we simply averaged the change in strabismus angle after injection over all of the acquired data (Figure 5.3.4). Figure 5.3.5 summarizes the change in strabismus angles before and after injections in the superior colliculus of both monkeys. Figure 5.3.5A shows change in horizontal strabismus angle, arranged in increasing order of convergence, during right eye viewing in both the monkeys. The same injection order is used in all figures. Mean change in horizontal strabismus angle during control injection while the monkey was fixating with the right eye was 0.2°±0.7° (Fig 5.3.5A, Inj 7 and 9) and with the left eye was -1.6°±2.7° (Fig 5.3.5C, Inj 7 and 9). There was significant difference in change in horizontal strabismus angle between saline control injection and muscimol injection in most experiments during right eye viewing (Fig 5.3.5A; M1: 7/8 Kruskal-Wallis One way ANOVA on ranks $H[8] = 561.28$, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$; M2: 1/3 Kruskal-Wallis One way ANOVA on ranks $H[3] = 36.07$, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$). During right eye viewing, 5 sites (all in M1) yielded a divergent change in strabismus angle (Mean change in horizontal strabismus angle: -5.5°±2.7°) while 3 sites (M1-1; M2-2) yielded a convergent change in strabismus angle (Mean change in horizontal strabismus angle: 2.3°±1.1°). Both convergent and divergent changes were statistically different from control.

During left eye viewing (Figure 5.3.5C), 4/8 sites in M1 (Kruskal-Wallis One way ANOVA on ranks H [8] = 540.47, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$) and 3/3 sites in M2 (Kruskal-Wallis One way ANOVA on ranks H [3] = 65.0, $P < 0.001$; Dunn's method for multiple comparisons versus control $P < 0.05$) showed significant changes in strabismus angle out of which only 1 site showed a divergent change (-0.49°) and 6 sites showed a convergent change (1.4°±1.0°). Part of the apparent difference in right and left eye viewing conditions was the small divergent change in strabismus angle observed following the control injection during left eye viewing conditions only.

There was also significant difference in change in vertical strabismus angle during 10/11 right eye viewing conditions (Fig 5.3.5B; M1: 7/8 Kruskal-Wallis One way ANOVA on ranks H[8] = 575.76, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$); M2: 3/3 Kruskal-Wallis One way ANOVA on ranks H[3] = 200.85, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$) and during 8/11 left eye viewing conditions (Fig 5.3.5D; M1: 5/8 Kruskal-Wallis One way ANOVA on ranks H[8] = 593.52, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$; M2: 3/3 Kruskal-Wallis One way ANOVA on ranks H[4] = 65.0, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$). However, the mean absolute vertical strabismus angle change was only 0.8°±0.9° for right eye viewing and 0.7°±0.8° for left eye viewing. These differences are small in comparison to difference in horizontal strabismus angle and are likely to be functionally insignificant.

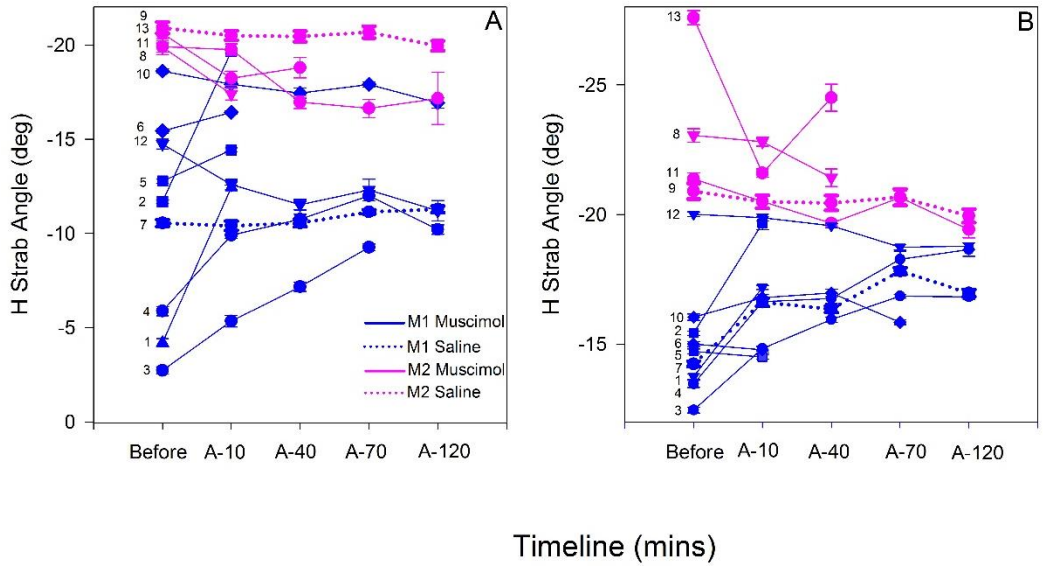

Figure 5.3.4. Timeline showing change in horizontal strabismus angle during the experiment

Figure 5.3.4. Line and scatter plot showing strabismus angle at different time points during each experiment. More negative strabismus angle indicates divergent change and less negative strabismus angle is a convergent change. Panel A shows strabismus angle during right eye viewing and panel B shows strabismus angle during left eye viewing.

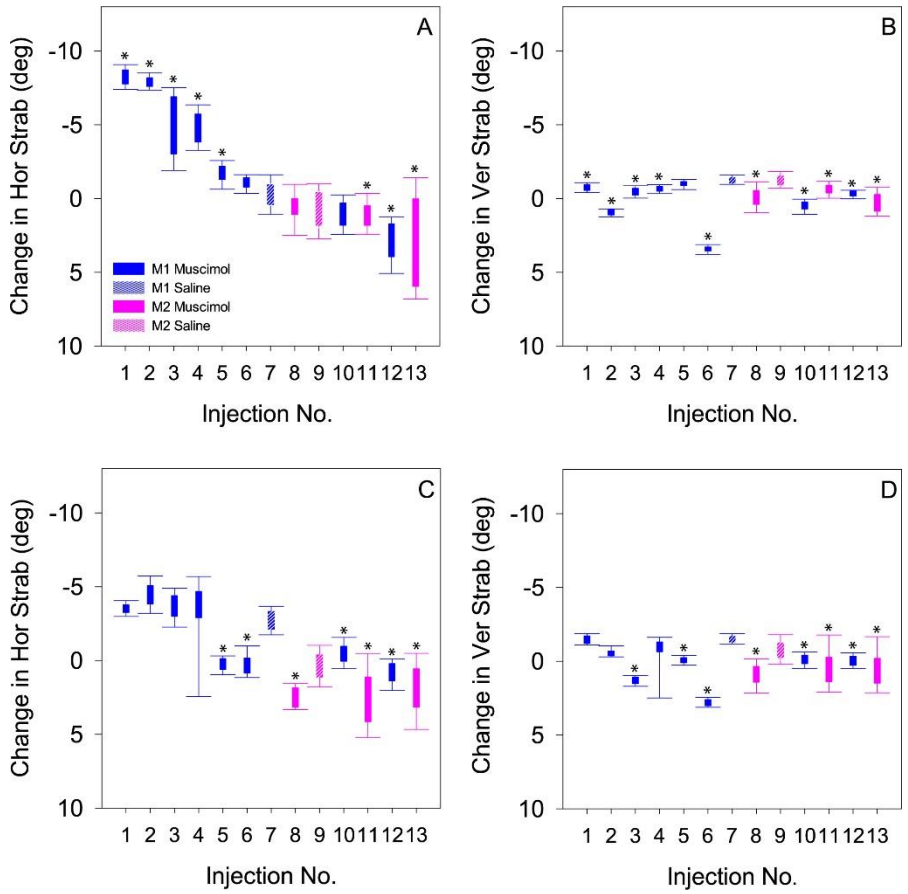

Figure 5.3.5. Box plot summarizing change in strabismus angle in all the injections in two monkeys

Figure 5.3.5 Change in horizontal (Panel A – right eye viewing; Panel C – left eye viewing) and vertical (Panel B – right eye viewing; Panel D – left eye viewing) strabismus angle due to muscimol and saline injections. Data in all plots are arranged in increasing order of convergence change obtained in right eye viewing (Panel A). Negative values indicate divergent change and positive values indicate convergent change in strabismus angle. Control injection in M1 is Inj7 and in M2 is Inj9.

5.3.2 Analysis of fixation instability after muscimol injection

Fixation data were collected when the monkeys viewed a straight-ahead target for 60 sec. Dispersion of eye position during fixation was calculated using the BCEA metric described earlier. Figure 5.3.6A, C, and E show horizontal and vertical dispersion of eye position during fixation before injection, and panels B, D, and F show dispersion of eye positions after injection. Data in the top and bottom rows were from muscimol injections Inj4 and Inj9 in right SC of M1 and the middle row data were from the control (saline) injection Inj7 also in right SC of M1. Data in Figure 5.3.6A, B suggests there was a decrease in BCEA following SC inactivation (improved fixation stability). However, other injections provided opposite results (Inj9; Fig 5.3.6E, F). There was no significant change in BCEA during control injection. Convergent or divergent changes in strabismus angle as discussed in the earlier section are also visible in data shown in figure 5.3.6.

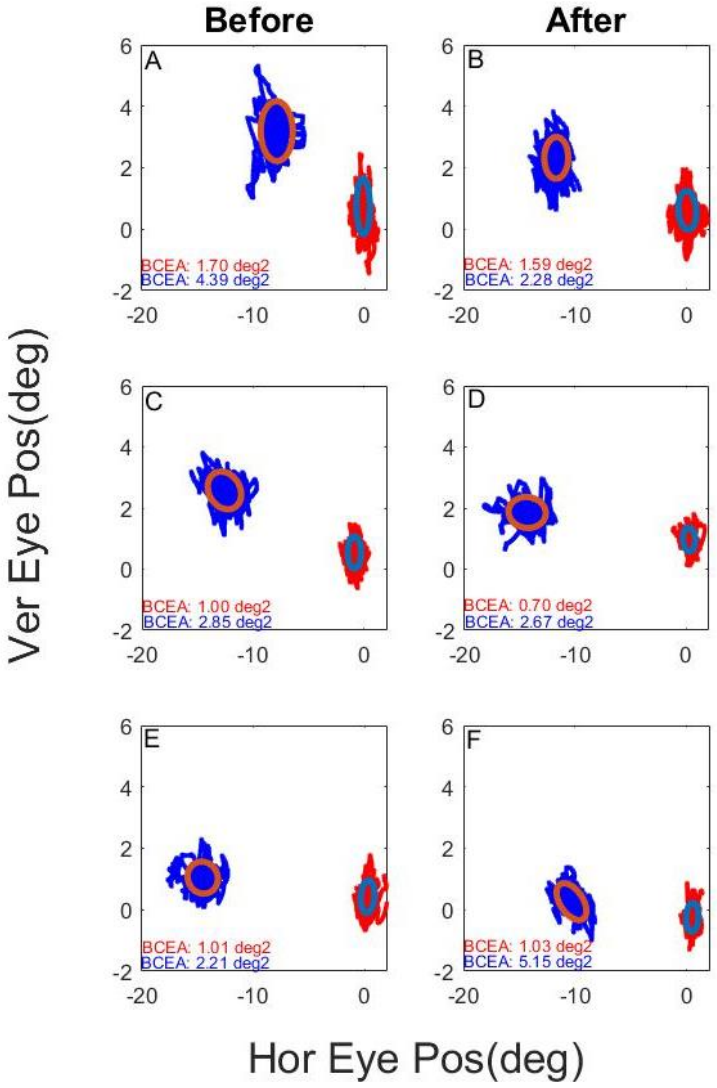

Figure 5.3.6. Raw fixation data before and after muscimol injections

Figure 5.3.6. Dispersion of left and right eye positions during fixation. The ellipse represents 68% of fixation locations and area of ellipse is a measure of fixation stability (BCEA). Panel A, C, and E shows data before injection and panel B, D, and F shows corresponding fixation data after injection. All of the injection shown in this figure were performed in right superior colliculus. Top row and bottom row show effect of muscimol injection (Inj 4 and Inj 23) whereas middle row shows effect of control (saline) injection 7.

Although individual measures of fixation instability suggests that muscimol inactivation could improve (Figure 5.3.6A, B) or worsen (Figure 5.3.6E, F) fixation stability, there was no significant change in fixation instability in comparison to control when the data were considered as a group except for the viewing eye of M1 during right eye viewing conditions (M1-left eye viewing: viewing eye, $t=1.76$, $df=10$, $P=0.11$; non-viewing eye, , $t=1.44$, $df=10$, $P=0.18$; M2-left eye viewing: viewing eye, $t=1.08$, $df=5$, $P=0.33$; non-viewing eye, Mann-Whitney rank sum test, $P=0.40$; M1-right eye viewing: viewing eye, Mann-Whitney rank sum test, $P=0.004$; non-viewing eye, $t=0.48$, $df=10$, $P=0.64$; M2-right eye viewing: viewing eye, $t=0.53$, $df=5$,$P=0.62$; non-viewing eye, $t=0.62$, $df=5$, $P=0.56$). Figure 5.3.7 summarizes the fixation instability data from all the injections. Inset box plots in this figure show the median values of the group. Figure 5.3.7A&C show change in BCEA in the viewing eye during right eye viewing and left eye viewing, while figure 5.3.7B&D show corresponding changes in the non-viewing eye.

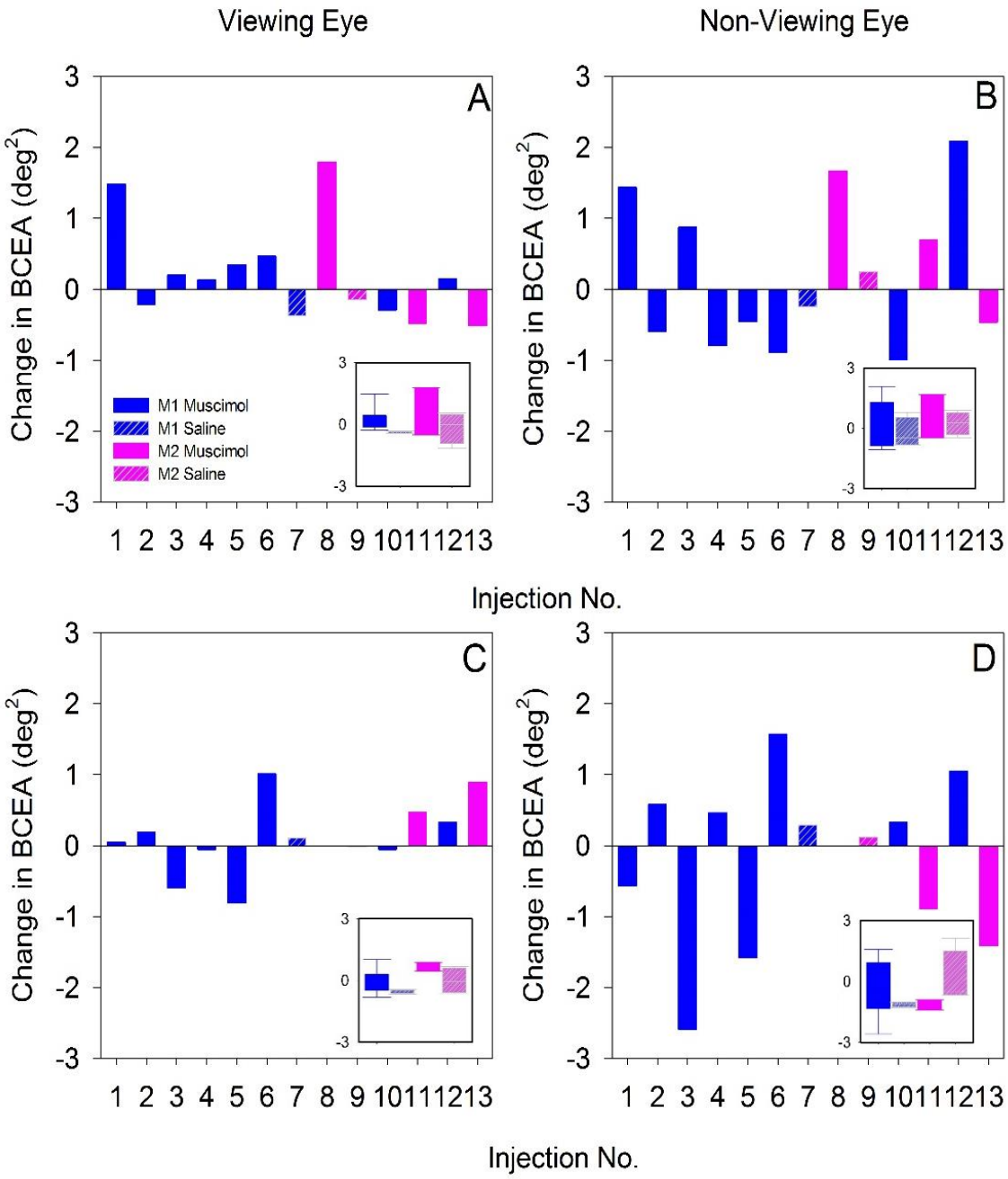

Figure 5.3.7. Bar and box plot showing effect of change in BCEA after injections

Figure 5.3.7. Change in BCEA due to muscimol inactivation of SC. Panel A and B are data from right eye viewing conditions and panel C and D are data from left eye viewing conditions. Order of injection is same as shown in figure 6. Positive number indicates

increase in BCEA and negative number indicate decrease in BCEA due to muscimol injection. Inset at the bottom of each panel shown the distribution of combined data.

In addition to conventional fixation stability of right and left eyes, we have also measured fixation stability in depth (vergence BCEA). Our previous study has shown that vergence BCEA in strabismic monkeys and humans was larger and more variable in comparison to version BCEA (Upadhyaya et al. 2017, Kelly et al. 2018). Figure 5.3.8 shows summary of change (BECA after – BCEA before) in version and vergence BCEA in all injections. There were both increases and decreases in version and vergence BCEA similar to what we observed in fixation instability of viewing and non-viewing eye. Seven muscimol injections resulted in an increase in version instability (Fig 5.3.8A; mean 0.5 ±0.5 deg^2) whereas four injections showed decrease in version instability (mean -0.3 ±0.2 deg^2). Similarly, vergence BCEA increased in seven muscimol injections (Fig 5.3.8B; mean 0.7 ±0.3 deg^2) and decreased in six injections (mean -0.4 ±0.4deg^2). Although there was small difference in change of vergence and version BCEA after muscimol injections, none of these differences reached statistical significance (M1-version BCEA: t=0.60, df=10, P=0.56; M2-version BCEA: t=1.03, df=5, P=0.35; M1-vergence BCEA: Mann-Whitney rank sum test, P=0.28; M2-vergence BCEA: t= -1.30, df=5, P=0.24).

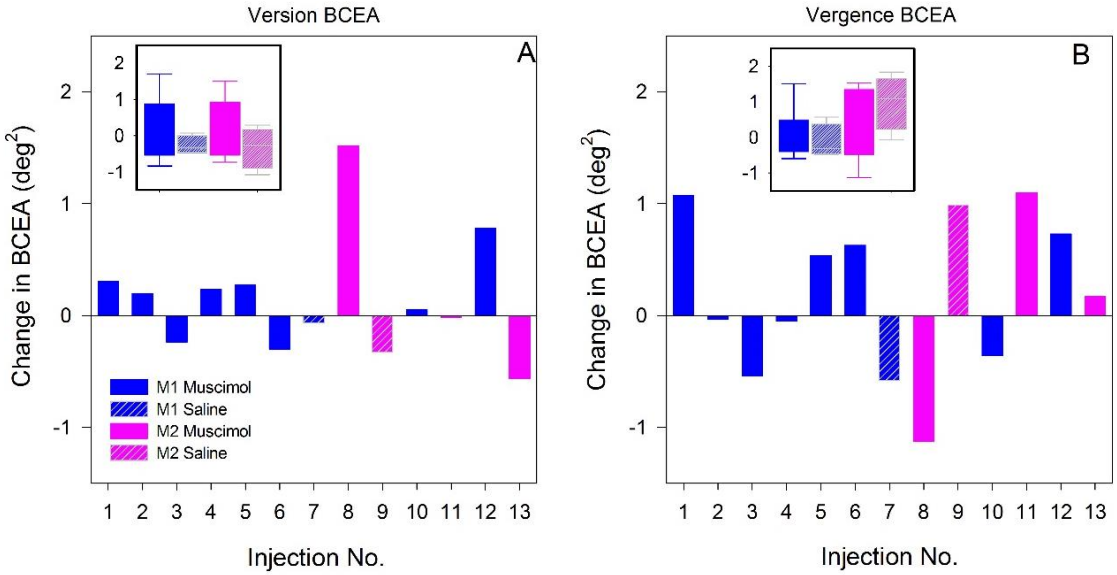

Figure 5.3.8 Bar and box plot showing change in vergence and version BCEA after injections

Figure 5.3.8. Bar plot showing effect of muscimol and control injection on vergence (difference between left and right eye positions) and version (average of left and right eye positions) BCEA. Same order and color coding as previous figures. Inset at the top of each panel shown the distribution of combined data.

5.3.3 Analysis of changes in characteristics of fixational saccades after muscimol injection

Fixational saccades were detected in the fixation data using a clustering method as described earlier. Figure 5.3.9 summarizes the amplitude of fixational saccades from all the injections during right eye viewing conditions. There was a trend for an increase in fixational saccade amplitude in both animals following all muscimol injections. However the inherent variability in the amplitude of fixational saccades resulted in statistical significance being attained in 6/8 experiments in M1 and none in M2 (M1: 6/8 Kruskal-Wallis One way ANOVA on ranks $H[8] = 297.0$, $P < 0.001$; Dunn's method for multiple comparison versus control $P < 0.05$; M2: 0/3 Kruskal-Wallis One way ANOVA on ranks $H[3] = 9.25$, $P = 0.02$; Dunn's method for multiple comparison versus control $P > 0.05$). On average the change in fixation saccade amplitude in muscimol injection was $0.07° \pm 0.42°$ and saline was $-0.22° \pm 0.32°$. In our view, these changes are too small to be functionally significant. Similarly, there were small changes in non-viewing eye fixational saccade amplitude (Fig 5.3.9B) and amplitude of fixational saccades during left eye viewing.

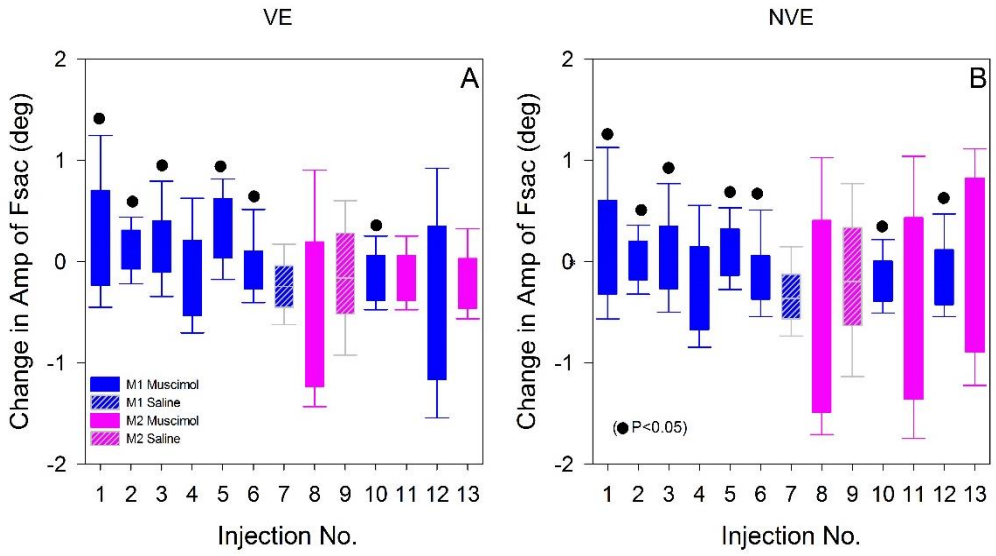

Figure 5.3.9. Box plot showing change in amplitude of fixational saccades

Figure 5.3.9 Change in amplitude of fixational saccades in viewing (panel A) and non-viewing eye (panel B) as a consequence of muscimol inactivation of SC. Order and color of bars is same as in other figures. Positive numbers on the y-axis indicates an increase in amplitude of fixational saccade and negative numbers indicates a decrease in amplitude of fixational saccades.

Finally, we also analyzed the frequency of fixational saccades and Figure 5.3.10 summarizes the change in frequency of fixational saccades due to muscimol injections. Note that the cohort of fixational saccades in strabismic monkeys includes both microsaccades and quick phase of nystagmus (Upadhyaya et al. 2017). Hafed and colleagues have shown that inactivation of rostral superior colliculus in normal monkeys decreased the frequency of microsaccades (Goffart et al. 2012). Figure 5.3.10A&B showed that there was generally a small increase in frequency of fixational saccades following muscimol injection (REV: mean increase of 0.41±0.25 Hz due to muscimol injection; LEV: 0.53±0.32 Hz) but these did not achieve statistically significance (REV - M1 Mann-Whitney rank sum test, P=1.0; M2 t=2.23, df =5, P=0.08; LEV - M1 t=0.66, df = 10, P=0.52; M2 t=-1.3, df =5, P=0.25) .

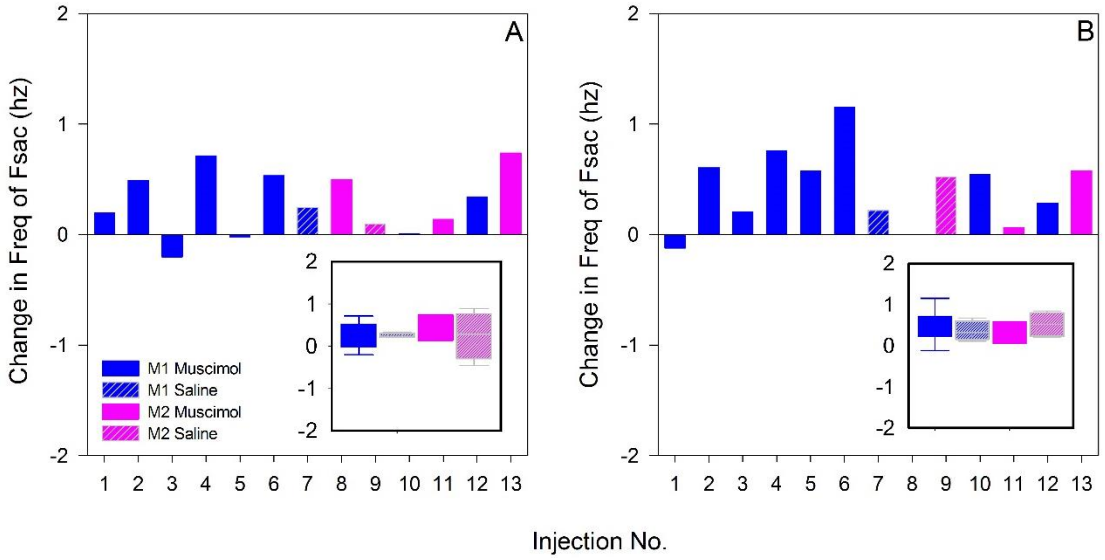

Figure 5.3.10. Vertical bar and box plot showing effect on frequency of fixational saccades

Figure 5.3.10. Change in frequency of fixational saccades in viewing eye during right eye viewing (panel A) or left eye viewing (Panel B). The order of injection and bar colors as same as in previous figures. Positive numbers indicate increase in frequency of fixational saccades and negative numbers indicate decrease in frequency of fixational saccades. Inset at the bottom of each panel shown the distribution of combined data.

5.4 Discussion

In this study, we have used the strategy of pharmacological inactivation to study the role of the superior colliculus in two properties of strabismus, one driving eye misalignment and the other fixation instability. The main findings in our study were 1) Muscimol inactivation of SC significantly changes the static angle of misalignment in convergent or divergent direction depending on inactivation site. 2) There was no consistent effect of muscimol inactivation on overall fixation instability including version and vergence instability. 3) Inactivation of SC also had no effect on frequency of fixational saccades, although there was sometimes a small increase in amplitude of fixational saccades. Below we examine these findings in context of superior colliculus and strabismus properties, specifically alignment and fixation stability.

5.4.1 Role of SC in Eye Misalignment

We have been systematically studying the role of the superior colliculus in maintaining static misalignment in monkey models of strabismus. In our first study we performed electrical stimulation of superior colliculus in strabismic monkeys and showed that there was a change in strabismus angle that could be either divergent or convergent (Upadhyaya et al. 2017). Subsequently we found both near response and far response cells in rostral superior colliculus of strabismic monkeys (Upadhyaya and Das 2018). The current study was to determine whether muscimol inactivation of the superior colliculus could substantially disrupt angle of misalignment in strabismus. We found that SC inactivation was able to change the strabismus angle in either convergent or divergent

directions but the changes were generally small. Perhaps this result is due to focal disturbance within the area (e.g., inactivation of a subset of convergent or divergent cell) that shifts the static balance towards increased convergence or divergence, i.e., cause convergent change if more divergent cells are inactivated or divergent change if more convergent cells are inactivated.

5.4.2 Role of SC in Fixation Stability

Another important question this study addressed was whether SC played a role in the fixation instability of strabismic monkeys. We found that the inactivation of SC did not change fixation stability (BCEA metric) by a large amount. There was a small increase in amplitude of fixational saccades after muscimol inactivation of superior colliculus although the frequency of fixational saccades remained unchanged. The slight increase in fixational saccade amplitude could be due to the fact that the focal point of the muscimol inactivation was quite rostral leading to the reduction or elimination of small amplitude fixational saccades.

As noted in our behavioral study (Upadhyaya et al. 2017), there was a nonlinear relationship between amplitude of fixational saccades and the fixation stability BCEA metrics, with fixational saccade amplitude saturating for larger values of BCEA. We suggested that due to the saturation of fixational saccade amplitude, drifts must be the main contributing factor for larger fixational instability in strabismic monkeys. Therefore, it could either be that a) the slight increase in amplitude of fixational saccades observed following muscimol inactivation is insufficient to significantly influence fixation

instability or that b) SC inactivation does not affect fixation instability because drifts are the predominant component of fixation instability in strabismus.

5.4.3 Role of SC on vergence stability and accommodation

As we have seen previously in our behavioral study (Upadhyaya et al. 2017), vergence stability (BCEA) for normal monkeys was within the panum's fusional area but that of strabismic monkeys was larger and more variable. A similar finding of disruption in vergence control was noted in strabismic and anisometropic children (Kelly et al. 2018). In this study we found significant change in strabismus angle but only small changes in vergence instability during muscimol injection in comparison to saline injection. This suggests that SC was involved in vergence but not in vergence instability.

Another aspect to consider is the link between vergence and accommodation. SC has also been shown as a structure involved in accommodation. In cats, it has been shown that there was change in accommodation due to electrical stimulation of SC (Sawa and Ohtsuka 1994). In humans, accommodation palsy was notice after ablation of both colliculi (Ohtsuka et al. 2002). In monkeys, an anatomical connection was found between SC and EW through cMRF (May et al. 2015). As preliminary data from Joshi has shown, in strabismic monkeys higher accommodative fluctuation was positively correlated with vergence instability (Joshi 2016). Although, little is known about the interaction of vergence and accommodative in strabismic monkeys, it is possible that any change in vergence stability following inactivation would have been due to change in accommodative fluctuation in strabismic monkeys. One hypothesis for the lack of change

in vergence stability following inactivation could be due to inactivation of relatively equal numbers of accommodative and disacommodative cells.

Chapter-6: Discussion

The overall goal for this series of experiments was to understand the role of the superior colliculus in strabismus. This series of behavioral, electrical stimulation, neurophysiology, and pharmacological inactivation studies has provided valuable information about two fundamental properties of strabismus: strabismus angle and fixation instability. In this chapter, I will first briefly mention the novel findings of each experiment, then provide an overall summary of the project, and finally, propose research that will help in further understanding strabismus.

Behavioral study: The aim of the study was to determine the role of fixational saccades on fixation instability of strabismic monkeys. The main findings were i) there was a non-linear relationship between amplitude of fixational saccades and fixation instability (Fig 2.3.6). Fixation instability increases with an increase in fixational saccades amplitude, but after a certain point, it saturates ii) amplitude and frequency of fixational saccades in strabismic monkeys were larger than that of normal monkeys iii) vergence stability was poor in strabismic monkeys in comparison to normal monkeys.

Electrical stimulation experiment: We found that electrical stimulation of SC in developmental strabismic monkeys resulted in a change in strabismus angle. These changes could be either divergent and convergent depending on stimulation site (Figure 3.3.4). This study provided the first concrete evidence that the SC was part of a disrupted vergence circuit in monkey models of strabismus. Further analysis showed that disconjugate saccades evoked due to electrical stimulation of SC insufficiently accounted

for the total change seen in strabismus angle, implicating the superior colliculus in disconjugate post saccadic movements possibly through activation of a specific sub-population of the SC, the recently identified vergence (misalignment) cells.

Single cell recording experiment: These experiments showed that there were indeed misalignment-related cells within the SC of strabismic monkeys, as predicted by the previous electrical stimulation study. We found both convergent type cells (near response cells that showed increased responses for smaller angles of exotropia) and divergent type cells (far response cells that showed increased responses for larger angles of exotropia), as shown in Figure 4.3.3. Further analysis of the cell properties showed that sensitivity of near and far response cells in strabismic monkeys was similar to convergence and divergence cells found in rSC of normal monkeys, but thresholds differed significantly.

Muscimol experiment: This experiment was designed to test for a causal role of superior colliculus in two strabismic properties—misalignment angle and fixation instability. Unilateral inactivation of superior colliculus neither resulted in normal ocular alignment nor completely helped stabilize or further destabilize fixation. However, muscimol inactivation of SC induced convergent and divergent changes in strabismus angle, as shown in Figure 5.3.5. The effect of inactivation on BCEA (fixation instability metric) was not significant but increased in amplitude of fixational saccades was observed.

Overall summary and Discussion:

Behavioral studies have shown that fixation stability is poor in strabismic humans and monkeys but have not discovered what determines the larger fixation instability in this disorder. To address this issue, we first conducted a behavioral study and showed that fixation instability in strabismic monkeys is due to larger amplitude of fixational saccades. A nonlinear relationship exists between amplitude of fixational saccades and fixation instability meaning that drifts also contribute towards fixation instability. This study has also shown that there is significantly larger vergence instability in strabismic monkeys in comparison to normal monkeys. The SC of cats, monkeys, and humans has been implicated in vergence. From previous studies, we knew that disrupted vergence circuits cause strabismus, but not whether the SC is a part of this circuitry. Disruption of binocular vision during critical periods of development leads to a cascade of events, ultimately disrupting vergence circuits. In normals, both eyes are properly aligned when looking at distance. Over or under convergence and divergence while fixating at a distant object is called strabismus. To determine whether SC is involved in strabismus, we began with an electrical stimulation study. If the superior colliculus does not affect strabismus, then there should be no change in strabismus angle. However, we saw that there was significant change in strabismus angle during micro-stimulation of SC. Further analysis of the change in strabismus showed that disconjugate staircase saccades produced due to electrical stimulation were unable to fully account for the change seen in the strabismus angle leading to the hypothesis that disconjugate post-saccadic drift could be due to activation of vergence related cells within the SC. To find these cells, we performed a single unit recording study in strabismic monkeys. We changed the vergence angle by

taking advantage of dissociated horizontal deviation in our strabismic monkeys. We found convergence and divergence cells that modulate their firing in correlation with changes in the strabismus angle. When comparing the similar population of convergence and divergence cells recorded from rostral colliculus of strabismic and normal monkeys, we found that the sensitivity of these cells were similar, but the thresholds differed. These thresholds shifted toward the habitual strabismic state of experimental monkeys. Other properties of these misalignment-related cells were similar to fixation cells, which decrease their discharge during saccades and are not affected by the presence of a visual stimulus. A subset of these cells showed responses correlated with the quick phase of nystagmus, suggesting that the cells also encode saccade like events of specific amplitude and direction including quick phases. To follow up on the single-unit recording experiments, we also performed unilateral inactivation of superior colliculus and examined its effect on misalignment. If SC was primarily responsible for misalignment and if we removed this structure from brain circuitry, then we should have found normal ocular alignment. However, unilateral inactivation of SC resulted only in small convergent and divergent changes in strabismus angle. We concluded that SC is not the central source of disrupted vergence circuits in strabismus, but that SC is a part of a larger disruption in neural networks during the development of strabismus, i.e., the SC was partially responsible in ocular misalignment. In summary, the SC is part of the vergence circuitry that is contributing to the maintenance of ocular misalignment.

These findings have the potential to expand onto a new series of studies that I describe in the next section.

Proposed Experiments

1) **Does the caudal superior colliculus also have misalignment cells?**

Thus far, we have explored the rostral superior colliculus of strabismic monkeys in search of misalignment-related cells. This study was specifically focused in the rostral region, because a recent study in normal monkeys showed that there are convergent and divergent-related cells in rostral superior colliculus of normal monkeys. However, the change in strabismus angle we observed in our electrical stimulation study was similar in rostral and caudal sites of stimulation. Similarly, in the muscimol inactivation experiments, inactivating some caudal sites resulted in a significant divergent effect. This suggests that caudal SC may also have vergence (misalignment) related cells. Additional investigation is necessary to learn whether vergence (misalignment) cells are more densely packed in the rostral area or if they are present in a rostral to caudal continuum like saccadic cells in SC.

2) **Investigating the role of the FEF in strabismus**

The SC and the frontal eye field (FEF) are closely linked for the control of saccades (Stanton et al. 1988, Hanes and Wurtz 2001, Gandhi and Katnani 2011). Like the SC, the FEF also seems to play a role in control of vergence eye movements (Gamlin and Yoon 2000). Convergence and divergence neurons are present in the FEF, which modulates during 3D smooth pursuits(Fukushima et al. 2002). Anatomical studies have shown that a connection exists between FEF and SC (Komatsu and Suzuki 1985). In addition, near response cells in nucleus reticularis tegmenti pontis (NRTP) also receive input from the frontal eye field (Buttner-Ennever and Buttner 1988). We have shown the role of the SC

in strabismus. Then, the question is whether there are any other higher structures involved in strabismus, with the FEF being an obvious choice for future investigation. The first experiment could be to investigate whether electrical stimulation of FEF can change the strabismus angle in strabismic monkeys. Comparing changes in the strabismus angle due to electrical stimulation of SC with that from the FEF will help in understanding the relative extent to which SC and FEF contribute towards strabismus maintenance.

The next phase of FEF investigation could be to perform single cell recording studies and comparing FEF and SC responses. For example, sensitivity of misalignment-related cells in SC can be compared to that of the FEF. As FEF feeds input to SC, there might be differences in misalignment-related cell sensitivity. A similar comparison of firing rate thresholds between SC and FEF cells can be performed. Finally, FEF sensitivity and threshold properties can be compared to that of the normal, similar to our analysis of the SC cells. Although the involvement of the FEF is unclear, a comprehensive modeling of structures in the brain known to be important for vergence could explain how much neural alteration in each structure is responsible for what amount of strabismus.

If the superior colliculus was the primary structure that results in strabismus, then when we temporarily remove the SC from the vergence circuitry, it should result in normal ocular alignment. Our study has shown that this was not the case. Our inactivation study also showed that there could be a convergent or divergent effect following application of muscimol into the SC, although the effects were relatively small. This could mean that there is a balance in the population of convergent and divergent cell output from SC in

order to maintain habitual ocular alignment in normal or misalignment in strabismic monkeys. Studies have shown that lesions of SC and FEF together will hamper the generation of saccades because each plays a complementary role for saccade generation(Schiller et al. 1980). As we learn more about the role of superior colliculus in vergence, the question is whether it will be possible to generate vergence without SC and FEF. What will be the effect on strabismus angle if we simultaneously inactivate the two major structures involved in vergence?

Although not a main focus of our study, we observed that some of the misalignment-related cells found in the SC of strabismic monkeys encode the nystagmus quick phase when the electrode was placed in the topographic location that matches the direction and amplitude of nystagmus. Fundamentally, this reveals that microsaccades and nystagmus quick phases are driven by the same saccade circuitry. The other important finding is that it appears that the same cell could encode vergence and also microsaccades or the nystagmus quick phase. The same questions and analysis about control of nystagmus quick phases can be carried out within the FEF.

3) Fixational eye movements, refractive error, and strabismus

Fixational saccades could possibly also give new insight into refractive error and the development of strabismus as we are still learning how the brain distinguishes between hyperopic blur from myopic blur. The temporal dynamics of fixational eye movements have shown that blur image shows more luminance modulation than static image when eyes are constantly moving the image in the retina (Rucci and Victor 2018). More precisely, the retina does not have an image but only a spatiotemporal flow of luminance.

Even if human eyes are a perfect optical system like a camera, if the camera shakes, then the image will be blurred unless there is corollary discharges of these movements that are counterbalanced by optical or neural components of the optical system. As strabismic individuals have disconjugate eye movements, it will be interesting to investigate how the onset of strabismic affects refractive error and fixational eye movements.

4) Can fixation metrics be used as a clinical tool?

Humans are constantly making fixational eye movements even when fixating on a stationary target. These fixational eye movements can be broadly classified into microsaccades, drifts, and tremors. Overall, fixation stability is poor in many diseases, such as strabismus, amblyopia, age-related macular degeneration, diabetic retinopathy, and other neurological disorders. One of the quantitative metrics used to measure fixation stability is the bivariate contour ellipse area (BCEA), which measures the dispersion of eye position in two dimensions. In disorders such as strabismus, the ability to perceive depth (stereopsis) is impaired. Thus, it is crucial to know how fixation stability changes in depth (vergence BCEA). The use of BCEA is relatively rare in mainstream optometry and ophthalmology practice. BCEA can be used as a guide for diagnosis, prognosis, and monitoring interventions in a variety of conditions. The fundamental question is whether fixational eye movements can serve as precise and quantitative biomarkers for disease detection and measuring efficacy of treatment in various neurological diseases?

5) **Is ocular accommodation evoked via electrical stimulation of SC in normal and strabismic monkeys?**

When humans look at near objects, there is convergence, accommodation, and pupillary constriction (near triad). Usually, convergence and accommodation accompany each other. In strabismus, however, it is unknown how these two components interact. It would be particularly interesting to study the SC because micro-stimulation and pharmacological inactivation studies in cats have suggested the role of rSC in accommodation (Ohtsuka & Sato, 1997). Sawa and Ohtsuka showed that lens accommodation could be evoked with weak current from superficial and intermediate layers of the rostral portion of the SC in cats (Sawa and Ohtsuka 1994). In 2015, May et al. showed that, in monkeys, the preganglionic motor neurons of the Edinger-Westphal nucleus receive synaptic input from cMRF(May et al. 2015). Ostrin and Glasser stimulated the Edinger-Westphal nucleus and were able to measure change in accommodation in normal rhesus monkeys (Ostrin and Glasser 2007). Ohtsuka's case report showed that bilateral superior colliculus lesions in humans cause accommodation palsy, leaving accommodation of just 2D at the age of 30 years (Ohtsuka et al. 2002). We were unfortunately not able to record accommodative responses in our studies and so adding accommodation as a measured variable in similar future studies could provide new insight into vergence and accommodation control in strabismus and their relative roles in maintenance of the disease condition.

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