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Spatial and state-dependent effects of transcranial magnetic stimulation of prefrontal cortex in non-human primates

Chao Gu, *The University of Western Ontario*

Supervisor: Dr. Brian D. Corneil, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

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by

Chao Gu

Graduate Program in Neuroscience

A thesis submitted in partial fulfillment
of the requirements for the degree of
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The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

Abstract

The indirect effects of transcranial magnetic stimulation (TMS) within a distributed neural network are still largely unknown. Here we propose to use the non-human primate (NHP) oculomotor system as an animal model for investigating the effects of TMS. Across three animals, single pulses of TMS to the prefrontal cortex (PFC), including the frontal eye fields (FEF), reliably evoked a contralateral head turning synergy, similar to what is seen following intracortical microstimulation. Furthermore, double pulses of TMS paired with the memory-guided saccade paradigm only evoked neck muscle activity preceding contralateral saccades, showing similar state-dependency as previously observed in human TMS studies. These results indicate that the NHP oculomotor system is a feasible model to study the distributed effects of TMS outside of the stimulated area, and motivates future studies pairing TMS and neurophysiological recordings.

Keywords

Neck muscles, electromyography, prefrontal cortex, TMS, frontal eye fields, NHP

Co-Authorship Statement

I, Chao Gu, am submitting this research project as partial fulfillment of the Master of Science Degree in the discipline of Neuroscience. As such, I have assume the primary role in all aspect of this document including, but not limited to, laboratory setup, experimental design, data collection and analysis, as well as producing the initial draft of the thesis. I collected and analysis all of the data from monkeys *al* and *sp*. I preformed all of the statistical analysis from all of the animals. I'll also help in the surgical preparation from monkey *al*, as well as training the monkey on the the memory-guided saccade task. Dr. Brian D. Corneil acted as my supervisor for this project. He provided the framework for this project as well as providing critical advice throughout all stages, and also acted as an editor to the subsequent drafts of this thesis. He also collected all of the data from monkey *zn*. Dr. Sharon Cushing performed the electromyographic electrode surgery for all of the animals used in this thesis.

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List of Abbreviations

CI	Confidence interval
dlPFC	Dorsal lateral prefrontal cortex
EMG	Electromyographic
FEF	Frontal eye fields
FP	Fixation period
ICMS	Intracortical microstimulation
LED	Light emitting diode
LIP	Lateral intraparietal area
M1	Primary motor cortex
MEP	Motor evoked potential
MSO	Maximum stimulator output
NHP	Non-human primate
OCI	Obliquus capitis inferior
OPN	Omnipause neurons
PFC	Prefrontal Cortex
PPRF	Paramedian pontine reticular formation
RCM	Rectus capitis posterior major
RF	Response field
riMLF	Rostral interstitial nucleus of medial longitudinal fasciculus
RSN	Reticulospinal neurons
SC	Superior colliculus
SEF	Supplementary eye fields
SP	Splenius capitis
SRT	Saccadic reaction time
TMS	Transcrianal magnetic stimulation

List of Symbols

~	Approximately
dB	Decibel
°	Degree
=	Equal to
>	Greater than
Hz	Hertz
kg	Kilogram
kHz	Kilohertz
<	Less than
μA	Microamperes
mm	Millimeter
ms	Millisecond
%	Percentage
±	Plus or minus

Chapter 1 – Introduction

1.1 Transcranial Magnetic Stimulation

In 1954, Penfield and Jasper first demonstrated that passing an electrical current through exposed human brain tissue could induce changes in behavior, which was consistent with previous findings of similar results within animals (Ferrier, 1874). In 1980, based on this understanding of the brain, Merton and Morton demonstrated that electrical stimulation on an intact scalp over primary motor cortex (M1) could evoke a contralateral muscle response. However, one major drawback of this technique was the activation of pain receptors over the scalp from the electrical current, which caused this technique to be not practical for further investigation. In 1985, Barker and colleagues delivered transcranial magnetic stimulation (TMS), a relatively painless technique, to M1 to evoke a contralateral motor evoked potential (MEP) similar to the response elicited from electrical stimulation (Barker et al., 1985). TMS works by passing a rapid current through a tightly wound coil, this causes lines of magnetic flux perpendicular to the plane of the coil. Based on Faraday's law, the induction of the magnetic flux creates an electrical field parallel and in the opposite direction to the plane of the coil (Fig. 1A). A single circular coil will induce the highest electrical field density directly below the coil and a null electrical field at the center of the coil (Fig. 1B). In present day, figure-eight coils are most commonly used with TMS (two circular coils combined together, where electrical currents passes through in opposite directions), as this reduces the spread of the electrical field and also produces the largest electrical field density directly under the intersection

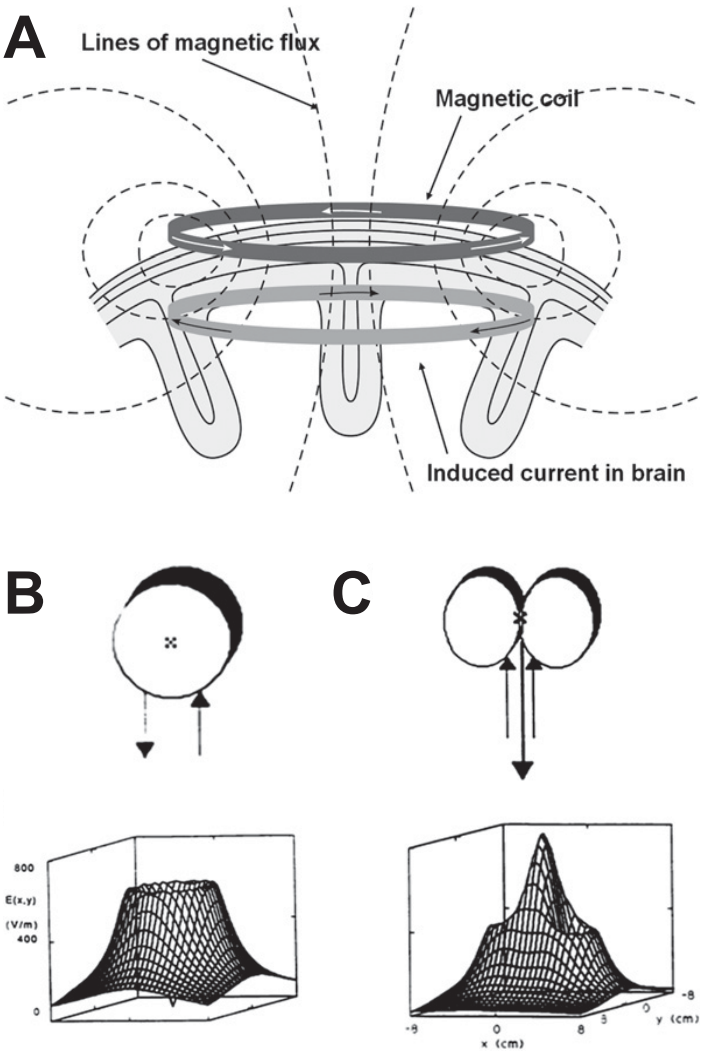

Figure 1: Principal of transcranial magnetic stimulation.

(A) Electrical current is passed through a magnetic coil in a counterclockwise direction (dark ring). This generates lines of magnetic flux perpendicular to the magnetic coil (dash line). With the generation of the magnetic flux it also produces an electrical fields that is induced in a clockwise manner (light ring), which is thought to perturb brain activity. (B) A round magnetic coil (top) and the electrical field that it generates (bottom), note the absence of an electrical field at the center of the coil. (C) A figure-eight coil (top) and the electrical fields it generates (bottom). (Taken from Hallett, 2007).

of the two coils (Cohen et al., 1990) (Fig. 1C).

Currently TMS is extensively used in a research setting to perturb brain function (for review, see Hallett, 2007). There are two main types of TMS applications: on-line and off-line TMS. On-line TMS are short trains of TMS applied concurrently with a behavioral task. This allows for specific temporal perturbation of the brain during a task. This type of TMS can produce both facilitative and disruptive effects. For example in a simple reaction time task where subjects flex their arm, compared to intermixed non-TMS control trials, high intensity TMS-M1 increased reaction time (disruption), while low intensity TMS to the same site decreased reaction time (facilitation) (Pascual-Leone et al., 1992). In off-line TMS, a high number of rhythmic pulses of TMS (rTMS) are applied to the brain independent of a task. Depending on the frequency and protocol, this form of TMS can have prolonged suppressive or excitatory effects on the brain, which is thought to arise from long-term depression or long-term potentiation of the stimulated cortex (for review, see Hoogendam et al., 2010). To determine the effects of rTMS, a baseline period of behavior before rTMS is used to compare to the performance after rTMS application. Additionally, the frequency of the pulses of TMS can selectively enhance the natural brain oscillations of the same frequency and may be able to mimic specific task modulation effects (Thut et al., 2011). The ability to have persistent suppressive and/or excitatory effects on specific cortical regions or even specific neural networks can be a powerful non-pharmacological tool for treatment of both psychological and neurological disorders.

Despite the widespread use of TMS, the underlying mechanisms of TMS are still largely unknown. Most of our understanding of TMS on human behavior has come from work done on the M1; this is primarily due to its simple and direct circuitry. Corticospinal neurons within M1 project down the spinal column via the corticospinal tract to the efferent motor neurons. TMS is thought to activate corticospinal neurons at the axon of these neurons (Day et al., 1989). This activation propagates down to the efferent motor neurons and can be recorded with surface electromyographic (EMG) recordings. This straightforward monosynaptic response allows for easy quantification of TMS effects on behavior and suggests that the effects of TMS may also be propagating to other connected regions within the network.

Currently there are both rodent (Ji et al., 1998) and feline (Allen et al., 2007) animal models that investigate the neural effects of TMS. However these models use anesthetized animals and only study the direct effect of TMS on the underlying stimulated cortex. They cannot properly study the effects of TMS within a distributed network in an awake and behaving animal. Furthermore the physical size of these animal cortices as well as the inability to train both rodents and felines on complex cognitive tasks ultimately limits the ability to fully advance our understanding of TMS effects on behavior changes in humans.

Monkeys have a homologous cortical architecture as humans and they are also able to perform complex tasks. Previous TMS-M1 studies of monkeys have shown that TMS has a very spatially defined region of activation (Amaya et al., 2010) and can elicit a similar MEP response on contralateral hand muscles as in humans studies (Baker et al.,

1994). A non-human primate (NHP) animal model could potentially be the link required to better our understanding of the effects of TMS within a distributed network. An ideal target can be the oculomotor system; it has been studied extensively in monkeys. The draw of the oculomotor system is its simplistic output, a movement of the eyes. Monkeys are able to perform similar oculomotor and cognitive tasks as humans and the underlying neural activities have been studied extensively. The rapid and succinct output of the eyes, has allowed for this system to precisely measurement of cognitively demanding tasks. Nevertheless the oculomotor system itself involves multiple brain structures in both the cortex and other subcortical regions and has outputs to both the eyes and the neck (Fig. 2A). Thus the oculomotor system could be an ideal model to investigate TMS effects on a distributed network.

1.2 The Oculomotor System

Humans and monkeys rely heavily on vision for sensory information about the surrounding environment. We have evolved to have a highly efficient oculomotor system to acquire visual information via the retina, more specifically at the fovea where there is a highly dense population of photoreceptors. To fully utilize the fovea we have developed rapid movements to change our line of sight, through either saccades or gaze shifts. Saccades are small changes in line of sight made with eye movements without movement of the head (i.e. eye-in-head movements), while gaze shifts are usually larger line of sight changes made by coordinated eye and head movements (i.e. both an eye-in-head and head-in-space movements) (Guitton and Volle, 1987). Both saccades and gaze shifts serve fundamentally the same purpose, to overtly orient our line of sight towards a new

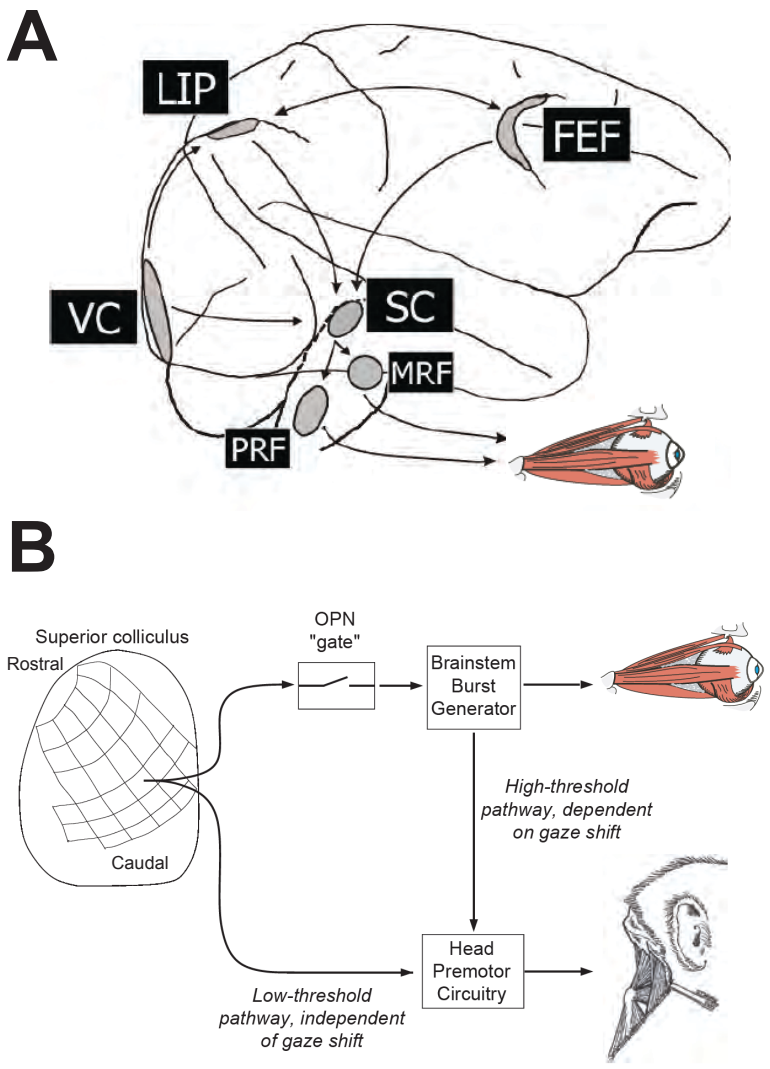

Figure 2: Simplified overall view of the NHP oculomotor system.

(A) Visual information enters the visual cortex (VC) and is fed to the superior colliculus (SC) and to the frontal eye fields (FEF) via the lateral intraparietal area (LIP). The motor command is consolidated at the SC from both LIP and FEF, and passed down to the brainstem (PRF and MRF) to generate saccadic eye movements. (B) Saccade and gaze command pathways. The signal to generate a saccade is tightly regulated by omni-pause neurons (OPNs) (top) before being sent downstream to the eye. However a copy of the same command from SC that is not controlled by the OPNs, is sent to the premotor circuitry down to the neck muscles.

region of interest. Changes in line of sight involve a complex network within the brain that includes multiple cortical structures, basal ganglia, thalamus, brainstem and cerebellum (Leigh and Zee, 2006) (Fig. 2A).

1.3 Superior Colliculus and Brainstem

Within the intermediate layers of the superior colliculus (SC) there is a retinotopic organization of neurons that encode for both visual information and/or motor output (Goldberg and Wurtz, 1972a; Wurtz and Goldberg, 1972). Neurons within the SC have a defined response field (RF), which encodes a specific area within the contralateral visual hemisphere. These neurons increase their firing rate for either a visual stimulus in their RF and/or a changes in line of sight into the RF. Neurons at the rostral end of the SC encode for fixation and small RF around the fovea, while neurons at the caudal end encode for larger peripheral RF in the contralateral hemisphere. Focal lesions of the SC disrupt saccadic generation to the corresponding retinotopic location (Goldberg and Wurtz, 1972b; Schiller et al., 1980), whereas electrical stimulation of the SC can evoke saccades (Robinson, 1972) or gaze shifts (Freedman et al., 1996; Klier et al., 2001) in the RF. Both of these lines of work demonstrate that the SC is a critical component for the initiating changes in line of sight. For the same magnitude of change in line of sight, despite difference in head and eye kinematics depending on the initial starting position, there is a consistent firing rate of SC (Freedman and Sparks, 1997), suggesting that the SC's signal is a common command that is dissociated downstream.

Downstream of the SC, the firing rate of neurons in both the paramedian pontine reticular formation (PPRF) and the rostral interstitial nucleus of medial longitudinal fasciculus (riMLF) encode the horizontal and vertical component of the ensuing saccadic eye movement, respectively (Büttner et al., 1977; Sasaki and Shimazu, 1981). Both the PPRF and riMLF are potently inhibited by omni-pause neurons (OPNs) within the nucleus raphe interpositus. It is believed that to initiate a saccadic eye movement, the SC silences the OPNs, possibly through an inhibitory intermediate region within the central mesencephalic reticular formation (Wang et al., 2013). The SC also sends the change in line of sight command to both the PPRF and riMLF neurons to evoke the desired saccadic eye movement portion of the gaze command (Luschei and Fuchs, 1972) (Fig. 2B).

The SC also projects down to the reticulospinal neurons (RSNs) and through the reticulospinal tract down to the spinal cord to the efferent motor neurons (Fig. 2B). Electrical stimulation of the OPNs during a change in line of sight interrupts both eye and gaze movements, but the head continues along its intended trajectory (Gandhi and Sparks, 2007). Suggests that OPNs control eye-in-head portion and does influence head-in-space movements during changes in line of sight. Unlike the eye, the visual-related activity of the SC can be measured through neck muscle EMG (Corneil et al., 2004), and neck muscle activity has also been correlated to low frequency SC activity independent of saccade (Rezvani and Corneil, 2008). Furthermore electrical stimulation of the SC can evoke changes in neck muscle EMG activity even in the absence of a saccade (Corneil et al., 2002a). Based on the previous results, the RSNs seem not be influenced by the OPNs,

and neck muscle EMG activity appears to provide a more sensitive indicator of the underlying SC activity.

1.4 Frontal Cortex

The frontal eye fields (FEF), supplementary eye fields (SEF) and the dorsolateral prefrontal cortex (dlPFC) are key cortical regions within the frontal cortex that are associated with oculomotor control. In monkeys the FEF (Brodmann Area 8) is located on the anterior bank of the arcuate sulcus (Fig. 2A), the SEF (Brodmann Area 6) is located medially to the FEF, posterior of the medial arm of the arcuate sulcus and the dlPFC (Brodmann area 9 and 46) is the cortical region around the principal sulcus, just anterior of the FEF. There are both inter and intra-hemispheric projections between the FEF, SEF and dlPFC (Barbas and Pandya, 1984; 1989; Huerta and Kaas, 1990; Schall et al., 1993). Additionally, all three of these regions projected to the SC (Kuypers and Lawrence, 1967; Goldman and Nauta, 1976; Stanton et al., 1988; Shook et al., 1990). Similar to the SC, neurons in FEF, SEF, and dlPFC have activity related to visual stimulus and/or saccades into specific RFs (Bruce and Goldberg, 1985; Schlag and Schlag-Rey, 1985; Boch and Goldberg, 1989). However the neural activity in the dlPFC is variable and may occur after saccade onset, suggesting that dlPFC modulates oculomotor commands rather than generating these commands (Johnston et al., 2009). Previous intracortical miscrostimulation (ICMS) of both FEF and SEF were able to evoke contralateral saccadic eye movements (Bruce et al., 1985; Schlag and Schlag-Rey, 1987), moreover like the SC, ICMS within these regions evokes gaze shifts (Tu and Keating, 2000; Martinez-Trujillo et al., 2004; Chen and Walton, 2005; Knight and Fuchs, 2007).

Additionally with ICMS-FEF, neck EMG activity can be evoked without a corresponding saccadic eye movement like with electrical stimulation in the SC (Corneil et al., 2010). The threshold for evoking a reliable neck muscle response is at a lower current than that to evoke a saccadic eye movement.

While FEF has anatomical projections down to both the PPRF and riMLF (Kuypers and Lawrence, 1967; Stanton et al., 1988), and these projections are thought to be critical in recovery of saccade generation after ipsilateral SC lesion (Goldberg and Wurtz, 1972b; Schiller et al., 1980), the functionality of these projections are unknown in an intact oculomotor system. Insight to the direct pathway functionality may be gleaned from a study conducted by Hanes and Wurtz (2001), where they temporarily inactivated a localized region of the SC, and were not able to evoke saccades with ICMS-FEF with the corresponding RF. This suggests that the FEF's direct connections to the premotor circuitry are weak and become only functionally relevant after ablation of SC due to plasticity. This result suggests that the SC mediates FEF's signals in an intact and functional oculomotor system and that neck muscle activity is a more sensitive indicator of both FEF and SC activity compared to saccadic eye movements.

1.5 Lateral Intraparietal Area

The lateral intraparietal area (LIP) in the posterior parietal cortex is another cortical region involved within the oculomotor system. A retrograde tracing study of the LIP has shown projections to both the SC and FEF (Lynch et al., 1985), furthermore tracing and antidromic activation studies have linked both FEF and SC to LIP (Schall et al., 1995;

Ferraina et al., 2002). Neurons within LIP have both visual-related and saccade-related activity similar to SC and FEF neurons (Paré and Wurtz, 1997). However the behavioral effects following ablation of LIP are less severe than following either ablation of SC and FEF, with only an increase in saccadic reaction time (SRT) and reduction in saccade accuracy (Lynch and McLaren, 1989). Currently the LIP has been hypothesized to play a role in combining top-down and bottom-up signals to produce a priority map (Bisley and Goldberg, 2010). This priority map is used to allocate attention and similar to the dlPFC, the LIP may play a role in saccade modulation.

1.6 TMS in the Human Oculomotor System

Previous human TMS experiments have failed to elicit saccadic eye movements with TMS applied to any cortical region (Wessel and Kömpf, 1991). However TMS paired with different oculomotor tasks has demonstrated modulations in saccadic behavior. During the memory-guided saccade task, where subjects maintain the spatial location of a remembered target for up to a few seconds in working memory and saccade to the remembered target after the offset of a central fixation point, single pulses of TMS to LIP immediately after central fixation offset (100 ms after offset) increased saccade latencies bilaterally (Müri et al., 2000). The same study also found that single pulse of TMS to dlPFC during the memory period (700-1500 ms after target onset) decreased the accuracy of contralateral saccades. The dlPFC plays a critical role in spatial memory, and TMS is thought to disrupt the encoding of the spatial location of the contralateral target. In the same memory-saccade paradigm, double pulses of TMS (20 Hz, 50 ms apart) were applied to the FEF with the 1st pulses concurrent with the offset of the central fixation

(i.e. GO cue). TMS selectively decreased the saccadic reaction time for only contralateral saccades, and had no effects on ipsilateral saccades (Wipfli et al., 2001). The underlying FEF and dlPFC were more engaged during contralateral memory-guided saccades compared to ipsilateral saccades. Both of these previous two studies demonstrate that TMS to either FEF or dlPFC selectively affects contralateral saccades and not ipsilateral saccades. This selective enhancement of TMS based on underlying cortical activity is known as the state-dependent effect.

Based on the state-dependent effects of double pulse TMS-FEF on SRT and the previous knowledge from animal work that neck muscle activity is a more sensitive indicator of oculomotor activity, Goonetilleke and colleagues (2011) performed the same double pulse TMS-FEF experiment while also recording neck muscle activity. Consistent with the previous TMS-FEF, they reported a state-dependency of TMS; with only decreased SRT in contralateral saccades. They also reported an increase in EMG activity of contralateral neck muscles time-locked to TMS for only contralateral saccade trials (Fig. 3). Moreover the decrease in SRT was also correlated with an increase in EMG activity between the subjects. The increase in neck muscle EMG response suggests that neck muscle EMG can also be as an alternative readout of oculomotor activity to the SRT. In addition, based on the previous monkey neck muscle EMG studies, it may be used as a more precise, rapid gauge of TMS effects on the oculomotor system, even potentially on a trial-by-trial basis.

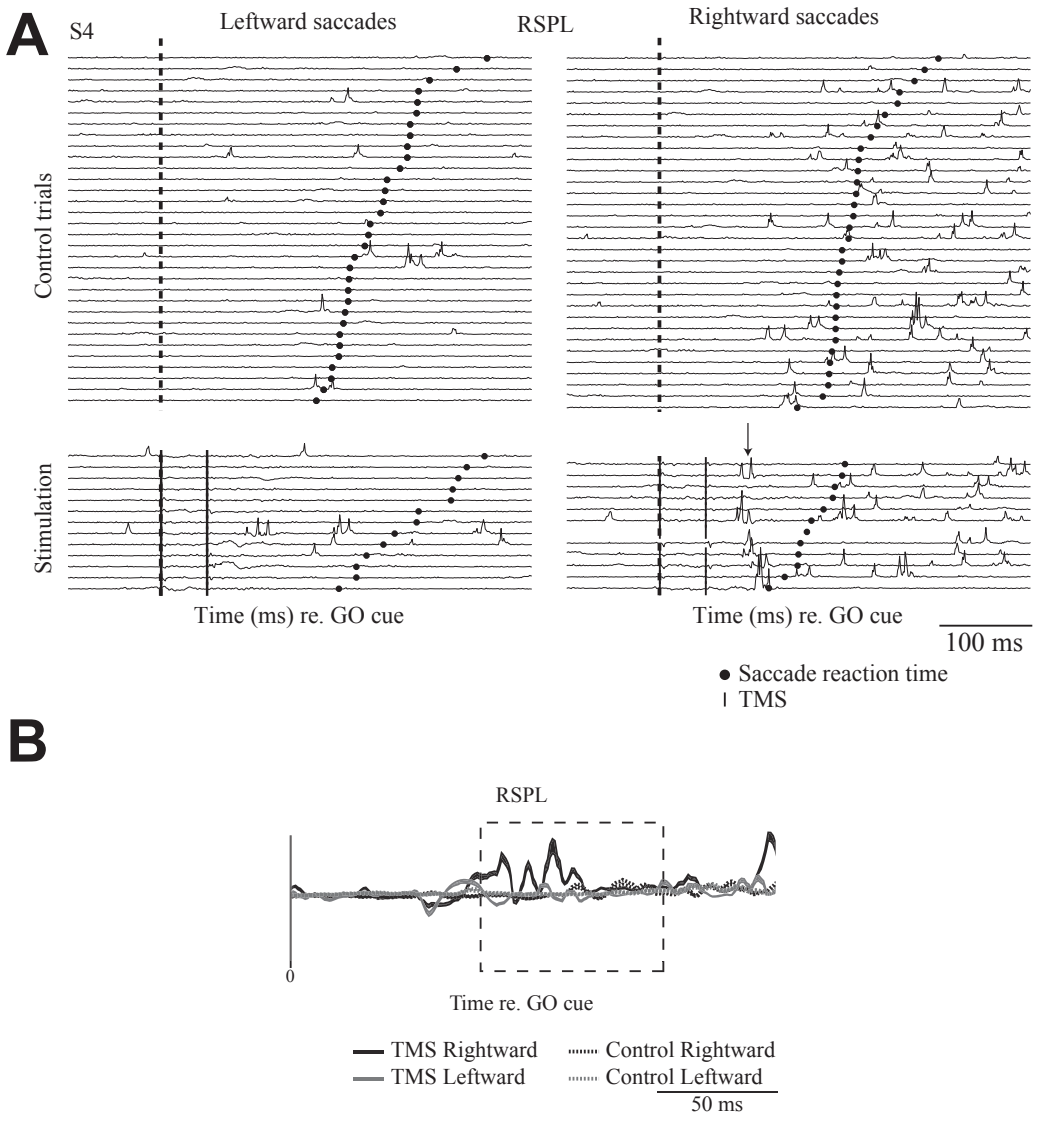

Figure 3: Human TMS-FEF Study

Single subject results from human TMS-to left FEF during the memory-guided saccade task. (A) Individual EMG traces from the right splenius neck muscle, a rightward head turner muscle, separated by non-TMS (top), TMS (bottom) and leftward (ipsilateral, left) or rightward (contralateral, right) saccade trials aligned to GO cue (vertical dash line). Note the increased EMG activity time-locked to TMS (vertical solid line) on only contralateral TMS saccade trials (bottom right, arrow). (B) Average EMG activity from right splenius neck muscles. (Taken from Goonetilleke et al., 2011).

1.7 TMS in the Monkey Oculomotor System

Currently there are two studies that have combined TMS with oculomotor tasks in monkeys. Gerits and colleagues (2011) performed rTMS over either left or right FEF or as a control to M1, which was only 10 mm away from the FEF. They found no difference on SRT after rTMS-M1, demonstrating the spatial dependency of TMS on the oculomotor system. For both left and right rTMS-FEF they found a small (7 ms) decrease in visually guided saccade latency for both contralateral and ipsilateral saccades, suggesting an enhancement of the FEF. However the rTMS protocol used has previously been demonstrated to have suppressive effects in human M1 (Huang et al., 2005). The authors proposed that TMS was selectively suppressing fixation neurons rather than saccadic-related neurons. This study indicates that TMS can have a very specific localized effect in awake and behaving monkey similar to what has been reported in humans. The other study investigated on-line TMS with the anti-saccade task, where monkeys must look 180° diametrically away from the visual stimulus, with single pulse of TMS-FEF delivered around the time of the visual stimulus (Valero-Cabré et al., 2012). They demonstrated that on-line TMS could be performed in awake and behaving monkeys, and found a state-dependent effect of TMS in monkeys with only a decreased SRT in ipsilateral anti-saccades. However their results were also very small and only apparent when the results were pooled together. Both of these studies demonstrated the feasibility of the NHP oculomotor system as a potential animal model for the investigation of TMS, however both studies only reported modest change in SRT over multiple different sessions. The primary goal of this thesis is to investigate whether neck

muscle activity evoked by TMS may provide a more sensitive indicator of TMS effects on the primate oculomotor system.

1.8 Hypothesis and Predictions

Overall the effects of TMS on the oculomotor system are not well understood partly due to the tightly regulated nature of saccadic eye movements, which prevents a more sensitive behavioral measurement of the system. Based on the previous studies that have suggested neck muscle EMG activity could be a more sensitive indicator of FEF activity, we hypothesize that TMS to the monkey FEF will evoke transient neck muscle activity similar to that evoked from sub-saccadic ICMS of FEF (Corneil et al., 2010). If so, the effects of TMS should depend on both the intensity and location of the TMS coil. Secondly we hypothesize that double pulse TMS (20 Hz) paired with the memory guided saccade task will increase neck muscle EMG activity and decrease SRT in only contralateral saccade trials and have no effects on ipsilateral saccade trials in the same state-dependent manner of TMS as previous human TMS-FEF studies (Wipfli et al., 2001; Goonetilleke et al., 2011). The outcome of this study will therefore help establish and cross validate our NHP model with previous human TMS studies.

Chapter 2 – Methods

2.1 Animal Preparation

Three male macaque monkeys (two *Macaca mulatta*, monkeys *sp* and *zn*, and one *Macaca fascicularis*, monkey *al*) weighing approximately 13, 12 and 9 kg, respectively, performed in these experiments. All training, surgical, and experimental procedures were approved by the Animal Use Subcommittee of the University of Western Ontario, University Council on Animal Care (Appendix 1), and conducted in accordance with the Canadian Council on Animal Care policy on the use of laboratory animals which conforms to the guidelines laid down the National Institutes of Health regarding the care and use of animals for experimental procedures. The monkeys' health and weight were monitored daily.

Each animal underwent two surgeries. In the first surgery, a titanium head post for head restraint and a grid of receptacles that served as fiducial markers (10 mm spacing) were imbedded within an acrylic implant. A mixture of titanium and ceramic screws were used to secure the acrylic, with ceramic screws placed in the vicinity of where the TMS would be delivered. The ceramic screws were used to prevent distortion of the anatomical MRI images. The grid of receptacles was placed directly on the left (monkeys *sp* and *al*) or the right (monkey *zn*) anterior half the skull, covering all cortical areas anterior to the central sulcus. Each receptacle was filled with a 2-gram/liter copper (II) sulfate solution (monkeys *sp* [9 receptacles] and *zn* [6 receptacles]) or threaded to receive rods filled with the same solution (monkey *al* [10 receptacles]) (see Fig. 4B). This solution was highly

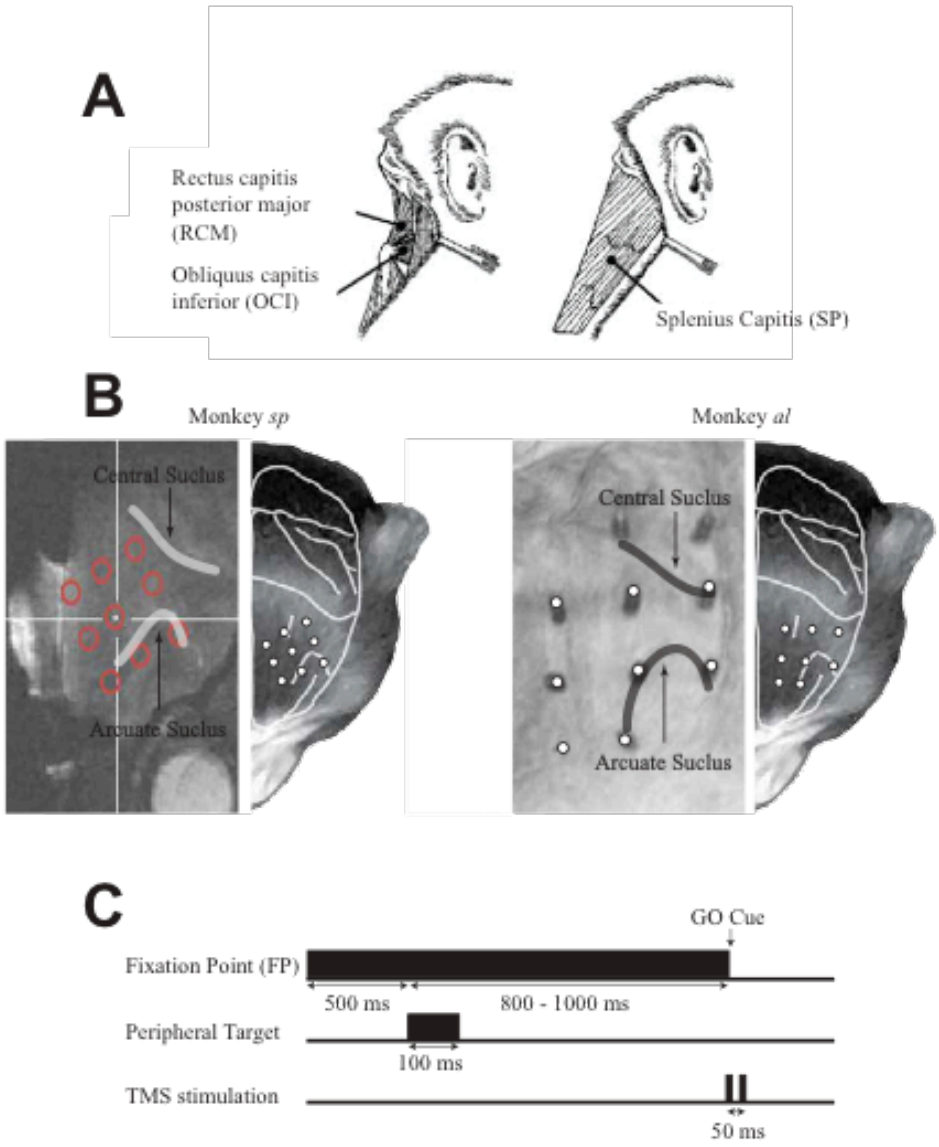

Figure 4: Methods

(A) Schematic line drawing of the targeted muscles for chronic EMG implant, bilateral implant of both deep, rectus capitis posterior major (RCM) and obliquus capitis inferior (OCI), muscles and the more superficial splenius capitis (SP) muscle. All three of these muscles are responsible for horizontal head turns. (B) The locations of the fiducial markers for both monkeys *sp* and *al* overlaid of the anatomical MRI scans, with the central and arcuate sulcus highlighted (left), and a representative head of a monkey with the estimated locations of the fiducial makers (right). (C) Schematic representation of the memory-guided saccade paradigm: The fixation (FP) was illuminated prior, during and after the flash of the peripheral target. The peripheral target was flashed 20° left or right of the FP for 100 ms. Then on ⅓ of all trials, double pulses of TMS (20 Hz, 50 ms apart) was delivered concurrently with the offset of the FP (also served as the GO cue).

visible during an anatomical MRI scan conducted for each monkey, permitting straightforward referencing of the receptacle locations with underlying cortical landmarks. In monkey *al*, the grid of receptacles was designed to also mesh with a mating plastic mold fit to the bottom of the TMS coil, offering a simple means of consistent TMS location day-to-day. In all monkeys, the thickness of the acrylic was kept as thin as possible over the intended locations of TMS (<10 mm, which was the height of the receptacle).

In the second surgery, chronically-indwelling bipolar hook electromyography (EMG) electrodes were implanted bilaterally into three dorsal neck muscles responsible for horizontal head turning (see (Elsley et al., 2007) for surgical details). The implanted muscles included two deep sub-occipital muscles: obliquus capitis inferior (OCI) and rectus capitis posterior major (RCM), and the more superficial splenius capitis (SP) muscle (see Fig. 4A). These muscles are responsible for the horizontal head turning synergy (Corneil et al., 2002b) and are robustly recruited by extracellular stimulation of the FEF (Elsley et al., 2007) and the SEF (Chapman et al., 2012). Leads from these electrodes were tunneled subcutaneously up to the skull and connected to a connector embedded within the acrylic.

2.2 Transcranial Magnetic Stimulation

TMS was applied over the acrylic implant using a MagStim Rapid Transcranial Magnetic Stimulator with a 25-mm radius per coil, figure-eight coil designed for peripheral nerve stimulation (MagStim Company, Spring Gardens, UK), with a peak

magnetic field of 2 Telsa. This coil has been previously used by other TMS studies with monkeys (Amaya et al., 2010; Gerits et al., 2011; Valero-Cabré et al., 2012). The TMS coil was held in position by a customized clamp anchored to the head post. The center of the TMS coil was placed directly on top of the receptacles or dental acrylic for monkeys *sp* and *zn*, or set by positioning a customized plastic mold on the bottom of the coil into the grid receptacles for monkey *al*. The coil was placed surface normal to the acrylic and rotated 45° clockwise from anterior-posterior directional current flow, to induce a posterior-medial to anterior-lateral direction current flow. Pilot results showed that coil orientation had a negligible effect on the evoked neck muscles response.

2.3 Behavioral Paradigm

The monkeys were placed in a customized primate chair (Crist Instruments, Hagertown, MD, USA). All experiments were conducted head-restrained in a dark room. Monkeys were placed 0.6 meters away from an array of red LEDs.

We delivered TMS in two experimental contexts. In the first context, single pulses of TMS were delivered when monkeys simply fixated at a fixation point (FP). Neck muscle responses were measured while TMS coil locations were varied systematically over the frontal cortex (the *mapping* experiment) or while the levels of TMS output were varied (the *intensity* experiment). Our rationale for requiring the monkeys to fixate straight ahead during TMS was because tonic neck muscle activity varies with eye-in-head position (Stuphorn et al., 1999; Corneil et al., 2002b). In the *mapping* experiment, the intensity of TMS was set to the lowest levels capable of reliably recruiting

contralateral neck muscle activity (45%, 40%, 35% of maximum stimulation output (MSO) for monkeys *sp*, *zn* and *al*, respectively). The coil was moved systematically to different locations, similar to a recent monkey TMS-M1 study (Amaya et al., 2010), covering different locations separated by either 5 or 10 mm. At each location on a given day, we delivered single pulse of TMS 25 times: the TMS was triggered manually while the monkey fixated at a central FP. For a given mapping session conducted within a single day, TMS was delivered up to 20 different locations, with the order of locations randomly selected at the beginning of the day. A total of 10 complete mapping sessions were collected for both monkeys *sp* and *al*; a total of 3 mapping sessions were collected for monkey *zn*.

Based on the results of the *mapping* experiment, the TMS location with the largest evoked contralateral neck muscle response was identified for monkeys *sp* and *al*. Using these locations, we examined the effect of systematic variations of the different levels of stimulator output (*intensity* experiment). First, we examined contralateral neck muscle recruitment, varying the intensity of TMS in 5% increments from 5% below to 15% above the TMS intensity level used in the *mapping* experiment (40-60% and 30-50% MSO for monkeys *sp* and *al* respectively). All other experimental details were the same as the *mapping* experiment. A total of 10 sessions were conducted for both monkeys, with the order of intensity settings within a single day selected randomly. Second, based on previous reports that TMS-M1 can suppress the activity of antagonist muscles at lower stimulation levels than that required to excite agonist muscles (Kimiskidis et al., 2005; Werhahn et al., 2007), we collected an additional series of 10 sessions with stimulator

output varying in 5% increments from 5% below to 25% below the *mapping* experiment (20-35% MSO and 15-30% MSO for monkeys *sp* and *al* respectively). During this experiment the monkeys fixated at a FP positioned 20° horizontally ipsilateral to the side of TMS coil, which increased the background EMG activity on the antagonist muscles of interest; all other experimental details were the same as in the *mapping* experiment.

In the second context, we delivered TMS in conjunction with a behavioral task. To facilitate comparisons, monkeys performed a memory-guided saccade task (Fig. 4C), with the timing of TMS matching that performed in previous human studies (Wipfli et al., 2001; Goonetilleke et al., 2011). To achieve a liquid reward, the monkeys first had to look at a central fixation point (FP) within a 6° radius window, maintaining central fixation before (500 ms), during (100 ms) and after (700-900 ms, varied randomly amongst 4 equally-spaced intervals) presentation of a peripheral visual target. The peripheral target was flashed 20° to the left or right of the FP. The monkey was allowed to saccade to the remembered location of the peripheral target within 800 ms after the disappearance of the FP (i.e., the disappearance of the FP served as the GO cue), within an 8° radius window. The peripheral target reappeared 100 ms after the monkey entered the window, and the monkey maintained fixation in the target window for an additional 300 ms. Two pulses of TMS (20 Hz, 50 ms apart) were delivered on one-third of all trials, with the first pulse of TMS coinciding with FP disappearance (Fig. 4C). Window size remained the same on trials with or without TMS. All trial conditions (left or right cue, with or without TMS) were pseudo-randomly interleaved within a session of at least 240 successful trials. Within such a session, the monkey had to complete a block of 30

trials (5 or 10 trials with or without TMS in each direction) before moving to the next block. The intensities of TMS was set at 20%, 25% and 25% for monkeys *al*, *sp*, and *zn*, respectively; these intensity were based on the lowest stimulator intensity that evoked an antagonist muscle response from the *intensity* experiment.

Across different sessions, the location of TMS was varied amongst three distinct groups. In the first group of sites, the *PFC group*, TMS was applied to sites on or anterior to the arcuate sulcus, where TMS in the *mapping* experiment evoked a contralateral head turning synergy (3, 4 and 1 different sites in monkeys *al*, *sp*, and *zn* respectively; similar EMG and behavioral results were obtained at all locations, and hence the data were pooled together in the results). This group of sites allowed us to test whether neck muscle responses evoked by TMS varied with an oculomotor task. In the second group of sites, the *auditory control group*, TMS was delivered 5 cm above the scalp (monkeys *al* and *sp*) directly over the PFC sites, to control for the acoustic noise of TMS pulses. In the third group of sites, the *brain control group*, TMS was applied to a site posterior to the arcuate sulcus (monkeys *al* and *sp*), where TMS was not able to evoke a contralateral head turning synergy in the *mapping* experiment. This group served to control for any tactile sensation associated with TMS, and also tested for spatial specificity of any effects seen with the *PFC group*. Importantly, the site tested in monkey *al* was the location from where TMS was capable of evoking a different profile of neck muscle activity, which we attributed to the delivery of TMS-M1 (see results). We observed no difference in the neck EMG or behavioral results obtained between the auditory control group and brain control

group, and hence have pooled the results from these two control groups together in the results.

2.4 Data Acquisition

Eye-in-head position was tracked with an eye-tracking system (ETL-200, iScan, Woburn, MA, USA) at 120 Hz. The processing of the EMG signals commenced at the headstage (Plexon, Dallas, TX, USA), which was plugged into the EMG connector embedded within the acrylic implant. The headstage performed differential amplification of the EMG signals (20× gain) and filtering (bandwidth, 20 Hz to 17 kHz). A flexible ribbon cable linked the headstage to the Plexon preamplifier, which contained a signal processing board customized for EMG recording (50× gain bandwidth, 100 Hz to 4 kHz). All analog signals were digitized to 10 kHz.

Off-line analyses were conducted with customized Matlab (The Mathworks, Nantick, MA, USA) programs. Further details regarding analysis windows are given in the Results. EMG signals were rectified and downsized into 1-ms bins, as previously described (Elsley et al., 2007). For the *mapping* and *intensity* experiments, trials with baseline EMG activity greater than 3 standard deviations away from the mean the pooled baseline were rejected. For the memory-guided saccade paradigm, a customized graphical user interface permitted trial-by-trial inspection. Trials with SRTs < 80 ms relative to the GO cue were rejected for being anticipatory saccades, whereas SRTs > 500 ms relative to the GO cue were rejected for presumed inattention. All trials with blinks were rejected for monkey *al*; while trials with blinks were accepted for both monkeys *sp* and *zn* (see

results for justification). Blinks had very distinct characteristic eye traces; both horizontal and vertical eye position changed instantaneously by > than 30°, and blinks were automatically marked and no SRT were given for those trials.

Chapter 3 – Results

All three monkeys acclimatized to delivery of TMS within the first day of application and showed no signs of discomfort. This allowed us to collect a substantial dataset from two monkeys (*sp* and *al*), and a smaller dataset from a third monkey (*zn*). Overall there was no different between the three difference muscles we recorded from; for the results we used the OCI muscles for monkeys *al* and *zn*, while for monkey *sp* we used SP muscles. For simplicity, we define a contralateral neck muscle as any muscle that turned the head away from the TMS coil, and an ipsilateral neck muscle as any muscle that turned the head towards the TMS coil. In the first set of experiments, we studied the effects of TMS while the monkeys simply maintained stable fixation. We collected a total of 10 mapping sessions from monkeys *sp* and *al*, and 3 sessions from monkey *zn*. From these mapping sessions, we identified the location evoking the largest neck muscle response, and used this location to study the effects of manipulating stimulator output on both agonist (contralateral) neck muscle recruitment and antagonist (ipsilateral) neck muscle inhibition (20 sessions total for both monkey *sp* and *al*). In the second set of experiments, across all three monkeys, we collected a total of 110 sessions consisting of at least 240 trials each in the memory-guided saccade paradigm: 73 sessions were the PFC group, 19 sessions were the auditory control group and 18 sessions were brain control group.

3.1 Single Pulse TMS

Despite head restraint, TMS applied to the frontal cortex over and anterior of the arcuate sulcus, near the FEF, in all three monkeys reliably increased the activity of contralateral neck muscles, and/or decreased the activity of ipsilateral neck muscle (Fig. 5). This evoked response began ~20 ms after the TMS pulse, and persisted for another ~30 ms (see shaded regions). Neck muscle activity then either returned to pre-stimulation levels, or rebounded above the pre-stimulation levels of activity on the ipsilateral muscles (e.g., 1st and 3rd rows of Fig. 5). In this figure, we have purposely retained the stimulation artifact on the EMG traces to show that it did not extend into the response window (lighter portion left of the shaded regions in Fig. 5; the artifact in monkey *al* were negligible). The EMG responses evoked by TMS evolved simultaneously on ipsilateral and contralateral neck muscles when both responses were present, but the decrease in ipsilateral neck muscle activity tended to be more reliable. To determine whether TMS evoked a significant response averaging across all trials, we used the 50 ms interval preceding TMS to define a 99% confidence interval (CI) for baseline activity for each session. For the examples shown in Fig. 5, the activity of contralateral and ipsilateral neck muscles within the response window of 20-50 ms after TMS for all three monkeys were significantly greater and lower than the 99% CI, respectively. Overall, the synergy and timing of the neck muscle responses evoked by TMS resembled that evoked by ICMS of both FEF (Elsley et al., 2007) and SEF (Chapman et al., 2012). Importantly, single pulse TMS to the frontal cortex never evoked a saccadic eye movement.

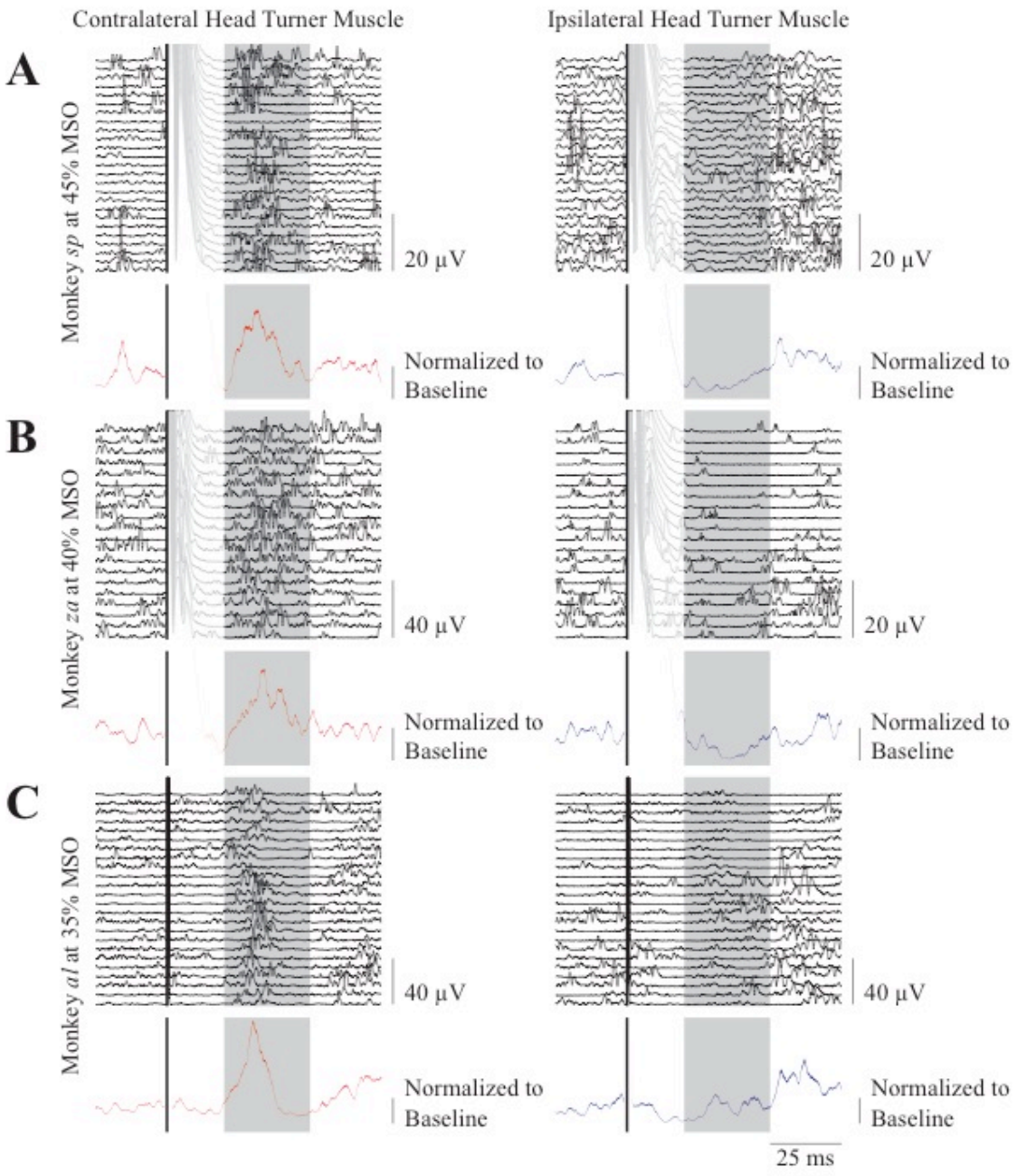

Figure 5: Contralateral head turning synergy from TMS

Sample EMG activity with single pulses of TMS to prefrontal cortex evoked a contralateral head turning synergy. Individual raw rectified EMG activity of the contralateral (left) and ipsilateral (right) neck head tuner muscles from a specific session aligned to TMS (black line). The mean ± standard error of the session normalized to baseline (mean activity −50 to −1 ms prior to TMS) within each session (bottom). There was an increase and decrease in activity for contralateral and ipsilateral head tuner muscle activity 20-50 ms after TMS (shaded box) for all three monkeys (above or below the 99% CI of baseline). For monkeys *sp* and *zn*, the TMS artifact occurred 0-20 ms after TMS (white box), but the artifact was outside of our response window.

3.2 Change in Response Over Frontal Cortex

We sought to determine how contralateral head turning response evoked by TMS changed with systematic changes in TMS location. In all three monkeys, we moved the TMS coil based on the grid provided by the fiducial markers embedded in the acrylic, this allowed us to map the evoked neck muscle response with a 5 mm resolution. As shown in Fig. 6, for monkeys *sp* and *al*, TMS applied progressively more anterior evoked larger excitation in contralateral neck muscles and more prominent inhibition on ipsilateral neck muscles. Thus, over a wide expansion of the frontal cortex, anterior to and including the arcuate sulcus, TMS evoked a contralateral head turning synergy. In both monkeys *sp* and *al*, the locations evoking the largest increases in contralateral neck muscles resided over the superior arm of the arcuate sulcus (asterisks in Fig. 6). At both of these sites, on a trial-by-trial basis TMS evoked an excitatory contralateral neck muscle response (greater than 3 standard errors above the trial baseline) on 40% and 45% of all trials for monkeys *sp* and *al*, respectively.

Although the increase and decrease on the contralateral and ipsilateral neck muscle activity appeared to evolve simultaneously, there were some subtle differences particularly for TMS at or slightly posterior to the arcuate sulcus. TMS at these locations occasionally evoked prominent decreases in ipsilateral neck muscle activity without changing contralateral neck muscle activity (e.g. 2nd row for monkey *sp* in Fig. 6A and 2nd and 3rd row for monkey *al* in Fig. 6B). Accordingly, the areas over which a reliable decrease in ipsilateral neck muscle activity could be evoked were larger than the areas over which an increase in contralateral neck muscle activity could be evoked (Fig. 6).

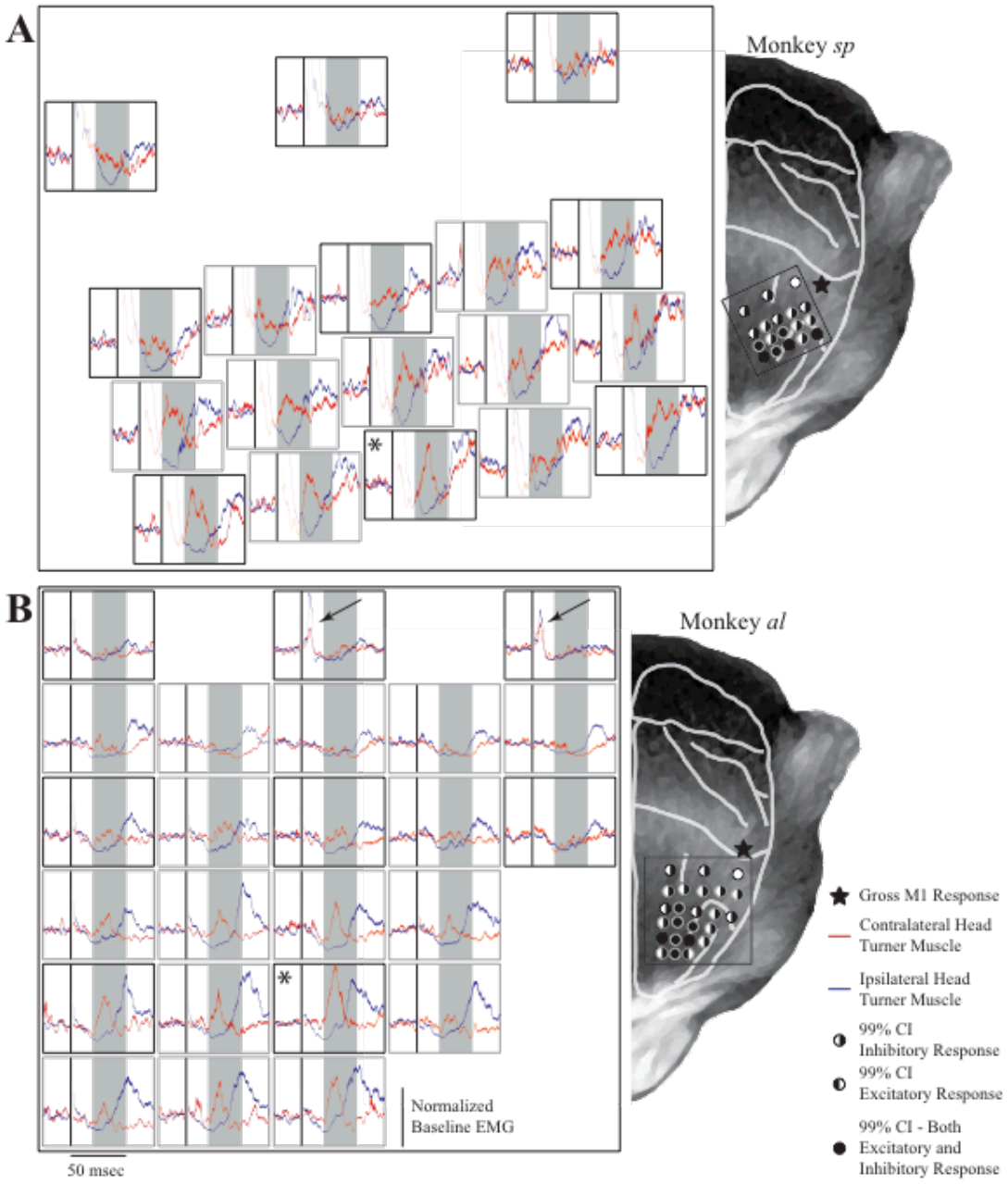

Figure 6: Spatial Specificity of TMS

The mean ± standard error of contralateral (red) and ipsilateral (blue) neck muscle activity aligned to TMS (black line), normalized to baseline activity at each site (left). The response window of the contralateral head turning synergy occurred at 20-50 ms after TMS (shaded box). The depicted sites were approximate locations of markers projected onto a representation of a monkey's head for both monkeys *sp* and *al* (right), filled left and right semi-circles indicate below and above 99% CI of baseline activity for ipsilateral and contralateral neck muscle. The largest evoked response was identified (asterisk). Location where TMS evoke a gross contralateral and twitch (star), note for monkey *al* the bilateral co-contraction of neck muscle ~5 ms after TMS (arrow).

3.3 TMS-M1 MEP Response

In monkeys *sp* and *al*, TMS 10% higher than the intensity used for the *mapping* experiment evoked an observable twitch on the contralateral hand when applied near the central sulcus (stars in Fig. 6). We presumed that these locations corresponded to the hand representation of M1, which have been previously reported in monkey TMS studies (Edgley et al., 1997; Amaya et al., 2010; Valero-Cabré et al., 2012). During our *mapping* experiment in monkey *al*, we encountered locations slightly anterior and medial to the hand representation where TMS evoked a distinct response consisting of bilateral neck muscle recruitment (arrows in the 1st row in Fig. 6). This bilateral response began within ~5 ms of the TMS pulse, and lasted only ~5 ms in total. Following this response both contra- and ipsilateral neck muscles exhibited a brief period of reduced activity for another ~20 ms before returning to baseline activity. As we will expand upon in the Discussion, the time and profile of this response are consistent with a different pathway than the oculomotor system, possibly through either a cortico-spinal or cortico-reticulospinal pathway. Unfortunately, the longer stimulation artifacts in both monkeys *sp* and *zn* obscured our ability to replicate this observation. Note however in monkey *sp* that TMS at the most posterior locations (1st row in Fig. 6A) did produce a hint of bilateral suppression that differed from the other neck muscle responses evoked at more anterior locations; such bilateral suppresion may correspond to the brief period of reduced activity observed in monkey *al* after bilateral co-contraction.

3.4 Change in Response with TMS intensity

Having established the TMS locations that recruit a contralateral head turning synergy, we next examined the role of TMS intensity on the response. For both monkey *sp* and *al*, we modulated stimulator output while delivering TMS to the sites with the largest and most reliable response (asterisks in Fig. 6). Increasing TMS intensity increased the magnitude of the contralateral neck muscle recruitment, but did not noticeably decrease the onset time of evoked response (Fig. 7A). Previous studies of human TMS to M1 have shown that TMS can inhibit antagonist muscle activity at lower levels than that required to excite agonist muscle activity (Kimiskidis et al., 2005; Werhahn et al., 2007). We therefore separately determined the lowest TMS intensity capable of suppressing the activity of ipsilateral neck muscles. For this experiment, monkeys fixated 20° ipsilateral to the side of TMS, increasing tonic background activity of ipsilateral neck muscle. In both monkeys *sp* and *al*, a substantially lower TMS intensity could inhibit ipsilateral neck muscle activity, compared to that required to evoke excitation of contralateral neck muscle (Fig. 7B). Based on these results, we determined that MSO settings of 25% and 15% were capable of evoking ipsilateral neck muscle response for monkeys *sp* and *al* respectively, analogous to the active motor threshold.

3.5 TMS During Memory-Guided Saccade Task

We now turn to the effects of TMS to the frontal cortex during an oculomotor task. A central tenet of TMS in humans is state-dependency, wherein the effects of TMS vary

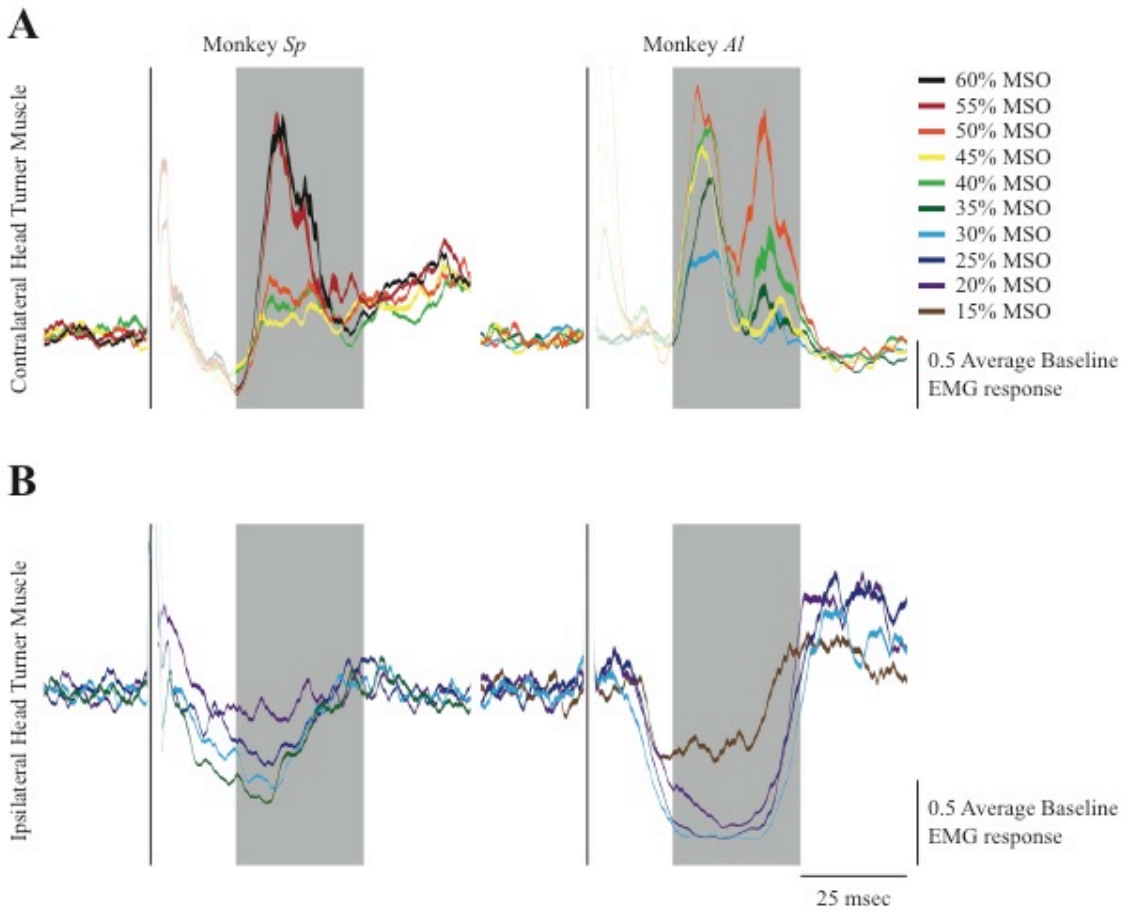

Figure 7: Intensity modulation of TMS

(A) Contralateral neck muscles response aligned to TMS pulse (back line), with a central FP at various TMS intensity for monkey *sp* (left) and monkey *al* (right). Increases in TMS intensity increased contralateral neck muscle activity within the response window (20-50 ms after TMS, shaded box). (B) Ipsilateral neck muscle response aligned to TMS, with FP at 20° ipsilateral to the side of TMS for monkeys *sp* and *al*. The color of asterick respresents the lowest intensity to evoke a response (outside of the 99% CI from baseline). Not the difference in the TMS intensity to evoke an excitatory response on the contralateral neck muscle muslce and the intensity for an inhibitory response (40% MSO vs. 25% MSO for monkey *sp* and 30% MSO vs. 15% MSO for monkey *al*).

with the endogenous activity in an area at the time of stimulation (for review Silvanto et al., 2008).To test state-dependency, we occasionally delivered low-intensity TMS while monkeys performed both contralateral and ipsilateral memory-guided saccades, with respect to the TMS coil. Much of the PFC, including the FEF, is more activity before contralaterally-directed saccades (Bruce and Goldberg, 1985; Funahashi et al., 1989; Tsujimoto and Sawaguchi, 2004), hence TMS should evoke greater levels of neck muscle activity when delivered before contralateral compared to ipsilateral saccades. For this experiment similar to the TMS protocol of the previous human TMS-FEF studies (Wipfli et al., 2001; Goonetilleke et al., 2011), we delivered 2 pulses of TMS (20 Hz) aligned to the GO cue, at the active motor threshold MSO setting as determined from the *intensity* experiments described above (25% MSO for monkeys *sp* and *zn*, 20% MSO for monkey *al*).

3.6 Increase in EMG activity Only for Contralateral Saccades

Figure 8A shows contralateral neck muscle activity for individual trials from a single session for monkey *sp*, segregated by saccade direction and whether TMS (blue squares) were delivered to the PFC or not. All trials are aligned to the GO cue (green square; FP offset) and further sorted based on saccadic reaction time (red circle). Even in the absence of TMS, contralateral neck muscle activity increased just before contralateral saccades and remained tonically elevated, and decreased just before ipsilateral saccades and remained tonically suppressed. Such phasic and tonic coupling of neck muscle activity with saccades and eccentric eye positions have been previously described (Werner et al., 1997; Corneil et al., 2002a). However, TMS evoked a further increase in

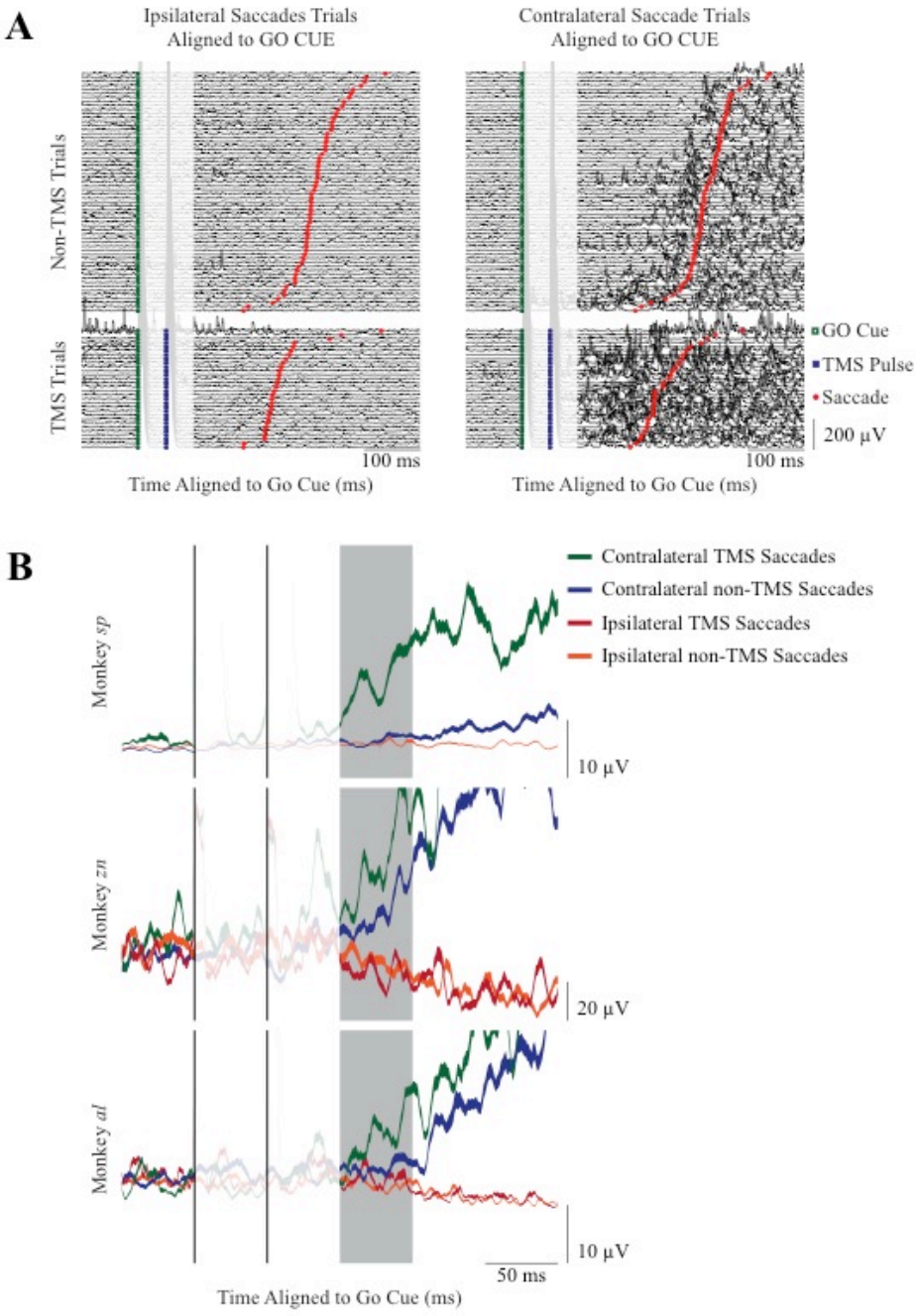

Figure 8: Single session TMS with memory-guided saccade task

(A) Individual trials from a single session of double pulse TMS (20 Hz, 50 ms apart) during the memory-guided saccade task from monkey *sp*, aligned to the GO cue (green square). Trials were separated by ipsilateral (left) or contralateral (right) saccades, and either non-TMS (top) or TMS (bottom, blue square). Trials were sorted by SRT (red circle). (B) The mean ± standard error of EMG activity for all four conditions from single sessions for all three monkeys. The plots are aligned to the GO cue, and the window of from 100 ms – 150 ms after GO cue (shaded box).

contralateral neck muscle activity only when delivered before contralateral saccades (lower-right plot in Fig. 8A); note the activity ~100-150 ms after the GO cue that is not present before contralateral saccades in the absence of TMS, nor before ipsilateral saccade whether TMS was delivered or not. Thus, as predicted by state-dependency, TMS only increased contralateral neck muscle activity when delivered to the PFC that is presumably more active for the contralateral memory-guided saccade.

Representative session examples from all three monkeys are shown in Fig. 8B (using the session shown in Fig. 8A for monkey *sp*). To quantify such state-dependency, we first calculated the average total EMG activity from 100-150 ms after the GO cue for all 4 conditions within a session, excluding any trials with SRTs < 175 ms to exclude phasic changes in neck EMG associated with saccade onset. For the representative examples for all three monkeys, the greatest level of EMG activity in the window of interest occurred on trials where TMS preceded contralateral saccades. Consistent with state-dependency, such evoked activity was a function of both saccade direction and TMS.

Across our sessions, we first compared the effects of the presence or absence of TMS before contralateral saccades. For TMS-PFC sessions, neck muscle activity was consistently larger 100-150 ms after GO cue, for TMS trials compared to non-TMS trials (Fig. 9A; 17% median increase, $p < 1 \times 10^{-7}$, paired t-test), note that sessions clustered above the line of unity (dash line). In contrast, when TMS was applied to control sites either posterior to the arcuate (brain control site) or into the air (sound control), we

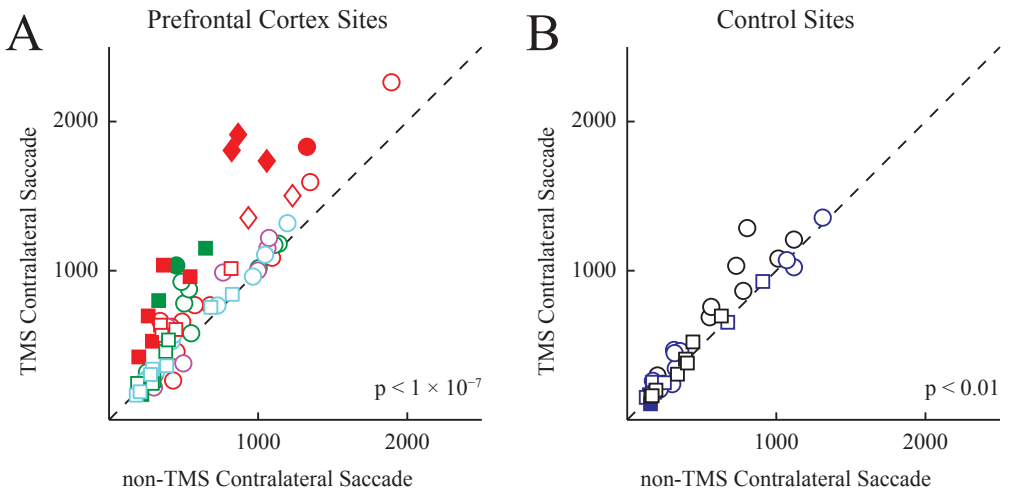

Figure 9: Group comparison of TMS vs. non-TMS contralateral saccade trials

Comparisons between the mean EMG activities of contralateral neck muscle within the window of interest (100-150 ms after GO cue) for TMS vs. non-TMS contralateral saccade trials fro PFC sites (A) and control sites, plotted with the line of unity (dashed line). There was a significant greater median increase for PFC (17% increase, $p < 1 \times 10^{-7}$) sites compared to control sites (6% median increase, $p < 0.01$). Each symbol represents a single sessions, different color represents a different location, red and green – anterior of arcuate sulcus, purple and light blue – slightly posterior to arcuate sulcus, black – brain control site, dark blue – sound control. Different shape represents a different monkey, square – monkey *sp*, circle – monkey *al*, diamond – monkey *zn*. Filled symbol represents significant within a session Bonferroni corrected.

observed much more modest increases in neck EMG before contralateral saccades when TMS was applied (Fig. 9B; 6% median increase, $p < 0.01$). The significant that was seen on the control may be due to the bilateral decrease in SRT on TMS trials compared to non-TMS trials, the increase EMG may just be associated with an saccade-related EMG activity. Importantly, some of the sites visited for the brain control group were the sites from where bilateral neck EMG could be evoked; TMS at such sites did not evoke a state-dependent effect in this oculomotor task, emphasizing the spatial specificity of the effect of TMS-PFC on neck EMG. Finally, there was no effect of TMS before ipsilateral saccades regardless of whether TMS was applied to the PFC or to control sites (data not shown; TMS-PFC: 1% median decrease, $p = 0.43$: TMS-control: 1% median decrease, $p = 0.85$).

Next, we compared the effects of TMS delivered before contralateral versus ipsilateral saccades. Since the prefrontal cortex is more actively engaged before a contralateral versus ipsilateral memory-guided saccade, state-dependency predicts larger neck EMG responses when TMS precedes contralateral saccades. Figure 10 plots the mean EMG activity of contralateral vs. ipsilateral TMS saccade trials from each session, for both PFC sites (A) and control sites (B). For the PFC sites, there was a significant deviation above the line of unity; representing a larger average EMG activity for TMS contralateral saccades compared TMS ipsilateral saccades (24% median increase, $p < 1\times10^{-7}$). For TMS to control sites, we also observed greater activity when TMS preceded contralateral versus ipsilateral saccades (13% median increase, $p < 1\times10^{-3}$). However, we suspected that the effect of TMS at control sites were mainly associated with the

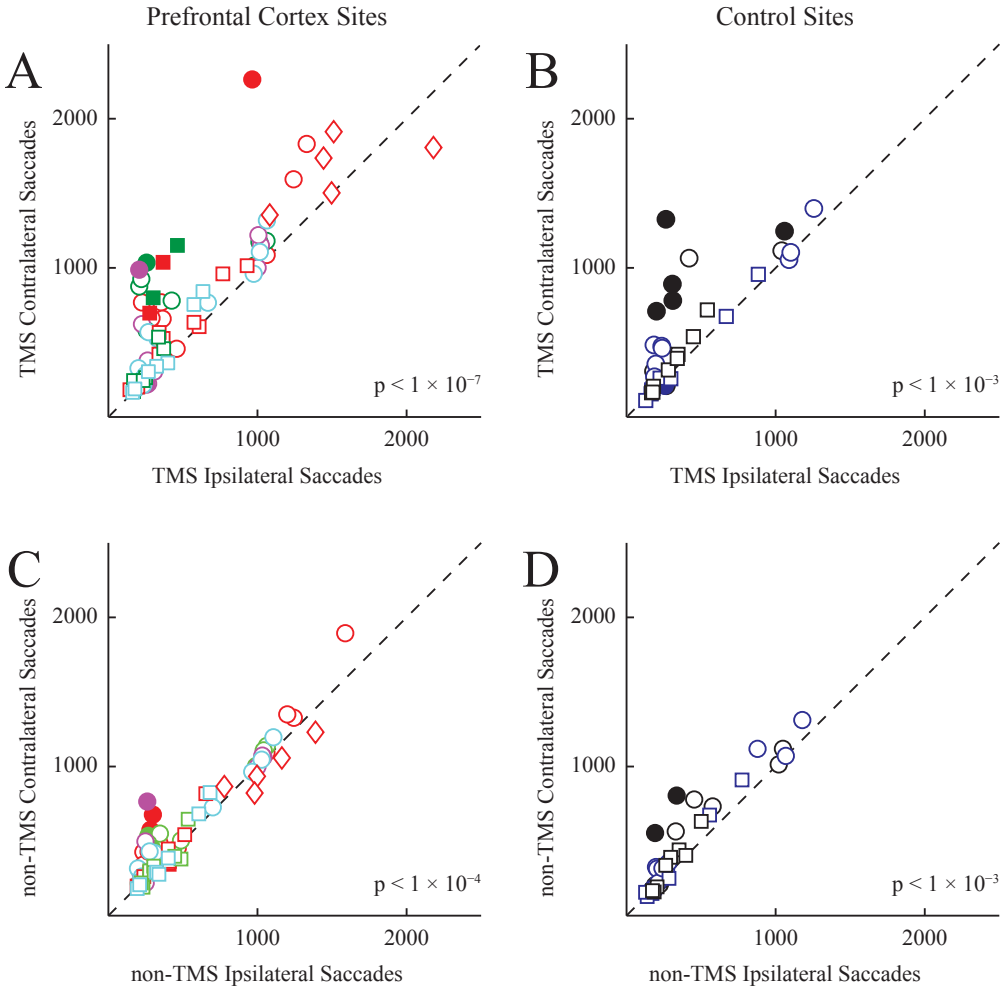

Figure 10: Group comparison of TMS contralateral vs. TMS ipsilateral saccade trials

Comparisons between the mean EMG activities of contralateral neck muscle within the window of interest (100-150 ms after GO cue) for TMS contralateral vs. TMS ipsilateral saccade trials for PFC sites (A) and control sites, plotted with the line of unity (dashed line). Once again there was a significantly greater median increase for PFC (24% median increase, $p < 1\times10^{-7}$) compared to control sites (13% median increase, $p < 1\times10^{-3}$). The increase in the TMS contralateral vs. ipsilateral saccades for the control sites was most likely due to movement related activity. Comparing the non-TMS contralateral vs. non-TMS ipsilateral saccade trials for both PFC and control sites, there was a similar increase for contralateral saccades (6% median increase, $p < 1\times10^{-4}$, and 11% median increase, $p < 1\times10^{-3}$, respectively). Each symbol represents a single session; different color represents a different location, red and green – anterior of arcuate sulcus, purple and light blue – slightly posterior to arcuate sulcus, black – brain control site, dark blue – sound control. Different shape represents a different monkey, square – monkey *sp*, circle – monkey *al*, diamond – monkey *zn*. Filled symbol represents significant within a session Bonferroni corrected.

preparation of contralateral saccades, as the same effects were observed for trials without TMS for both PFC and control sites (Fig. 10C, D; 6% median increase, $p < 1\times10^{-4}$, and 11% median increase, $p < 1\times10^{-3}$, for the non-TMS PFC and non-TMS control sites, respectively). Nevertheless, the effects seen on contralateral saccade are much greater than the non-TMS control trials and suggests that the increased in EMG activity is due to TMS effects on the PFC. For monkey *sp*, TMS-PFC often induced blinks (59% of all TMS-PFC trials), which obscured our ability to extract SRT from such trials. Blink trials from monkey *sp* were included in the above EMG analysis provided the eye movements after the blink attained to the target. To ensure that blinks did not confound our results from this monkey, we compared the evoked neck EMG response preceding contralateral saccades 100-150 ms after the GO cue on TMS trials with and without blinks, and found no differences ($p = 0.52$, t-test). For monkey *al*, blinks rarely occurred on TMS-PFC (<1%), and hence we simply discarded blink trials as errors.

3.7 Decrease in Contralateral Saccade Reaction Time

Next we examined whether TMS influenced SRT. In humans, TMS-FEF in this task decreased contralateral but not ipsilateral SRT (Wipfli et al., 2001; Goonetilleke et al., 2011). We pooled all trials without blinks for monkeys *sp* (Fig. 11A) and *al* (Fig. 11B), and segregated the data by saccade direction, presence or absence of TMS, and whether TMS was applied to the PFC sites or the control sites. Across all conditions and in both monkeys, TMS shortened SRT compared to non-TMS trials. For monkey *sp*, TMS decreased contralateral SRT 62 ms for PFC sites and 44 ms for control sites. TMS decreased ipsilateral SRT 14 ms for PFC sites and 19 ms for control sites. For monkey *al*,

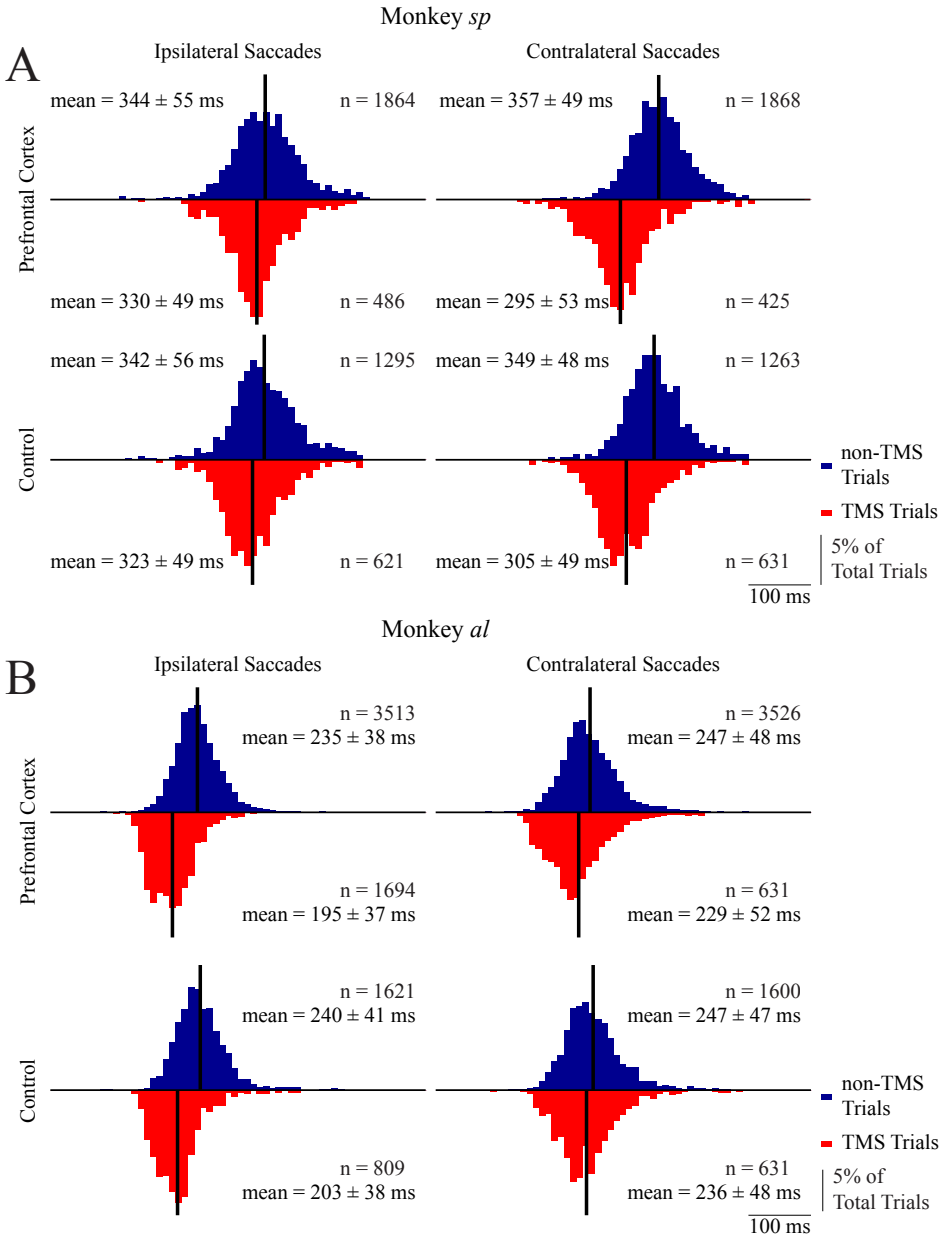

Figure 11: Saccadic reaction time for TMS memory-guided saccade task

SRT for monkeys *sp* (A) and *al* (B) in the memory guided saccade task. The SRT were separated based on ipsilateral (left) or contralateral (right) saccades to the TMS coil; stimulation site, PFC (top) or control sites (bottom), and whether TMS was applied (red) or not (blue). The SRT was binned in 8 ms bins. The mean for each of the conditions were also plotted (black vertical line). There was an interaction between TMS and location of TMS coil for only contralateral saccades ($p < 1\times10^{-5}$, and $p < 0.01$ for monkeys *sp* and *al*) but not for ipsilateral saccades ($p = 0.15$ and $p = 0.07$ for monkeys *sp* and *al*).

TMS decreased contralateral SRT 18 ms for PFC sites and 11 ms for control sites. TMS decreased ipsilateral SRT 40 ms for PFC sites and 37 ms for control sites. We wanted to test if TMS would decrease contralateral saccade trials only at PFC sites and not affect ipsilateral saccade trials at PFC or either contralateral or ipsilateral saccade trials at Control sites. We therefore separated the contralateral and ipsilateral saccade trials and performed 2 different 2-way ANOVA's based on TMS and location. Based on our hypothesis we expected an interaction between TMS and location for only contralateral saccade trials but not ipsilateral saccade trials. For both monkeys *sp* and *al* there was a significant interaction between TMS and location for contralateral saccade trials ($p < 1\times10^{-5}$ and $p < 0.01$, respectively) but no significant interaction between TMS and location for ipsilateral saccade trials ($p = 0.15$ and $p = 0.06$, respectively). This suggests that TMS had an interaction with site location for contralateral SRT but TMS had no interaction with site location for ipsilateral SRT, in a similar fashion as the previous human TMS-FEF studies.

3.8 Increase in EMG Response Correlates to Decrease in Reaction Time

We wanted to investigate if there was a correlation between greater EMG activity and greater decrease in SRT within each session, which was weakly correlated in the human TMS-FEF study (Goonetilleke et al., 2011). Other studies have correlated greater amount of EMG activity and SC activity with decreased SRT (Dorris et al., 1997; Rezvani and Corneil, 2008). With the high variance of EMG activity from trial-to-trial, we had to pool across sessions. We were only able to use the data from monkey *al*, due to an incomplete dataset from prevalence of blinking trials with monkey *sp*. For monkey *al*, we rank

ordered the difference between the mean SRT of TMS contralateral saccade trials and non-TMS contralateral saccade trials, we then ranked ordered the mean total EMG activity on TMS contralateral saccade trials. We tallied up the absolute difference between the two ranks for each session, and compared the difference to 1000 randomly shuffled pairs of ranking. The actual sum difference of our ranking compared to the randomly shuffled pairs was statically significant ($p < 0.05$) for TMS to PFC sites, but not significant ($p = 0.24$) for TMS to Control sites. This indicates that there was a weak correlation between larger EMG activity for contralateral TMS saccade trials and a greater decrease in contralateral SRT between TMS and non-TMS trials for only PFC sites but not control sites for one monkey.

Chapter 4 – Discussion

With TMS we were able to evoke a contralateral head turning synergy, which involved an increase and/or decrease in the activity of contralateral and/or ipsilateral neck muscles, respectively. This orienting response on the neck was prominent throughout the PFC but was not seen over premotor or motor cortex. In addition the response was task modulated, with TMS evoking a larger response on the contralateral neck muscles before contralateral saccades. Both the spatial specificity and state-dependency of TMS were consistent with previous human TMS-FEF studies and confirms the validity of our NHP oculomotor system model. Neck muscle recording response to TMS provide a simple and objective indicator of the activation of the oculomotor system, which can be considered in some ways analogous to an MEP response of TMS-M1.

4.1 TMS Over PFC Evoked Contralateral Head Turning Synergy

The contralateral head turning synergy, an increase in contralateral neck muscle activity concurrent with a decrease in ipsilateral neck muscle activity, can be considered a hallmark of neck muscle response to oculomotor activity. ICMS of different oculomotor structure all elicit this response; from the FEF (Elsley et al., 2007), and the supplementary eye fields (SEF) (Chapman et al., 2012) within the frontal cortex, to both the SC (Corneil et al., 2002a) and the interstitial nucleus of Cajal (Farshadmanesh et al., 2008) within the brainstem. ICMS-FEF evoked responses 20-25 ms after stimulation onset, even when stimulation failed to evoke a saccade (Corneil et al., 2010). Here with

TMS we evoked neck muscle response starting at ~20 ms after TMS onset, suggesting that we may also be activating FEF at a sub-saccadic level.

One surprising result of TMS to the PFC was an apparent dissociation between contralateral neck muscle facilitation and ipsilateral neck muscle inhibition; inhibitory responses could be evoked over a wider area of PFC (Fig. 6), and persisted at lower TMS intensities (Fig. 7). Dissociation of excitatory and inhibitory effects has been previously reported in human TMS-M1 studies, with inhibition persisting at lower TMS intensities compared to that required to evoke an MEP (Triggs et al., 1992; Werhahn et al., 2007), and over a larger area of cortex compared to that capable of evoking an MEP (Wassermann et al., 1993; Wilson et al., 1993). A proposed hypothesis suggests that this could be attributed to decrease in spinal excitability (Chen et al., 1999). However these observations differed from that obtained with ICMS-FEF, where no obvious dissociation between the two responses was noted, even at sub-saccadic levels of stimulation current (Corneil et al., 2010). Nevertheless the ICMS-FEF study did not actively load tonic background activity like we did, so it could still possible that there was a dissociation that was not readily observable.

4.2 Contralateral Head Turning Synergy Evoked Only Over PFC

Although TMS was able to evoke a contralateral head turning synergy response over a large area, TMS to many sites was still incapable of evoking this response. The vast area of the monkey's head in which we applied TMS allows us to rule out many of the non-specific effects of TMS that could have explained the neck muscle responses. For

example if it was either an acoustic startle or tactile stimulation that evoked the contralateral head turning synergy, we would have observed this response regardless of where we TMS. However we only found this neck muscle response over PFC and not over motor or premotor cortex. Furthermore, TMS to monkey *al* at sites medial and anterior to the site capable of evoking an observable hand twitch (Fig. 6B) evoked a very distinct profile of rapid (5-10 ms) bilateral neck muscle contraction followed by a longer period (10-30 ms) of inhibition. We suspect that such bilateral contraction may originate from M1 through a corticospinal pathway, since the neck representation of M1 in humans lies medial to that of the upper limb and because TMS-M1 in humans produces bilateral MEPs on neck muscles within ~9 ms after TMS (Thompson et al., 1997). In addition the period of inhibition that follows the initial excitation has also been reported after hand muscle MEPs in humans (Wilson et al., 1993). An alternative pathway for this neck response may be a cortico-reticulospinal pathway. Recent work done in anaesthetized monkeys has also demonstrated robust activation of the pontomedullary reticular formation in the brainstem from TMS-M1 (Fisher et al., 2012). Importantly, the application of TMS to these putative M1 locations during the memory-guided saccade task did not evoke the state-dependent profiles of neck muscle recruitment seen with TMS-PFC. This observation reinforces the spatial specificity of the effects we observed, implicating a descending signal through the oculomotor pathway rather than a motor pathway.

The ability to distinguish these two different profile of neck muscle activity demonstrates that any neck muscle activity evoked by TMS were not due to a simple

startle response. The profile of neck EMG evoked by TMS at latency < 25 ms was distinctly different than that associated with startle evoked by loud (113 dB) acoustic stimuli, which in humans evokes bilateral neck muscle co-contraction after ~60 ms of the stimulus onset (Oude Nijhuis et al., 2007). Additionally, such spatial specificity also differs from a recent report wherein TMS could perturb ongoing saccadic trajectory regardless of the site of TMS application (Xu-Wilson et al., 2011).

4.3 TMS-PFC Affects Multiple Cortical Regions of Oculomotor System

Given the extent of the area from which TMS evoked a contralateral head turning synergy, we suspect that the biophysical effects of TMS are not limited to the FEF, but may additionally include the dlPFC located just anterior of the FEF and on the gyral surface. Modeling studies in humans suggest that the highest electrical field strengths occur at gyral crowns, and drop off substantially in the sulcus (Thielscher et al., 2011). In monkeys, the FEF is situated on the anterior bank of arcuate sulcus and can extend up to ~7 mm below cortical surface (Bruce and Goldberg, 1985).

Historically the FEF has been defined as any region evokes an saccade with ICMS at 50 μA of electrical current (Bruce et al., 1985). However anatomical tracer studies (Barbas and Pandya, 1989) and functional connectivity studies (Hutchison and Everling, 2013) have shown that dlPFC is highly connected with both FEF and SEF, from where robust recruitment of contralateral head turning synergy can be evoked via ICMS (Elsley et al., 2007; Chapman et al., 2012). dlPFC neurons also project directly to the SC (Kuypers and Lawrence, 1967; Goldman and Nauta, 1976). Anecdotally, ICMS of the

region just anterior of the FEF, presumably in dlPFC, also evokes the same neck muscle response, although this observation was not systematically investigated (Elsley et al., 2007). Taken together with these anatomical findings, our results suggest that dlPFC may have a role in contributing to head orienting during gaze shifts.

Like the FEF, neurons in the dlPFC are also activated during memory-guided saccades. Neurophysiological recordings have found similar sustained increased firing rate during the delay period for both FEF (Bruce and Goldberg, 1985) and dlPFC (Tsujimoto and Sawaguchi, 2004). High-intensity TMS to human dlPFC during the memory-guided paradigm has also shown to increase contralateral saccade errors (Müri et al., 1996), it suggest that TMS was disrupting working spatial memory causing the increase error rates. Here we delivered low-intensity TMS to PFC and found facilitation on neck muscle EMG on contralateral saccades, suggesting that TMS enhanced both the FEF and dlPFC. While seemingly paradoxical, where TMS to similar regions of the brain can have both facilitative and disruptive effects, this effect has also been seen with ICMS in the SEF. ICMS-SEF have shown to increase error rates for anti-saccades bilaterally, while at the same facilitating contralateral neck muscle activity (Chapman and Corneil, 2014), demonstrating the multi-faceted effects of stimulation to the oculomotor network.

4.4 General Conclusion

Our results demonstrate that neck muscle EMG activity provides a rapid assessment of oculomotor activity. Thus, neck muscle response to TMS may provide a functional localizer for frontal oculomotor structures for TMS in both humans and

monkeys, analogous to an MEP response from TMS-M1. This rapid assessment has been lacking for TMS in the oculomotor system for both humans and monkeys. We were able to demonstrate on a trial-by-trial basis of transient activation of the oculomotor system. Furthermore, based on the memory-guided saccade task, we demonstrate that while there was only a minor change in saccadic behavior when we pooled all of our trials together, we were able to see more prominent changes in neck muscle responses on a session-by-session basis.

Both the transient TMS application and TMS application with the memory-guided saccade task results strengthen the feasibility of NHP oculomotor system for investigating TMS along with previous NHP studies (Gerits et al., 2011; Valero-Cabré et al., 2012). We demonstrated that TMS selectively enhanced contralateral neck muscle activity for only contralateral memory-guided saccade trials, time-locked to GO cue (100-150 ms). From previous studies we know that the underlying PFC, both dlPFC and FEF, are more engaged during contralateral memory-guided saccades compared to ipsilateral memory-guided saccades. This suggests that TMS summated with endogenous activity of both FEF and dlPFC to facilitate neck muscle responses. Moreover the neck muscle response that we evoked during the memory-guided saccade task in our NHP model shows strong similarity with the previous human TMS-FEF study (Goonetilleke et al., 2011), therefore cross validating our animal model for future neurophysiological studies.

Based on the prior knowledge of the oculomotor pathway, we suspect that the TMS activates PFC and the signal is fed downstream to the head premotor circuitry, via the superior colliculus, and then down to the neck muscle. Future directions of this TMS

animal model will be to study the neurophysiological effects of the direct downstream (SC) and cortical (LIP and contralateral FEF, dlPFC) connections during both online and offline TMS. This result also demonstrates that neck muscle activity can be used as a rapid assessment of TMS for not only the oculomotor system with FEF, but also potentially for TMS on dlPFC, which is involved in higher-level cognitive tasks.

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Appendix

Appendix 1 – Ethic Protocol

2007-099-10::6:

AUP Number: 2007-099-10
AUP Title: Sensory and Motor Roles for Neck Muscles in Visually-Guided Actions: Neural Mechanisms Underlying Recruitment and Kinesthesia

Yearly Renewal Date: 01/01/2014
The YEARLY RENEWAL to Animal Use Protocol (AUP) 2007-099-10 has been approved, and will be approved for one year following the above review date.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office.
   Health certificates will be required.

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.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Savage, Colleen
on behalf of the Animal Use Subcommittee

Curriculum Vitae

Chao Gu

DATE OF BIRTH
November 10th, 1990. Shanghai, China

CITIZENSHIP
Canadian

PROFESSIONAL STATUS
Graduate Student, Graduate Program in Neuroscience, Western University

EDUCATION
Ph.D. Psychology – Western University, London, Ontario, Canada. 2014-present
M.Sc. Neuroscience – Western University, London, Ontario, Canada. 2012-2014
B.Sc. Life Science – Queen's University, Kingston, Ontario, Canada. 2008-2012

TEACHING EXPERIENCE
2012-2013: Teaching assistant, Western University: Undergraduate Psychology 1000
2014: Teaching assistant, Western University: Graduate Neuroscience 9500

MEMBERSHIP IN PROFESSIONAL SOCIETIES
Society for Neuroscience
Society for Neural Control of Movement

ABSTRACTS
Gu, C., Stevens, T., Thielscher, A., Bell, A.H., and Corneil, B.D. (2013) Development on an animal model for the effects of transcranial magnetic stimulation on the primate oculomotor system. *Gordon Research Conference on Eye Movements.*

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