TIME-DEPENDENT CONTRIBUTION OF PRIMARY MOTOR CORTEX TO VISUOMOTOR MEMORY RETENTION

by

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Abstract

The acquisition and retention of a visuomotor skill, such as accurate arm reaching to visual targets, involves a distributed network which includes the primary motor cortex (M1). While studies using electrophysiology, functional imaging and transcranial magnetic stimulation have revealed evidence for the role of M1 in motor learning and memory, little is known about the details of the processing occurring in M1 which underlies this role. In the work described in this thesis, we attempted to investigate the nature of that processing by a temporally specific disruption of M1 activity using single-pulse transcranial magnetic stimulation (TMS). The formation of an internal model of a novel visuomotor relationship requires the processing of error information to update the model, resulting in improvement in performance over time. It is likely that disruption of this processing would result in impaired performance. Thus, we hypothesized that during adaptation to a novel visuomotor relation, disruption of activity in M1 when it is responding to error but not at other times would impair the process that learns from error. To study this, we applied single-pulse TMS to M1 during adaptation of rapid reaching movements (~150ms duration) to a gradual visuomotor transformation. M1 was stimulated either immediately after the end of the trial, which was estimated to be the time when a significant amount of feedback was arriving from the periphery, or with a 700 ms delay following the trial end. Subjects who received immediate-TMS and delayed-TMS both exhibited normal adaptation. However, while the subjects who received delayed-TMS showed normal rates of forgetting during de-adaptation, those who received immediate-TMS showed a significantly faster rate of forgetting suggesting that the retention of the formed motor memory was impaired in these subjects. These results support the role of M1 in the formation of motor memory in a time-dependent manner relative to task events.

Advisor: Dr. Reza Shadmehr

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To Dr. Reza Shadmehr and members of the Shadmehr lab for a scientifically and personally enriching two years, and to my parents and my brother for their love and enduring support.

Contributions of Collaborators

The research presented here was a collaborative effort between Dr. Arash Hadipour-Niktarash and the author. Conceptualization of the project, collection of the experimental data, and analysis of the data were conducted jointly with Dr. Hadipour-Niktarash.

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1 Introduction

Acquiring and retaining a motor skill involves a distributed network in the brain. Experimental data from electrophysiology, imaging and clinical studies have gradually revealed the involvement of different areas of the brain in motor learning in increasing detail. Meanwhile, mathematical theories of motor control, combined with psychophysics studies, have generated models of the computations which underlie motor learning. One concept that is commonly shared between these computational models is the idea of a system that receives both internally-generated predictions of sensory (e.g. visual, proprioceptive) feedback and actual sensory information from the periphery (Shadmehr & Wise, 2005). The predictions are compared against the actual feedback, and the error signal subsequently derived from this comparison is used by the system to update the internal model, essentially the basis of learning. While this concept of feedback processing for error correction is a crucial one in models of motor learning, the neural correlate of the locus in which processing occurs is still a subject of inquiry. It is reasonable that there is no single area which serves in this role; rather, it seems that there are different areas that are involved in such processing depending on the specifics of the task being learnt. For example, the cerebellum is thought to be an error signal generator for tasks such as saccade adaptation. In addition, recording experiments on non-human primates have found evidence for neural correlates of internal models of reaching movements in the supplementary motor area (Padoa-Schioppa et al., 2004), premotor cortex (Padoa-Schioppa et al., 2002), and primary motor cortex (Li et al., 2001; Paz et al., 2003).

Recent experimental work has shown that the primary motor cortex (M1) is likely to be involved in various aspects of motor learning. Previously, findings such as the discovery of plastic changes in M1 following amputations hinted at the possibility of its involvement in higher order functions (Sanes & Donoghue, 2003), and motivated a shift away from the classical

viewpoint that M1 functions solely as an "output" motor execution structure (Scott, 2003). Evidence for changes in M1 during adaptation to force-field and visuomotor perturbations were found in primate electrophysiology studies (Li et al., 2001; Paz et al., 2003), providing a stronger basis for inferring the necessity of M1 in learning. Recent data from repetitive transcranial magnetic stimulation (rTMS) studies (Muellbacher et al., 2000; Baraduc et al., 2004; Richardson et al., 2006) have begun to support the causal nature of M1 involvement in motor learning. However, much remains unclear about the exact role of M1 in motor adaptation and retention.

In this work, we explored the temporal specificity of M1 involvement in learning and retention of a visuomotor skill by transiently disrupting activity in the M1 of normal human subjects in a temporally-controlled manner using single-pulse transcranial magnetic stimulation (TMS). We hypothesized that M1 may contribute to the processing of feedback received from the periphery to update an internal model, resulting in learning and retention of the skill. In addition, we predicted that this contribution of M1 occurs in a time-dependent manner, likely taking place when the brain is responding to error information but not at other times. In our experiments, we found that disruption of M1 did not affect adaptation to a gradually implemented visuomotor rotation. However, we found evidence for impaired motor memory retention when M1 was disrupted immediately after the end of each trial, but not when disruption occurred with a 700 ms delay following trial end. These results and the implications of this work are discussed in the following chapters.

1.1 Motor learning

Motor learning as an expansive term refers to the achievement of a new level or new quality of performance by the motor system through the use of information about movements and the effect of such movements on the interaction of the system with the environment. This broad description of motor learning can be narrowed down in scope depending on the features of interest. For example, motor learning may entail the procedural learning of a motor skill to fine-tune

spatiotemporal muscle-activation patterns, or it may involve the acquisition of novel associations between environmental cues and motor actions (Sanes & Donoghue, 2000). It may be categorized according to other sensory modalities involved, such as vision or audition. It can also be differentiated into different types depending on whether the learning requires a change in existing internal models, or whether it requires the formation of new models (Shadmehr & Wise, 2005).

In this work, we define motor learning in accordance with the task that was undertaken in our experiments. First, we specifically studied visuomotor learning, as a variant of sensorimotor learning which involves movements guided by vision. Sensory feedback is visually presented in the task, requiring the learning of a changing relationship between visual inputs and motor command outputs. In other words, motor learning in this case entailed updating of the visuomotor map involved in the task (Ghahramani & Wolpert, 1997). Second, the task involved goal-directed arm reaching movements. These movements to a visually represented target required the brain to convert spatial information on target location in retinal coordinates to produce patterns of arm muscle activity in joint coordinates. This complex process of sensorimotor transformation in preparation for movement generation is known to involve regions such as the posterior parietal cortex and dorsal premotor cortex (Scott, 2003). Furthermore, during the learning of a new visuomotor transformation, visual and proprioceptive feedback in different coordinate systems must be combined and processed for use by the internal model to learn, a process which also involves multiple areas in the brain including M1, cerebellum, and posterior parietal cortex (Shadmehr & Wise, 2005). We postulated that M1 is one such area which contributes to the processing underlying such learning, and in the current study we have focused exclusively on this contribution.

1.2 Primary motor cortex (M1)

1.2.1 Anatomy and physiology

The primary motor cortex (M1) is located in the anterior bank of the central sulcus and on the adjacent dorsal portion of the precentral gyrus (Dum & Strick 2005). It was proposed in 1935 by Fulton that the motor cortex could be divided into such a primary motor area and a premotor area. This division was based on cytoarchitectonic differences, electrical thresholds, and evidence from clinical observations and cortical ablation experiments on monkeys (Chouinard & Paus, 2006). The threshold for evoking movement with electrical stimulation is lower in M1 than in any other cortical region (Dum & Strick, 2005). Anatomically, M1 corresponds to Brodmann area 4, which is characterized by the presence of giant pyramidal cells in cortical layer V (Graziano et al., 2002). Unique to M1 is the high proportion of large corticospinal neurons, which are thought to be important for mediating corticomotoneuronal synapses; 31% of corticospinal neurons that arise from M1 are large, and these represent 79% of all large corticospinal neurons (Dum & Strick, 1991), making M1 the source of the largest contribution to the descending corticospinal tract. Besides projecting to the corticospinal tract, M1 also sends input into the basal ganglia via the striatum and to the cerebellum via the pontine nuclei. M1 in turn receives projections from the basal ganglia and cerebellum. Cortical input to M1 is confined to regions in the frontal and parietal lobes that are the origins of projections to the spinal cord, similar to M1. These include premotor areas in the frontal lobe (ventral premotor area, dorsal premotor area, supplementary motor area, cingulate motor areas), parts of the superior parietal lobe (SPL), and primary and secondary somatosensory cortex.

The spatial organization of M1, relevant to our study as we were targeting specific muscles of the forearms, has been described as a highly ordered somatotopy occurring in a discrete and topographically segregated fashion across the motor cortex. This early concept, exemplified by the well-known homunculus schema, is now considered inaccurate. More recent experimental data from electrical stimulation, pharmacological inactivation, and neuroimaging methods have

revealed the organization in M1 to have discrete gross subdivisions, while each subdivision has an internal distributed network in which control emerges from broad activity patterns (Sanes & Donoghue, 2000).

1.2.2 Functional properties and relation to other motor areas

Classically, the generation of motor commands was considered in a hierarchical and serial framework, with M1 thought to receive motor execution messages from upstream cortical areas and send corresponding output to the corticospinal tract (Scott, 2003). It has thus been widely accepted that M1 plays a crucial role in voluntary motor control. However, it has also been found that premotor areas, in addition to having indirect access through M1, also directly access spinal cord mechanisms involved in movement generation and control. As an example, intracortical stimulation of each premotor area can evoke body movements. It is now thought that independent and parallel pathways for generating and controlling movement originate in M1 and other premotor areas, which may vary in specific aspects of motor behavior (Dum & Strick, 2005).

Moving beyond the role of M1 as an information processing structure for the planning and control of movements, recent evidence has implicated M1 in learning and cognition as well (Sanes & Donoghue, 2000). Neural activity in M1 is reflective of a range from high-level goals to low-level details of motor execution (Scott, 2003). M1 neurons are sensitive to changes in limb posture or position, the global goal of the task such as desired movement direction, to changes in muscle force. Some neurons receive strong sensory input while others do not respond to any sensory stimuli. This wide range of M1 neuron functionality is reflective of the complexity of M1 function underlying its role in behavior. At a behavioral level, the repertoire of complex motor functions in which M1 is involved includes complex trajectory planning (Ashe et al., 1993), mental rotation (Georgopoulos et al., 1989), and movement sequence production (Lu & Ashe., 2005). More recently, there has been growing support for the idea that M1 is involved in learning of motor skills, but the extent of this role is still being elucidated.

1.3 Transcranial magnetic stimulation

1.3.1 Description and background

Transcranial magnetic stimulation (TMS) is a technique involving noninvasive electromagnetic stimulation of cortical sites to influence cortical activity for the study of brain function. First introduced by Barker et al. in 1985, it has commonly been used as a tool in neuroscience research owing to its relatively non-invasive quality and capacity to study functional connectivity of different brain areas or probe the causal contribution of these areas to behavior (Robertson et al., 2003). TMS is based on the principle of electromagnetic induction; a current flowing through a coil of wire generates a magnetic field, and if the strength of the magnetic field is changing over time, the field in turn induces a secondary current in a nearby conductor. The TMS device essentially comprises a capacitor and stimulating coil. The coil is placed on a subject's head and as a brief pulse of current flows through it, a time-varying magnetic field is generated which passes through the subject's skull and induces a current in the conductive brain tissue (Pascual-Leone et al., 2000). Because significant currents can be induced without applying large voltages across the skull, this minimizes the activation of pain fibers (Robertson et al., 2003). The precise effect of this stimulation on neurons is still unclear; however, it is hypothesized that the large magnetic stimulus (duration of ~100μs) synchronously excites a population of neurons, which fire a rapid series of impulses for a few milliseconds after which the entire activity is suppressed by a long-lasting period of GABAergic inhibition (Pascual-Leone et al., 2000). This process is thought to last between 20 and 200 ms depending on stimulus intensity. The area of stimulation depends on coil geometry and stimulation intensity (Thielscher and Kammer, 2004), and for a 70 mm figure-of-eight coil is considered to be approximately 1 cm^2.

There are currently a few different types of TMS commonly used. Repetitive TMS (rTMS) involves trains of pulses occurring for relatively long periods of time (typically 10-15 minutes). A period of rTMS results in a subsequent period (~15 minutes) of modulated cortical excitability. Generally, low-frequency rTMS (<1 Hz) results in decreased cortical excitability, while high-

frequency (>5 Hz) results in increased cortical excitability (Sack, 2006). Due to these effects lasting beyond the stimulation session itself, rTMS is employed in "off-line" paradigms in which intervention occurs before beginning a behavioral task session (Robertson et al., 2003). Single-pulse TMS usually refers to stimulation that occurs at a rate less than 1 Hz and is non-periodic, and it is generally used in event-related protocols in which the stimulation pulse is time-locked to a certain temporal feature of the behavioral task. By applying single-pulse TMS at various times during task execution, it is possible to examine the time point at which the neural activity at the stimulation site is necessary for task performance. Paired-pulse TMS protocols involve a subthreshold conditioning stimulus closely followed by a suprathreshold test stimulus, generally with a 1-15 ms inter-stimulus interval (Rossi et al., 2004). By observing changes in the amplitude of the test stimulus, the presence of inhibitory (at shorter intervals) or facilitatory (at longer intervals) phenomena taking place intra-cortically can be evaluated.

1.3.2 Usage of single-pulse TMS in current study

The purpose of the current work was to study the time-specific contribution of M1 in visuomotor adaptation and retention. As mentioned above, a defining characteristic of TMS as a research tool is that one can use it to demonstrate a causal link between a brain area and a behavior, by disrupting the processing occurring in a local area and observing the subsequent effects. This is in contrast to other brain activity measures such as functional magnetic resonance imaging or event-related potentials which are limited to correlative conclusions (Robertson et al., 2003). Furthermore, single-pulse TMS enables the user to obtain information on the precise time point at which activity contributes to task performance. We therefore used single-pulse TMS to disrupt M1 activity at specific time points following a reaching movement task to examine the chronometry of contribution to visuomotor learning.

In particular, motor cortex stimulation results in an overt response in the form of a muscle twitch, which can be quantified using surface electromyography (EMG). This conveniently

sidesteps a frequent problem in TMS studies of localizing the precise site of stimulation, especially if there are no overt responses to TMS elicited (such as the muscle twitch after mortor cortex stimulation or phosphenes by visual cortex stimulation). Variability in scalp and bony landmarks across subjects preclude the use of these for site localization, requiring techniques such as MRI and fMRI guided targeting of the association cortex. Furthermore, for motor cortex stimulation as in our experiments, the site of stimulation can be checked prior to a block of movement trials and monitored throughout the block by observing motor evoked potentials (MEPs) recorded by EMG. While variability in MEP amplitude across time can occur due to many possible task-related and non-specific factors and thus there is a limit to how much one can infer about the consistency of stimulation location, even a rough monitoring of MEPs during a task helps to decrease the uncertainty regarding consistency of stimulation site.

1.4 Involvement of M1 in motor learning

1.4.1 Plasticity in M1

The surprising capacity of M1 to undergo extensive physiological and functional plastic changes in response to changes in sensory input has been a reasonable basis for inferring the possible role of M1 in complex brain functions such as learning and memory. This idea of a dynamic organization in the motor cortex is an old concept that has been experimentally studied in primates as early as the mid-20th century (Gellhorn and Hyde, 1953). More recently, intracortical electrical stimulation mapping in both animals and humans has clearly shown that maps in M1 can undergo rapid and long-lasting reorganization following peripheral nerve lesions or limb amputations (Chen et al., 2002). It has been postulated that the extensive horizontal connections which span M1 are the substrate for M1 plasticity, while evidence for activity-dependent synaptic plasticity has also been found in M1. However, the propensity of M1 to undergo plastic changes of map representations in response to changes in the body and environment did not necessarily

implicate it as having an active role in plasticity which specifically underlies motor learning, though it was suggestive of this capacity.

1.4.2 Changes in M1 neuronal properties during learning

Changes in M1 maps have been observed in relation to the acquisition of motor skills. For example, electrical stimulation mapping demonstrated changes in monkeys after learning a precision grasping task (Nudo et al., 1996), as well as during an association task which required establishing an association between an arbitrary visual cue and a well-learned movement (Wise et al., 1998). Recent evidence from electrophysiological studies have shown that learning- and memory-related changes occur in the activity of a subpopulation of neurons in the primary motor cortex (M1) while monkeys adapt to force-fields or visuomotor transformations (Li et al., 2001; Paz et al., 2003). In these studies, it was found that the activity of subsets of M1 neurons reflects a memory trace of novel movement dynamics, but the population activity reflects only task execution (Li et al. 2001). Despite the clear evidence for learning-related changes recorded at the neuronal level, it was unclear whether the observed changes were the cause of changes in behavior or were merely reflective of the learning.

1.4.3 Evidence from repetitive TMS studies

Recently, studies using transcranial magnetic stimulation have begun to probe whether M1 has a pivotal role in motor learning. Off-line experimental paradigms utilizing repetitive TMS (rTMS), in which intervention occurs before beginning a behavioral task session, have linked the early phase of motor memory consolidation to M1. Muellbacher et al. (2002) showed that a period of rTMS following training of ballistic finger movements resulted in a return of performance (peak pinch acceleration) back to baseline. It was suggested that this was due to interference of early motor consolidation, rather than impaired recall of the learned motor memory, as rTMS of M1 given 6 hours after the initial training session did not affect performance immediately afterwards

in a second testing period. Also, this effect was specific to M1, as stimulation of other areas (dorsolateral prefrontal cortex and occipital cortex) did not affect performance. Baraduc et al. (2004) confirmed this disruptive effect of rTMS of M1 in retention of a learned ballistic movement task but failed to find an effect in a force-field adaptation task, suggesting the variable involvement of M1 in different types of motor learning (namely, lack of involvement in the motor memory retention of novel dynamics). Most recently, Richardson et al. (2006) showed that M1 disruption by 1 Hz rTMS for a period of 15 minutes prior to adaptation resulted in a normal rate of adaptation of reaching movements to a perturbing force-field. However, they found that the prior stimulation resulted in impaired performance when tested 24 hours following the training session.

Taken together, the latter two studies suggest that M1 is not necessary in the process of adaptation to visuomotor and force fields (Paz et al., 2005; Richardson et al., 2006), but is necessary for retention of motor memory formation. While these studies begin to answer the question of whether there is a causal relationship between M1 activity and behavior, the shortcomings of these studies owes to the fact that rTMS is done "off-line" to the adaptation period and so its disruptive effect can last relatively uniformly for many minutes. Because of this, rTMS experiments fail to give insight into the chronometry of M1 contribution to memory during this period.

2 Methods

2.1 Subjects

43 right-handed subjects (mean age ± s.d., 23.3 ± 5.0, range 18-41 years old, 18 females) participated after providing written informed consent. Protocols were approved by the Johns Hopkins Medicine Institutional Review Board. All subjects were screened for history of seizures, strokes, neurological disorders, respiratory illness, metal implants and other contraindications for TMS. 27 subjects participated in Experiment 1 (9 in control group, 9 each in two TMS stimulation groups) and 16 participated in Experiment 2 (8 in control group, 8 in TMS group).

2.2 Experimental procedures

2.2.1 Apparatus and general task procedure

The subject was seated in front of a vertically oriented monitor with the center of the monitor aligned with the subject's midline and eye level (Figure 2.1A). The subject's forearm was supported in the horizontal plane with a sling placed just distal to the elbow, and the wrist was immobilized by a splint. Head movements were restrained with the use of a custom-molded bite bar, and a shield placed around the bite bar prevented vision of the hand and arm. Movement of the right shoulder was minimized by a restraining strap placed over the right shoulder and across the chest. While performing movements, the subject grasped a robot manipulandum handle. Optical encoders located on the motors of the robot recorded the two-dimensional position of the center of the manipulandum handle at 200 Hz.

Subjects performed rapid "shooting" reaching movements towards and through targets displayed on the monitor (Figure 2.1B). They were instructed to move a cursor from a central start position to the center of the target in a straight line and with an appropriate speed. The start position was represented by a yellow cross (length/height, 1 cm) that was continuously displayed

at the center of the screen, and the handle position was displayed as a white cursor (diameter, 0.5 cm). There was a 1:1 mapping between cursor and hand displacement. The trial began with the cursor at the start position. A warning tone sounded when the cursor was more than 0.7 cm away from the center of the cross.

After a variable wait time uniformly distributed between 2.5 and 3.5 sec, a beep sounded as a square target (length, 1 cm) appeared. The target was located at one of three positions along a continuously-displayed boundary circle with a 10 cm radius and the cross as the center (see below). As the subject moved the cursor towards the target, a trace of the cursor path was displayed on the screen in the wake of the cursor. At the moment the cursor passed through the boundary circle (trial end), the cursor was hidden, the boundary pass point (endpoint) was marked with a dot, and the robot exerted a velocity-dependent dampening field to slow the hand motion. After the handle stopped moving (defined as the time when velocity fell below 1 cm/s), visual feedback was displayed for 200 ms. If the cursor passed through the target, peak tangential velocity was within 130±12.5 cm/s, and path curvature index (movement path length divided by the distance between cursor position at target onset and endpoint) was ≤1.01, an animation of target explosion and a tone were given as reward. The target turned red or blue if peak velocity was higher or lower than the desired range, respectively. If the reaction time (time between target onset and movement onset) was greater than 400 ms, a yellow box (length, 1cm) appeared to the left of the target as a warning signal. The trace of the movement (up until endpoint) also remained displayed during this feedback period. At the end of the feedback period, the visual feedback was removed and the robot moved the manipulandum handle back towards the start position in preparation for the next trial. When the distance between the cursor and the center of the cross became less than 2 cm, the cursor reappeared and the robot released the handle, allowing the

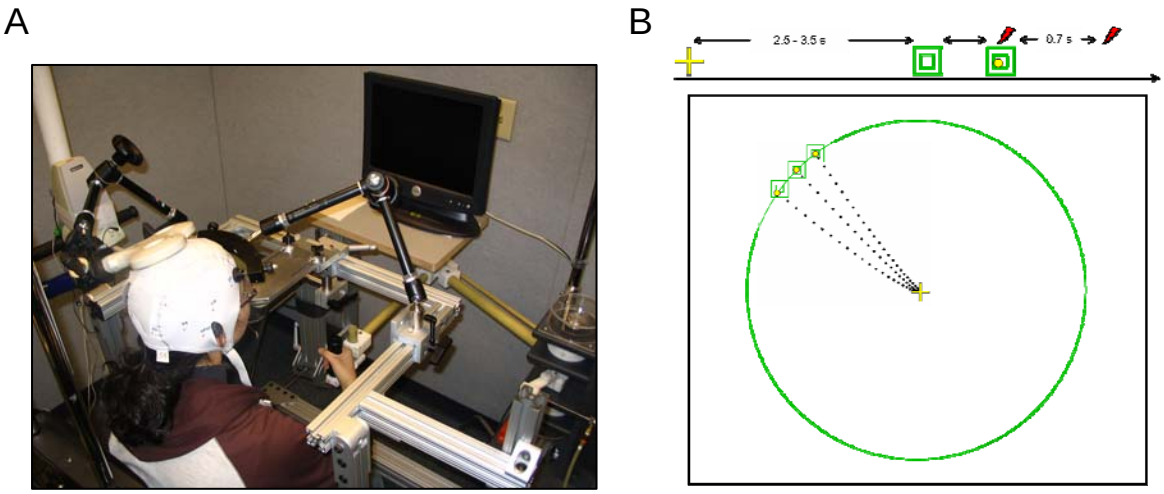

Figure 2.1 Experimental setup and screen display. A, Experimental apparatus. The subject gripped the handle of the robot while biting on a bite-bar attached to the frame and with the TMS coil on the head. The forearm was supported by a sling (not shown). A shield around the bite-bar prevented the subject from seeing their hand and arm. B, Example of screen display and timeline of events. The boundary circle displayed on the screen had a 10 cm radius and was centered on the start position cross. Targets were located at three possible positions (shown here at 127.5°, 135°, or 142.5° for Experiment 1) with a "bulls-eye" shape to encourage subjects to aim at the center of the target. Target onset occurred after a random wait time (uniform distribution between 2.5 and 3.5 s. As the cursor moved, a trace of the cursor path was displayed in the wake of the cursor until the endpoint. TMS onset occurred either at trial end (for immediate-TMS group) or 700 ms after trial end (for delayed-TMS group).

subject to place the cursor at the center of the cross.

In addition, subjects were asked to pay attention if the reaction time warning signal appeared and to begin subsequent movements sooner. Subjects were also asked to fixate on the center cross at the beginning of each trial in the absence of targets, and when a target appeared to look and move naturally towards the target. Proper fixation and saccades were confirmed by observation.

2.2.2 Experiment 1 paradigm

The experiment consisted of blocks of 48 trials, with a timed period of rest (1 min) between all blocks (Figure 2.2). Prior to the experimental session, subjects performed three blocks of movements in the null condition (i.e. without any visuomotor transformation) to familiarize themselves with the task.

The session began with a pre-adaptation block in the null condition. Subjects then performed four blocks of adaptation as a rotational transformation was applied to the cursor direction with respect to the hand direction. The first 12 trials of the first adaptation block were performed with 0° rotation, after which the magnitude increased by 1° clockwise for every 6 trials, so that the last 6 trials of the fourth block were performed with the maximal 30° clockwise rotation. The visual rotation was implemented gradually to decrease the likelihood that subjects would use cognitive strategies to improve performance (Mazzoni and Krakauer, 2006). Subjects were divided into three groups (n=9 per group). For the immediate-TMS group, a single TMS pulse was given at the end of each trial (the time at which the cursor passed the boundary circle). For the delayed-TMS group, a single pulse was given with a 700 ms delay after each trial end. A third group of subjects did not receive TMS. After the adaptation period, subjects underwent a block of de-adaptation in the null condition, in which the subjects de-adapted back to baseline as they performed movements without receiving TMS.

For Experiment 1, targets were located at 127.5°, 135°, or 142.5° along the boundary circle. Target locations were slightly varied as such to prevent stereotyped movements that would preclude actual planning of movements. Targets were presented in a pseudo-random order, such that every block of movements (48 trials/block) would contain 16 trials for each target, and every set of three movements (e.g. Trials 1-3, 4-6, 7-9) would include one each of the three possible targets. These sets in turn were pseudo-randomly ordered within a block so that there would never be any two consecutive trials with the same target direction, thus decreasing the likelihood of unequal adaptation in any one direction.

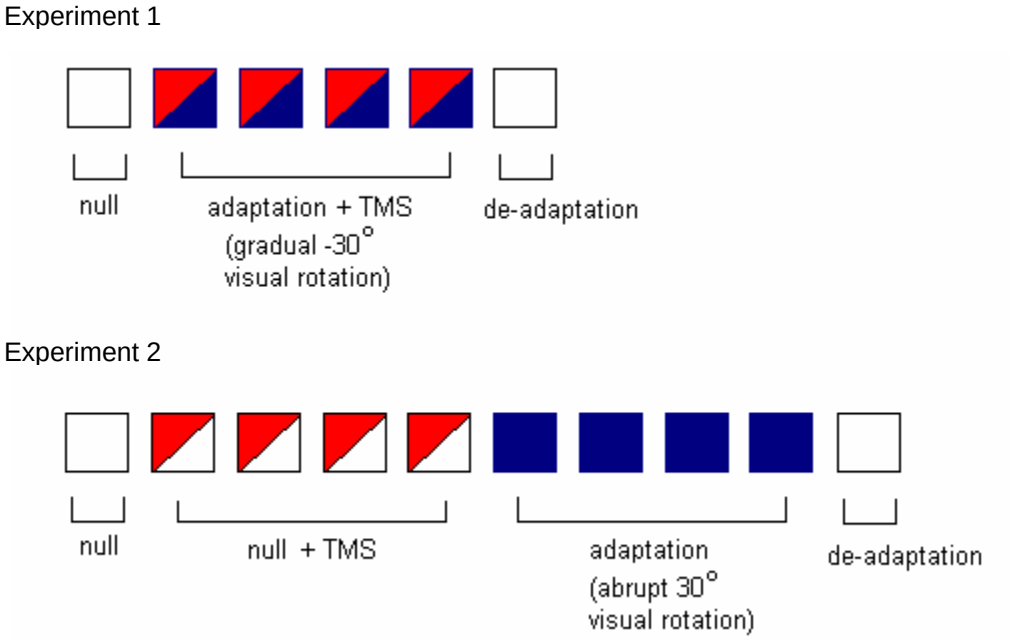

Figure 2.2 Experimental procedure. Red indicates blocks with TMS, blue indicates blocks with adaptation, and white indicates blocks in the null condition.

2.2.3 Experiment 2 paradigm

The aim of this second experiment was two-fold. First, we wished to consider whether a period of TMS preceding a sudden large error (e.g. initially in the de-adaptation period of Experiment 1) induces a general increase in sensitivity to the error (i.e. both faster adaptation and deadaptation), rather than a specific increase in deadaptation. Second, we wished to examine whether Experiment 1 was influenced by other non-specific effects such as tactile or auditory sensations that were induced by the TMS preceding the de-adaptation period.

As in Experiment 1, subjects underwent familiarization training prior to the experimental session. The session was composed of four periods: a pre-adaptation period, a null period with TMS, an adaptation period, and a de-adaptation period. The experiment began with a pre-

adaptation block (48 trials/block) performed in the null condition (Figure 2.2). For one group of subjects (n=8), this was followed by four blocks with a single pulse of TMS applied at the time of each trial end, but unlike Experiment 1, these blocks occurred in the null condition rather than with visual rotation. In another group of subjects (n=8), no TMS was applied. After this period, subjects underwent four blocks of adaptation where from the first trial onwards, a 30° counter-clockwise visual rotation was present. Finally, subjects performed one de-adaptation block in the null field and in the absence of TMS. The rest time between blocks was kept constant for all subjects as in Experiment 1.

In Experiment 2, targets were located 30° counter-clockwise relative to the targets in Experiment 1, at 157.5°, 165°, and 172.5° along the boundary circle. The target locations and the counter-clockwise direction of visual rotation were chosen so that the kinematics of the movements made by subjects during the adaptation period of Experiment 2 would parallel those of the movements made by subjects during the de-adaptation period in Experiment 1. We carefully matched the movements so that, given the fixed cortical area being stimulated by TMS, the results of this experiment could not be attributed to the use of different muscles from those in Experiment 1. Figure 2.3 details this pattern of movements in Experiment 1 and 2.

2.3 TMS and localization of stimulation sites

The stimulation site and intensity for each subject in the TMS groups was determined at the beginning of the session before the experimental task. A Magstim 200 monophasic stimulator (Magstim, Whitland, UK) and a standard figure-of-eight coil with 70 mm wing diameter were used to deliver TMS. Surface electromyogram (EMG) recordings were taken from the right biceps brachii, deltoid, and first dorsal interosseous (FDI) muscles. The coil was placed tangentially to the scalp with the handle pointed backwards and at a 45° angle away from the anterior-posterior axis, as motor threshold is minimized when the induced electrical current is perpendicular to the central sulcus (Brasil-Neto et al., 1992; Mills et al., 1992). Single pulses of

TMS were applied to the left motor cortex to localize the FDI motor hotspot, defined as the site which required the lowest stimulation intensity for eliciting MEPs in the FDI. The resting motor threshold (RMT) was determined to the nearest 1% of maximum stimulator output and defined as

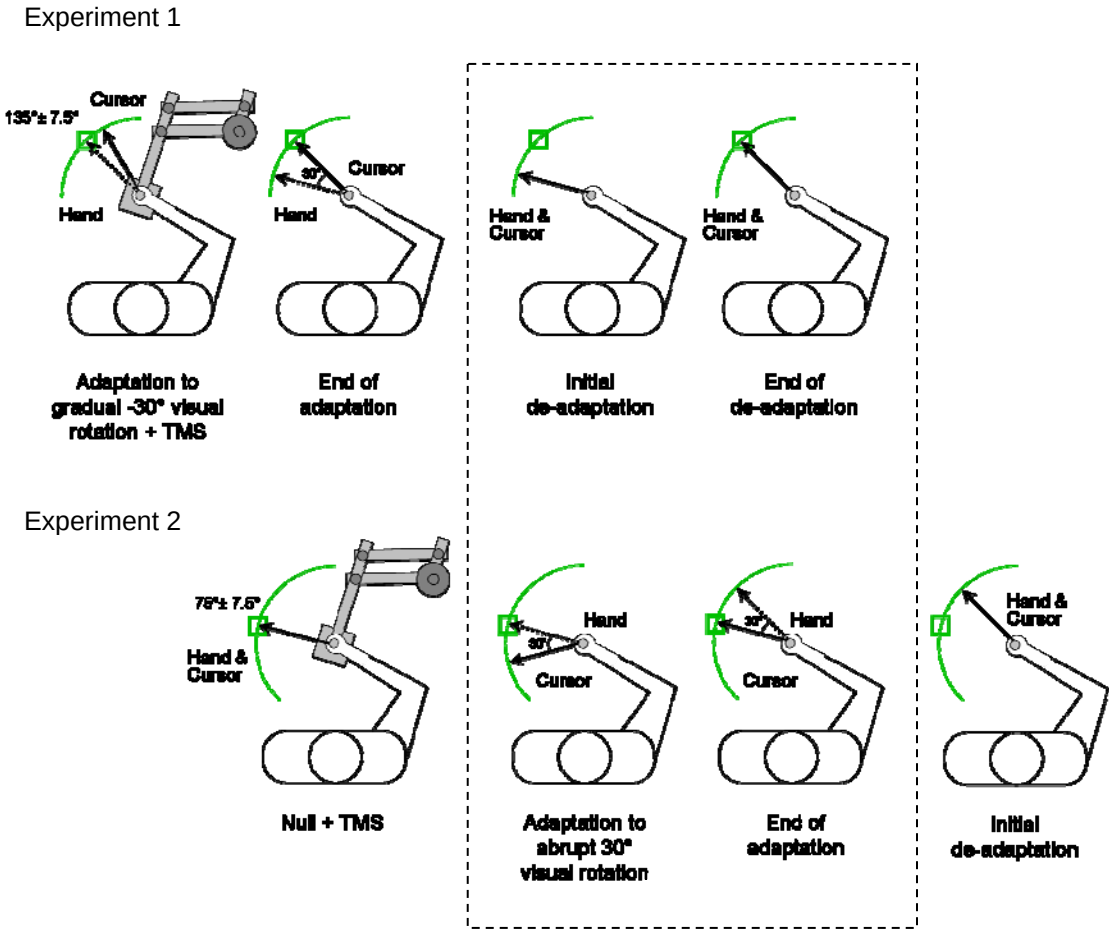

Figure 2.3 Comparison of Experiment 1 and Experiment 2 hand and cursor movement directions. The de-adaptation period of Experiment 1 and the adaptation period of Experiment 2 (specifically, the first block of adaptation), as denoted by the box, had the same pattern of changes in hand movement direction. The direction of the initial hand movements in the de-adaptation period of Experiment 1 were 30° counter-clockwise relative to the targets (i.e. towards 157.5°, 165°, and 172.5°) since subjects had previously adapted to the visual rotation. This was the same direction as the initial movements of the adaptation period of Experiment 2. Over the course of de-adaptation trials in Experiment 1, the direction of movement gradually shifted in a clockwise manner to return to the normal baseline directions (towards the targets at 127.5°, 135°, or 142.5°). This clockwise shift was again preserved during adaptation in Experiment 2.

the minimum intensity which elicited motor evoked potentials (MEPs) of ≥50 μV amplitude in the FDI in ≥5 of 10 consecutive pulses applied to the FDI hotspot. We found that the mean RMT for the immediate-TMS group and the delayed-TMS group were 41.78 +/- 3.11 s.d. % and 40.11 +/- 4.73 s.d. % respectively. Subsequently, we localized the motor hotspots for the biceps and the deltoid using single pulses of TMS.

Given the proximity of M1 to other motor areas, it was necessary to minimize the possibility of spread of stimulation to these areas. In particular, we wished to avoid stimulating the dorsal premotor cortex (PMd) which is located just anterior and medial to M1. In our study, prior to the experimental session, 10 pulses at 120% of RMT were applied to the PMd (defined as the point 2.5 cm anterior and 1 cm medial to the FDI hotspot) and the EMG recordings were checked for any MEPs which could indicate a spread of current from the PMd to the M1. An absence of MEPs implied that TMS on M1 would be unlikely to spread to the PMd during the experiment.

For the TMS applied during the experiments, the intensity of stimulation was set at 120% of FDI RMT. The center of the coil was positioned at the midpoint between the biceps and deltoid hotspots in order to stimulate the representation of muscles chiefly used in the present task (Thoroughman and Shadmehr, 1999). A single-pulse of TMS was given for every movement, which corresponded to ~0.2 Hz. It has previously been shown that 250 pulses of repetitive TMS on M1 at a frequency of 0.2 Hz does not affect cortical excitability as measured by MEP amplitudes (Murase et al., 2005).

2.4 Data analysis

The performance in each trial was quantified using the angular endpoint error, defined as the angle between the line connecting the initial cursor position to the center of the target and the line connecting the initial cursor position to the endpoint. Trials were binned by three, and statistics were carried out on the binned data using repeated-measures analysis of variance (ANOVA) with Tukey correction for multiple comparisons. For calculating mean cursor trajectories during the

duration of the movement (movement onset to trial end), the cursor position data for each movement was resampled at 20 points evenly spaced over the movement duration (from movement onset to trial end), with linear interpolation between adjacent time points. For mean cursor trajectories after the trial end, the cursor position data was resampled at 15 points evenly spaced over the period of 100 ms after trial end, with linear interpolation between adjacent time points.

3 Results

3.1 Experiment 1 results

3.1.1 Disruption of M1 does not affect movement execution

None of the subjects experienced any of the known side effects from TMS during the experiments. In Experiment 1, we did not observe a significant effect of TMS on either execution or adaptation of movements (Figure 3.1). Hand trajectories appeared indistinguishable between groups for both null trials and visual rotation trials. Mean error of the first 12 trials of the first adaptation block was compared with mean error of the first 12 trials of the pre-adaptation null block to assess the effect of TMS on movement execution. We found no main effect of TMS group ($F_{(2,24)}$=0.082; p=0.921) or interaction effect of group and block ($F_{(2,24)}$=0.004; p=0.996). In addition, we found no significant main effects of group on movement kinematics, which included peak velocity ('PV': $F_{(2,24)}$=0.144; p=0.867), movement duration ('MD': $F_{(2,24)}$=1.309; p=0.289), path curvature index ('PC': $F_{(2,24)}$=0.038; p=0.962), and reaction time ('RT': $F_{(2,24)}$=0.289; p=0.752). The values of the kinematic variables are given in Table 3.1.

3.1.2 Disruption of M1 does not affect adaptation to visual rotation

Over the four adaptation blocks, errors gradually increased but remained within the target range (dotted lines at ±2.862° in Figure 3.1). During post-experiment debriefing, we confirmed that subjects had been unaware of the gradual visual rotation, implying that the errors were attributed to self-generated variability in movements. Notably, we saw no significant effect of immediate-TMS or delayed-TMS on the extent of adaptation. First, performance of the immediate-TMS and delayed-TMS groups did not differ from that of the no-TMS group during the adaptation period. There was no main effect of group ($F_{(2,24)}$=0.190; p=0.828) or interaction effect of group and time ($F_{(126,1512)}$=0.772; p=0.969) on errors of the four blocks. Similarly,

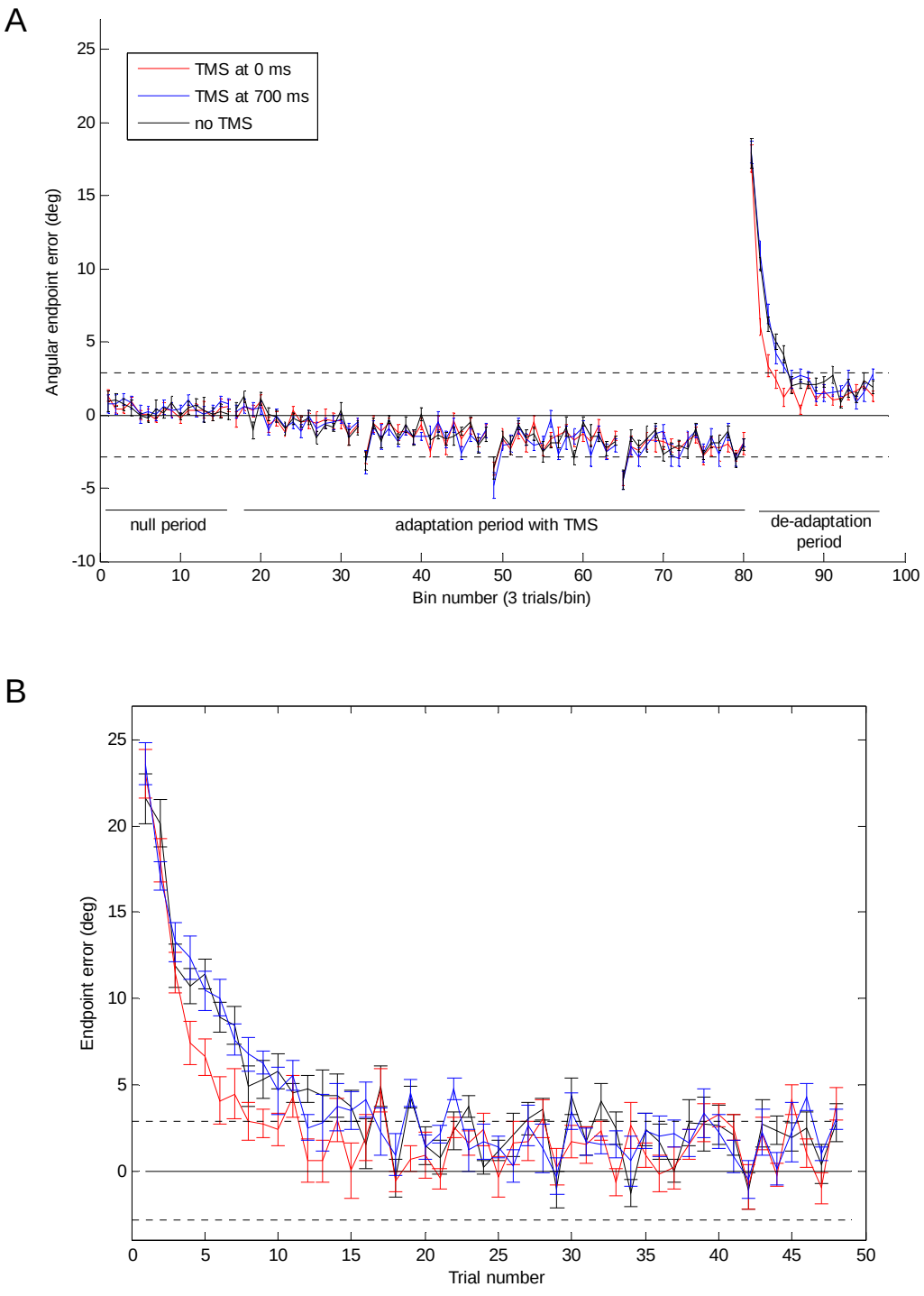

Figure 3.1 Angular endpoint errors in Experiment 1. A, Endpoint errors (mean±SEM of 3-trial bins) during pre-adaptation, adaptation and de-adaptation periods of Experiment 1. Positive values indicate counter-clockwise deviation. TMS immediately after trial end impaired performance during de-adaptation but not adaptation in Experiment 1. B, Expansion of de-adaptation block showing trial-by-trial errors (mean±SEM).

kinematics remained indistinguishable between the groups (PV: $F_{(2,24)}$=0.066; p=0.936, MD: $F_{(2,24)}$=1.854; p=0.178, PC: $F_{(2,24)}$=0.157; p=0.855, RT: $F_{(2,24)}$=0.799; p=0.461). Second, while the 1 min rest between blocks produced forgetting in all groups, the two types of TMS did not affect this time-dependent process of forgetting, as the errors of the first trials of blocks 2 to 4 were similar across groups (block 2: $F_{(2,24)}$=0.249; p=0.781, block 3: $F_{(2,24)}$=0.040; p=0.961, block 4: $F_{(2,24)}$=0.133; p=0.876). Third, when subjects performed movements during the de-adaptation block after removal of the visual rotation (Figure 3.1) the magnitudes of the initial three aftereffects were not significantly different between groups ($F_{(2,24)}$=0.073; p=0.930). This is also observed in the movement trajectories presented in Figure 3.2. Therefore, we could not detect any effects of either immediate-TMS or delayed-TMS on how the motor system adapted to the gradual perturbations.

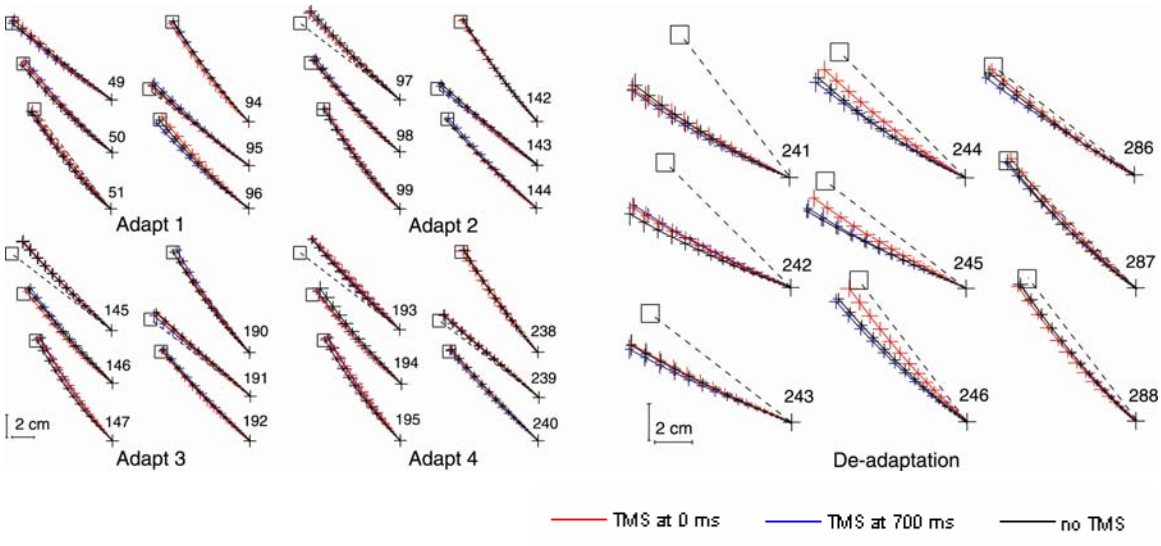

Figure 3.2 Cursor trajectories in Experiment 1. Trajectories are shown (mean±SD) for first three and last three trials of each adaptation block, and for first six trials and last three trials of de-adaptation period in Experiment 1. Trajectory labels correspond to trial numbers.

		Peak velocity (cm/s)	Movement duration (ms)	Path curvature index	Reaction time (ms)
Null	Immediate-TMS	128 ± 4.6	146 ± 16	1.0025 ± 0.0016	297 ± 29
	Delayed-TMS	129 ± 5.6	156 ± 9	1.0021 ± 0.0014	281 ± 39
	No-TMS	126 ± 6.0	155 ± 11	1.0025 ± 0.0016	280 ± 36
Adaptation	Immediate-TMS	125 ± 5.6	147 ± 15	1.0022 ± 0.0019	296 ± 34
	Delayed-TMS	126 ± 4.7	157 ± 9	1.0018 ± 0.0012	287 ± 39
	No-TMS	125 ± 4.8	149 ± 9	1.002 ± 0.0012	273 ± 39
De-adaptation	Immediate-TMS	125 ± 4.5	149 ± 18	1.003 ± 0.0018	321 ± 31
	Delayed-TMS	127 ± 5.8	160 ± 11	1.0024 ± 0.0014	296 ± 40
	No-TMS	128 ± 6.7	154 ± 9	1.0032 ± 0.002	293 ± 42

Table 3.1 Experiment 1 movement variable values. Values are mean ± SD across subjects.

3.1.3 Immediate disruption of M1 results in faster rate of de-adaptation

However, in the post-adaptation period, the de-adaptation rate of the immediate-TMS group was significantly faster than the delayed-TMS group and the no-TMS group, while there was no difference between the delayed-TMS and no-TMS groups (Figure 3.1). Here we found a main effect of group ($F_{(2,24)}$=12.899; p<0.0005) as well as an interaction effect of group and time ($F_{(30,360)}$=2.830; p<0.0005). Post-hoc analysis revealed a significant difference of the immediate-TMS group from the delayed-TMS group (p=0.001) and the no-TMS group (p<0.0005), while the delayed-TMS and no-TMS groups did not differ (p=0.997). However, we noted no significant effect of group on peak velocity 'PV' ($F_{(2,24)}$=0.723; p=0.496), movement duration 'MD' ($F_{(2,24)}$=1.505; p=0.242), path curvature index 'PC' ($F_{(2,24)}$=0.427; p=0.657), and reaction time 'RT' ($F_{(2,24)}$=1.501; p=0.243).

To examine direction-dependency of errors during de-adaptation, we computed the mean error for each target direction across the trials in the first half of the block, which captured the initial rapid rate of de-adaptation. A repeated-measures ANOVA with direction as within-subjects factor again found a main effect of group ($F_{(2,24)}$=13.395, p<0.0005), with post-hoc analysis revealing that mean error of the immediate-TMS group was significantly different from the delayed-TMS group (p<0.0005) and the no-TMS group (p=0.001) while delayed-TMS and no-TMS groups did not differ (p=0.937). There was no main effect of direction ($F_{(2,48)}$=2.382;

p=0.103) or interaction effect of group and direction ($F_{(4,48)}$=0.098, p=0.982) on the error, implying that the effect of group was robust regardless of movement direction.

Given the effect of immediate-TMS on the rate of change of endpoint errors during the de-adaptation period, we also wished to examine whether immediate-TMS induced any changes for the portion of movement trajectories after trial end (i.e. after the cursor passed the boundary circle). In particular, we wished to see whether any changes occurred during the adaptation period in this late portion of the trajectory that was not represented in the endpoint errors, which were not different across groups. We found that the trajectories of immediate-TMS subjects and no-TMS subject overlapped during the first twelve trials of the first adaptation block (which occurred without any visual rotation), during the adaptation trials in which the gradual visual rotation was implemented, and also during the de-adaptation period. From the overlap, we concluded that the TMS is not affecting the movement trajectories after trial end. Representative trajectories are shown in Figure 3.3.

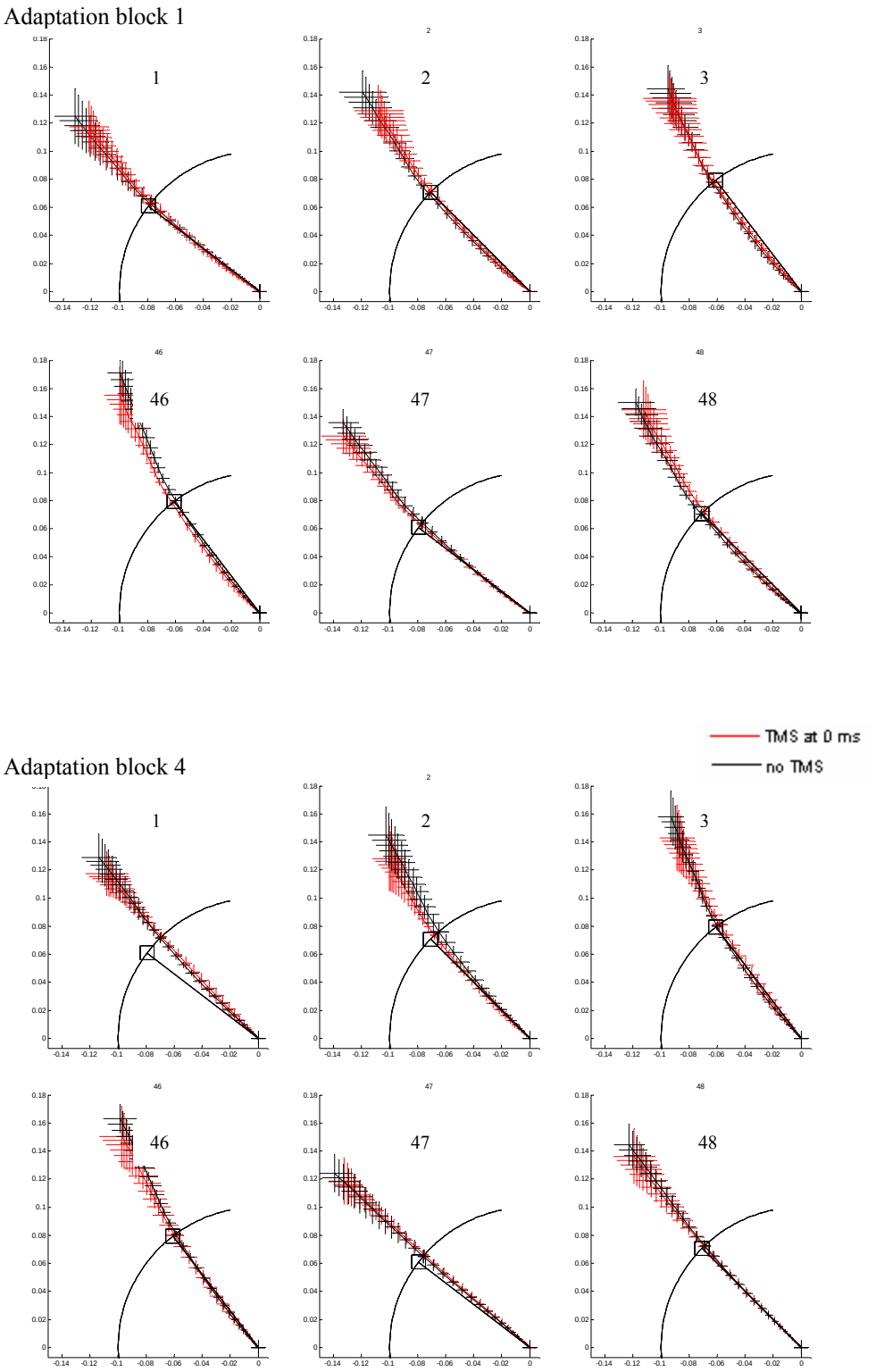

Figure 3.3 Cursor trajectories after trial end in Experiment 1 of immediate-TMS and no-TMS groups during adaptation. Representative trajectories are shown (mean±SD) for first three and last three trials of adaptation blocks 1 and 4. These graphs illustrate the similarity in the movement trajectories after trial end of the immediate-TMS and non-TMS groups, which was the case for movements throughout the adaptation period.

3.2 Experiment 2 results

Conceivably, the rapid de-adaptation of the immediate-TMS group in Experiment 1 could have been due to a non-specific attentional or behavioral effect of the TMS (Robertson et al., 2003) rather than an effect of the TMS to specifically increase the deadaptation rate. One possibility is that the TMS somehow caused a heightened sensitivity to error in the immediate-TMS group, enabling them to respond more quickly to the initial large errors during de-adaptation and thereby resulting in a faster rate of de-adaptation. If this were the case, then the TMS group in Experiment 2 should have also learned more rapidly during the adaptation period due to the TMS received during the preceding null period. However, we found that during adaptation (Figure 3.4), there was no main effect of TMS on errors ($F_{(1,14)}$=0.014; p=0.907) or movement variables (PV: $F_{(1,14)}$=0.046; p=0.834, MD: $F_{(1,14)}$=2.155; p=0.164, PC: $F_{(1,14)}$=0.056; p=0.817, RT: $F_{(1,14)}$=0.921; p=0.354). There was no interaction effect of TMS and time ($F_{(63,882)}$=0.885; p=0.725) on errors during adaptation. Additionally, the performance of the TMS group was not affected during the preceding null period (Figure 3.4), as there was no main effect of TMS ($F_{(1,14)}$=1.248; p=0.283) or interaction effect of TMS and time ($F_{(63,882)}$=0.610; p=0.993) on errors, and no main effect of TMS on movement variables (PV: $F_{(1,14)}$=0.022; p=0.883, MD: $F_{(1,14)}$=0.134, p=0.719, PC: $F_{(1,14)}$=0.103, p=0.753, RT: $F_{(1,14)}$=1.003; p=0.333). There was also no main effect of TMS on errors ($F_{(1,14)}$=0.650; p=0.434) or movement variables (PV: $F_{(1,14)}$=0.400; p=0.537, MD: $F_{(1,14)}$=2.339; p=0.148, PC: $F_{(1,14)}$=1.953; p=0.184, RT: $F_{(1,14)}$=1.829; p=0.198), as well as no interaction of TMS and time on errors ($F_{(15,210)}$=1.225; p=0.255) for the de-adaptation block. Values of movement variables are shown in Table 3.2.

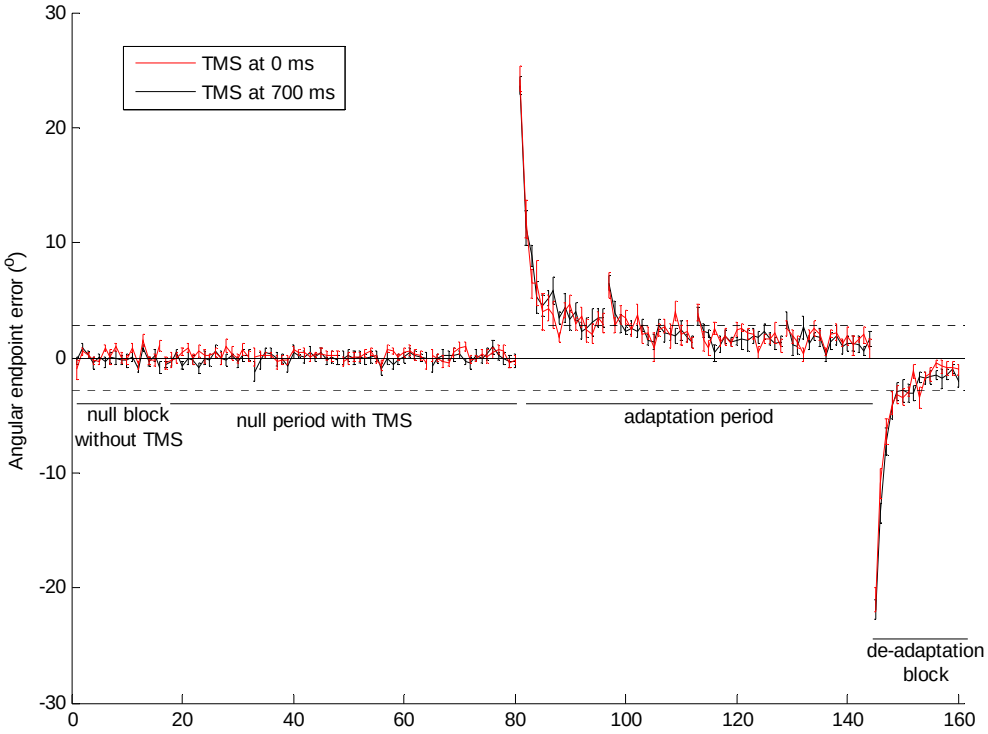

Figure 3.4 Angular endpoint errors in Experiment 2. TMS immediately after trial end during null blocks did not affect subsequent adaptation to abrupt counter-clockwise 30° visual rotation. Endpoint errors are shown (mean±SEM of 3-trial bins) for pre-adaptation, null with TMS, adaptation, and de-adaptation periods of Experiment 2.

		Peak velocity (cm/s)	Movement duration (ms)	Path curvature index	Reaction time (ms)
Null	Immediate-TMS	127 ± 4.5	144 ± 14	1.001 ± 0.0006	286 ± 73
	No-TMS	128 ± 11.7	145 ± 5	1.0008 ± 0.0003	290 ± 28
Null+TMS	Immediate-TMS	127 ± 4.7	142 ± 11	1.0008 ± 0.0005	269 ± 45
	No-TMS	128 ± 6.7	144 ± 6	1.0009 ± 0.0006	290 ± 38
Adaptation	Immediate-TMS	128 ± 6.4	147 ± 11	1.0024 ± 0.0018	290 ± 54
	No-TMS	128 ± 8.6	155 ± 10	1.0026 ± 0.0018	313 ± 39
De-adaptation	Immediate-TMS	123 ± 5.9	145 ± 10	1.001 ± 0.0006	287 ± 53
	No-TMS	126 ± 11.2	154 ± 14	1.0014 ± 0.0005	322 ± 50

Table 3.2 Experiment 2 movement variable values. Values presented are mean ± SD across subjects.

4 Discussion and Conclusions

In this study, we used single-pulse TMS to disrupt M1 activity around the time of error feedback from the periphery in order to interfere with the process that learned from this error. Because TMS of M1 can affect movement execution by inducing a muscle twitch, it was important to apply TMS at a time point after the movement was effectively over. We therefore required an adaptation task that would allow a significant percentage of feedback to be received by M1 after the trial end, thus allowing a time window of disruption of the processing of this feedback. Considering the latency in the response of M1 cells to visual feedback (e.g. 112-192 ms; Riehle 1991), we designed a task which involved a rapid "shooting" movement that had a duration of ~150 ms until the task goal (i.e. hitting the target) was met. We found that a single-pulse of TMS at either 0 ms (immediate-TMS) or 700 ms later (delayed-TMS) relative to the trial end during gradual visuomotor adaptation did not affect the rate of adaptation. However, immediate-TMS resulted in a faster de-adaptation rate than the no-TMS group. In addition, this effect on de-adaptation rate was time-dependent as it did not occur when TMS was applied with a 700 ms delay.

The identical adaptation rates in the immediate-TMS, delayed-TMS and no-TMS groups in Experiment 1 demonstrate that disruption of M1 does not interfere with adaptation to a visuomotor transformation. This suggests either that M1 is not necessary for visuomotor adaptation, or that the impairment of M1 can be acutely compensated by other parts of the motor system (Lee et al., 2003). As previously mentioned, other studies using visuomotor and force-field adaptations (Paz et al., 2005; Richardson et al., 2006) have also postulated the non-necessity of M1 in initial visuomotor adaptation. In addition, one feature of Experiment 1 was that the visuomotor transformation was gradually implemented without the subjects' awareness. Had the visual rotation been abrupt and thus detected by the subjects, the similarity in endpoint errors of

the groups during adaptation could have been attributed to a similarity in cognitive strategies that had overshadowed an actual difference in adaptation. The similar rates of adaptation which occurred subconsciously, combined with the similar initial aftereffects seen in the de-adaptation period, strongly support our interpretation that adaptation that was not impaired by either immediate- or delayed-TMS.

The faster de-adaptation rate of the immediate-TMS group implies that proper activity in M1 is necessary for motor memory retention. This is in some respects in agreement with previous TMS studies. Muellbacher et al. (2002) used rTMS to disrupt M1 after ballistic finger movement learning to probe the necessity of M1 in early memory consolidation, while Richardson et al. (2006) performed rTMS before force-field learning so that M1 activity would be in a disrupted state throughout the learning period. Both of these studies found evidence for a disruption of retention of the acquired motor memory. Our study differs in several ways from these studies. First, the task to be learnt was different. Considering that the distributed brain network involved in motor learning is task-dependent, our use of a visuomotor learning task was a possible factor in the extent to which TMS disruption impairs memory of task (Baraduc et al., 2004). Second, we disrupted M1 during the course of adaptation rather than in an "off-line" fashion before or after the learning period. Third and most important, disruption of M1 occurred in a time-specific manner in our experiments. The main novel result of our study was that the effect of TMS on motor memory retention was time-dependent, as it no longer occurred if TMS was applied late enough in the inter-trial interval (700 ms in present study). From this, we can infer that the neural processing underlying the retention of an acquired visuomotor skill is ongoing at the end of the trial but is diminished at 700 ms later. Alternatively, it is possible that the processing continues to take place at later times, but at that point the processing is resistant to disruption by TMS or the disruption can be compensated by other parts of the motor system (Lee et al., 2003).

The identical adaptation rates and initial aftereffect magnitudes of control and immediate-TMS groups suggest that both groups equally acquired knowledge of the field as an internal

model (Shadmehr et al., 1994; Wolpert et al., 2000). However, the immediate-TMS group underwent some process that resulted in impaired retention. It is unclear what is the neural mechanism underlying this selective contribution of M1 to retention. One example of a possible neural mechanism for this result of normal adaptation and impaired retention of the immediate-TMS group are the "memory cells" which were observed by Li et al. (2001) in electrophysiological experiments with monkeys learning force-field perturbations. According to this study, Class I memory cells normally retain the adaptive response to a force-field perturbation. During washout, an opposite adaptive response of Class II cells balance out the activity of the Class I cells, resulting in a population response that is identical to baseline. Cast in this framework, the disruption of M1 by TMS in our study may have impaired the proper development of plastic changes underlying the Class I memory cells in the immediate-TMS group, causing the activity to be balanced out faster during the de-adaptation period compared with the no-TMS and delayed-TMS groups.

As TMS is a strong contextual cue with nonspecific attentional and behavioral effects (Robertson et al., 2003), it was important to consider and rule out the possibility that the results we observed were due to these nonspecific effects. The main effect of the increased rate of de-adaptation in the immediate-TMS group occurred during a period when TMS was not present, making it unlikely that the result was due to a direct effect of TMS such as distractibility or attentional changes. However, there was a change in context from the adaptation period to the deadaptation period. Because we applied TMS during a period of subconscious adaptation, (i.e. a period when errors were so small as to be within the target size of the reaching movements), the change in the contextual cue (i.e. transfer from period with TMS to without TMS) was coincident with the arrival of very large error initially in the de-adaptation period. Was the more rapid de-adaptation of the immediate-TMS group due to this change in context? Two pieces of evidence argue against this interpretation. First, TMS at 700 ms is also a contextual cue, but it did not affect the de-adaptation rate. Second, in Experiment 2, we found that TMS during null blocks did

not alter subsequent response to sudden large errors occurring in the absence of TMS. Therefore, it is unlikely that the faster de-adaptation of the immediate-TMS group in Experiment 1 was due to a contextual change or, by the same reasoning, other nonspecific effects of TMS. In addition, it might be argued that immediate-TMS caused a change in cortical excitability which affected the subsequent de-adaptation period. While this is already unlikely given the low frequency (~0.2 Hz) and number of pulses (196) (Murase et al., 2005), it is ruled out by the result that the delayed-TMS group was not similarly affected by the TMS.

In conclusion, the present results demonstrate that disruption of M1 during adaptation does not critically alter the adaptive response to error. However, if the disruption selectively occurs early following the end of the trial, it produces a more fragile motor memory that shows poor retention. This suggests that processing in M1 contributes to retention of an acquired visuomotor skill in a time-dependent manner, with a strong contribution early in the inter-trial interval when there is a high probability of receiving error feedback, and with weaker contribution at later times. Given the variety of complex motor behaviors and the distributed brain networks involved, it remains to be determined to what extent our results on the involvement of M1 in visuomotor memory retention can be generalized to other types of motor learning.

References

Baraduc P, Lang N, Rothwell JC, Wolpert DM (2004) Consolidation of dynamic motor learning is not disrupted by rTMS of primary motor cortex. Curr Biol 14:252-256.

Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex. Lancet 1:1106-1107.

Brasil-Neto JP, Cohen LG, Panizza M, Nilsson J, Roth BJ, Hallett M (1992) Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J Clin Neurophysiol 9:132-6.

Chen R, Cohen LG, Hallett M (2002) Nervous system reorganization following injury. Neuroscience. 111:761-73.

Chouinard PA, Paus T (2006) The primary motor and premotor areas of the human cerebral cortex. Neuroscientist 12:143-52.

Dum RP, Strick PL (1991) The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci 11:667-89.

Dum RP, Strick PL (2005) Motor areas in the frontal lobe: the anatomical substrate for the central control of movement. *Motor Cortex in Voluntary Movements: A distributed system for distributed functions*, A. Riehle and E. Vaadia (eds), CRC Press, pp. 297-328.

Fulton J (1935) A note on the definition of the "motor" and "premotor" areas. Brain 58:311-6.

Gellhorn E, Hyde J (1953) Influence of proprioception on map of cortical responses. J Physiol 122:371-85.

Georgopoulos AP, Lurito JT, Petrides M, Schwartz AB, Massey JT (1989) Mental rotation of the neuronal population vector. Science 243:234-6.

Ghahramani Z, Wolpert DM (1997) Modular decomposition in visuomotor learning. Nature 386:392-5.

Graziano MS, Taylor CS, Moore T, Cooke DF (2002) The cortical control of movement revisited. Neuron 36:349-62.

Lee L, Siebner HR, Rowe JB, Rizzo V, Rothwell JC, Frackowiak RS, Friston KJ (2003) Acute remapping within the motor system induced by low-frequency repetitive transcranial magnetic stimulation. J Neurosci 23:5308-5318.

Lu X, Ashe J (2005) Anticipatory activity in primary motor cortex codes memorized movement sequences. Neuron 45:967-973.

Mills KR, Boniface SJ, Schubert M (1992) Magnetic brain stimulation with a double coil: the importance of coil orientation. Electroencephalogr Clin Neurophysiol 85:17-21.

Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, Boroojerdi B, Poewe W, Hallett M (2002) Early consolidation in human primary motor cortex. Nature 415:640-644.

Murase N, Rothwell JC, Kaji R, Urushihara R, Nakamura K, Murayama N, Igasaki T, Sakata-Igasaki M, Mima T, Ikeda A, Shibasaki H (2005) Subthreshold low-frequency repetitive transcranial magnetic stimulation over the premotor cortex modulates writer's cramp. Brain 128:104-115.

Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM (1996) Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. J Neurosci 16:785-807.

Pascual-Leone A, Walsh V, Rothwell J (2000) Transcranial magnetic stimulation in cognitive neuroscience--virtual lesion, chronometry, and functional connectivity. Curr Opin Neurobiol 10:232-7.

Paus T (1999) Imaging the brain before, during, and after transcranial magnetic stimulation. Neuropsychologia 37:219-24.

Richardson AG, Overduin SA, Valero-Cabré A, Padoa-Schioppa C, Pascual-Leone A, Bizzi E, Press DZ (2006) Disruption of primary motor cortex before learning impairs memory of movement dynamics. J Neurosci 26:12466-12470.

Riehle A (1991) Visually induced signal-locked neuronal activity changes in precentral motor areas of the monkey: hierarchical progression of signal processing. Brain Res 540:131-137.

Robertson EM, Théoret H, Pascual-Leone A (2003) Studies in cognition: the problems solved and created by transcranial magnetic stimulation. J Cogn Neurosci 15:948-60.

Rossi S, Rossini PM (2004) TMS in cognitive plasticity and the potential for rehabilitation. Trends Cogn Sci 8:273-9.

Sack AT (2006) Transcranial magnetic stimulation, causal structure-function mapping and networks of functional relevance. Curr Opin Neurobiol 16:593-99.

Sanes JN (2003) Neocortical mechanisms in motor learning. Curr Opin Neurobiol 13:225-31.

Scott SH (2003) The role of primary motor cortex in goal-directed movements: insights from neurophysiological studies on non-human primates. Curr Opin Neurobiol 13:671-7.

Shadmehr R, Wise SP (2005) *Computational Neurobiology of Reaching and Pointing: A Foundation for Motor Learning*, MIT Press, Cambridge MA.

Thielscher, A., Kammer, T (2004) Electric field properties of two commercial figure-8 coils in TMS: calculation of focality and efficiency. Clin Neurophysiol 115:1697-708.

Thoroughman KA, Shadmehr R (1999) Electromyographic correlates of learning an internal model of reaching movements. J Neurosci 19:8573– 8588.

Wise SP, Moody SL, Blomstrom KJ, Mitz AR (1998) Changes in motor cortical activity during visuomotor adaptation. Exp Brain Res 121:285-99.

Wolpert DM, Ghahramani Z (2000) Computational principles of movement neuroscience. Nat Neurosci 3(Suppl):1212-1217.