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A biomechanical explanation for convergent head movements evoked by stimulation of the primate supplementary eye fields

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By

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fulfilment of the degree of
Master of Science

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CERTIFICATE OF EXAMINATION

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requirements for the degree of
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Abstract

Cortical microstimulation has played an important role in the investigation of movement coding in the frontal and supplementary eye fields (FEF and SEF). Recent evidence has shown that microstimulation of the SEF produces eye head gaze shifts whose kinematics depend on initial position. Across different stimulation sites in the SEF, evoked movements converge in a variety of reference frames, possibly reflecting the SEF's role in complex sensory-motor transformations. Here, we examine neck muscle activity evoked by SEF stimulation while monkeys attained a range of different initial positions through electromyographic (EMG) recordings of the neck muscles. A similar approach targeting the FEF and superior colliculus (SC) has revealed a counter-intuitive trend where increasing levels of agonist (contralateral) neck muscle activity are associated with the smallest evoked movements.

Monkeys were trained to look to one of nine different fixation points prior to SEF microstimulation (100 µA, 300 Hz, 200 ms) with stimulation was passed on half of all trials. SEF stimulation evoked a rapid facilitation (18 ± 5.5 ms ms) of EMG activity on contralateral agonist neck muscles and a simultaneous suppression of EMG activity on the antagonist neck muscles. Importantly, the expression of this generic evoked response depended on the positionally-dependent level of background EMG activity attained prior to stimulation. As in the SC and FEF, we observed a counter-intuitive trend where the smallest amplitude movements, which occurred for initial positions contralateral to the side of stimulation, were associated with the largest increases in evoked neck muscle activity. Our results suggest that the apparent convergence of the head following stimulation in a variety of oculomotor areas may result more because of biomechanical considerations consequent to the initial positions, as opposed to a centrally-programmed strategy reflective of reference frame coding.

Key words: Supplementary eye fields, electrical stimulation, electromyography, gaze shifts, frames of reference, rhesus monkey, motor control

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I, Michael Pace, am submitting this research project as partial fulfillment of the Master of Science Degree in the discipline of Neuroscience. As such, I have assumed a primary role in all aspects of this document including, but not limited to, design aspects, data collection and analysis as well as producing the initial draft of the thesis. Dr. Brian D. Corneil acted as my supervisor for this project. He provided the framework for this project as well as providing critical advice and guidance throughout all stages of the project. He also acted as an editor to the subsequent drafts of this thesis. Brendan Chapman performed initial functional mapping of the supplementary eye fields of one of the monkeys and Dr. Sharon Cushing performed the electromyographic electrode surgery on the animals used in this thesis.

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List of Abbreviations

CI	Convergence index
SC	Superior Colliculus
FEF	Frontal Eye Fields
SEF	Supplementary Eye Fields
PPRF	Paramedian Pontine Reticular Formation
NRPc	Nucleus Reticularis Pontis Caudalis
NRGc	Nucleus Reticularis Gigantocellularis
EMG	Electromyography
OCI	Obliquus Capitis Inferior
RCM	Rectus Capitis Posterior Major
SP	Splenius Capitis
BC	Biventer Cervicis
COM	Complexus
LED	Light-emitting diode
FP	Fixation point
GUI	Graphical user interface
hCI	Head convergence index
AI	Activity index
V1	Primary Visual Cortex

List of Symbols

kg	Kilogram
mm	Millimeter
°	Degree
ft^3	Cubic foot
ft	Foot
kHz	Kilohertz
MΩ	Megaohm
g	Gram
Hz	Hertz
ms	Millisecond
μA	Microampere
cd	Candela
m^2	Square meter
°/s	Degree per second
±	Plus or minus
sd	Standard deviation
μV	Microvolt

Chapter 1 – Introduction

1.1 - Gaze Shifts

Humans are foveate animals, meaning that all high acuity vision is restricted to a small area in the centre of the retina known as the fovea. As a consequence, humans can only view images in detail when they are in the centre of our vision. This is why, for example, one cannot read a book out of the corner of one's eye, nor can one watch TV while focusing on the cat sleeping on the couch. Humans are thus required to shift their gaze to any object they need to view in detail, and in order to build up a holistic view of the world around us, humans need to make hundreds of thousands of gaze shifts every day.

Larger gaze shifts involve rapid and coordinated movement of the eyes and head and can be separated into an eye and a head component. The eye component consists of the eyes moving rapidly within their orbits and the head component of the neck turning the head about the body (Fig. 1). During a gaze shift, the eye component and head component can move in the same direction at the same time to complete larger gaze shifts. After the rapid movement of the eyes and the gaze falling on the endpoint, the vestibulo-ocular reflex maintains gaze stability for the duration of the head movement through a counter-rotation of the eyes. The overall gaze amplitude is a summation of the displacement of the eye-in-head and the head-in-space during the gaze shift.

No one predetermined pattern of eye and head contributions exists for gaze shifts; equal amplitude gaze shifts can be accomplished by an essentially infinite combination of eye and head contributions. Thus, appropriate eye and head contributions must be

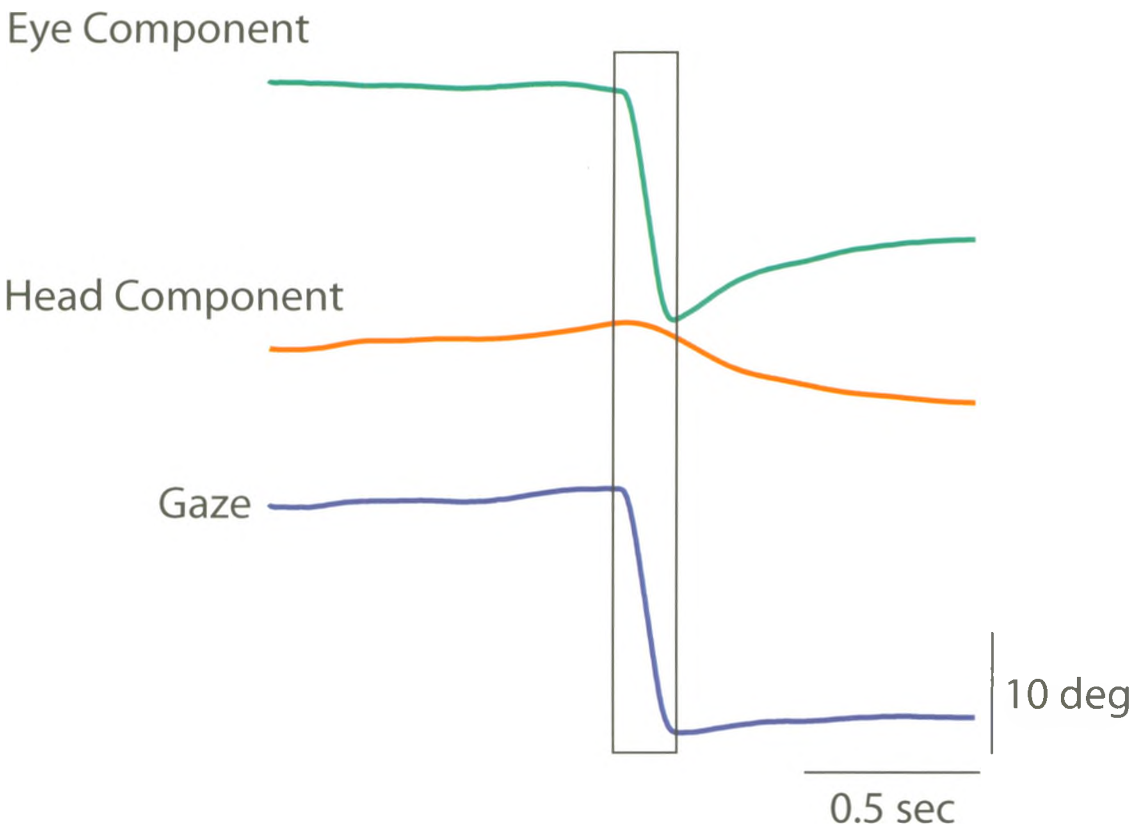

Figure 1. Head free gaze shift. The gaze shift occurs during the time period contained within the box. During this time both the eye and the head component contribute to gaze amplitude. Gaze is stable following the gaze shift due to the counter rotation of the eye component as the head component finishes moving.

selected to perform accurate gaze shifts. A number of factors including current posture, idiosyncratic dispositions and expected subsequent movements can influence the pattern of eye-head coordination, resulting in a wide variety of different eye and head contributions (Bard et al. 1992; Fuller 1992a; Fuller 1992b; Stahl 1999).

1.2 - Biomechanics of the eyes and head

The head and eyes require completely different signals in order to accomplish a given movement. The eyes are small, light and are characterized by a relatively low inertia. Due to these biomechanics, eye motion is relatively responsive to changes in activity on the extraocular muscles (Sindermann et al. 1978). The eye musculature is also relatively simple, with three agonist-antagonist muscle pairs responsible for the movement of the eye within the fixed orbit of the skull. The low inertia, fixed position relative to the head, and extraocular musculature make the eye relatively simple from a biomechanical perspective. Head biomechanics are quite different from those of the eyes. The inertia required to move the more bulky head is much larger and must be overcome by the forces developed by the neck muscles. There are also a large number of neck muscles of various sizes responsible for rotating, pitching and tilting the head. The increased inertia of the head and complex musculature of the neck make the head plant a much more biomechanically complicated system than the eyes. Despite the biomechanical differences between the eyes and the head, the brain must control both these systems in unison to create rapid and accurate eye-head gaze shifts.

1.3 - Reference Frames

The oculomotor system is a collection of brain structures responsible for the generation of gaze shifts and the selection of potential gaze targets. The different structures of the oculomotor system span from lower-level areas involved in the immediate generation of movement to higher-level areas involved in abstract goal selection. Lower-level areas are involved in the transformation of movement signals into the appropriate commands issued to the muscles. Thus, the activity of these low-level areas must accomodate the biomechanics of the components of the oculomotor system to ensure appropriate commands are sent to the muscles to produce an accurate movement. Intermediate areas are involved in the transformation of spatially coded signals of the high-level areas into the temporal code of the lower-level areas and act as a relay between the oculomotor areas. High-level areas encode goal-directed movement signals in that the signal sent by a high-level area encodes the desired gaze shift rather than individual component contributions. One of the challenges within motor control research is understanding how information encoded by high-level areas is transformed into the appropriate muscle based commands to achieve a desired goal.

One way of describing the nature of coding within a particular brain region is to consider the reference frame in which the region operates. At the lowest levels, motoneurons innervate muscles and hence operate in a muscle-based frame of reference. A step up from the motoneurons are the pre-motor areas, some of which encode movements via the frequency and duration of neural activity. Since the temporal coding of these areas is responsible for the transmission of the movement signal, these areas can be considered as operating within a temporal frame of reference. Moving progressively up through the levels of the oculomotor system, movements are encoded in more spatially-based patterns of activation. In spatially coded areas, movements are encoded based on which neurons are activated rather than the temporal components of neural activity. Higher-level areas generally encode spatial movement with respect to the position of various things, such as the components of the body. For example, high-level oculomotor areas can fall into eye-centred, head-centred and body-centred frames of reference as outlined in Fig. 2A-C. Other frames of reference also exist, such as space and limb-centred as well as object-centred reference frames.

Due to the spatial coding scheme of higher-level areas, neurons in these areas are optimally active for a gaze shift driven to a preferred movement goal. This movement goal is based on the initial orientation of the oculomotor component corresponding to the reference frame of that brain area. For example, in an eye-centred reference frame the preferred movement goal is a fixed vector based on the initial position of the eyes. Thus, for a neuron with the preferred goal outlined in Fig. 2A, any of the movements shown in Fig. 2D would be paired with the same level of neural activation. This is because each movement outlined in Fig. 2D has the same movement goal based on the initial position of the eyes.

For a neuron coding in a head-centred frame of reference the preferred movement goal is a fixed vector from the initial orientation of the head such as that outlined in Fig. 2B. Neurons in a head-centre reference frame fire optimally for movements made to a goal based on initial head orientation. Head orientation is a significant part of gaze orientation, so when the head is free to move it will assume different initial orientations based on the initial gaze position. The result of different initial head orientations is a shift

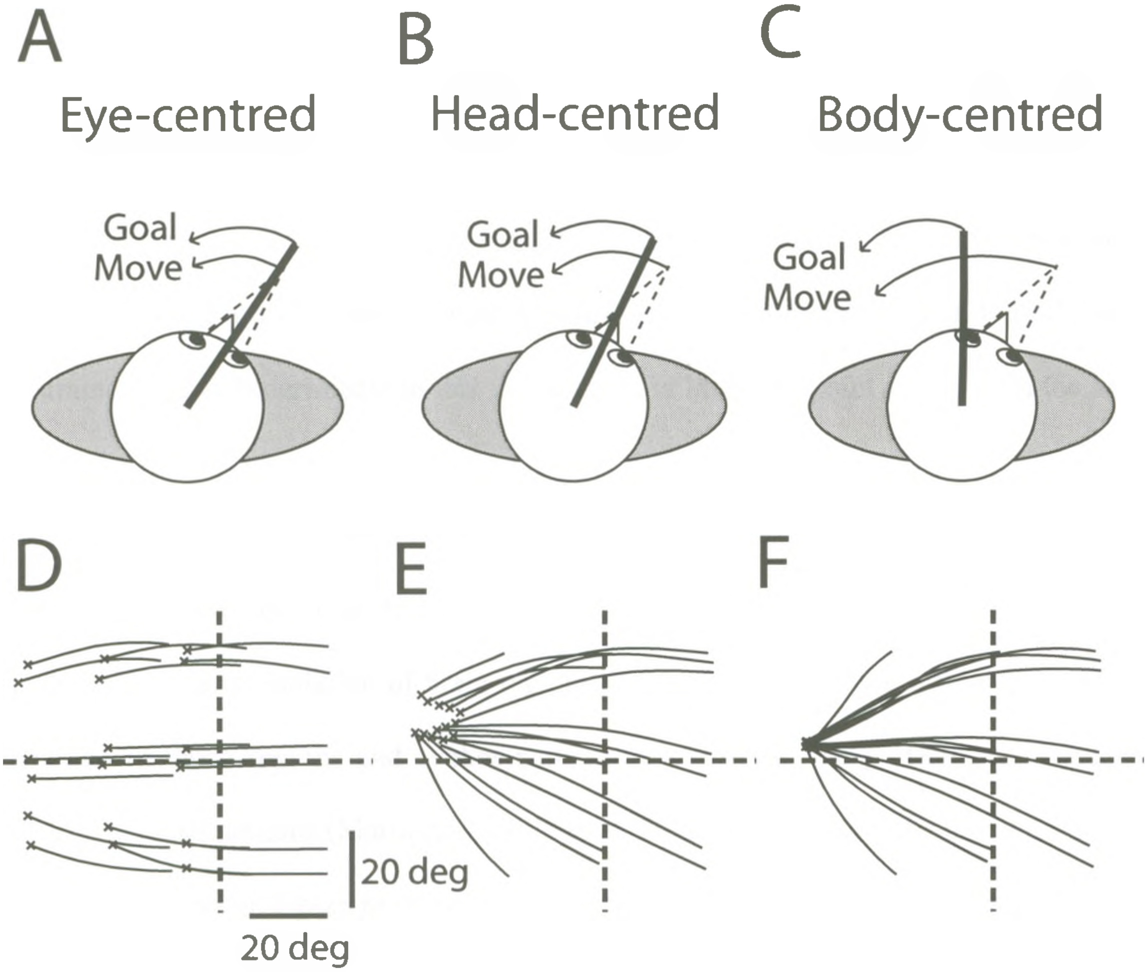

Figure 2. Models of eye-centred (A), head-centred (B), and body-centred (C) frames of reference. Goal position is based on the initial orientation of the body part around which the reference frame is centred (A-C). Movement is always based on the initial gaze position resulting in different preferred movements for each frame of reference. Gaze traces (x denotes endpoint) for which a neuron would fire equally when coding in an eye-centred (D), head-centred (E), or body-centred (F) frame of reference.

in the preferred goal location with the head. Thus, for neurons in a head-centred reference frame, all the movements shown in Fig. 2E could be paired with the same level of neural activation. Neurons encoding in a body-centred reference frame will fire optimally for movements made to a goal based on the orientation of the body as shown in Fig. 2C. These neurons discharge maximally for movements made to the body based goal location such as the movements outlined in Fig. 2F. As the trunk is not involved to a significant degree in the relatively small changes in initial gaze orientation shown in Fig. 2F, and is restrained in the experiments in this thesis, there is little end point variation in the body-centred goal location.

The theory of reference frame coding and transformation in the oculomotor system has been supported by the use of intracortical microstimulation. The assumption is that electrical stimulation of brain regions within the oculomotor system activates the neurons in those regions and evokes natural gaze shifts to the preferred goal location coded by those neurons (Martinez-Trujillo et al. 2003a). By varying the initial orientation of the eye, head and gaze prior to stimulation, the thinking is that the pattern of evoked movements reveals the nature of the area's frame of reference. One method of qualifying the pattern of stimulation evoked movements is to measure how those movements fall, or converge, onto a goal location (Martinez-Trujillo et al. 2004).

If the stimulated area is operating in an eye-centred frame of reference, then stimulation-evoked movements will be directed to the same goal location relative to the initial orientation of the eyes. Thus, the vector of the evoked movements will be the same at all initial gaze positions producing a pattern of evoked movements similar to that shown in Fig. 3A. Because movements evoked by stimulation of eye-centred reference

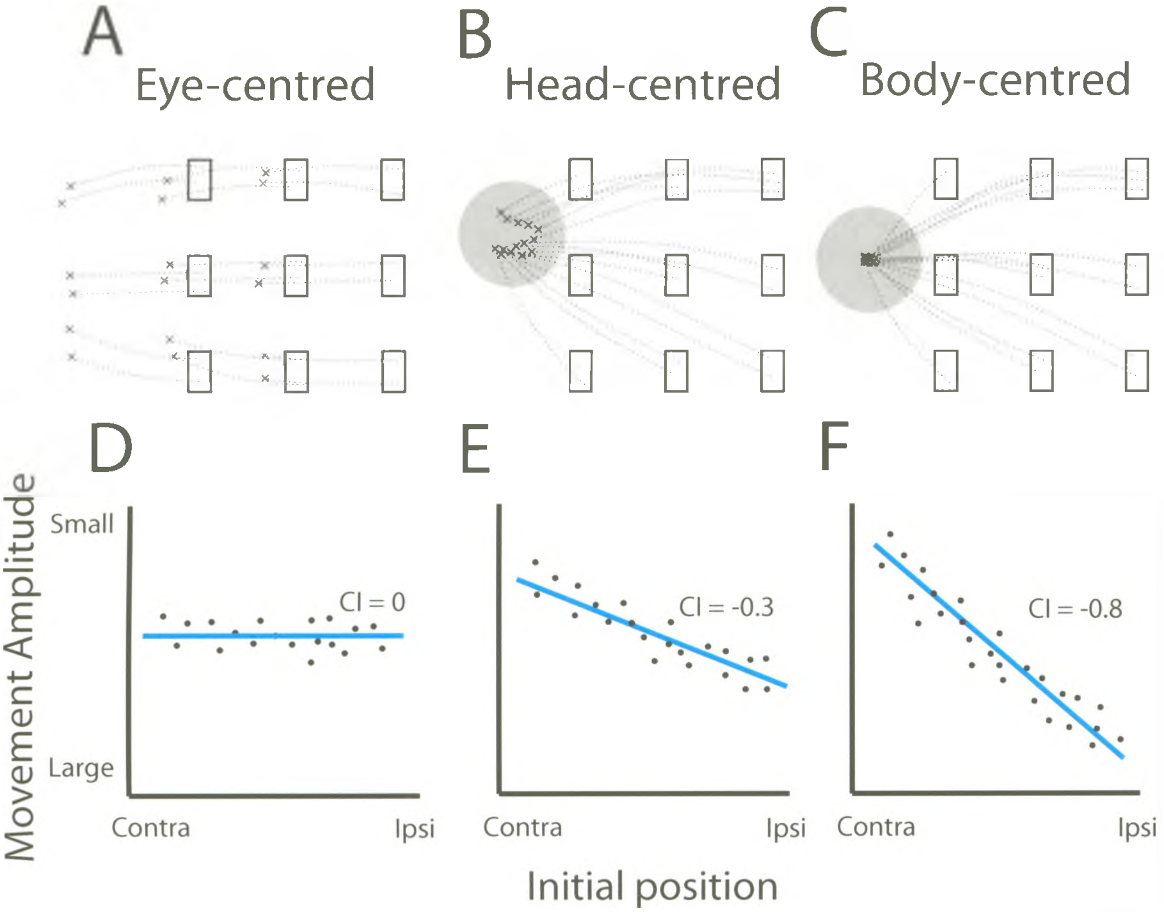

Figure 3. Stimulation evoked gaze shifts from different initial fixation positions, boxes denote fixation windows and x denotes gaze endpoint, for eye-centred (A), head-centred (B), and body-centred (C) frames of reference. Filled grey circle in B and C corresponds to the observed termination zone of evoked movements. Theoretical stimulation-evoked movement amplitudes are plotted from contralateral to ipsilateral initial gaze positions for eye-centred (D), head-centred (E), and body-centred (F) frames of reference. Blue linear regression lines are fit to the theoretical movement amplitudes with the slope being the convergence index (CI).

frame areas do not converge on a goal location, these stimulation sites are said to have low convergence. To quantify the convergence of evoked movements a convergence index (CI) is calculated. The CI corresponds to how tightly the evoked movements converge. CI is calculated as the slope of the regression line fit to the amplitude of the evoked movements plotted against the initial gaze position. A CI of zero indicates no convergence of evoked movements while a CI of -1 indicates complete convergence of evoked movements. One example of the CI for a theoretical stimulation site operating in an eye-centred frame of reference is shown in Fig. 3D. The calculated slope of the regression line (CI) is near zero since evoked movement amplitude varies little with initial gaze position.

For stimulation sites operating in a head-centred reference frame, the evoked movements are driven close together to a point in space relative to initial head orientation. Because different initial gaze positions result in different initial head orientations in head free animals, the goal position will vary in space with initial head orientation as shown in Fig. 3B. The result is more variability in evoked movement amplitude across initial gaze positions as shown in Fig. 3E, leading to the observation of increased convergence. Because the goal position varies with the changing of initial head position, convergence is not complete. Thus, the calculated CI (eg Fig. 3E) for a stimulation site operating in a head-centred reference frame will be of medium magnitude. For stimulation sites operating in body-centred reference frames, stimulation-evoked movements will be driven to the same spatial goal location relative to the body regardless of initial gaze position, as shown in Fig. 3C. Since there is little movement of the body across relatively small differences in initial gaze position, the goal location remains stable. The result is the significant variability in the amplitude of evoked movements across initial gaze positions resulting in large convergence as shown in Fig. 3F. The calculated CI for a stimulation site operating in a body-centred reference frame will be of large magnitude and close to -1.

Having introduced the concept of reference frames, I will now examine reference frames of the different structures in the oculomotor system. I will consider the coding schemes of the low-level pre-motor areas followed by the intermediate level superior colliculus (SC) and finally the high-level cortical structures of the frontal eye fields (FEF) and supplementary eye fields (SEF).

1.4 - Low-level: Brainstem oculomotor circuits

At the base of the oculomotor system are the low-level brainstem circuits responsible for the transformation of movement goal signal into the motor commands required to execute that movement. The areas responsible for the generation of horizontal gaze shifts are divided between the eye circuits located in the paramedian pontine reticular formation (PPRF) and the head circuits located in the nucleus reticularis pontis caudalis (NRPc) and the nucleus reticularis gigantocellularis (NRGc), all of which receive monosynaptic input from the superior colliculus (SC) and project directly to the motoneuron pool for the eyes and neck respectively [see Scudder et al. (2002) and Isa and Sasaki (2002) for review]. Stimulation of these areas produces continuous movement that persists for the duration of stimulation (Quessy and Freedman 2004; Gandhi et al. 2008). Such persistent motion during stimulation is characteristic of a low-level temporally-centred reference frame. Operating in a temporally-centred reference frame allows these brain areas to interface with the motor periphery using hardwired patterns that underlie muscle synergies (Shinoda et al. 1996). This is especially important in head movements due to the apparent redundancy of the neck musclulature. The hardwired inputs from the brainstem define the synergies used to activate the neck musculature and resolve the issue of appropriate drive signal selection.

1.5 - Intermediate-level: Superior Colliculus (SC)

Major players in the generation of gaze shifts are the intermediate and deep layers of the SC. Located in the dorsal mesencephalon, the SC receives input from all of the cortical oculomotor centres and is thought to act as a hub where orienting commands converge from across the brain (Scudder et al. 2002; Platt et al. 2003). The SC projects heavily to the brainstem oculomotor circuits and begins the transformation of spatially encoded signals into a muscular command through the pattern of axonal projections to the brainstem oculomotor circuits (Scudder et al. 2002; Isa and Sasaki 2002; Sparks 2002). The intermediate and deep output layers of the SC are arranged topographically in eye-centred coordinates with respect to saccade size and direction into the contralateral hemifield (Wurtz and Goldberg 1971; Robinson 1972). Stimulation of the intermediate and deep layers of the SC evokes contralateral eye-head gaze shifts in head-free monkeys (Freedman et al. 1996; Klier et al. 2001). The topographical organization of the SC results in different levels of activation of the brainstem oculomotor circuits produced by stimulation, corresponding to the evoked movement vector (Izawa et al. 1999). Stimulation of the SC has traditionally been viewed as evoking fixed vector gaze shifts from different initial gaze positions (Robinson 1972). The evoked movements display little convergence supporting the theory that the SC operates in an eye centred reference frame. Although earlier studies (Robinson 1972) observed some convergent movements evoked by SC stimulation, later studies offered explanations that such apparent convergence arose due to the restraint of the head not allowing the full gaze shift to be observed (Freedman et al. 1996; Martinez-Trujillo et al. 2003b). Other studies also put forth that the convergent pattern of evoked movements could be produced if the SC encodes in retinal rather than eye coordinates (Klier et al. 2001). The consensus view is that the SC is positioned as an intermediary between the abstract spatial coding of the high-level brain areas and the temporal coding of the lower-level areas. The SC begins these spatiotemporal transformations of the movement signal as a result of the pattern of SC projections to the lower-level areas.

1.6 - High-level: Frontal Eye Fields

Since the late nineteenth century scientists have known of the involvement of the frontal lobes in the generation of gaze shifts. In a set of seminal experiments, Ferrier (1875) demonstrated that stimulation of the primate frontal lobes around the arcuate sulcus and the dorsomedial frontal cortex with a ball electrode produced contralateral eye and head movements. The FEFs have since been identified as a region located on the anterior bank of the arcuate sulcus involved in the generation of gaze shifts. Recordings of FEF neurons have shown saccade related activity and stimulation of the FEF evokes contralateral gaze shifts (Bruce et al. 1985; Schall 1991a). The involvement of the FEF in the generation of gaze shifts is likely mediated by the dense projections from the FEF to the SC (Sommer and Wurtz 1998). Gaze shifts can still be evoked by stimulation following ablation of the SC via direct projections from the FEF to the brainstem oculomotor circuits (Schiller et al. 1980), however these projections are not sufficient to drive gaze shifts when the SC is reversibly inactivated (Hanes and Wurtz 2001).

Most stimulation sites in the FEF in head free primates display little convergence of evoked movements, implicating an eye-centred reference frame (Russo and Bruce 1993). There are however, a number of observed sites that display moderate convergence of evoked movements consistent with a head-centred frame of reference (Russo and Bruce 1993; Knight and Fuchs 2007). While the observed function and reference frame of the SC is fairly congruous, it appears that stimulation and recording studies in the FEF have observed multiple functions and reference frames.

1.7 - Higher-level: Supplementary Eye Fields

The supplementary eye fields (SEF) are areas located on the dorsomedial frontal cortex, medial and slightly anterior of the horn of the arcuate sulcus close to midline, in which electrical stimulation readily evokes eye-head gaze shifts (Schlag and Schlag-Rey 1987; Tehovnik and Lee 1993; Sparks et al. 2001). Neural recordings in the SEF indicate that the SEF appears to be related to a wide variety of oculomotor functions, including fixation, saccade generation, attention and even smooth pursuit (Schall 1991b; Bon and Lucchetti 1992; Bon and Lucchetti 1997; Heinen 1995). The SEF is intimately linked with the FEF and also projects heavily to the SC (Huerta and Kaas 1990; Parthasarathy et al. 1992; Schall et al. 1993). The SEF also appears to have connections to the brainstem oculomotor circuits, with electrical stimulation of the SEF driving eye movements in the absence of the FEF and SC (Tehovnik et al. 1994; Shook et al. 1990). The wide gamut of oculomotor functions in which the SEF is involved, as well as the intimate connections shared with other oculomotor structures, suggests the SEF is a higher-level structure related to target selection and high-level modulation of oculomotor control, initiation and planning.

Intracortical microstimulation in the SEF evokes gaze shifts from sites appearing to code in a variety of reference frames. One of the defining characteristics of the SEF is the presence of a goal or termination zone: an area in the visual field where the endpoints of stimulation-evoked gaze shifts converge (Schlag and Schlag-Rey 1987; Tehovnik and Lee 1993; Bon and Lucchetti 1992; Mitz and Godschalk 1989). Recordings of the response field of some SEF neurons have indicated, however, that neuronal response fields are invariant of initial gaze position (Russo and Bruce 1996; Russo and Bruce 2000). These SEF neurons thus appear to code target and desired gaze shifts in an eye-centred fashion. While stimulation of some sites in the SEF does appear to produce gaze shifts with little convergence (Martinez-Trujillo et al. 2004; Russo and Bruce 1993), these low convergence sites appear to be in the minority, with most SEF stimulation sites displaying large degrees of convergence (Martinez-Trujillo et al. 2004; Russo and Bruce 1993). The absence of convergent response fields for recorded neurons while the majority of stimulation sites produce convergent movements is an interesting observation. Russo and Bruce (1993) concluded that stimulation-evoked movements may override a circuit to compensate for variation in initial gaze position and thus are observed to converge. When examining the differences between recording and stimulation in the SEF, Russo and Bruce (2000) came to the conclusion that the SEF encodes movements in a similar fashion to the SC and FEF. The rational for this conclusion being that in order to facilitate the large interconnectivity of the oculomotor areas, these areas are required to encode movements in a similar fashion.

The presence of these convergent stimulation-evoked movements is still seen by many to imply a head or body-centred reference frame (Martinez-Trujillo et al. 2004; Tehovnik et al. 1998) and studies have been performed to fully differentiate between head and body-centred reference frames using head unrestrained animals. Tehovnik et. al. (1998) put forth that the termination zone for SEF stimulation follows the head as it is rotated about the trunk and gravity in head restrained monkeys, suggesting head-centred reference frame coding in the primate SEF. More recent evidence from analysis of kinematics and convergence of eye and head movements in head unrestrained preparations shows a number of stimulation sites with convergence corresponding to a body-centred frame of reference in addition to the eye and head-centred reference frames previously seen (Martinez-Trujillo et al. 2004). Taken together, these studies suggest there is no single discernable pattern of convergence. The use of stimulation appears to illustrate a patchwork of responses in the SEF, making the true functions of the SEF difficult to elucidate with this technique (Martinez-Trujillo et al. 2004). The conflicting conclusions of recording and stimulation studies in the SEF provide a useful opportunity to examine the use of stimulation as a valid technique for analyzing reference frame coding.

1.8 - Examining reference frames through neck muscle activity recordings

An underlying assumption in the investigation of reference frame coding is that stimulation reveals the inherent reference frame of a brain region. If the movements evoked by stimulation accurately reflect the reference frame organization of an area, then intracortical microstimulation of a brain region would be expected to engage lower-level areas in a naturalistic way, thus evoking natural patterns of activity. Interestingly, one correlate of reference frame coding that has not been directly examined in the SEF, at least for the head component of a gaze shift, is the nature of the signal sent to the motor periphery. Moreover, the complex biomechanics of the head preclude the use of head movement kinematics as an effective means to draw conclusions about the pattern of evoked activity on the motor periphery. These conclusions cannot be drawn because the biomechanics of the head act as a low-pass filter. Theoretically, due to the filtering nature of the head biomechanics, the same output can be generated by many different patterns of input.

An advantage of studying head movements is the relative accessibility of the neck muscles. Recording neck muscle activity allows direct measurement of the stimulation-evoked activity patterns on the motor periphery. If stimulation produces a natural signal sent to the motor periphery, activity recorded from the neck muscles following stimulation should reflect a natural pattern of activity. For convergent stimulation-evoked head movements, a natural pattern of neck muscle activity would be appropriate for the amplitude of the evoked head movements. Larger movements of the head would be expected to be paired with increased neck muscle drive in order to accomplish large amplitude movements of the head. Similarly, small head movements would be expected to be accompanied by lower levels of neck muscle drive. This “neural” mechanism for convergence is illustrated in Fig. 4A where neck muscle activity is colour-coded onto hypothetical convergent head movements. If stimulation-evoked movements follow this

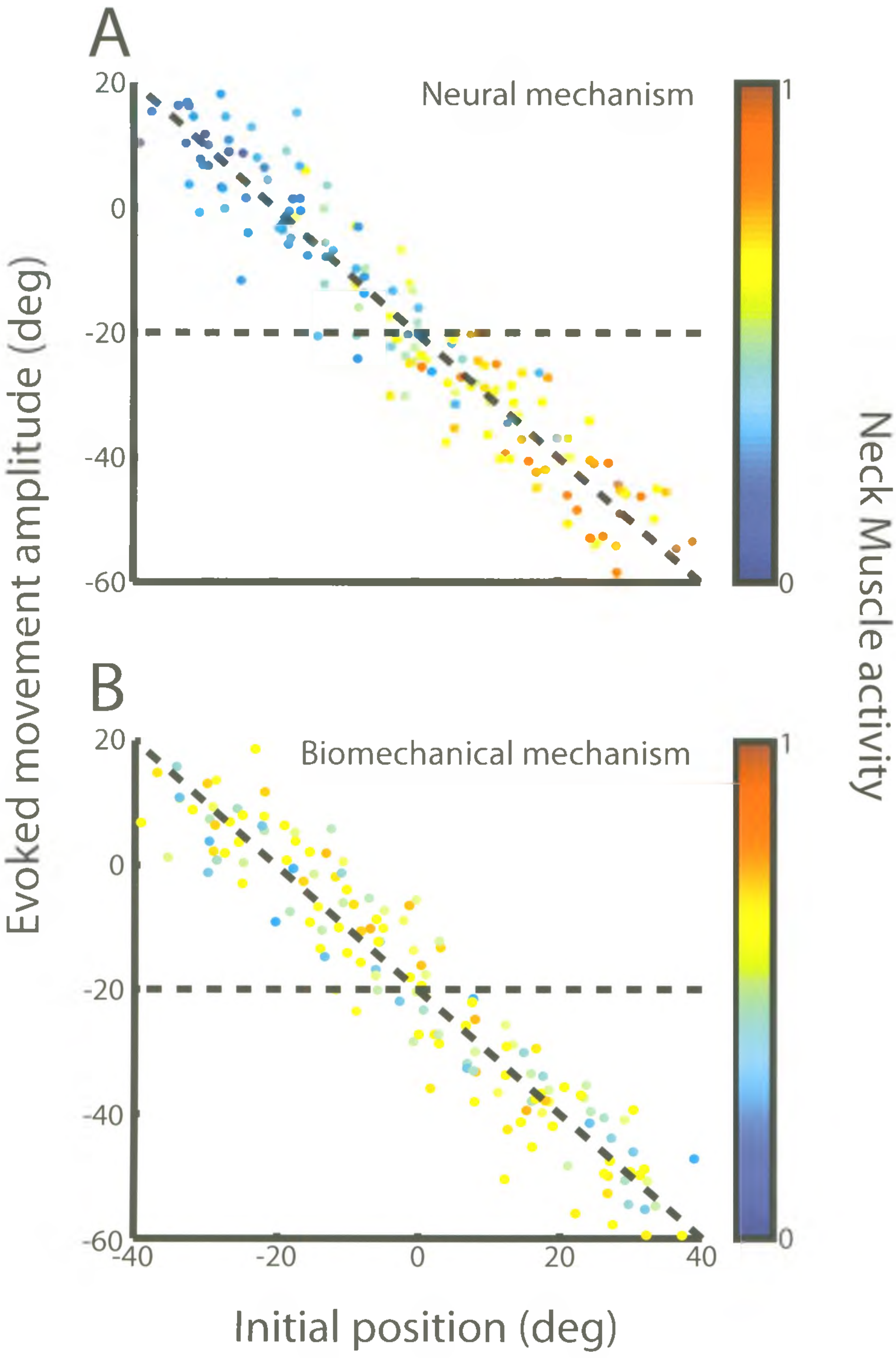

Figure 4. A. Theoretical stimulation-evoked movements with a CI of -1. Dashed lines represent CI of 0 and -1. Predicted neck muscle activity associated with each movement is superimposed as colour from low activity (blue) to high activity (red). For the neural mechanism, larger movements are associated with increased neck muscle activity as compared to smaller movements. B. Predicted pattern of neck muscle activity associated with convergent stimulation evoked movements for a biomechanical mechanism. Theoretical highly convergent stimulation evoked movements, CI = -1, are colour coded with the predicted neck muscle activity. For the biomechanical mechanism, all movement amplitudes are associated with similar levels of neck muscle activity.

neural pattern of activity, larger amplitude head movements should be associated with increased levels of neck muscle activity which is consistent with volitional movements (Corneil et al. 2001).

However, since the biomechanics of the head allow for multiple different inputs to produce the same output, stimulation could produce different patterns of neck muscle activity and still result in a seemingly normal head movement. If stimulation is not producing an amplitude appropriate signal at the level of the neck muscles but a generic overriding signal as some studies suggest (Russo and Bruce 1993; Chapman et al. 2008; Corneil et al. 2002a, 2002b), convergent head movements may arise due to the complex biomechanical factors of the head. Perhaps biomechanical factors of the head resist the turning of the head when the head is oriented in the direction of movement and facilitate the turning of the head when the head is oriented opposite to the direction of movement. Theoretically, if stimulation delivers an equal neck muscle drive at a variety of initial head orientations, the biomechanics of the head and neck may be sufficient to produce convergent movements. This "biomechanical" mechanism would manifest as the observation of an equivalent signal sent to the neck muscles by stimulation for all observed head movements as illustrated in Fig. 4B. The observation of this biomechanical mechanism would suggest that convergence is a product of intracortical microstimulation and would challenge the assumption that convergence is reflective of the intrinsic reference frame coding.

1.9 - Goal of this thesis

It has generally been the assumption that intracortical microstimulation is an appropriate method to reveal the intrinsic organization of the SEF in terms of reference frames (Martinez-Trujillo et al. 2003a; Martinez-Trujillo et al. 2004; Tehovnik et al. 1998). Surprisingly, the activity of the motor periphery evoked by stimulation has never been directly analyzed in terms of reference frames.

The goal of this thesis is to examine the validity of intracortical microstimulation as a technique to examine reference frame coding in the brain. Observations that stimulation evokes a generic activation of neck muscles during convergent movements in the SC (Corneil et al. 2002a; Corneil et al. 2002b) suggest that stimulation does not engage lower-level oculomotor structures in a natural manner. I will combine intracortical electrical microstimulation in the primate SEF with electromyography (EMG) in the dorsal neck muscles in an attempt to perform a more comprehensive analysis of the pattern of neck muscle activity accompanying convergent head movements. EMG recordings of the neck muscles will allow me to determine if stimulation in a higher cortical area sends an appropriate naturalistic signal to the motor periphery to generate convergent movements or whether stimulation evokes a generic activation of the musculature with convergence arising from biomechanical factors. If stimulation does not engage lower-level oculomotor structures in a natural manner, the use of stimulation evoked convergence may not be an appropriate method for examining inherent reference frame coding in the brain.

Chapter 2 – Methods

2.1 - Surgical Procedures

Two male monkeys (*Macaca mulatta*, monkey *S* and *Z*), weighing 12.5 and 15.0 kg were used in these experiments. All procedures were approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care in compliance with the Canadian Council on Animal Care policy on the use of laboratory animals (appendix 1). The monkeys' weights were monitored daily, and their general health was under the close supervision of the university veterinarians.

Each monkey underwent two surgeries. The first surgery enabled chronic recording of gaze position. A prefabricated eye coil [3 turns of stainless steel Bairdwire 18 mm in diameter] was implanted subconjunctivally into one eye (Judge et al. 1980) to measure gaze shifts using the magnetic search coil technique (Fuchs and Robinson 1966). A head implant constructed from dental acrylic was anchored to the skull using titanium screws and provided a secure foundation for a titanium head post used to restrain the head.

A second surgery was performed to allow bilateral access to the SEF. A recording cylinder (Crist Instruments, Maryland, USA) was positioned stereotaxically over a 19 mm craniotomy made over midline biased slightly to the right (interaural coordinates: A35.0, R3.0), and angled to lie flush with the skull to permit a surface normal approach to the frontal cortex.

During this second surgery, obliquus capitis inferior (OCI), rectus capitis posterior major (RCM), splenius capitis (SP), biventer cervicis (BC) and complexus

(COM) were implanted bilaterally with chronically-indwelling biopolar hook electrodes to facilitate the recording of neck muscle EMG activity. The muscles were approached dorsally and isolated by separating the muscle layers from the dorsal midline raphe to gain access to the cleavage planes between the muscles. Intramuscular hook electrodes constructed from Teflon-coated wire (Cooner wire: AS-631), as previously described (Goslow 1987), were implanted into the targeted muscles. The electrode recording contacts were 3 mm long, staggered by 3 mm, and were oriented perpendicular to the long axis of the muscle fiber fascicles. The ground was taken from a wire crimped into a gold pin and secured into the skull. The leads from all electrodes were tunneled subcutaneously to the dental acrylic head implant and connected to a 26-pin connector. By the first postoperative day both animals appeared to be making normal head movements.

2.2 - Experimental Setup

Prior to EMG recording, the monkey was placed in a custom-built primate chair designed to allow for both head restrained and unrestrained preparations. Both monkeys wore customized primate vests (Lomir Biomedical, Quebec, Canada) which allowed them to be tethered to the primate chair, limiting trunk rotation to a maximum of ±10° and permitting unimpaired head unrestrained experiments. Once in the primate chair, the monkey was placed in a dark, sound-attenuated room in the center of a 3 ft^3 acquisition search coil system (CNC Engineering). The monkey faced an array of tricolor light-emitting diodes (LEDs; Fairchild Semiconductors MV5437) arranged such that they covered ±35° of the horizontal and vertical visual fields. LEDs were located along a flat horizontal–vertical rectilinear grid, 1.5 ft from the subjects' head, and were spaced 2° apart from the center of the grid to a horizontal and vertical eccentricity of 20° and 5° apart between 20° and 35° eccentricity. All aspects of the experiment were controlled at 1 kHz by customized real-time LabView programs interfacing with the hardware through a PXI controller (National Instruments, Texas, USA). The monkeys were monitored throughout the experimental session by infrared cameras positioned outside their line of sight.

2.3 - Microstimulation Parameters

Stimulation was generated by a stimulator and two constant-current stimulus isolation units (model S88 and PSIU-6; Grass Instruments, Rhode Island, USA) and delivered through a tungsten microelectrode (resistance of ~0.3 MΩ at 1KHz; Frederick Haer and Co., Maine, USA). The SEF cylinder was fitted with a delrin grid (1 mm spacing; Crist Instruments, Maryland, USA) which standardized electrode penetrations. A custom built low-weight (100 g) electric microdrive was anchored to the SEF cylinder, and electrodes were lowered through guide tubes secured inside the cylinder. Electrode movement was computer controlled. Stimulation consisted of biphasic cathodal-first pulses delivered at a pulse rate of 300 Hz, with individual pulse duration of 0.3 ms per phase. Stimulation duration was controlled by an experimental computer, and was set at 300 ms to ensure that evoked head movements were realized.

Before data collection commenced, stimulation was passed (100 μA, 300 Hz, 300 ms) to ensure that each stimulation site produced consistent EMG activation and contralateral gaze shifts at either central or off-centre fixation position. The endpoints of evoked gaze shifts were estimated online to set the target position for non-stimulation control trials (see below). If no saccade was evoked at a given fixation location then the target was set at the same location as the fixation point leading to the animal performing a fixation task from that fixation location. This allowed us to develop a database of volitional movements that were of approximately the same amplitude as the movements evoked by stimulation.

2.4 - Behavioural Paradigm

Monkeys were trained in a gap saccade task involving eight off-centre and one central fixation point. Trials began with the extinguishing of a diffuse background light (1.0 cd/m^2) and the illumination of a fixation point (FP) 300 ms later. The FP was presented pseudorandomly at either a central location or one of eight possible locations 28° from centre. The monkey was required to acquire the FP within 2000 ms and hold his gaze within a computer controlled window (8°) for a random interval between 750 ms and 1250 ms. The FP was then extinguished and the monkey was required to maintain his gaze at the FP during a gap period of 500 ms. Stimulation was passed on fifty percent of all trials 200 ms after the FP was extinguished. Immediately following the stimulation, or gap period, a visual target was presented at the previously determined evoked movement endpoint (see above). The monkey was required to fixate within a computer controlled window (8°) around the target for 300 ms to obtain a liquid reward. Fifteen stimulation trials were pseudorandomly intermixed with 15 control trials for each fixation point within a stimulation site, producing 270 total trials from each stimulation site.

2.5 - Data analysis

The processing of the EMG signals began at a headstage plugged directly onto the EMG connector in the head implant. The headstage (Plexon Inc, Texas, USA) differentially amplified (20x gain) and filtered (bandwidth 20Hz - 17 kHz) the EMG signals. The headstage was connected to a Plexon pre-amplifier, which housed a signal processing board customized for EMG recordings (50x gains, bandwidth 100Hz – 4 kHz). All analog signals (EMG and coil signals) were digitized at a fixed rate of 10 kHz. Off line, coil signals were downsampled by a factor of 10 to 1 kHz. EMG signals were notch filtered to remove 60 Hz noise, rectified, and then integrated into 1 ms bins, using a rationale described previously (Bak and Loeb 1979). These steps attenuated the digitized peak-to-peak amplitudes by a factor of ~3x.

Offline, computer software determined the beginning and end of evoked movements using a velocity criterion (velocity greater than 30°/s for gaze and eye movements; greater than 10°/s for head movements). This was later confirmed by an experimenter who verified, and if necessary, corrected the beginning and end points of movements using a customized graphical user interface (GUI) written in Matlab (the Mathworks, Massachusetts, USA). Using the GUI the experimenter inspected all trials, and if necessary discarded trials with irregular movements or excessive background EMG activity. Using customized Matlab programs evoked amplitudes were extracted for both gaze and head movements. Only those movements directed in the proper direction and evoked during the duration of stimulation were included for further analysis.

Chapter 3 – Results

We stimulated in a number of sites in the dorsomedial frontal cortex of two monkeys, slightly lateral of midline and anterior of the horn of the arcuate sulcus. Movement sites were identified as those sites where stimulation parameters commonly used to localize the SEF (eg. <50 μA, 300 Hz, 300ms) (Schlag and Schlag-Rey 1987) evoked rapid contralateral eye-head gaze shifts on at least half of all stimulation trials from a minimum of three fixation positions. Once a site was identified as a movement site, suprathreshold stimulation parameters (100 μA, 300 Hz, 300ms) were used for data collection to ensure contralateral gaze shifts were evoked on a large number of stimulation trials. These criteria resulted in a total of 40 stimulation sites (20 from monkey *Z*, 20 from monkey *S*) being studied in head unrestrained monkeys. Our analysis focused on horizontal movements and the associated lateral head turning muscles OCI, RCM and SP. Electrode penetration locations for each monkey are illustrated in Fig. 5.

3.1 - Description of SEF stimulation sites

Consistent with previous results (Schlag and Schlag-Rey 1987), we observed no topographical organization in the SEF. During initial functional mapping, the posterior and lateral borders of what was later determined to be the SEF were explored. Stimulation in a number of these sites elicited small smooth pursuit eye movements that persisted for the duration of stimulation, as seen previously in the posterior regions of the SEF (Missal and Heinen 2001). Stimulation with the head unrestrained in penetrations at the lateral margins of the dorsomedial frontal cortex failed to evoke gaze shifts.

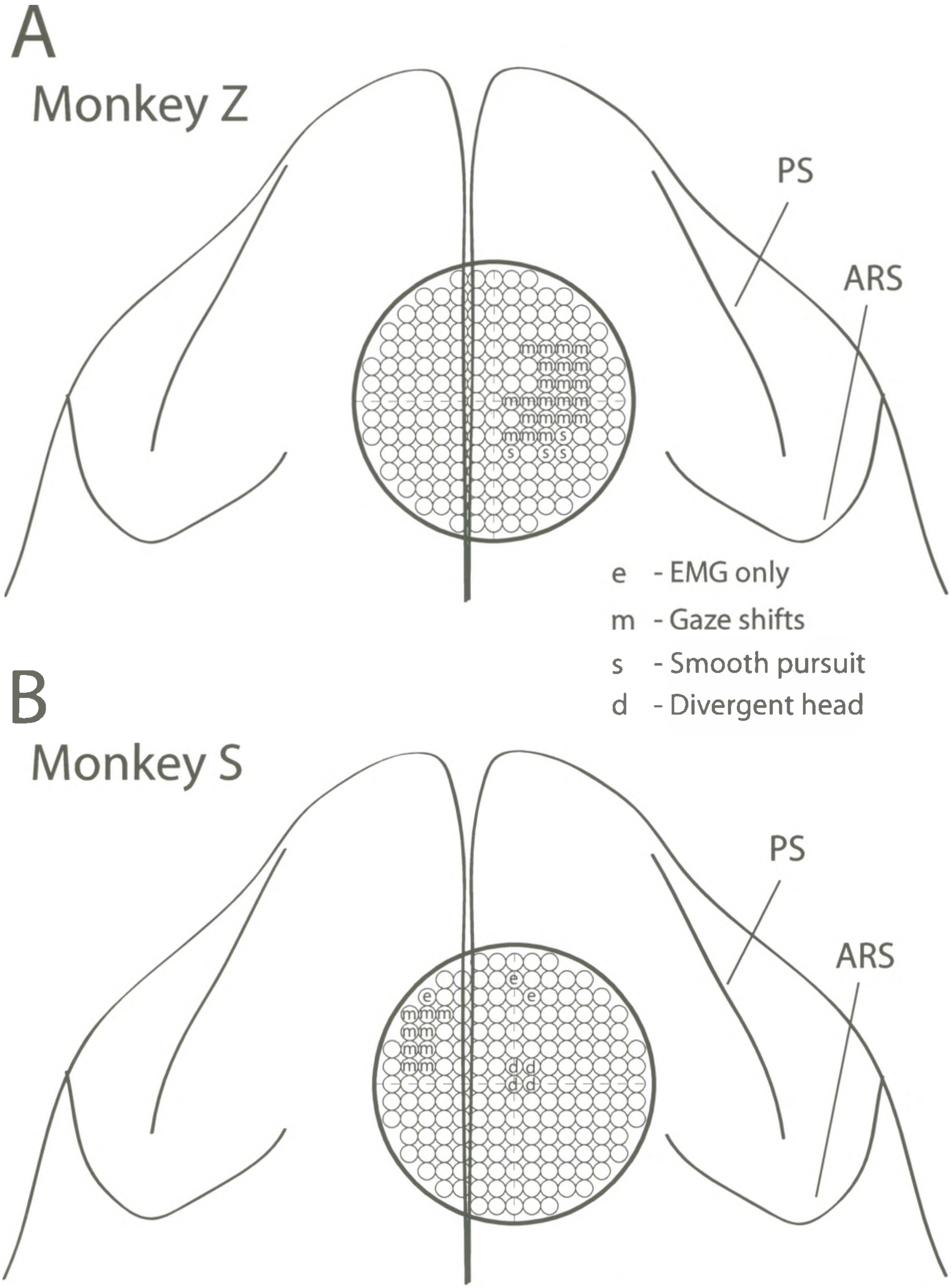

Figure 5. Depiction of grid locations explored in monkey Z and S (A and B). Positioning of the chambers relative to the diagrammatic rendition of the arcuate (ARS) and principle (PS) sulci estimated from anatomical magnetic resonance image. Penetrations are shown that evoked: EMG activity only (e), gaze shifts (m), smooth pursuit (s), or divergent head movements (d) at 100 μA.

Stimulation did, however, evoke divergent movements of the head from the centre towards the current gaze location, similar to the divergence seen by (Sparks et al. 2001) in the cortex between the SEF and FEF.

The most recognizable product of SEF stimulation is the high degree of convergence seen across gaze shifts evoked from different initial gaze positions from a single stimulation site (Schlag and Schlag-Rey 1987; Tehovnik and Lee 1993; Tehovnik et al. 1998). Stimulation evoked gaze shifts that converged in a manner qualitatively similar to previous head unrestrained observations in the SEF (Martinez-Trujillo et al. 2003a; Martinez-Trujillo et al. 2004; Chen and Walton 2005). A CI was calculated for each stimulation site as the slope of a linear regression line fit to the gaze shift amplitude plotted as a function of initial gaze position (Fig. 6A). Initial fixation positions where stimulation did not evoke gaze shifts are included in CI calculations using gaze amplitudes of zero degrees. CI values ranged from -0.02 to -0.59 (-0.30 ± 0.19, mean ± sd) for monkey *Z* and from -0.02 to -0.80 (-0.36 ± 0.19, mean ± sd) for monkey *S* (Fig. 7A) and were not significantly different between monkeys (P=0.34, t-test; P=0.87, F-test). These calculated values of CI closely mirrored previous studies which examined the CI of gaze shifts in the SEF (Martinez-Trujillo et al. 2004; Russo and Bruce 1993).

A CI based on evoked head movement (hCI) was also calculated (Fig. 6B). Unlike gaze shifts, head movements were consistently evoked from almost all initial fixation positions (Fig. 6). Due to the component nature of gaze, monkeys were able to adopt a variety of initial head orientations to achieve the same gaze location during fixation. The additional initial head orientations produced a more complete range of initial head orientations as compared to the narrower range of initial gaze positions.

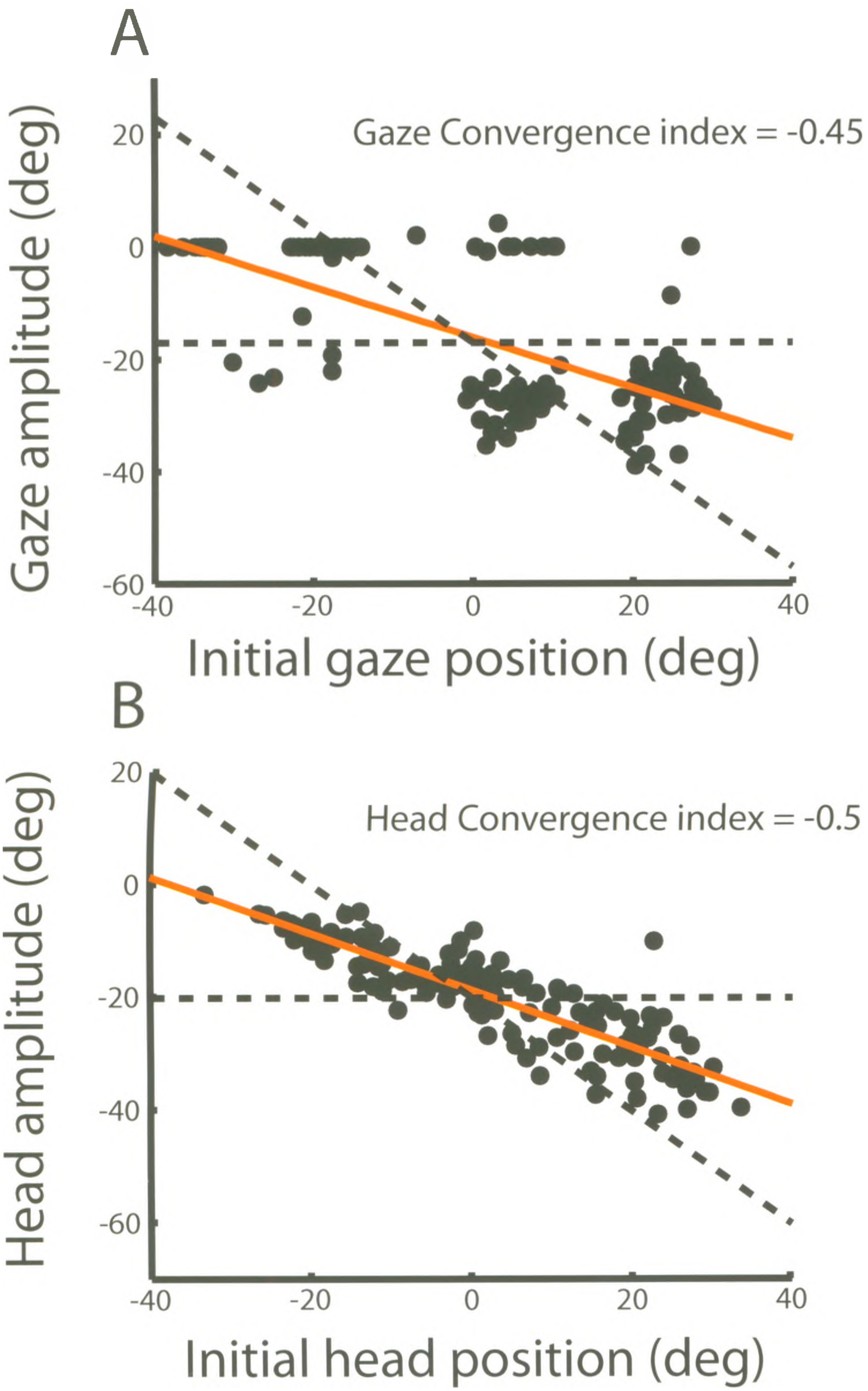

Figure 6. A. Convergence of gaze shifts evoked by stimulation of a single site. Trials where no movement was evoked were included as zero amplitude movements. Slope of the red linear regression line fit to the gaze amplitude plotted as a function of initial gaze position corresponds to CI (-0.45). B. Convergence of head movements evoked by stimulation of a single site. Head movements were evoked on all trials. Slope of the blue linear regression line fit to the head amplitude plotted as a function of initial head position corresponds to hCI (-0.5). Dashed lines indicate CI and hCI of 0 (horizontal line) and -1 (sloped line).

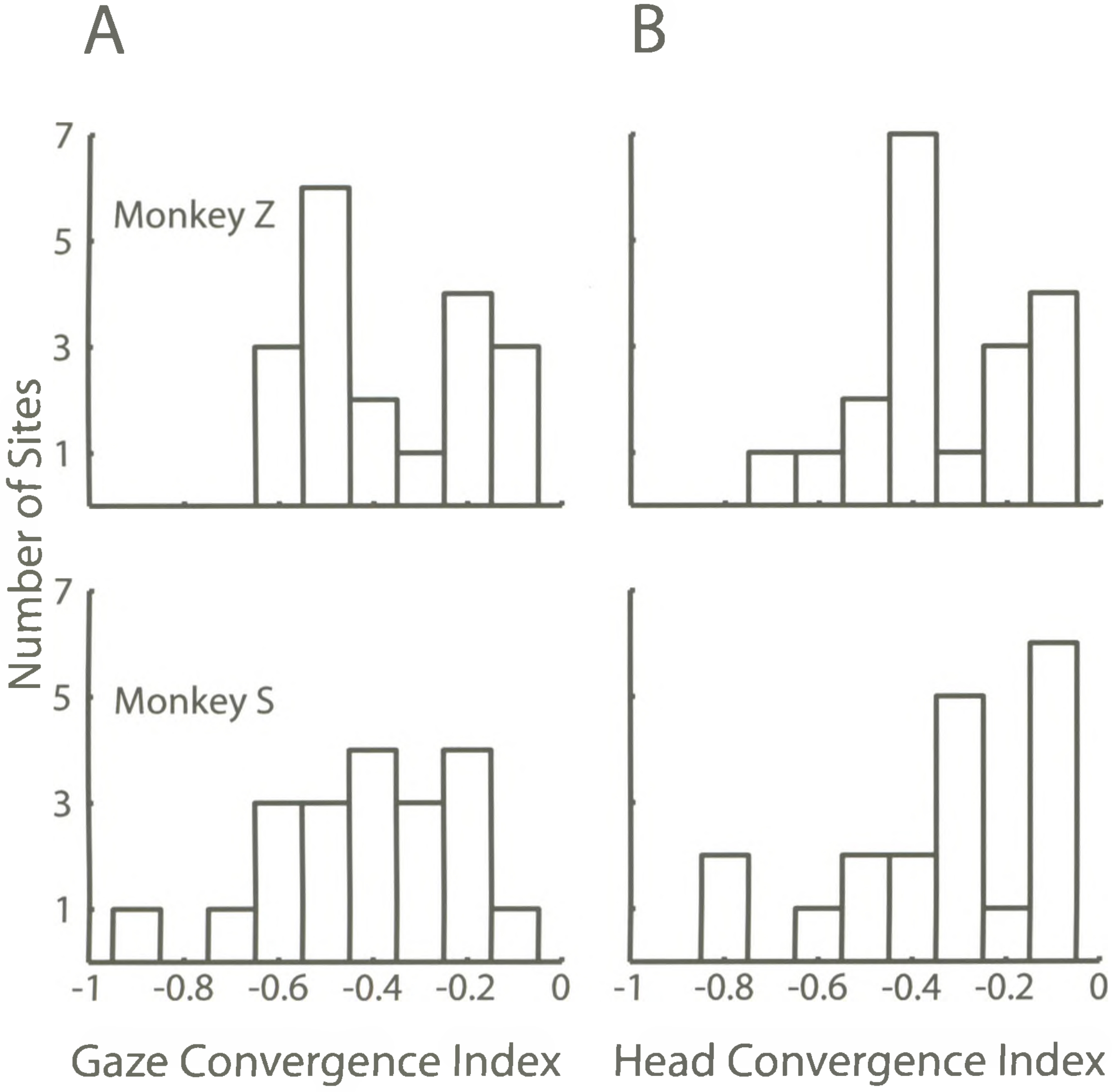

Figure 7. A. Distribution of gaze CI for all stimulation sites for monkey Z and S. Distribution of gaze CI was not significantly different between monkeys (P=0.34). B. Distribution of hCI for all stimulation sites for monkey Z and S, and was not significantly different between monkeys (P=0.92). The distribution of hCI was not significantly different than the distribution of gaze CI (P=0.16).

Calculated hCI values ranged from -0.01 to -0.68 (-0.27 ± 0.19, mean ± sd) for monkey *Z* and from 0.01 to -0.74 (-0.27 ± 0.23, mean ± sd) for monkey *S* (Fig. 7B) and were not significantly different between monkeys (P=0.92, t-test; P=0.37, F-test). Calculated hCI values were not significantly different from gaze CI (P=0.16, paired t-test, P=0.66, F-test).

Having established the similarity between these results and other studies in the SEF, the analysis will now focus on the pattern of neck EMG activity accompanying convergent evoked movements of the head.

3.2 - Neck muscle EMG evoked by stimulation of the SEF

Figure 8 shows the horizontal gaze and head movements and accompanying patterns of neck muscle EMG activity observed at a single representative site. For simplicity, evoked movements and activity are only shown for those sites on the horizontal meridian. As suggested by the analysis of CI above, the amplitude of evoked head movements and the propensity of gaze shifts decrease as gaze was positioned progressively more contralateral to the side of stimulation.

Surprisingly, the pattern of evoked neck muscle EMG activity followed the opposite pattern of that seen for the amplitude of the evoked movements. Agonist muscles displayed a rapid (18 ± 5.5 ms, mean ± sd) facilitating response following simulation onset, peaking within the first 50 ms of stimulation. The magnitude of this peak response was either relatively invariant across different initial positions (Fig. 8, RCM), or increased as gaze was initially deviated more contralaterally (Fig. 8, SP). After the initial peak of EMG activity, there was a short suppression followed by a smaller

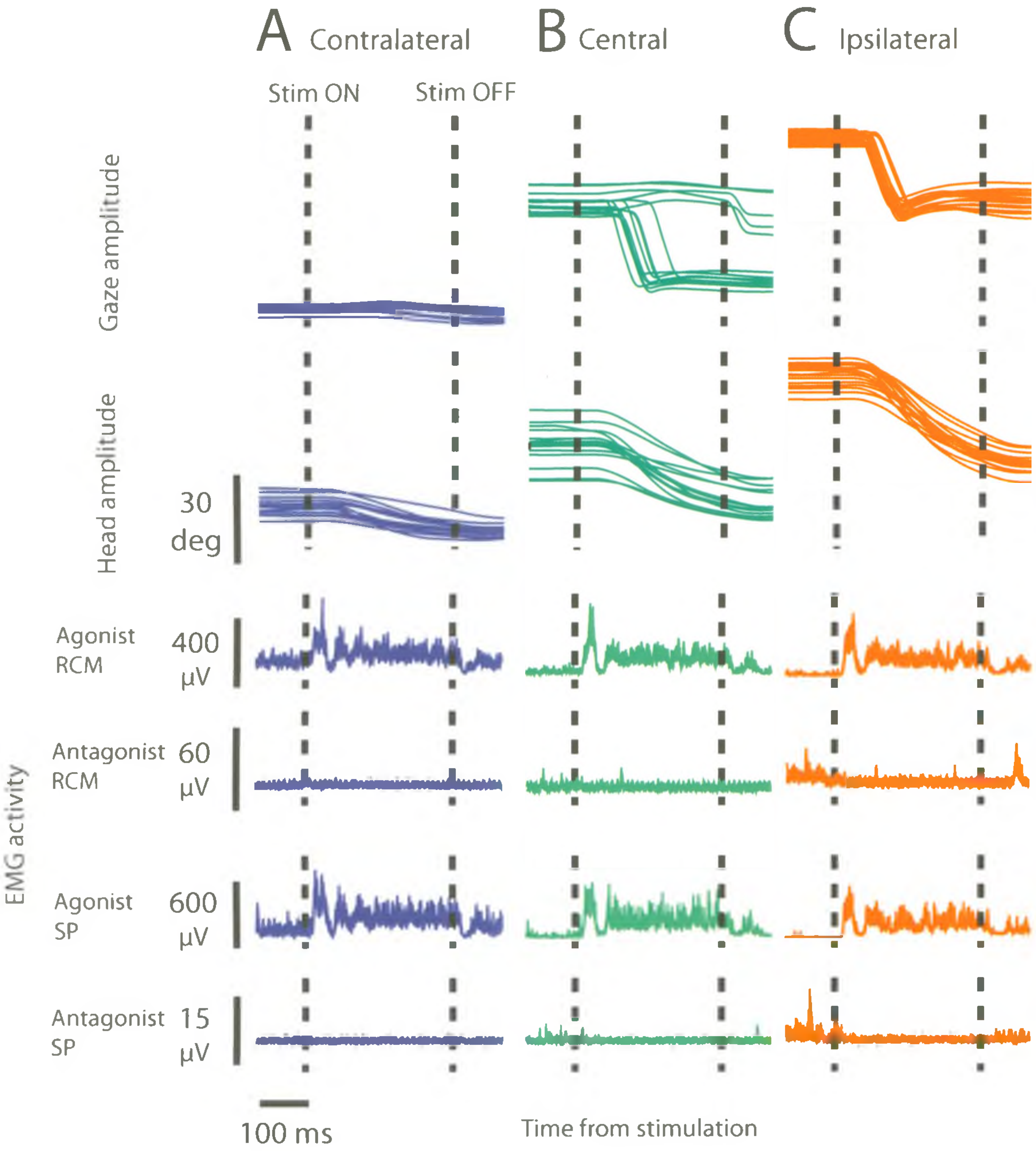

Figure 8. A number of stimulation trials from a single stimulation site at fixation positions contralateral (A, blue), central (B, green), and ipsilateral (C, red) to the side of stimulation. Gaze shifts are not evoked from contralateral fixation positions, evoked 50% of the time from central fixation and consistently evoked from ipsilateral fixation positions. Evoked head movements were small at contralateral fixation positions, medium at central fixation and large at ipsilateral fixation positions. Baseline EMG activity (activity before stimulation onset) on the agonist RCM and SP is increased at contralateral fixation positions and decreases with more ipsilateral fixation positions. Baseline EMG activity on antagonist RCM and SP increases with more ipsilateral fixation positions. Stimulation-evoked agonist RCM EMG activity varies little between fixation positions while stimulation-evoked agonist SP EMG activity decreases with more ipsilateral fixation positions. Stimulation-evoked antagonist suppression is most clearly seen on the antagonist SP at ipsilateral fixation positions (C, red). Stimulation-evoked agonist activation and antagonist suppression is rapid (~15 ms) and time locked to stimulation onset.

increase in EMG activity that persisted for the duration of stimulation. As is clear from Fig. 8, the background level of neck EMG prior to stimulation onset also increased for more contralateral initial gaze positions. For this site, the evoked EMG responses seem to be either saturating (Fig. 8, RCM), or are a function of initial gaze position (Fig. 8, SP). The antagonist muscles show marked suppression for the duration of stimulation. The background EMG activity seen on the antagonist muscles prior to stimulation onset increased for more ipsilateral gaze positions.

For this site, stimulation of the SEF appears not to evoke patterns of neck muscle EMG activity appropriate for the amplitude of the evoked head movement. Instead, SEF stimulation appears to be evoking a pattern of neck muscle EMG activity involving the generic activation of agonist neck muscles and the suppression of antagonist neck muscles. This agonist on / antagonist off pattern of neck muscle activation integrates with the tonic baseline activity present on the muscles and is the predominant evoked pattern at all initial gaze positions.

The profile of evoked neck EMG responses was quantified over the first 100 ms of stimulation. This time period allowed for the inclusion of the initial burst of neck muscle activity. The EMG activity of neck muscles was quantified by integrating the EMG activity to calculate the area under the curve during the first 100 ms of stimulation as denoted by the red fill under the raw EMG trace in Fig. 9. Evoked neck EMG responses were also quantified by the peak activity during the first 100 ms of stimulation and by integrating the EMG activity over the first 100 ms of stimulation after it had been normalized to the pre-stimulation period. The overall pattern of results across initial positions was qualitatively the same regardless of which quantification was used. An

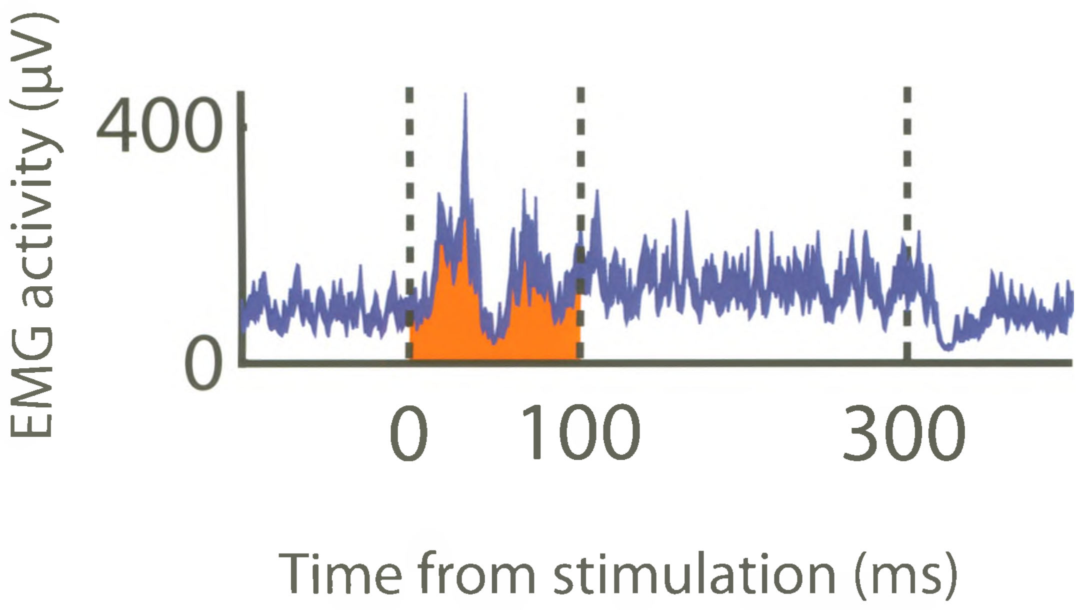

Figure 9. Quantification of EMG activity. The red area represents the quantified area under the curve calculated by integrating the EMG signal over the first 100 ms of stimulation.

integration of the raw neck EMG over the first 100 ms was therefore used as it included the influence of baseline activity.

In order to facilitate group comparison between stimulation sites, the quantified values of evoked EMG activity were normalized to the maximum value for each muscle at each stimulation site. Thus, per trial EMG activity is expressed as a percentage of the maximal evoked response for a given muscle at a given stimulation site.

3.3 - EMG as a function of initial head orientation

Figure 10 shows the convergence of head movements overlaid with a colour plot of the evoked EMG activity of the SP and RCM for the same site as Fig. 8. Neck muscle EMG for each evoked head movement is expressed as a percentage of the maximum level of EMG activity evoked for that stimulation site. The high level of convergence for this site translates into a wide variety of evoked head movement amplitudes. Evoked head movements for this site vary from almost negligible amplitudes when the head is initially oriented extremely contralaterally to the side of stimulation to evoked head movement amplitudes of more than 40 degrees when the head is initially oriented extremely ipsilaterally to the side of stimulation (See arrows, Fig. 10). Conversely, the level of evoked EMG activity is much less variable. For example, the level of evoked EMG activity associated with the evoked head movements indicated by the arrows in Fig. 10 was roughly equal despite a nearly 40-degree difference in the amplitudes of the evoked head movements. The low variability recorded from the neck muscle EMG as compared to the high variability of evoked head movement amplitudes suggests that stimulation of

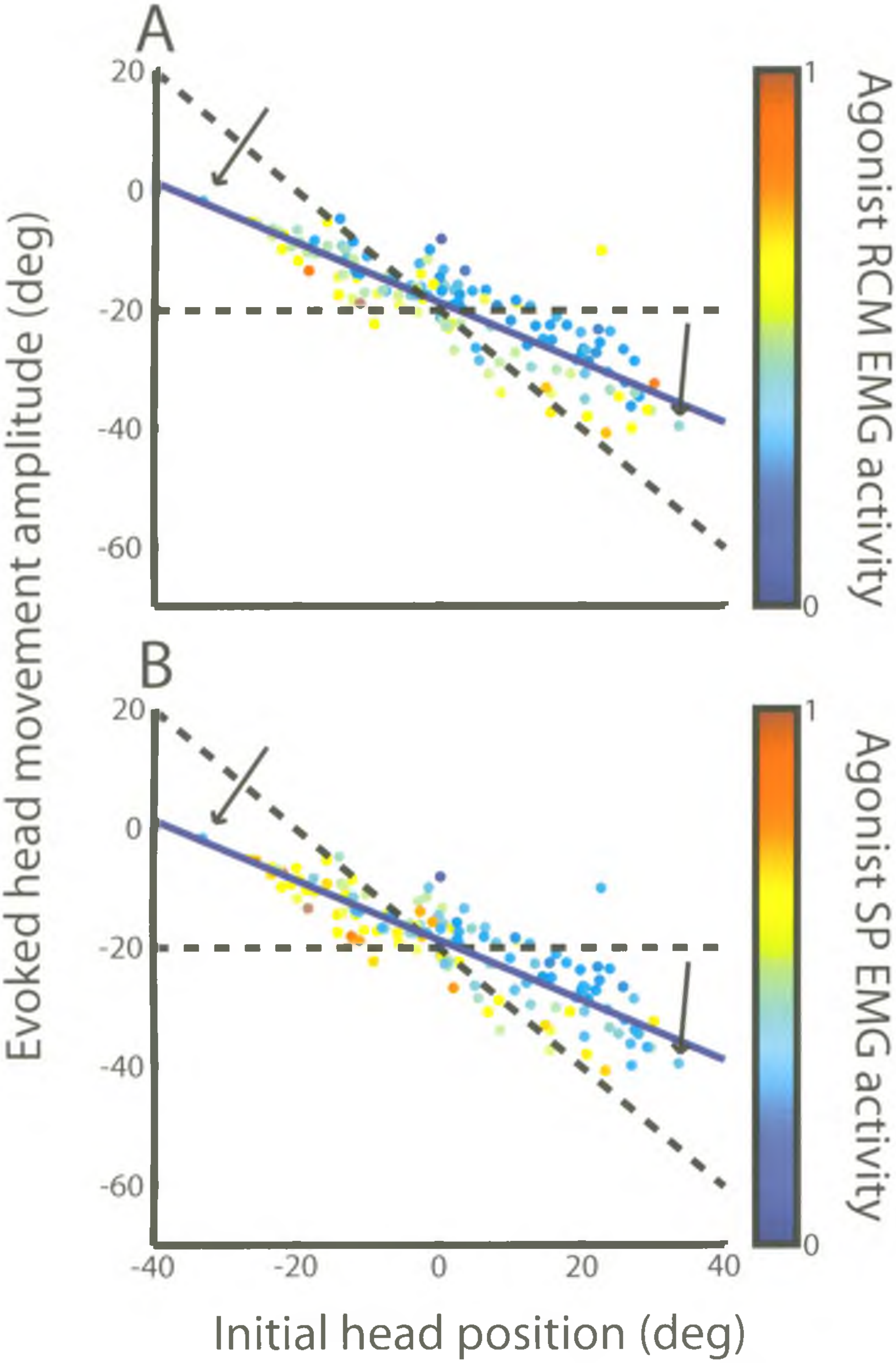

Figure 10. Stimulation evoked head movement amplitudes from a number of initial head orientations for a single stimulation site. The slope of the blue linear regression line represents the calculated hCI. Normalized integrated agonist RCM (A) and SP (B) EMG activity associated with each movement is superimposed as colour from low activity (blue) to high activity (red). Arrows indicate example movements where stimulation evoked similar agonist neck muscle activity (similar colour) but lead to extremely different evoked head movement amplitudes (~0° and ~40°). Dashed lines indicate slopes of 0 and -1.

the SEF evokes generic neck muscle EMG activity for all evoked head movement amplitudes.

To quantify the change in neck muscle EMG activity across initial head orientations an activity index (AI) was calculated for each muscle. AI is similar to hCI and corresponds to the slope of a regression line fit to the EMG activity plotted as a function of initial head position moving from contralateral to ipsilateral initial head orientations (Fig. 11). The magnitude of the AI is a measure of the variance of the evoked neck muscle EMG across initial head orientations, while the sign of the AI indicates whether the evoked neck muscle EMG activity is increasing (negative) or decreasing (positive) as initial head orientation begins more contralateral to the side of stimulation. The site shown in Fig. 11 (the same stimulation site as Fig. 8) has a negative AI for the RCM (AI = -0.0018, P=0.01) and SP (AI = -0.0046, P<0.0001). A negative AI indicates that the trend of evoked neck muscle EMG activity runs counter to what would be expected to occur naturally with convergence: smaller head movements are associated with larger increases in EMG activity.

3.4 - Population data

Figure 12 presents a comparison of the AI for the OCI, RCM and SP agonist neck muscles with the hCI for all stimulation sites. Data from monkey *S*'s agonist OCI are omitted due to degraded signal quality of the EMG recordings on that muscle experienced as the implanted electrodes aged. The black arrows indicate the agonist muscles for the representative site used throughout the results. Filled symbols indicate when the calculated AI is not significantly different from zero (regression, P>0.05). All

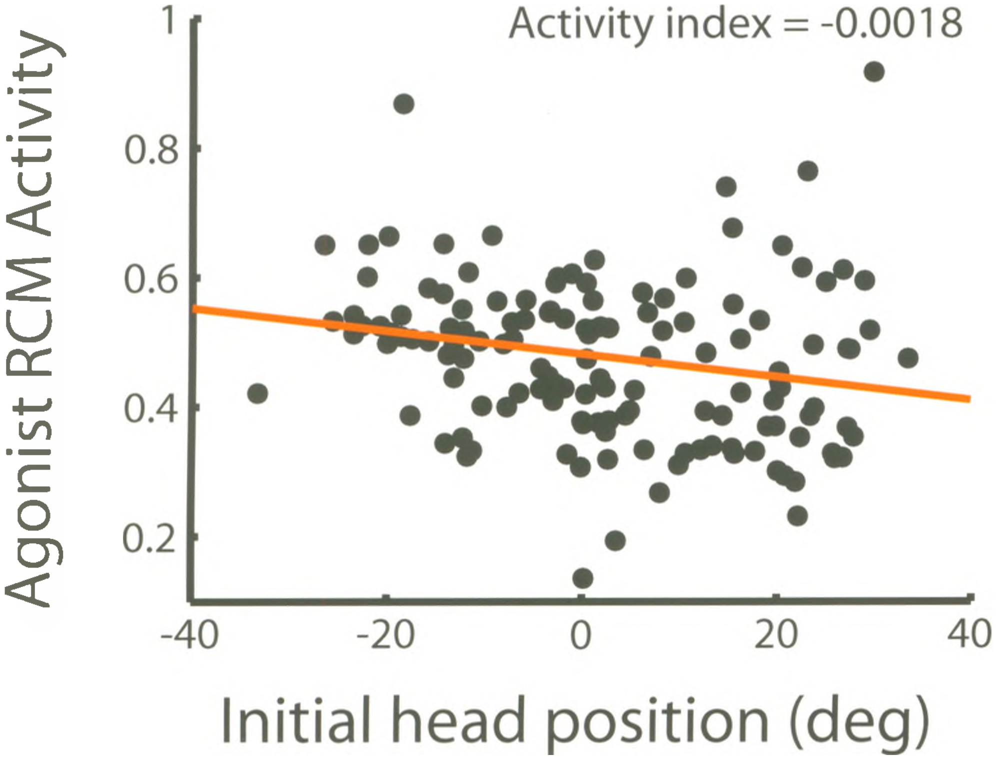

Figure 11. Change in agonist RCM EMG activity across initial head positions evoked by stimulation of a single site. The slope of the red linear regression line fit to the agonist RCM activity corresponds to the activity index (AI) of the agonist RCM for this site (-0.0018).

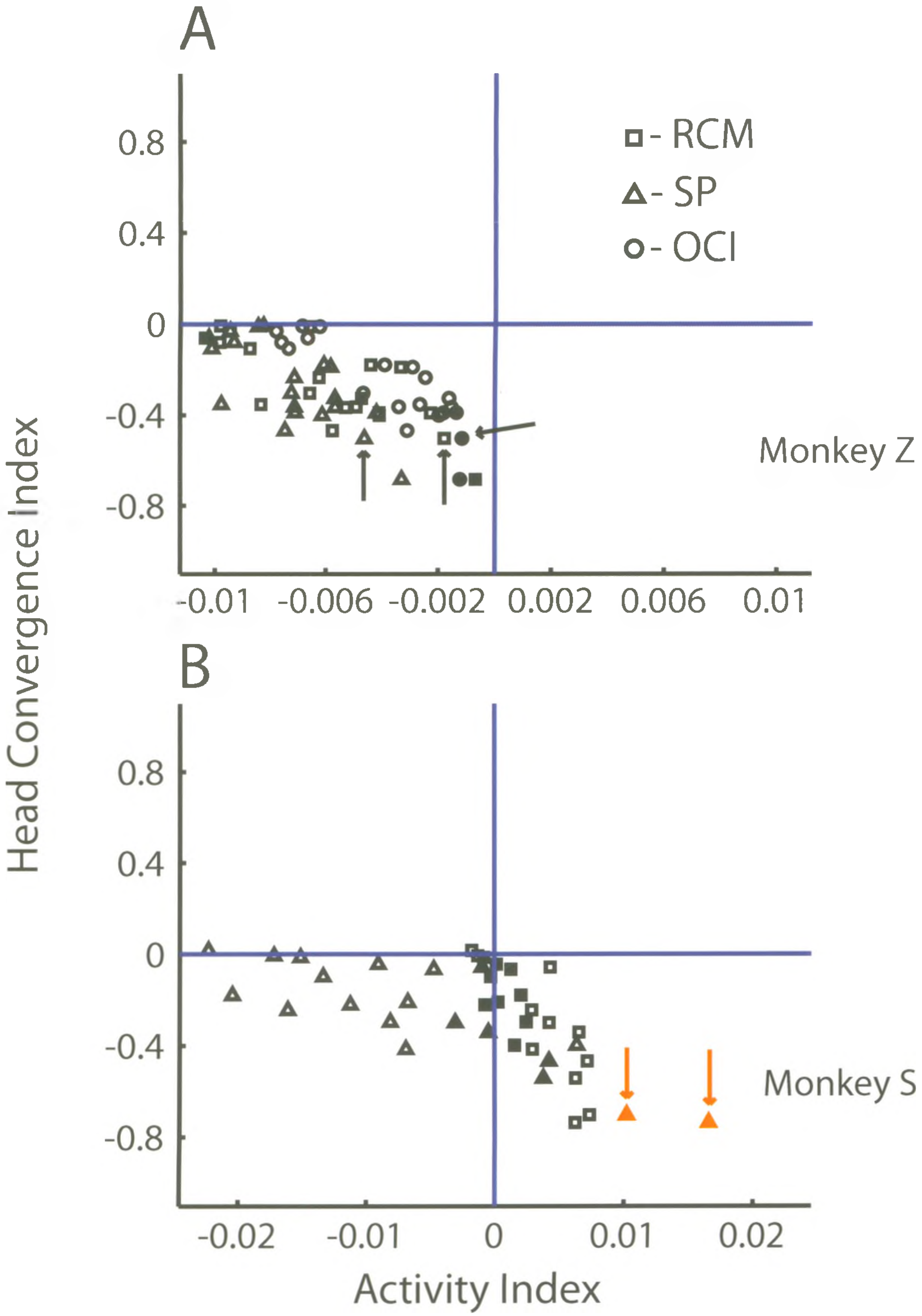

Figure 12. Comparison of hCI and AI for agonist RCM (square), SP (triangle), and OCI (circle) at all stimulation sites for monkey Z (A) and monkey (S). Monkey S agonist OCI data not shown. Filled symbols indicate an AI not significantly different from zero (P>0.05). Black arrows (A) indicate the example stimulation site used previously. Red arrows pointing to red filled symbols (B) outline two sites with alternative patterns of evoked neck muscle activity.

the data from monkey *Z* fall into the bottom left quadrant indicating that, for all agonist muscle activity at all stimulation sites, AI was always negative (OCI -0.004 ± 0.002, RCM -0.006 ± 0.003, SP -0.007 ± 0.002, mean ± sd). This implies that for monkey *Z*, the evoked neck muscle EMG activity always displayed a pattern counter to that which would be expected to occur naturally with convergent movements. For monkey *Z*, smaller evoked head movements were always associated with at least the same if not more neck EMG activity than larger evoked head movements. With the exception of the two sites discussed below indicated by the red arrows, EMG recorded from SP in monkey *S* generally produced negative AI (-0.006 ± 0.01, mean ± sd) producing patterns of neck muscle EMG activity where smaller evoked head movements are associated with increased SP EMG activity.

We performed an analysis of the neck muscle EMG activity accompanying negligible (<1°) head movement amplitudes. We calculated a contrast index comparing the relative increase in neck muscle activity for the first 100 ms of stimulation over the 100 ms of EMG activity preceding stimulation onset:

$$Contrast\ Index = \frac{(EMG_{post-stim}) - (EMG_{pre-stim})}{(EMG_{post-stim}) + (EMG_{pre-stim})}$$

The skew towards positive contrast indices shown in Fig. 13 (0.19 ± 0.20, mean ± sd) indicates that negligible head movements (<1°) were associated with a significant ($P<0.0001$, t-test) increase in neck EMG activity following stimulation onset. Even in the absence of an observed head movement, stimulation continued to evoke neck muscle EMG activity.

For all neck muscle EMG recorded, increasing CI magnitude was correlated with more positive AI (Monkey *Z*: OCI R=0.86, RCM R=0.79, SP R=0.73, Monkey *S*: RCM

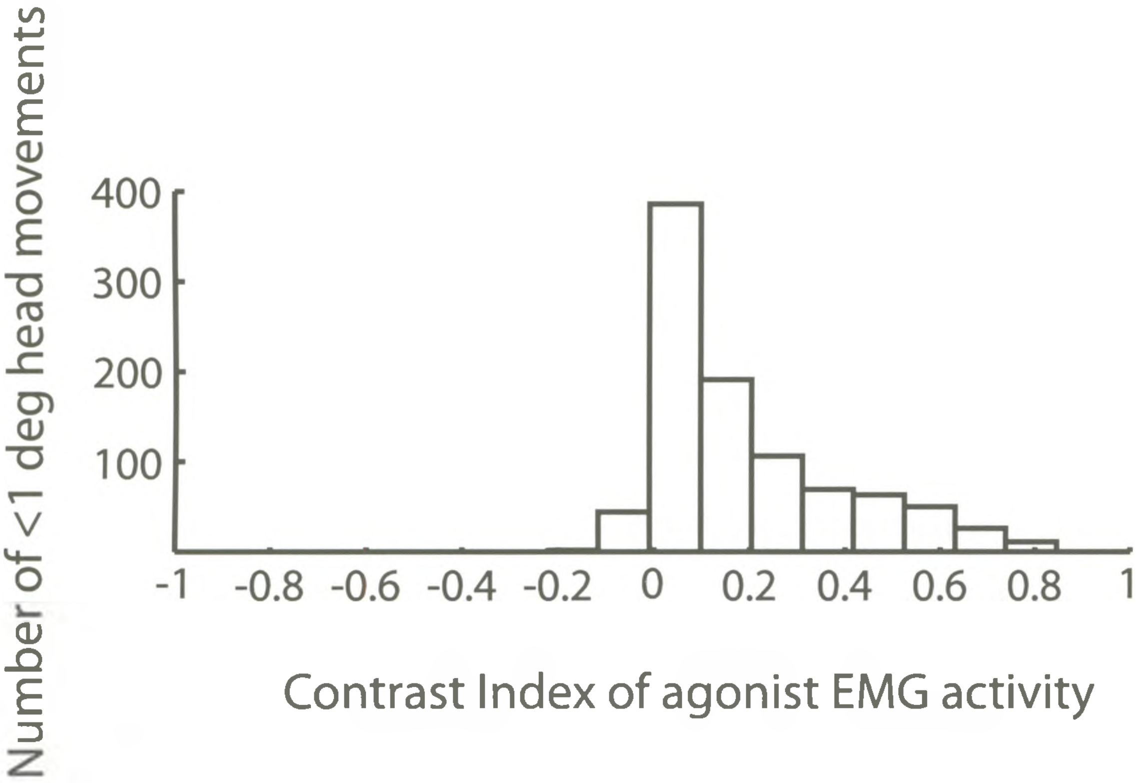

Figure 13. Contrast index of agonist EMG activity evoked during the first 100 ms of stimulation against the 100 ms preceding stimulation for negligible (<1°) evoked head movements. A positive contrast index indicates an increase in agonist EMG activity evoked by stimulation over the agonist EMG activity preceding stimulation. Mean contrast index (0.19 ± 0.20, mean ± sd) was significantly greater than zero ($P<0.0001$).

R=0.81, SP R=0.82, P<0.001). Since almost all stimulation sites had negative AI, CI magnitude correlated with more positive AI indicates that more convergent evoked head movement sites are associated with less variation in evoked neck muscle EMG activity. The difference in distribution of sites between the two monkeys (Fig. 12) is likely due to the slight differences in chamber placements between the two monkeys. Differences between monkeys are not evident for the EMG activity accompanying negligible head movements (Fig. 13, P>0.05, t-test), with stimulation evoking the same generic response in both monkeys.

The two sites indicated by the red arrows in Fig. 12 posses interesting qualities when compared with the population of stimulation sites as a whole. These are sites that, contrary to most other sites recorded, display increased evoked EMG activity with larger amplitude evoked head movements. The raw SP EMG traces for these two sites are presented in Fig. 14. Note the increase in neck muscle EMG associated with increased head movement amplitude. These two sites also possessed the largest magnitude CI of any sites recorded (0.70 and 0.74) and no sites recorded in Monkey *Z* had a CI of this magnitude. While these sites may display an atypical pattern of evoked neck muscle activity across initial head orientations, negligible evoked head movement amplitudes from these sites were associated with considerable agonist neck muscle drive (Fig. 14). Although these sites are in the minority (two of the forty stimulation sites analyzed in this thesis), their presence suggests that certain cases of SEF stimulation may produce alternate patterns of evoked neck muscle EMG.

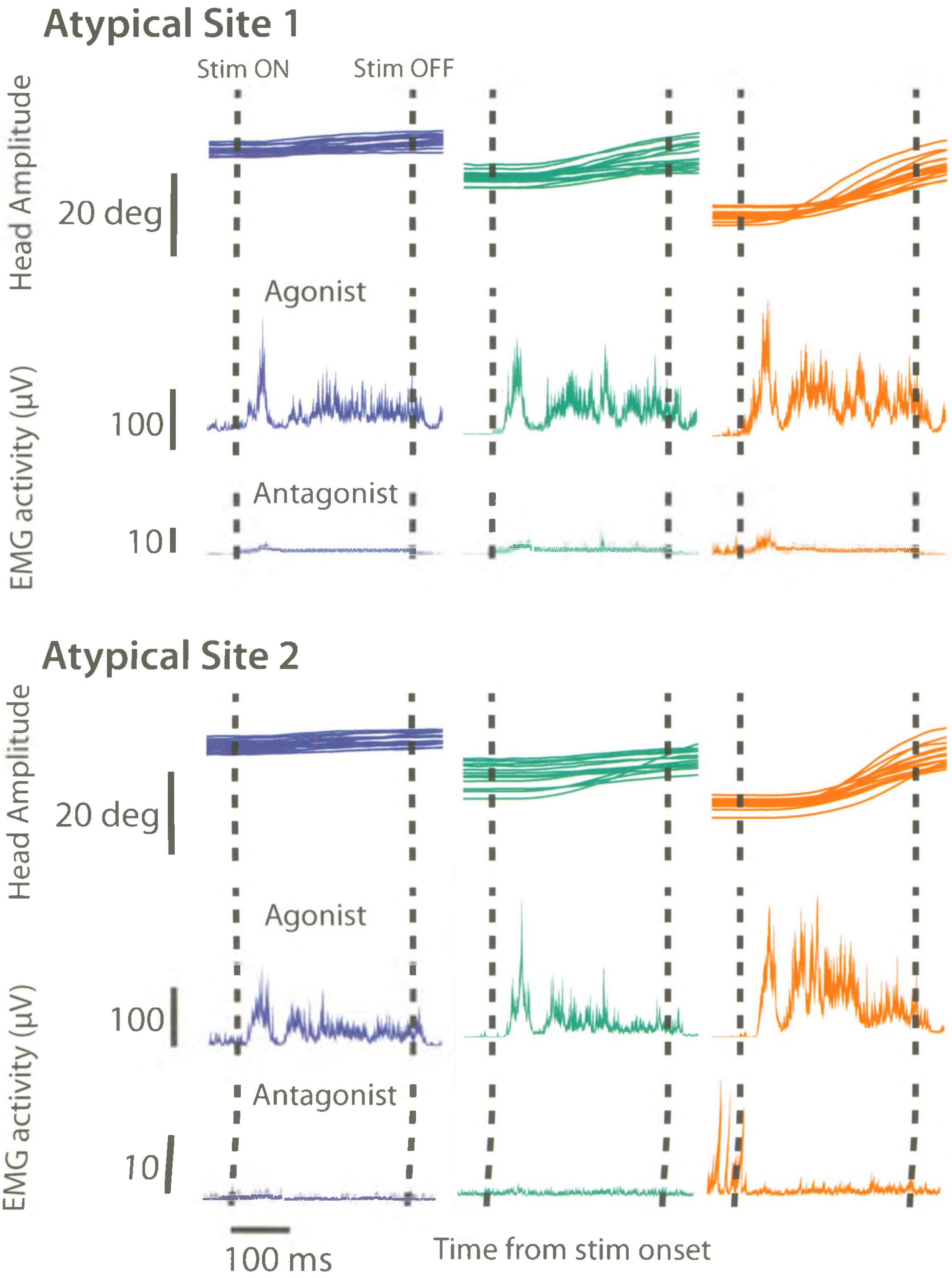

Figure 14. A number of stimulation trials from atypical sites seen in monkey S. Like typical stimulation sites, evoked head amplitude was small for contralateral fixation positions and increased with more ipsilateral fixation positions. Unlike typical stimulation sites, stimulation evoked agonist SP EMG activity increased with more ipsilateral fixation positions. The largest evoked head movements were thus associated with the greatest evoked agonist SP EMG activity. Agonist SP activation and antagonist SP suppression were rapid (~20 ms) and time locked to stimulation. Negligible evoked head movement amplitudes were associated with considerable agonist neck muscle drive (blue).

3.5 - Effect of evoked movement amplitude

Stimulation-evoked head movement amplitude from central initial head orientation was correlated with increased magnitude of CI (R=0.93, P<0.0001 for monkey *Z*, R=0.54, P=0.01 for monkey *S*) (Fig. 15). This correlation implies that stimulation sites that evoked small amplitude head movements showed little convergence of movement whereas sites evoking larger amplitude head movements displayed a greater degree of convergence. AI was correlated with evoked head movement amplitude for monkey *Z* (OCI R=0.74, RCM R=0.81, SP R=0.74, P<0.001) but not monkey *S* (P>0.05) (Fig. 15), indicating that stimulation sites with the largest evoked head movements displayed the least amount of variability (small AI) in neck muscle EMG signal for monkey *Z*.

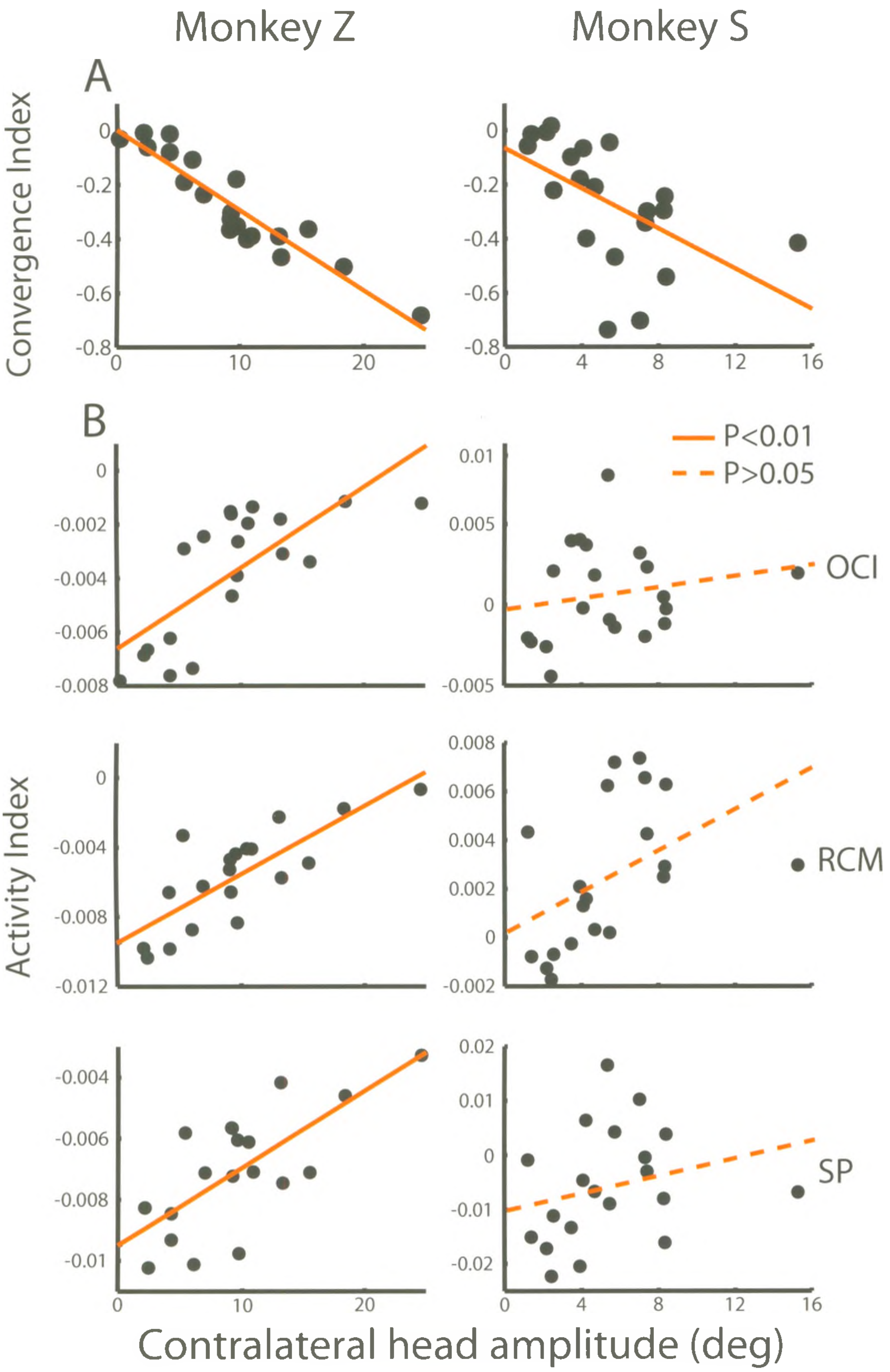

Figure 15. A. CI as a function of stimulation-evoked head movement amplitude for monkey Z and S. Increasing evoked head movement amplitude is correlated with increased magnitude CI, i.e. more convergent evoked head movements (R=0.93, P<0.0001 for monkey Z, R=0.54, P=0.01 for monkey S). B. AI for agonist OCI, RCM and SP as a function of evoked head movement amplitude. Increasing evoked head movement amplitude is correlated with decreasing magnitude AI for monkey Z (OCI R=0.74, RCM R=0.81, SP R=0.74, P<0.001) but not monkey S (OCI N.S., RCM N.S., SP N.S., P>0.05).

Chapter 4 - Discussion

In this thesis I have examined the neck muscle recruitment patterns accompanying convergent head movements evoked by cortical microstimulation of the primate SEF. Combining evoked head movements with the use of neck muscle EMG provides an accessible way to study the signal sent to the motor periphery. In the majority of SEF sites, stimulation elicited a counter intuitive trend of increased agonist muscle EMG activity accompanying smaller head movements. This trend appeared as the result of a generic agonist on / antagonist off pattern of neck muscle activation integrating with a posturally dependent level of activity on the neck muscle. These findings suggest that biomechanical factors, which have not previously been considered, significantly impact stimulation-evoked movements and are a major factor in the appearance of convergent movements.

I will begin by discussing how biomechanical factors influence stimulation-evoked movements to produce convergence of movement, then outline how these results fit with other literature on the convergence of movements in the SEF as well as other oculomotor areas. Lastly, I will examine the role cortical microstimulation has played in the analysis of brain reference frames and how my results contribute to the interpretation of these findings.

4.1 - Biomechanical factors influencing convergent movements

Muscles produce the forces required to accomplish active movement. They are activated via motoneurons that innervate the muscle and it is the activity of the motoneurons that is effectively measured by EMG. The neural drive to muscles is, however, only one of a number of factors that contribute to overall muscle force. Moreover, motoneuron activity does not translate linearly into muscle force. When considering an entire system, such as the head plant in the case of head turns, forces other than that produced by neck muscles also influence the resultant movement. The forces influencing a moving system, in this case the head, can be divided into two main categories: passive and active forces.

Passive forces arise from the resistive forces generated by muscles, tendons, ligaments and other tissues as they are stretched and moved. For horizontal movements of the head, passive forces are generally restoring, in that they act to return the head to a central orientation aligned to the orientation of the body. Passive forces oppose movements that deviate from centre. With respect to the methodology laid out in this thesis, passive forces resist active head turns when initial head orientation is central or deviated in the direction of movement (contralateral to the side of stimulation) and assist active head turns when initial head orientation is deviated in the direction opposite movement (ipsilateral to the side of stimulation). Once the head reaches centre from an initial head orientation ipsilateral to the direction of movement, the passive forces begin to resist the head turn. The passive restoring forces increase non-linearly as the ligaments, tendons and muscles become more stretched. The force exerted by passive forces thus increases non-linearly with more eccentric initial head orientations.

Active forces are the forces generated by the contraction of muscles in response to activation by the motoneurons. In the context of head movements, active forces provide the primary drive for head turns, but the active force generated by the neck muscles is also dependent on a number of factors other than motoneuron drive. A significant factor in muscular contraction is the relationship between the force generated by a muscle and the current length of the muscle. All muscles have an optimal length at which they develop a maximum amount of force for a given motoneuron drive. As muscle length deviates from this optimal point, the force generated for a given drive from the motoneurons decreases in a non-linear fashion. In the context of the head plant, the relationship between muscular length and force generated means that agonist neck muscles, given the same drive, will produce different force depending on the length of the muscles, which itself depends the orientation of the head. If it is assumed that the muscles are at an optimal length when the head is directed to centre, then agonist neck muscles produce less force when initial head orientation is deviated from centre. As the movement proceeds, muscle force for initial head orientations deviated in the direction opposite the movement (ipsilateral to the side of stimulation) will increase as the head moves to centre and the agonist muscle contracts to an optimal length. As initial head orientation deviates in the direction of movement (contralateral to the side of stimulation) the muscle contracts and shortens further from an optimal length and will experience a continued decrease in the force generated.

The active and passive forces acting together in the context of head movements have significant implications for the drive sent to the neck muscles during volitional movements. Different levels of neck muscle drive are needed to produce an equivalent amplitude head movement from different initial head orientations. Similarly, an equivalent level of neck muscle drive would produce larger head movements when the head is oriented in the direction opposite movement and smaller head movements when the head is oriented in the same direction as movement.

The data presented in this thesis show that stimulation of the SEF evokes a rapid, generic agonist on / antagonist off pattern of neck muscle drive at all initial head orientations (Fig. 8). This unnatural pattern of neck muscle recruitment lies in stark contrast to the elegance of natural neck muscle recruitment. Not only does natural neck muscle drive increase with larger amplitude movements, but the timing of the drive to various muscles is also tightly controlled to take full advantage of neck biomechanics (Corneil et al. 2001). In the case of natural head movements when the head is initially oriented in the direction opposite movement, the antagonist muscles are silenced prior to the activation of the agonist muscles, allowing the passive biomechanical forces alone to rotate the head to centre (Corneil et al. 2001). There is no such pattern during stimulation; agonist activation and antagonist suppression are time locked to the start of stimulation (Fig. 8). The differing biomechanical forces at each initial head orientation produce different amplitude head movements when given this generic neck muscle activation signal (Fig. 10). This suggests that these biomechanical forces combined with a generic stimulation-evoked drive to the neck muscles lead to the apparent convergence of evoked head movements. The biomechanical considerations for convergent movements have been generally neglected by studies examining the convergence of stimulation-evoked movements. If biomechanical forces combined with generic stimulation-evoked muscle drive are sufficient to produce convergence of movements, then using stimulation as a method for investigating convergence and the implications of such convergence may not be valid.

4.2 - Comparison to previous studies: SEF

The convergence of movements evoked by stimulation of the SEF is used as a characteristic trait of the SEF (Schlag and Schlag-Rey 1987; Mitz and Godschalk 1989). Recent studies have utilized the convergence of stimulation-evoked movements as a method to elucidate the organization and function of the SEF. A primary assumption of convergent stimulation-evoked movements in the SEF is that they indicate the reference frame in which the area encodes movement goals. The pattern of convergence seen during stimulation has been used as an indicator for different frames of reference in the SEF (Martinez-Trujillo et al. 2004; Tehovnik et al. 1998). The rationale behind basing reference frame coding on measured convergence is that stimulation in a region of cortex activates that area and evokes movements similar to those evoked by endogenous neural activity.

Neck muscle EMG affords the unique ability to test the signal sent to the motor periphery by stimulation. A logical expectation is that evoked neck muscle activity for the range of head movement amplitudes seen during SEF stimulation would be amplitude appropriate (i.e. larger recruitment for larger movements), as this is observed during volitional movements (Corneil et al. 2001). The neck muscle EMG data presented here do not support such a neural explanation for the head movement convergence evoked by SEF stimulation. The evoked EMG response varied little with changes in initial gaze position (Fig. 10) and many sites showed smaller head movements associated with increased levels of neck muscle recruitment.

When this neck muscle movement amplitude mismatch is taken to the extreme, the results are even more striking. During negligible head movements (<1°) evoked by stimulation there was still a significant and persistent pattern of evoked EMG on the agonist muscles (Fig. 13). If stimulation was evoking movements encoded within a reference frame and the head component of movement was already at the goal location, the brain would not issue a neural drive to move the head. The observation of evoked neck muscle drive during negligible movements of the head is completely incompatible with the idea that SEF stimulation evokes movements in a natural fashion.

Reference frames assigned by examining stimulation-evoked convergence also do not appear to exist as discrete patterns of movement. The approach taken by Martinez-Trujillo et al. (2004) was to plot gaze shifts in body-centred, head-centred and eye-centred coordinates and determine in which coordinate system gaze shifts were most convergent. Stimulation sites still converged in the other coordinate systems but to a lesser degree than in the reference frame to which that site was ultimately assigned. A site with a head-centred reference frame, for instance, does not present as only converging in head-centred coordinates, but still has a degree of convergence in eye- and body-centred coordinates. Moreover, stimulation sites do not fall into discrete groupings around ideal reference frames but are instead distributed over a range of convergence with a mean in what is interpreted as a head-centred reference frame.

The observation that convergence and the level of evoked EMG activity tightly co-vary with movement amplitude (Fig. 15) offers a simplified explanation of the distribution of convergence. If convergence is seen as simply a biomechanical product of stimulation then the distribution of convergence can be accounted for by movement amplitude. When stimulation is passed in a spatially encoded area, sites with larger characteristic evoked movement amplitudes are associated with an increase in the strength of the drive sent to the neck muscles (Corneil et al. 2002a). The generic stimulation-evoked activity on the neck muscles integrates with the tonic positionally dependent activity of the neck. Importantly, agonist neck muscle activity is actively suppressed at initial head orientations opposite the direction of movement (Fig. 8). Thus, in order to move the head, any stimulation-evoked neck muscle activity must overcome this tonic suppression. Since small amplitude stimulation sites produce less neck muscle drive, they are less able to overcome the tonic suppression than larger amplitude sites. The result is that small amplitude stimulation sites produce smaller evoked movements when the head is initially oriented opposite to the direction of movement. For initial head orientations in the same direction as movement, neck muscle drive has less effect, as the head is more difficult to move in these locations. This leads to less variation in evoked head movement amplitude from smaller amplitude stimulation sites. The consequence of this is that smaller movement amplitude stimulation sites will evoke less convergent movements, as seen in Fig. 15. The variety of evoked amplitudes seen here and in other studies of the SEF (Schlag and Schlag-Rey 1987) could be responsible for the distribution of convergence seen for evoked head movements.

4.3 - Comparison to previous studies: SC and FEF

Previous studies of neck muscle activity in the SC support the view of convergence as a biomechanical consequence of stimulation. The rapid latency of neck muscle EMG activity in response to SEF stimulation and previous anatomical studies is correlated with the effects of SEF stimulation being relayed through the SC (Shook et al. 1990). Due to the rapid latency of EMG activity, it is unlikely that signals compensating for initial head orientation would be able to influence the drive to the neck muscle in time to influence the head component of gaze. Observed neck muscle EMG latencies (18 ± 5.5 ms, mean ± sd) were slightly slower than those seen in response to SC stimulation (Corneil et al. 2002a). If the apparent convergence is a biomechanical consequence of stimulation, patterns of neck muscle activity evoked by stimulation of the SC are expected to be similar to those observed with simulation of the SEF. Convergence in the SC is less pronounced than in the SEF but still occurs at sufficiently eccentric initial gaze positions (Freedman et al. 1996; Klier et al. 2001; Corneil et al. 2002a; Corneil et al. 2002b). Neck muscle EMG recorded during convergent movements following stimulation of the SC displays a similar pattern to that observed following SEF stimulation. Stimulation of the SC results in the same generic agonist on / antagonist off pattern neck muscle activity as seen in the SEF (Corneil et al. 2002a; Corneil et al. 2002b). Many sites in the SC also show increased levels of neck muscle activity associated with smaller evoked movements consistent with a generic activation of the neck muscles integrating with positionally dependent tonic activity.

Neck muscle EMG latencies measured in the FEF show the same rapid response as those found in the SEF (Elsley et al. 2007). We suggest that stimulation in both the SEF and FEF is acting through the SC and provoking an immediate expression on the motor periphery. Such an immediate expression either overrides or is too rapid for the processes that compensate for head biomechanics during natural movements. Preliminary data show that stimulation in the FEF evokes convergent movements with the same basic pattern of neck EMG response seen in both the SC and here in the SEF (Chapman et al. 2008).

It is clear based on latency and EMG response that the signal sent to the motor periphery is essentially the same for stimulation in the SC, FEF and SEF. Stimulation in oculomotor areas therefore appears to be producing a rapid generic drive to the neck muscles regardless of which area is stimulated. Stimulation is not producing signals based on any inherent reference frame. If these oculomotor areas are operating in different frames of reference, then stimulation is clearly not an appropriate methodology for the study of reference frames.

4.4 - Cortical Stimulation

The data presented in this thesis reveal the importance of biomechanical factors previously neglected in the study of stimulation-evoked convergent movements. Based on the recorded EMG data from many oculomotor areas, stimulation appears to be producing a generic drive to the neck musculature regardless of initial gaze position (Results Fig. 8) (Chapman et al. 2008; Corneil et al. 2002a; Corneil et al. 2002b). This is in contrast to what would be expected if stimulation were producing convergent movements encoded based on an inherent reference frame of the stimulation site.

It is clear that stimulation in a range of oculomotor sites such as the SEF does not produce natural patterns of neck muscle activation. Is it possible, however, to find a brain area far back enough in the sensori-motor transformation system that stimulation would evoke natural gaze shifts? Primary visual cortex (V1), a preliminary area in visual sensori-motor transformation, provides a possible place to examine the effects of stimulation. Intracortical microstimulation of V1 induces a phosphene in the visual field and recent methodologies have trained primates to make gaze shifts to these stimulation evoked phosphenes (Tehovnik et al. 2005; Chen and Tehovnik 2007). Would the neck muscle activity evoked by these trained gaze shifts to phosphenes exhibit a natural pattern of activity due to their partially volitional nature? Or would the overriding signal sent by stimulation pass through the oculomotor system from V1 and evoke a pattern of neck muscle activity similar to that seen in this thesis? The results of such an experiment would provide insight into the effect of stimulation and the point at which motor command becomes divorced from sensory input.

Many studies have also seen an influence of initial position on the activity and preferred vectors of individually recorded neurons (Bon and Lucchetti 1992; Bon and Lucchetti 1997; Russo and Bruce 1996; Russo and Bruce 2000; Schlag et al. 1992). Since individual neural recordings are a direct measure of the endogenous activity of neurons and are not artificial stimuli playing out on a system with large biomechanical considerations, the present study shows that recordings are a more appropriate technique for elucidating the influence of reference frame coding in the oculomotor system.

4.5 - General conclusions

Previous observations show that convergent SEF stimulation-evoked gaze shifts are kinematically identical to volitional gaze shifts of the same amplitude (Martinez-Trujillo et al. 2003a). As mentioned above, however, the brainstem oculomotor circuits are a contained loop, faithfully transforming the spatial coding of higher brain areas into an appropriate temporal code for the movement signal. Because of the biomechanics of the head, many different patterns of neck muscle drive are able to produce equivalent movements. Thus, equivalent head kinematics does not imply an equivalent drive being sent to the neck musculature. The nature of the biomechanics of the head precludes the use of kinematics to compare the signal sent to the neck muscles between volitional and stimulation-evoked head movements.

The concept of convergence as an emergent property of stimulation is not novel. Russo and Bruce (1993) put forth the idea that stimulation is overriding the normal target selection circuit for generating saccades in higher order brain areas. Thus, eye position updating and compensation circuits (they hypothesized from the cerebellum) are not involved and saccades generated by stimulation of higher order brain areas would not contain a component to compensate for the tonic elastic centring forces inherent with different initial gaze positions. Constant stimulation parameters cause stimulation to evoke a generic neural activation. This generic response in the absence of gaze position compensation is responsible for the observed convergence of movements. Rather than eliciting saccades based on the reference frame coding of a brain region, the apparent convergence of movements is the biomechanical consequence of generic stimulation induced activation.

The overriding effect of stimulation, combined with the biomechanical factors of different initial gaze positions, appears to be responsible for producing the convergence of movements otherwise interpreted as arising due to the operation of different reference frames within the brain. Based on the evidence shown here and in other works, cortical microstimulation is not an appropriate method to examine the concept of reference frame coding in the SEF and other brain areas.

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Appendix 1

Western

October 25, 2007

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2011

Dear Dr Corneil:

Your Animal Use Protocol form entitled:
Sensory and motor roles for neck muscles in visually-guided actions: Neural mechanisms underlying recruitment and kinesthesia
Funding Agency CIHR - Grant #MOP 64202

has been approved by the University Council on Animal Care. This approval is valid from **October 25, 2007 to October 31, 2008**. The protocol number for this project is **2007-099-10 which replaces 2003-080-10**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 1 YR.

Species	Strain	Other Detail	Pain Level	Animal # Total for 1 Year
Other, add to detail	Rhesus - Macaca mulatta	3-8 yrs. 4-12 kg Male	D	4

STANDARD OPERATING PROCEDURES
Procedures in this protocol should be carried out according to the following SOPs. Please contact the Animal Use Subcommittee office (661-2111 ext. 86770) in case of difficulties or if you require copies
SOP's are also available at http://www.uwo.ca/animal/acvs
310 Holding Period Post-Admission
320 Euthanasia
322 Criteria for Early Euthanasia/Mammals/Non-rode
333 Post-Operative/Post-Anaesthetic Care-L3

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Protocol - B. Corneil, T. Admans
Approval Letter - T. Admans

Oct 25/07

The **University** *of* **Western Ontario**
Animal Use Subcommittee University Council on Animal Care
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