The motor system in healthy individuals and in stroke patients before and after rehabilitative therapy

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Abstract

A number of brain regions have been implicated in the recovery process after stroke. Yet, we still do not understand how these brain regions may influence each other. To overcome this problem, I used a *perturb-and-measure* approach, by combining transcranial magnetic stimulation (TMS) and positron emission tomography (PET), to examine effective connectivity of the motor system in healthy individuals and stroke patients. This doctorate thesis encompasses three studies. Together, the three studies provide new knowledge about effective connectivity and the function of the primary motor (M1) and dorsal premotor (PMd) cortices, and insights into rehabilitation-mediated recovery.

The first two studies examined effective connectivity and function of M1 and PMd in healthy individuals. Study 1 examined effective connectivity of M1 and PMd. This was achieved by stimulating the two cortical areas with repetitive TMS and measuring changes in cerebral blood-flow in the entire brain with PET. The results confirmed a hierarchical organization of the motor system. Repetitive TMS applied over M1 modulated a network of brain regions confined to the cortical and subcortical motor system. In contrast, repetitive TMS applied over PMd modulated a neural network that included several regions in the parietal and prefrontal cortices. Study 2 examined the role of M1 and PMd in the anticipatory scaling of forces during object lifting. This was achieved outside of the PET scanner by stimulating the two cortical areas using the same stimulation as in Study 1 and examining subsequent disruptions in the subjects' ability to lift different weights. The results demonstrated that M1 scales forces based on information acquired during a previous lift and PMd scales forces based on arbitrary cues.

Study 3 examined changes in the effective connectivity of M1 in stroke patients with chronic motor deficits (>1-year post-stroke) who underwent Constraint-Induced Movement Therapy (CI Therapy) for the affected arm. Improvements on various motor tests were observed immediately after therapy and were still present in most tests one-month afterwards. TMS / PET sessions were conducted before and immediately after therapy. During these sessions, I applied one-second trains of subthreshold 10-Hz

repetitive TMS over the probabilistic hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. The results demonstrated changes in the effective connectivity of the ipsilesional M1 with a number of motor regions in the brain including the contralesional M1, the non-primary motor areas in both hemispheres, and the basal ganglia in both hemispheres. I speculate that these results represent a rehabilitation-induced strengthening of a neural network necessary for the development of compensatory skills.

Résumé

Plusieurs régions cérébrales ont été impliquées dans le processus de récupération après un accident vasculaire cérébral (AVC). Mais nous ne comprenons pas encore comment ces régions cérébrales s'influencent les unes des autres. Pour résoudre ce problème, j'ai utilisé une approche *perturbe-et-mesure*, en combinant la stimulation magnétique transcrânienne (SMT) et la tomographie par émission de positons (TEP), afin d'examiner la connectivité effective du système moteur chez les individus sains et les victimes d'AVC. Cette thèse de doctorat comprend trois études. Ensemble, les trois études apportent des connaissances nouvelles au sujet de la connectivité effective et la fonction des cortex moteur primaire (M1) et prémoteur dorsal (PMd), et une certaine compréhension sur la récupération médiée par la réhabilitation.

Les deux premières études examinent la connectivité effective et la fonction de M1 et PMd chez les individus sains. L'étude 1 examine la connectivité effective de M1 et PMd. Ceci a été réalisé en stimulant les deux aires corticales avec la SMT répétitive et en mesurant les changements du flux sanguin dans le cerveau entier avec le TEP. Les résultats confirment l'organisation hiérarchique du système moteur. La SMT répétitive appliquée sur M1 module un réseau de régions cérébrales confiné au système moteur cortical et sous-cortical. Par contre, la SMT répétitive appliquée sur PMd module un réseau neural qui inclut plusieurs régions des cortex pariétaux et prémoteurs. L'étude 2 examine le rôle de M1 et PMd dans la gradation anticipée des forces à appliquer durant le soulèvement d'un objet. Ceci a été réalisé en dehors du scanner TEP en stimulant les 2 régions corticales avec les mêmes paramètres de l'étude 1 et en examinant les perturbations subséquentes dans l'habilité des sujets à soulever différents poids. Les résultats démontrent que M1 gradue les forces selon l'information acquise durant le soulèvement précédent et PMd selon des indices arbitraires.

L'étude 3 examine les changements dans la connectivité effective de M1 chez les patients avec AVC ayant un déficit moteur chronique (>1 an post AVC) qui ont suivi une thérapie-du-mouvement-par-contention pour le bras affecté. On observe des améliorations sur des tests moteurs variés immédiatement après la thérapie et ceux-ci persistent pour la plupart un mois plus tard. Des sessions SMT/TEP ont eu lieu avant et

immédiatement après la thérapie. Durant les scans, j'ai varié le nombre de trains de SMT (trains de 1 seconde à 10-Hz) appliqué au-dessus de la représentation probable de la main de M1 ipsilésionnel et contralésionnel. Les résultats démontrent des changements dans la connectivité effective de M1 ipsilésionnel avec un nombre de régions motrices dans le cerveau incluant M1 contralésionnel, les aires motrices non primaires des deux hémisphères et les noyaux de la base des deux hémisphères. Je propose que ces résultats représentent un renforcement induit par réhabilitation d'un réseau neural nécessaire pour le développement d'habilités compensatrices.

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Contribution of the Authors

Study 1: *Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. Journal of Neurophysiology 90: 1071-83.*

Authors: Philippe A. Chouinard, Ysbrand D. Van Der Werf, Gabriel Leonard, Tomáš Paus

I was responsible for designing, conducting, and analyzing the experiments. I also wrote the study for publication. Dr. Ysbrand Van Der Werf assisted me during the TMS / PET procedures and the data analysis. Dr. Gabriel Leonard and Dr. Tomáš Paus made important contributions to all aspects of my work, especially for the experimental design and manuscript preparation. This paper has been reprinted with copyright permission from the American Physiological Society.

Study 2: *Role of the primary motor and dorsal premotor cortices in the anticipation of forces during object lifting. Journal of Neuroscience 25: 2277-84.*

Authors: Philippe A. Chouinard, Gabriel Leonard, Tomáš Paus.

I was responsible for designing, conducting, and analyzing the experiments. I also wrote the study for publication. Dr. Gabriel Leonard and Dr. Tomáš Paus made important contributions to all aspects of my work, especially for the experimental design and manuscript preparation. This paper been reprinted with copyright permission from the Society for Neuroscience.

Study 3: *Changes in effective connectivity of the motor cortex in stroke patients after rehabilitative therapy (submitted).*

Authors: Philippe A. Chouinard, Gabriel Leonard, Tomáš Paus

I was responsible for designing, conducting, and analyzing the experiments. I also wrote the study for publication. Dr. Gabriel Leonard conducted the neuropsychological

evaluations and supervised me during the rehabilitation procedures. Dr. Tomáš Paus made important contributions to all aspects of my work, especially for the experimental design and manuscript preparation. This paper has been submitted for publication.

Chapter One

Introduction

I. Stroke

The World Health Organization defines a stroke as a sudden development of cerebral dysfunction that lasts more than 24 hours or leads to death with no apparent cause other than of vascular origin (Aho et al., 1980). Strokes occur when a blood clot interrupts the supply of nutrients to the brain or when a blood vessel in the brain ruptures and bleeds. As a result, brain cells affected by the stroke die.

Mortality due to stroke has declined in Canada by ~50% since 1969 to the current level of ~50 deaths per 100,000 people a year, which represents 7% of all causes of mortality (Heart and Stroke Foundation of Canada, 2003). This decline has been attributed both to an increase in the proportion of patients who survive stroke and to a decrease in the level of incidence (Bonita and Beaglehole, 1995). Health promotion has played a key role in the decline of incidence. Public health strategies targeted towards smoking cessation, diet, exercise, and weight control along with the management of hypertension, hyperlipidemia, diabetes, and heart disease have made substantial contributions (Bonita and Beaglehole, 1995).

Morbidity and the economic burden associated with stroke, however, remain high. Most stroke survivors are left with permanent disabilities, including hemiparesis, a condition characterized by motor weakness of one side of the body that impairs quality of life by restricting activity. In the year 2000, 72% of Canadians who have had a stroke reported that they required some form of assistance to perform activities of daily living (Heart and Stroke Foundation of Canada, 2003). Care for stroke patients accounts for 2.1% of all health care expenditures and with an increasingly elderly population, costs for the full range of health services required to manage the disease are projected to rise (Heart and Stroke Foundation of Canada, 2003).

During the first few months after stroke, a return of motor function can occur without therapeutic intervention (Nudo, 1999). This recovery is said to be spontaneous

and it involves the resolution of pathophysiological processes in the penumbra, a region of affected brain tissue around the core site of injury (Nudo, 1999). The amount of spontaneous recovery that occurs is related to the volume of penumbra that escapes infarction (Heiss and Graf, 1994; Furlan et al., 1996). Current notions consider motor deficits more or less permanent six months after stroke because of the diminishing possibility that brain tissue in the penumbra can regain function (Parker et al., 1986). Functional neuroimaging studies examining brain metabolism after stroke reveal that the resolution of diaschisis also contributes to recovery (Feeney and Baron, 1986; Seitz et al., 1999). Diaschisis refers to the suppression of intact brain regions that are remote from but anatomically connected to the area of primary injury (Von Monokow, 1914).

Longitudinal studies conclude that most motor recovery takes place during the first few months after stroke (Bonita and Beaglehole, 1988; Duncan et al., 1992; Nakayama et al., 1994; Jorgensen et al., 1995; Broeks et al., 1999). Nonetheless, some studies report that improvements in arm function can still occur many years after stroke (Duncan et al., 1992; Nakayama et al., 1994; Broeks et al., 1999). It is important to note that patients who demonstrate improvements in the affected arm in the later stages after stroke usually do so not because they can generate movements better but rather because of compensatory skills that enable them to maximize the residual control of the affected arm (Nakayama et al., 1994). This is not unlike the response of rats after the unilateral removal of the primary motor area (M1); it has been shown that these rats use postural compensation for retrieving food pellets rather than re-establishing normal strategies (Whishaw, 2000).

These observations correspond well with a theory of recovery proposed by John Hughlings Jackson, which he termed *Principles of Compensation*. Hughlings Jackson proposed his theory based on clinical observations he made with regards to cerebral localization (In: J Taylor, 1958). According to Hughlings Jackson, the central nervous system contains a number of hierarchical levels. Each level contains a complete set of representations of the next lower level that enables it to exert influence on motor behavior. If damage occurs in an area responsible for a particular movement, less heavily weighted areas for that same movement will become more involved at a higher level to compensate for damage at a lower level (In: J Taylor, 1958). This high-ordered re-

weighting of representation strengthens with time, amplifying further the degree of recovery.

Hughlings Jackson's hierarchical organization of the motor system was challenged in the 1990s with the emergence of anatomical studies in the macaque monkey that demonstrated a number of cortical areas other than M1 with direct projections to the spinal cord (Dum and Strick, 1991; He et al., 1993; 1995; Galea and Darian-Smith, 1994). Based on this evidence, some researchers proposed that these cortical areas had the capacity to act in parallel for the generation and control of distal arm movements (Fries et al., 1993; Dum and Strick, 1996). Fries et al. (1993) suggested further that these cortical areas had the capacity to substitute for each other functionally. Although corticospinal neurons project from areas other than M1, electrophysiological studies performed by Lemon and colleagues (1998; 2002) reveal that M1 is the only cortical area with a strong direct influence on lateral motor-neurons in the spinal cord and that this influence is important for the generation of distal arm movements. It is doubtful therefore that any other cortical area could substitute M1 in this respect.

There is evidence nonetheless that spared cortex adjacent to a damaged brain area can take over function. This theory, first described by Hermann Munk (1881), is known today as substitution. Munk's theory was disregarded for a long time as the result of a widely held notion that the brain was incapable of reorganization (Leyton and Sherrington, 1917). Substitution still remains controversial, but has acquired some acceptance after the following landmark studies were performed in the monkey. Merzenich and colleagues demonstrated that the somatotopic map of the primary somatosensory area can reorganize after peripheral nerve damage (1983), amputation of the fingers (1984), and after restricted lesions in the primary somatosensory area (Jenkins and Merzenich, 1987). In a series of additional studies, Nudo and colleagues demonstrated that the somatotopic map of M1 can expand after the acquisition of motor skills both in monkeys without lesions (1996a) and after movement therapy in monkeys with restricted lesions in M1 (1996b).

The degree to which compensation, substitution, or some combination of these two mechanisms takes place remains unclear. Brain mapping techniques in stroke patients may resolve this issue provided that information gained from these techniques is

interpreted within the scope of our knowledge about the anatomy and function of the motor system. In the next two sections, I will first discuss the anatomy and function of the motor system and then elaborate which brain regions could potentially underlie substitution and / or compensation.

II. Anatomy and function of the motor system

I will characterize the cortical motor system as a network of previously defined cortical areas in the frontal lobe that contain neurons projecting directly to the spinal cord (Dum and Strick, 2002). The cortical motor system can be separated into the primary (M1) and the non-primary motor areas. The non-primary motor areas encompass all areas in the frontal lobe that can influence motor output at the level of both M1 and the spinal cord (Dum and Strick, 1991).

Primary motor area

Mapping of M1 began in the late 19th century. The earliest experiments were performed on dogs. Fritsch and Hitzig (1870) demonstrated that electrical stimulation applied in the precentral region of the dog's brain could induce movement in the limbs. In non-human primates, Sherrington and colleagues later applied electrical stimulation to different locations in the precentral cortex and reported that they could induce movement for specific parts of the body by varying the location of stimulation (Grunbaum and Sherrington, 1908; Leyton and Sherrington, 1917). Penfield and colleagues also applied electrical stimulation along the precentral cortex in neurological patients during surgery for the removal of tumors and epileptic foci (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1952). Their results revealed a disproportionate somatotopic map of the body as shown in Penfield's drawing of a homunculus, which depicts the amount of area in M1 devoted to different parts of the body (Figure 1).

The composition of corticospinal neurons represents a key feature of M1. Axonal degeneration studies (Liu and Chambers, 1964) and later studies injecting anterograde tracers in M1 (Ralston and Ralston, 1985) reveal that ~75% of corticospinal neurons

decussate in the pyramids, ~15% decussate in the spinal cord, and the remaining ~10% do not cross. The strong presence of large corticospinal neurons is unique to M1; 31% of corticospinal neurons that arise from M1 are considered large and these neurons represent 79% of all large corticospinal neurons (Dum and Strick, 1991). Large corticospinal neurons are important for the generation of independent finger movements (Evarts, 1981). This is because they have a direct excitatory influence on the lateral motor-nuclei in the spinal cord (Muir and Lemon, 1983).

Another important feature of M1 is that it controls distal limb movements contralaterally. The complete unilateral removal of the sensorimotor cortex in the rhesus monkey results in sensorimotor impairments of the contralateral forelimb (Passingham et al., 1978; 1983). A detailed examination of motor dysfunction in these monkeys reveals permanent impairments of distal forelimb movements and a better recovery of more proximal forelimb movements (Passingham et al., 1978; 1983). After six months of recovery, these monkeys fail to grasp small objects between the two fingers and make isolated movements of the wrist. When attempting to grasp small objects, these monkeys use their hand as a shovel and contract all their fingers simultaneously around the object (Passingham et al., 1978; 1983).

Brinkman and Kuypers (1973) demonstrated in macaque monkeys with split-brains that each half of the brain controls distal movements of the forelimb contralaterally and proximal movements of the forelimb bilaterally. They examined movements of either forelimb in retrieving food pellets during visual input restricted to one half of the brain. The results indicated that the seeing half of the brain could control reaching and grasping movements of the contralateral forelimb and reaching movements but not grasping movements of the ipsilateral forelimb. This is because uncrossed corticospinal neurons terminate in either the medial motor-nuclei or intermediate zones of the spinal cord (Liu and Chambers, 1964; Ralston and Ralston, 1985) and innervate proximal forelimb muscles in the shoulder (Lawrence and Kuypers, 1968). Terminations of uncrossed corticospinal neurons in the lateral motor-nuclei, which innervate distal forelimb muscles, have yet to be demonstrated.

The corticospinal tract evolved as primates became more dexterous with their hands. Anatomical studies reveal that as primates developed precision grip, the

corticospinal tract increased in its overall size (Heffner and Masterton, 1983; Nudo et al., 1995) and was accompanied by the emergence of corticospinal terminations in the ventral horn where the lateral motor-nuclei are located (Bortoff and Strick, 1993). For example, cebus monkeys can use precision grip between the thumb and index finger to manipulate small objects and have abundant corticospinal terminations in the ventral horn. In contrast, squirrel monkeys cannot use precision grip and must instead use the whole hand to manipulate small objects; these monkeys have sparse corticospinal terminations in the ventral horn (Bortoff and Strick, 1993). Extra-cellular recordings in the spinal ventral horn in response to cortical stimulation reveal that excitatory post-synaptic potentials are smaller and rise more slowly in primate species that cannot use precision grip compared with those that do (Maier et al., 1997; 1998). Thus, corticospinal neurons in primate species that cannot use precision grip terminate further away from the soma of lateral motor-neurons.

Non-primary motor areas

Brodmann's cytoarchitectonic map of the human cortex designates area 4 as cortex in the anterior bank of the precentral sulcus and area 6 as cortex that encompasses the precentral gyrus and the posterior portion of the superior frontal gyrus on both the lateral and medial surfaces of the brain (1909). Based on clinical observations, Fulton (1935) proposed a functional distinction between two areas in the precentral cortex, coining the terms *primary motor area* for cortex in Brodmann area 4 and *premotor area* for cortex in the lateral part of Brodmann area 6. Penfield and Welch (1951) later used the term *supplementary motor area* to describe a functionally distinct area in the medial part of Brodmann area 6. The parcellation of the motor system has subsequently become more complex. Today, several distinct non-primary motor areas have been defined. Figure 2 illustrates the location of these areas in the brain of the macaque monkey and their respective homologues in the human brain (Adapted from: Rizzolatti et al., 1998; Picard and Strick, 2001).

Four distinct premotor areas have been identified on the lateral surface of the precentral cortex. The caudal dorsal and caudal ventral premotor areas have strong

connections with M1 (Dum and Strick, 1991; He et al., 1993) and the rostral dorsal and rostral ventral premotor areas have more robust connections with the prefrontal cortex (Barbas and Pandya, 1987; Lu et al., 1994). The caudal dorsal and rostral dorsal premotor areas are involved in the preparation of movements in response to external stimuli (Wise et al., 1997). Unlike the caudal dorsal premotor area, the rostral dorsal premotor area can also implement associations between arbitrary cues and motor responses (Petrides, 1982; Halsband and Passingham, 1982). The caudal ventral and rostral ventral premotor areas are also thought to mediate different aspects of motor behavior. The caudal ventral premotor area is involved in the transformation of specific positions in space into arm, neck, and face / mouth movements (Kakei et al., 2001). The rostral ventral premotor area is involved in the transformation of intrinsic properties of objects into hand actions (Rizzolatti et al., 1988; Rizzolatti and Luppino, 2001) and is thought to also underlie more cognitive-related functions including the understanding and imitation of actions (Rizzolatti et al., 1998; 2001).

The supplementary motor area, as originally described by Penfield and Welch (1951), has since been divided into two distinct areas. The supplementary motor area proper is located behind the vertical anterior-commissural line (Picard and Strick, 1996) and has strong connections with M1 (Dum and Strick, 1991; He et al., 1995). This area is involved in the initiation of actions and in the performance of sequential movements (Tanji et al., 1996; Gerloff et al., 1997). The pre-supplementary motor area is located in front of the vertical anterior-commissural line (Picard and Strick, 1996) and has more robust connections with the prefrontal cortex (Bates and Goldman-Rakic, 1993; Lu et al., 1994). This area is involved in learning sequential movements and is thought to also filter relevant sensory information for the production of actions (Nakamura et al., 1998; 1999; Nagahama et al., 1999).

Three distinct non-primary motor areas have been identified in the macaque monkey along the banks of the cingulate sulcus: the dorsal, ventral, and rostral cingulate motor areas (Luppino et al., 1991). The dorsal and ventral cingulate motor areas are located below the supplementary motor area on the dorsal and ventral banks of the cingulate sulcus and the rostral cingulate motor area spans both dorsal and ventral banks of the cingulate sulcus in front of the genu of the arcuate sulcus. The dorsal and ventral

cingulate motor areas have strong connections with M1 (Dum and Strick, 1991; He et al., 1995) and the ventral and rostral cingulate motor areas have more robust connections with the prefrontal cortex (Bates and Goldman-Rakic, 1993; Lu et al., 1994). The human homologues of these three cingulate motor areas are illustrated in Figure 2 and are involved in processes related to the voluntary control of movement (Paus et al., 1993; Picard and Strick, 1996; Paus, 2001).

III. Substitution versus compensation

The destruction of brain tissue is irreversible (Heiss and Graf, 1994). Substitution following any damage to M1 and / or its corticospinal fibers would have to involve alternative pathways that can access motor neurons in the spinal cord. Two mechanisms are proposed. The first mechanism is the substitution of spared M1. The second mechanism is the greater involvement of non-primary motor areas for the development of compensatory skills.

Substitution

Nudo and colleagues have used intra-cortical micro-stimulation to demonstrate that M1 can reorganize during motor skill acquisition. Movement representations in M1 of the squirrel monkey are larger in size and more complex for the dominant forelimb compared with the non-dominant forelimb (Nudo et al., 1992). In addition, movement representations in M1 can reorganize in squirrel monkeys that learned to retrieve food pellets from a small well (Nudo et al., 1996a; Plautz et al., 2000) and in rats that learned to retrieve food pellets from a rotating disk (Kleim et al., 1998). The representation for forelimb movements involved specifically in these tasks expanded at the expense of other representations. In contrast, M1 does not reorganize in control animals that performed unskilled movements repeatedly over an extended period of time (Kleim et al., 1998; Plautz et al., 2000).

When we consider that M1 can reorganize during motor-skill acquisition, it seems reasonable to suggest that it might retain some of this capacity after limited cortical

injury. In the chimpanzee, Leyton and Sherrington (1917) failed to demonstrate substitution of the adjacent intact cortex after having removed the forelimb representation of M1. In retrospect, this negative finding could have resulted from the large amount of cortex removed, as suggested by Nudo and colleagues (2001). Glees and Cole (1950) removed the thumb representation of M1 in the macaque monkey and reported that a new representation of the thumb emerged in the adjacent intact cortex. Intra-cortical micro-stimulation has firmly established that the hand representation of M1 can reorganize after ischemic lesions are made in ~30% of the cortical area devoted to the hand (Nudo et al., 1996b; Nudo and Milliken, 1996). This change, however, depends on the post-lesion experience. If squirrel monkeys undergo post-lesion movement therapy with shaping exercises for the affected hand, the amount of cortex devoted to representing the hand adjacent to the lesion can increase on average by 10% (Nudo et al., 1996b). In contrast, if squirrel monkeys are allowed to recover without any post-lesion training, the remaining undamaged representation of the hand retracts in size (Nudo and Milliken, 1996).

Compensation

The non-primary motor areas can also reorganize in response to injury in M1. Frost et al. (2003) demonstrated that the somatotopic map of the ipsilesional ventral premotor area in squirrel monkeys reorganizes after making ischemic lesions in the hand representation of M1. Both a return of motor function and a gradual expansion of the hand representation in the ventral premotor area occurred over a period of 3-months. Their results also demonstrated that a greater sparing of the hand representation in M1 resulted in less expansion of the hand representation in the ventral premotor area. The extent of reorganization in intact cortical areas that are remotely located from a lesion in M1 is thus greater as the damage in M1 increases.

The injection of retrograde tracers in the cervical spinal segments reveal that the premotor, supplementary motor, and cingulate motor areas collectively comprise more than 60% of the cortical area in the frontal lobe that projects fibers directly to the spinal cord (Dum and Strick, 1991; Galea and Darian-Smith, 1994). Fibers from corticospinal neurons in the non-primary motor areas descend in the anterior limb of the internal

capsule, passing more anteriorly than those from M1 that descend in the posterior limb of the internal capsule (Morecraft et al., 2002). A stroke may damage one or more of these pathways leaving others intact. A lesion that disables a population of neurons in a parallel system may impair but not abolish function (Fries et al., 1993).

Though parallel systems provide a safety factor to compensate for any transient or long-term failure of some of its components (Darian-Smith et al., 1999), the non-primary motor areas have nonetheless a weak direct influence on spinal motor-neurons. The injection of anterograde tracers in the forelimb representation of the non-primary motor areas reveal that the majority of their corticospinal neurons terminate in the intermediate zone of the spinal cord (Dum and Strick, 1996; 2002). Excitatory post-synaptic potentials recorded in the lateral motor-nuclei in response to electrical stimulation of the non-primary motor areas are much smaller and rise more slowly compared with electrical stimulation in M1 (Maier et al., 2002). It appears therefore that the influence of the non-primary motor areas on the spinal cord may reflect the preparation and modulation of intrinsic spinal circuitry (Prut and Fetz, 1999; Bizzi et al., 2000) rather than the generation of independent finger movements that requires a direct excitatory influence on spinal motor-neurons (Lemon et al., 2002; Maier et al., 2002). The non-primary motor areas could instead compensate for M1 damage; one possibility is by their role in the planning and maintenance of movements (York and Steinberg, 1995).

IV. Brain mapping techniques

Current brain mapping techniques enable us to examine anatomical, functional, and effective connectivity of the human brain. White-matter tracts can be mapped using voxel-wise analysis of structural magnetic-resonance images (Paus et al., 1999) and diffusion-tensor imaging (Behrens et al., 2003). These techniques provide valuable information about anatomical connectivity. Yet, a structural link between two brain regions does not always mandate a functional interaction.

Functional connectivity can be examined using a variety of brain mapping techniques, including positron emission tomography (Friston et al., 1994; Paus et al, 1996) and functional magnetic-resonance imaging (Buchel et al., 1999). In these studies,

statistical tools are used to determine the similarity in regional variations of task-related changes in cerebral activity. It is important to note that the engagement of the subject performing a task can nonetheless confound these types of analyses. Co-activations acquired with functional neuroimaging may reflect instead relationships between different task components rather than true connectivity.

Effective connectivity refers to the influence that one brain region exerts over another. For this thesis, I examine effective connectivity using a *perturb-and-measure* approach; namely, the physical perturbation of neural activity to evaluate cause-and-effect in the context of neural connectivity (reviewed in Paus, 2005). This is achieved by stimulating a target area of the cortex with transcranial magnetic stimulation (TMS) and measuring changes in activity in the entire brain with positron emission tomography (Paus et al., 1997; Fox et al., 1997; Siebner et al., 1998; reviewed in Siebner et al 2003a; Paus 2005; Figure 3). The advantage of combining TMS and positron emission tomography (PET) is that it serves as a behavior-independent assay of connectivity for a specific cortical area with other structures in the brain. The following sections describe briefly TMS and PET.

Transcranial magnetic stimulation

TMS is used to manipulate neural activity in space and time by inducing brief currents in restricted areas of the cortex. A brief current passes through a stimulating coil, which is placed over the scalp, that then induces a rapid rise of magnetic field, and this transient field in turn induces a current in the underlying brain tissue. The procedure is painless because the magnetic field passes through the scalp and skull virtually unattenuated. Barker et al. (1985) performed the first TMS experiment and the technique has since acquired importance as a method for non-invasive brain stimulation for examining motor, perceptual, and cognitive processes (Pascual-Leone et al., 1998; Walsh and Cowey, 2000; Chen, 2000; Siebner and Rothwell, 2003). TMS can be used to induce motor responses, interfere with neural processing, and modulate neural networks temporarily beyond the duration of stimulation (reviewed in Paus, 2002). To some extent, TMS can also be used to examine motor-output maps in a manner similar to the mapping of M1 in animals

(Wassermann et al., 1992). This is achieved by stimulating different locations on the scalp and mapping subsequently the positions on the scalp where stimulation evokes a response in a contralateral muscle.

Positron emission tomography

PET is based on a hypothesis advanced more than a century ago by Roy and Sherrington (1890) that changes in neural activity lead to changes in cerebral blood-flow and metabolism. PET allows the investigator to measure cerebral activity by injecting a small amount of radioactive tracer into the blood stream and measuring the amount of radioactivity that subsequently reaches the brain. The PET scanner consists of a ring of detectors that surrounds the subject's head and detects the coincident gamma rays produced by the annihilation of positron-electron pairs. The detection of coincident gamma rays allows one to determine the line along which the radioactive decay occurred. Following the administration of a positron-emitting radionuclide, an image of the distribution of radioactivity in the brain is generated by combining the coincidence detection of the annihilation gamma rays with the reconstruction algorithms of computed tomography.

The radioactive tracer ^{15}O-labeled H_2O is commonly used to measure cerebral blood-flow (CBF) in PET studies (Raichle et al., 1983). ^{15}O-labeled H_2O has a short half-life of approximately 2 minutes and allows the investigator to acquire multiple scans with short durations in the same scanning session. Studies performed in animals that combine electrophysiological recordings and functional neuroimaging reveal that the primary force for driving changes in regional CBF is excitatory post-synaptic activity (Mathiesen et al. 1998; Logothetis et al. 2001). A nitric-oxide based model has been proposed to explain the nature of this coupling. Essentially, the release of the excitatory neurotransmitter glutamate produces a cascade of events that increases the production of nitric oxide post-synaptically (Knowles et al., 1989). Nitric oxide then diffuses freely into the surrounding tissue and in turn signals blood vessels in the vicinity to dilate (Northington et al., 1992; Iadecola et al., 1993).

V. Brain mapping in recovered stroke patients

Functional neuroimaging has provided a wealth of knowledge about brain regions underlying stroke recovery. Table 1 provides a mini meta-analysis of functional neuroimaging studies conducted in stroke patients after recovery. Three robust findings emerge as recovered patients execute movements with their affected arm: 1) an increase and / or displacement of activity in the ipsilesional M1, 2) a greater involvement of the contralesional M1, and 3) a greater involvement of the non-primary motor areas in both hemispheres.

Changes in the ipsilesional M1

Previous studies reveal that the ipsilesional M1 can change in an adaptive manner provided that damage due to stroke is limited. In recovered patients with small subcortical infarcts, cerebral activity during simple hand movements increases and / or shifts location in the ipsilesional M1 (Weiller et al., 1992; 1993; Dettmers et al., 1997). Cramer and colleagues (1997) also report increases in activity along the rim of small infarcts in M1. Activity in the ipsilesional M1 can also increase disproportionately with the level of forces applied during key presses (Dettmers et al., 1997). A detailed analysis of the relationship between activity in the ipsilesional M1 and the level of forces applied reveals a linear relationship in healthy control subjects and an exponential relationship in recovered patients. These results suggest a greater recruitment of M1 neurons in recovered patients compared with healthy control subjects when exerting small amounts of force.

Some damage to the corticospinal fibers from M1 must be present for substitution to occur. In patients with lesions limited to the posterior limb of the internal capsule, activity in the ipsilesional M1 increases in location close to the face area during recovered hand movements, which suggests a lateral shift of the hand representation towards the face area (Weiller et al., 1993). This shift does not occur in patients with lesions limited to the anterior limb of the internal capsule (Weiller et al., 1993). In a TMS study, Byrnes and colleagues (1999) examined output maps for the abductor pollicis

brevis muscle in a group of patients with subcortical infarcts. The results of this study demonstrated that the reorganization of the ipsilesional M1 occurs in response to damage to its corticospinal fibers. Output maps for the affected thumb muscle shifted in location in patients with lesions in the posterior limb and not in the anterior limb of the internal capsule.

Other TMS studies demonstrate that responses evoked in affected arm muscles shortly after stroke can predict motor outcome. Patients who initially demonstrate motor evoked-potentials usually demonstrate good recovery afterwards (Heald et al., 1993; Binkofski et al., 1996; Rapisarda et al., 1996; Turton et al., 1996; Traversa et al., 1997; Byrnes et al., 1999; Delvaux et al., 2003). This finding indicates that some direct cortical influence on the spinal motor-neurons must be present for recovery to take place. TMS applied over M1 exerts its influence on corticospinal neurons that synapse with motor neurons in the spinal cord through activation of their afferents in the stimulated cortex (Day et al., 1989). The initial presence of motor evoked-potentials can also predict the extent that M1 can reorganize. A one-year longitudinal TMS mapping study demonstrates that both the amount of recovery and the extent with which the center of output maps for the first dorsal interosseus muscle shifts depends on whether TMS can evoke responses in this muscle one-day after stroke (Delvaux et al., 2003).

Changes in the contralesional M1

The role of the contralesional M1 during recovery remains unclear. The presence of ipsilateral motor evoked-potentials in the affected arm is associated with poor motor outcome (Turton et al., 1996; Netz et al., 1997). Ipsilateral motor evoked-potentials acquired in these patients have prolonged latencies and can be induced only during the voluntary contraction of arm muscles. These motor evoked-potentials are likely to reflect corticoreticulospinal pathways (Benecke et al., 1991). M1 has no direct access to the spinal motor-neurons that innervate distal arm muscles on the same side of the body. These observations rule out the possibility that uncrossed corticospinal fibers from the contralesional M1 could provide a substrate for substitution.

The contralesional M1 does nonetheless undergo adaptive changes during recovery. Patients who recover well from one stroke and have a second stroke in the opposite hemisphere will not only have a new contralateral hemiparesis, but will also have a reappearance of the original deficits caused by the first stroke (Fisher, 1992; Lee and van Donkelaar, 1995). Functional neuroimaging demonstrates increased activity in the contralesional M1 as recovered patients perform simple hand movements (Chollet et al., 1991; Weiller et al., 1993; Cramer et al., 1997; Honda et al., 1997; Cao et al., 1998). Cortical potentials related to simple hand movements also increase in the contralesional M1 (Honda et al., 1997). The increased activity in the contralesional M1 appears to emerge consistently with co-activity in the ipsilesional M1 provided that damage in the ipsilesional M1 is limited (Chollet et al., 1991; Weiller el al., 1993; Honda et al., 1997; Cao et al., 1998).

The involvement of the contralesional M1 could represent a compensatory mechanism. Direct connections between the M1s in the two hemispheres seem to exist in the human. In healthy individuals, TMS applied over M1 in one hemisphere reduces motor potentials in the hand evoked by TMS applied 6 to 11 ms later over the opposite M1 (Ferbert et al., 1992; Di Lazzaro et al., 1999). Studies combining TMS and PET report changes in cerebral activity in the right M1 as the result of repetitive TMS applied over the left M1 (Fox et al., 1997; Paus et al., 1998; Siebner et al. 2000). In healthy individuals, a balanced inter-hemispheric interaction between the two M1s is required for the generation of complex hand movements (Ferbert et al., 1992; Chen et al., 1997a). Cerebral activity increases in the M1 ipsilateral to the hand that performs a complex task compared with a simpler task (Rao et al., 1993; Shibaski et al., 1993). Repetitive TMS applied over M1 disrupts finger sequences as subjects play the piano with either hand (Chen et al., 1997a). The analogy in the amount of involvement of M1 in the two hemispheres during the performance of complex hand movements by healthy individuals and simple hand movements by stroke patients suggests that recovered stroke patients recruit additional resources in the intact hemisphere to execute simple motor tasks.

Changes in the non-primary motor areas

The non-primary motor areas, which exert a weak direct influence on spinal motor neurons (Maier et al., 2002; Lemon et al., 2002), are involved normally in the planning, selection, and maintenance of movements (Ashe and Ugurbil, 1994). These areas reside in what Hughlings Jackson called higher evolutionary levels of the central nervous system (In: J Taylor, 1958; York and Steinberg, 1995). As outlined earlier, Hughlings Jackson believed that if damage occurs in an area responsible for a particular movement, less heavily weighted areas for that same movement would become more involved at a higher level to compensate for damage at a lower level. This theory fits well with the fact that all non-primary areas have a somatotopic representation of the body and can influence movements via their connections with M1 and their projections to the intermediate zone of the spinal cord (Lemon et al., 2002).

Functional neuroimaging studies demonstrate increases in activity in the dorsal premotor area (PMd) in recovered patients who perform simple hand movements (Chollet et al., 1991; Weiller et al., 1992; 1993; Cramer et al., 1997; Cao et al., 1998; Sietz et al., 1998; Nelles et al., 1999b). In fact, a recent meta-analysis reveals that the contralesional PMd is the most frequently reported brain area involved in the execution of recovered movements after stroke (Calautti and Baron, 2003). Johansen-Berg and colleagues (2002a) provide further evidence that the contralesional PMd has an adaptive role. In an fMRI experiment, they demonstrated increased blood-oxygen level dependent (BOLD) signal in PMd in recovered stroke patients who performed a simple reaction task compared with healthy control subjects. In a separate experiment, single-pulse TMS applied over this area disrupted task performance and the degree of disruption correlated with the level of BOLD response.

PMd is involved normally in the selection of movements (Schluter et al., 1998) and may provide a compensatory mechanism to enable stroke patients to accomplish a motor task by selecting motor programs that they can perform. In healthy volunteers, Schulter and colleagues (1998) applied single-pulse TMS during a choice-reaction task to interfere with neural processing in M1 and PMd. During task performance, subjects selected an appropriate response based on visual cues. The authors compared the effects

of TMS applied at different time points after cue presentation. The results revealed that stimulation applied over PMd disrupted an early stage of movement selection and that stimulation applied over M1 disrupted a later stage of motor execution.

Functional neuroimaging studies also demonstrate increases in activity in the supplementary motor and cingulate motor areas in recovered patients who perform simple hand movements (Chollet et al., 1991; Weiller el al., 1992; 1993; Cramer et al., 1997; Honda et al., 1997; Cao et al., 1998). These responses may reflect the recruitment of additional resources necessary to accomplish motor tasks. The supplementary motor area is involved normally in the initiation of motor actions and the performance of sequential movements (Tanji et al., 1996; Gerloff et al., 1997). Permanent deficits in these two aspects of motor control can occur after the removal of the supplementary motor area in patients with tumors or epileptic foci (Brust, 1996). The cingulate motor areas are involved normally during motor tasks that require a greater level of voluntary control (Paus et al., 1993; Paus, 2001). Recovered patients may require a greater level of voluntary control to accomplish motor tasks that were previously automatic and / or effortless before stroke.

VI. Brain mapping before and after rehabilitative therapy

The review in the previous section consisted of brain mapping studies conducted in stroke patients with good recovery at a time when their motor deficits were considered permanent. These studies identified brain regions involved during the execution of recovered arm movements, but provide little information about the evolution of change that takes place. This section provides on overview of studies that demonstrate neural correlates of motor improvements acquired during rehabilitative therapy.

Changes take place in the ipsilesional M1 as patients improve motor function during conventional therapy (Liepert et al., 2000a; Traversa et al., 2000). Liepert and colleagues (2000a) demonstrated that the output map for the affected abductor pollicis brevis muscle enlarges temporarily following one session of conventional therapy. In this study, motor-output maps of patients one to two months after stroke were obtained before, one-hour after, and one-day after one session of therapy. The results demonstrated

an enlargement of the output map for the affected thumb muscle one-hour after therapy. This enlargement was not maintained one-day after therapy, which the authors proposed could indicate that one session of physiotherapy might not suffice for permanent enlargements of motor-output maps. Traversa and colleagues (2000) used TMS to examine motor-output maps of patients two to four months after stroke prior to and after the completion of a conventional rehabilitation program. The results demonstrated an enlargement of the output map for the affected abductor digiti minimi muscle; this change correlated further with the degree of clinical improvement. Similar changes take place with Constraint-Induced Movement Therapy (CI Therapy; Taub et al., 1993, 2002) more than six months after stroke (Liepert et al., 1998; 2000b).

The theoretical basis underlying CI Therapy arises from research in monkeys with unilateral forelimb deafferentation. Following this operation, monkeys fail to use the deafferented forelimb in the free situation (Lassek, 1953). Monkeys can however regain purposive use of the deafferented forelimb if the intact forelimb is restrained to *force* the use of the deafferented forelimb (Stein and Carpenter, 1965). This return in function can also be made permanent if the restraint is maintained for two weeks (Stein and Carpenter, 1965). These observations led Taub (1980) to propose that the non-use of an affected extremity after injury to the nervous system is partially the result of a learning phenomenon that involves a conditioned suppression of movement. Based on this theory, Taub et al. (1993, 2002) developed CI therapy for stroke patients with chronic motor deficits (>6 months post-stroke). During this therapy, patients wear a restraint on their good arm to discourage its use and perform exercises with the affected arm to improve its function. A number of studies have found that CI Therapy produces long-term improvements in the amount of use of the affected arm in the real-world environment (Taub et al., 1993; 2002; Liepert et al., 1998; 2000b; Miltner et al., 1999).

Liepert and colleagues (1998; 2000b) used TMS mapping to examine motor-output maps in patients >6 months after stroke before and after two weeks of CI Therapy. Before therapy, the output map for the affected abductor pollicis brevis muscle was smaller than the one for the unaffected abductor pollicis brevis muscle. Following functional improvements of the affected arm, the output map for the affected abductor pollicis brevis muscle increased in size and its center shifted in location. In contrast, the

output map for the unaffected abductor pollicis brevis muscle did not change in size and its center did not shift in location. In a different study, Johansen-Berg colleagues (2002b) used fMRI to examine neural correlates underlying motor improvements in patients >6 months after stroke before and after two weeks of a modified version of CI Therapy. Following functional improvements of the affected arm, the authors of this study correlated the change in BOLD response during a simple manual task with the ratio change in motor performance between the two hands. Their results revealed correlations in the premotor and secondary somatosensory cortices in the ipsilesional hemisphere and in the superior portions of the cerebellum bilaterally.

VII. Present investigation

1. The first study, entitled "*Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices*", examined effective connectivity of M1 and PMd by combining TMS and PET. This was achieved by stimulating the two cortical areas with low-frequency repetitive TMS at subthreshold intensity and measuring subsequent changes in cerebral blood-flow in the entire brain with PET. I hypothesized that repetitive TMS applied over M1 and PMd would influence CBF in different distal brain regions in a manner that would reflect their known anatomical connectivity in the monkey.

2. The second study, entitled "The role of the primary motor and dorsal premotor cortices in the anticipation of forces during object lifting", examined the role of M1 and PMd during motor control. This was achieved by stimulating the two cortical areas with the same stimulation used in Study 1 and examining subsequent disruptions in the subject's ability to scale forces during object lifting. When lifting small objects, people apply forces that match the expected weight of the object. I hypothesized that repetitive TMS applied over M1 would disrupt the generation of discrete forces when lifting different weights and that repetitive TMS applied over PMd would disrupt the scaling of forces based on arbitrary cues.

3. The third study, entitled "Changes in effective connectivity of the motor cortex in stroke patients after rehabilitative therapy", examined changes in the effective

connectivity of M1 in stroke patients who underwent CI Therapy more than one year after stroke. During TMS / PET sessions, I applied one-second trains of subthreshold 10-Hz repetitive TMS over the probabilistic hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. I hypothesized that the therapy would lead to a change in the local response of the ipsilesional M1 as well as changes in effective connectivity of the ipsilesional M1 with the non-primary motor areas and the basal ganglia.

VIII. Tables

Table 1. Mini meta-analysis of functional neuroimaging studies conducted in stroke patients after recovery.

A. Demographics

Study	Patients	Age (Years)	Location of Lesions	Time after Stroke (Months)	Degree of Recovery
Cao et al. (1998)	6 F, 2 M	19 to 70, Mean = 46	Cortical / Subcortical	5 to 43, Mean = 15.6	Variable
Chollet et al. (1991)	2 F, 4 M	25 to 71, Mean = 47	Cortical / Subcortical	> 2	Good
Cramer et al. (1997)	3 F, 7 M	55 to 86, Mean = 71	Cortical / Subcortical	0.4 to 15, Mean = 6.1	Good
Nelles et al. (1999a)	2 F, 4 M	52 to 75, Mean = 64	Subcortical	0.3 to 2.1, Mean = 0.7	Not yet recovered
Nelles et al. (1999b)	"	"	"	3 wks after Nelles et al (1999a)	Modest
Seitz et al. (1998)	1 F, 6 M	41 to 68, Mean = 54	Cortical	0.8 to 52, Mean = 10.5	Good
Weiller et al. (1992)	3 F, 7 M	21 to 62, Mean = 41	Subcortical	3 to 72, Mean = 14.6	Good
Weiller et al. (1993)	"	"	"	"	"

B. Methods

Study	Task	Mirror Movements (Ratio of Patients)	Contrast
Cao et al. (1998)	Finger-to-thumb opposition	2 / 8	Cluster analysis
Chollet et al. (1991)	Finger-to-thumb opposition	N / R	(Aff. Arm) - (Rest)
Cramer et al. (1997)	Tapping with index finger	1 / 10	Each patient vs. controls
Nelles et al. (1999a)	Passive elbow movements	N / A	$(\text{Aff. Arm - Rest})_{\text{Patients}}$ - $(\text{Aff. Arm – Rest})_{\text{Controls}}$
Nelles et al. (1999b)	Passive elbow movements	N / A	$(\text{Aff. Arm – Rest})_{\text{Ses2}}$ - $(\text{Aff. Arm – Rest})_{\text{Ses1}}$
Seitz et al. (1998)	Finger-to-thumb opposition	0 / 7	(Aff. Arm) - (Rest)
Weiller et al. (1992)	Finger-to-thumb opposition	4 / 10	Patients vs. controls
Weiller et al. (1993)	Finger-to-thumb opposition	"	Each patient vs. controls

C. Results

Study	SM1		SMA		PMC		CMA		Striatum	
	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
Cao et al. (1998)	7 / 8	6 / 8	3 / 8	2 / 8	1 / 8	6 / 8	3/ 8	0 / 8	N / R	N / R
Chollet et al. (1991)	Yes	Yes	Yes	Yes	Yes	Yes	N / R	N / R	No	No
Cramer et al. (1997)	3 / 9	6 / 9	4 / 9	3 / 9	1 / 9	4 / 9	N / R	N / R	0 / 9	2 / 9
Nelles et al. (1999a)	Yes	Yes	No	No	No	No	No	No	No	No
Nelles et al. (1999b)	No	No	No	No	No	Yes	No	No	No	No
Seitz et al. (1998)	No	No	Yes	No	Yes	Yes	Yes	No	N / R	N / R
Weiller et al. (1992)	No	No	No	No	No	Yes	Yes	Yes	No	Yes
Weiller et al. (1993)	4 / 8	4 / 8	5 / 8	5 / 8	1 / 8	8 / 8	3 / 8	3 / 8	0 / 8	4 / 8

Abbreviations: SM1 = primary sensorimotor cortex, SMA = supplementary motor areas, PMC = premotor cortex, CMA = cingulate motor areas, Ipsi = ipsilesional, and Contra = contralesional, N / R = not reported, N / A = not applicable.

IX. Figures

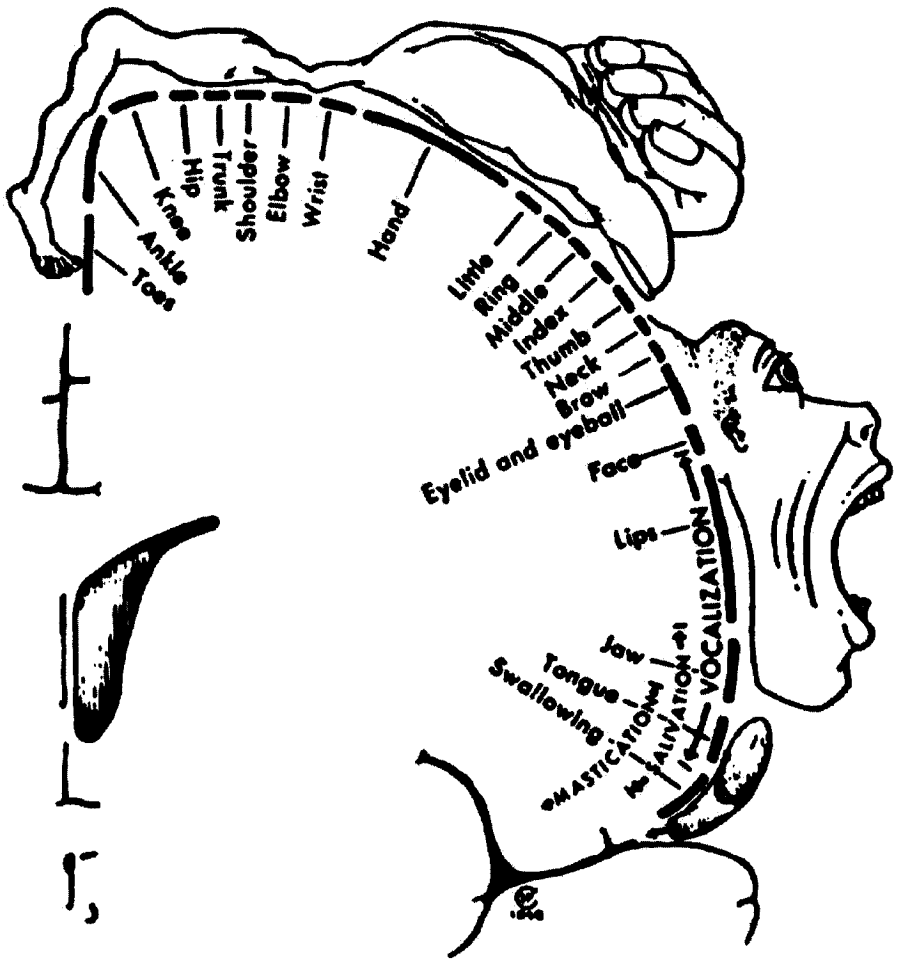

Figure 1. Penfield's Motor Homunculus. Penfield and colleagues revealed a disproportionate somatotopic map of the body in the primary motor cortex as shown in their drawing of a homunculus, which depicts the extent of primary motor cortex devoted to different parts of the body. Note how the cortical area devoted to the hand is much larger in comparison with the other parts of the body. Figure taken from: Penfield and Rasmussen (1952) The cerebral cortex of man. Macmillan: New York.

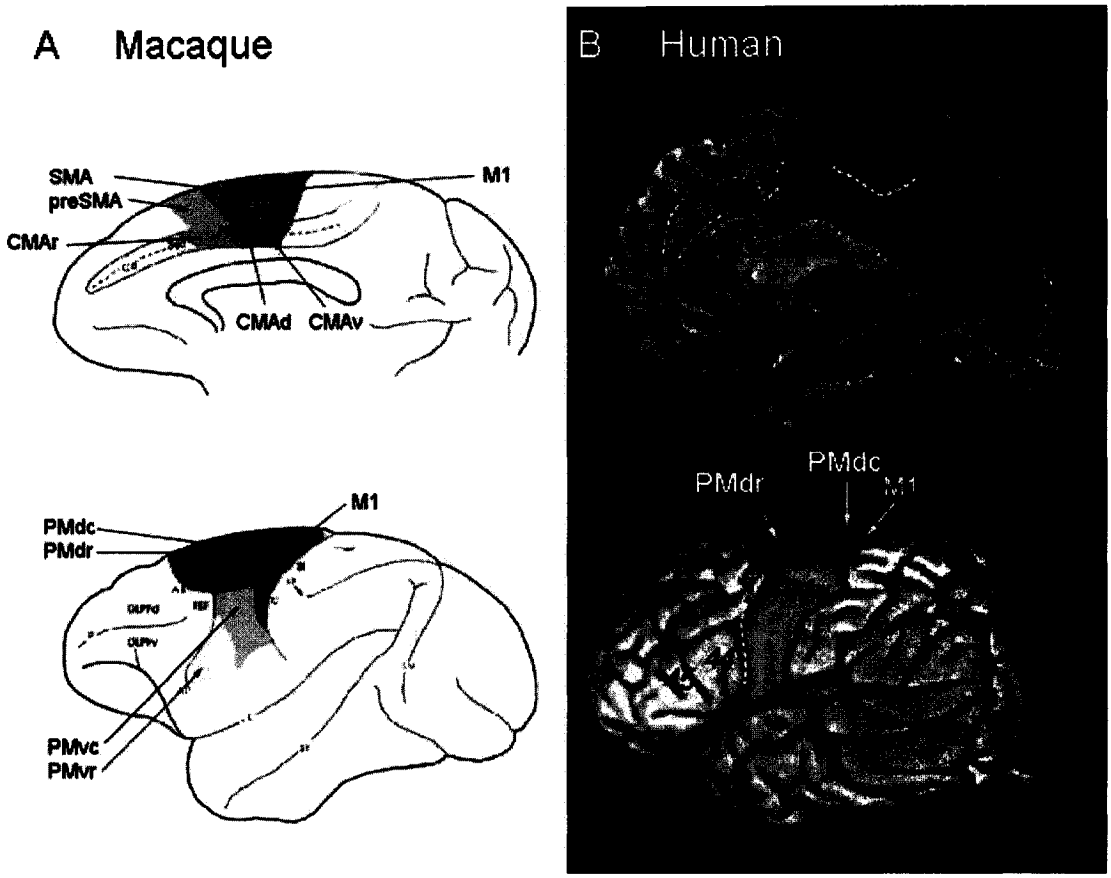

Figure 2. The Cortical Motor System. The cortical motor system can be separated into the primary motor and the non-primary motor areas. The non-primary motor areas are defined as all regions in the frontal lobe that can influence motor output at the level of both the primary motor cortex and the spinal cord. Several anatomically distinct areas constitute the non-primary motor cortex, each with a different specialization. The figure depicts A) the primary motor and non-primary motor areas in the brain of the macaque monkey and B) their respective homologues in the human brain. Abbreviations: M1 = primary motor area, PMdr = rostral dorsal premotor area, PMdc = caudal dorsal premotor area, SMA = supplementary motor area, CMAr = rostral cingulate motor area, CMAv = ventral cingulate motor area, CMAd = dorsal cingulate motor area, RCZa = anterior rostral cingulate zone, RCAp = posterior rostral cingulate zone, and CCZ = caudal cingulate zone. Adapted from: 1) Rizzolatti et al. (1998) Electroencephalography and Clinical Neurophysiology 106: 283-96, and 2) Picard and Strick (2001) Current Opinion in Neurobiology 11:663-72.

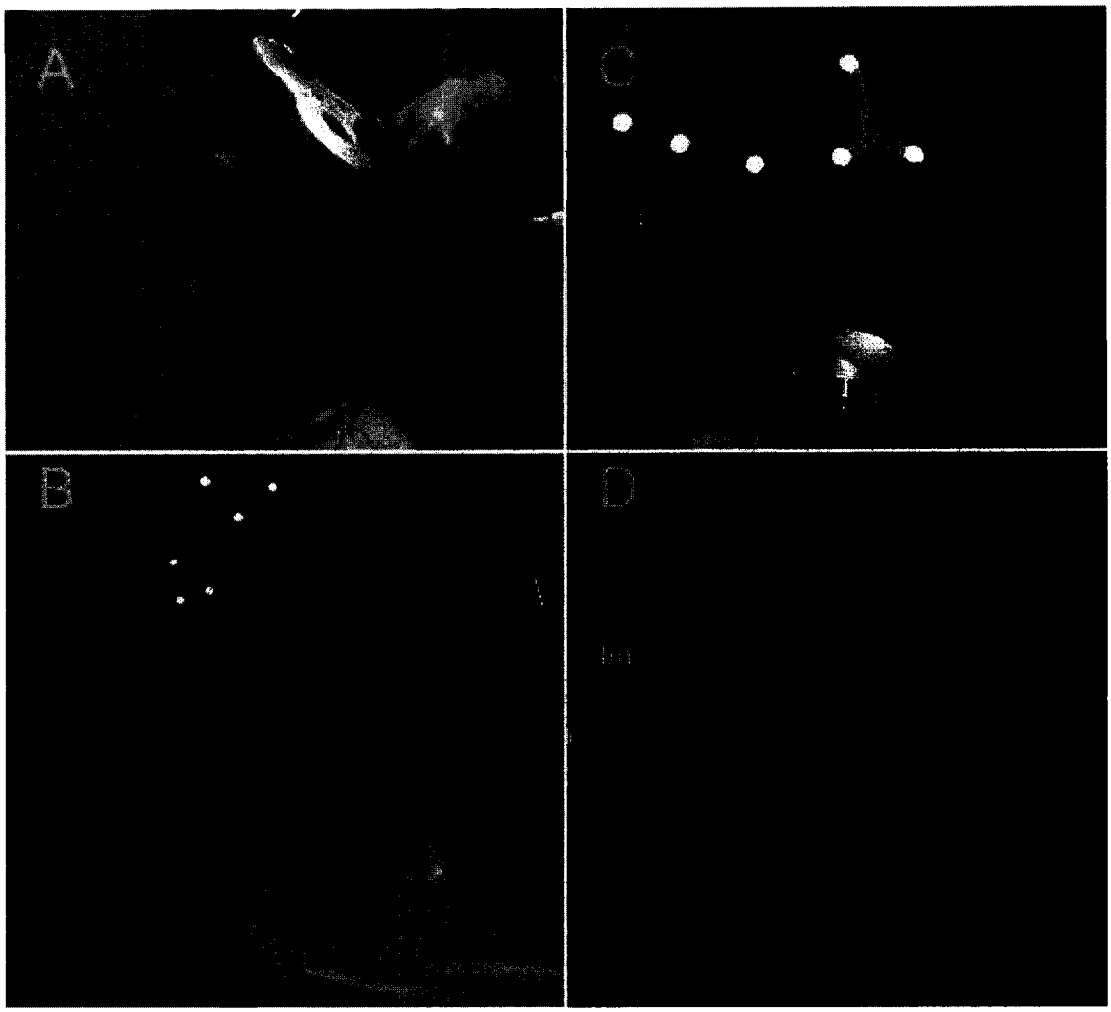

Figure 3. Overview of TMS / PET. I used PET in combination with TMS to map both local and distal changes in cerebral blood-flow. A) TMS was used to manipulate neural activity in space and time by inducing brief currents in a restricted cortical area. B) PET was used to measure cerebral blood-flow in the entire brain. This photograph shows the PET scanner, the TMS coil, a reference, and a tracker. With the subject lying on the bed of the scanner, I registered their head (C) with their MRIs (D) and tracked the TMS coil positions using an infrared optical-tracking system (Brainsight software: Rogue Research Inc., Montreal, Quebec, Canada; Polaris System: Northern Digital Inc., Waterloo, Ontario, Canada).

Chapter Two

Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices.[1]

Philippe A. Chouinard, Ysbrand D. Van Der Werf, Gabriel Leonard, Tomáš Paus.

I. Prelude

Study 1 examined effective connectivity of M1 and PMd by combining TMS and PET. This was achieved by stimulating the two cortical areas with low-frequency repetitive TMS at subthreshold intensity and measuring subsequent changes in cerebral blood-flow in the entire brain with PET. I hypothesized that repetitive TMS applied over M1 and PMd would influence CBF in different distal brain regions in a manner that would reflect their known anatomical connectivity in the monkey.

II. Abstract

Our study uses the combined transcranial magnetic stimulation / positron emission tomography (TMS/PET) method for elucidating neural connectivity of the human motor system. We first altered motor excitability by applying low-frequency repetitive TMS over two cortical motor regions in separate experiments: the dorsal premotor and primary motor cortices. We then assessed the consequences of modulating motor excitability by applying single-pulse TMS over the primary motor cortex and measuring: 1) muscle responses with electromyography and 2) cerebral blood-flow with PET. Low-frequency repetitive stimulation reduced muscle responses to a similar degree in both experiments. To map networks of brain regions in which activity changes reflected modulation of motor excitability, we generated t-statistical maps of correlations between reductions in muscle response and differences in cerebral blood-flow. Low-frequency repetitive stimulation altered neural activity differently in both experiments. Neural modulation

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occurred in multiple brain regions after dorsal premotor cortex stimulation; these included motor regions in the frontal cortex as well as more associational regions in the parietal and prefrontal cortices. In contrast, neural modulation occurred in a smaller number of brain regions after primary motor cortex stimulation, many of these confined to the motor system. These findings are consistent with the known differences between the dorsal premotor and primary motor cortices in the extent of cortico-cortical anatomical connectivity in the monkey.

III. Introduction

The cortical motor system can be separated into the primary motor and the non-primary motor areas. The non-primary motor areas are defined as all regions in the frontal lobe that have the potential to influence motor output at the level of both the primary motor cortex and the spinal cord (Dum and Strick, 1991); these include the premotor, supplementary motor, and cingulate motor areas. Transcranial magnetic stimulation (TMS) applied in trains of pulses can modulate the motor system in a temporary fashion lasting beyond the duration of stimulation. Studies that have examined these effects generally applied repetitive stimulation over the primary motor cortex and measured modulation of motor evoked potentials (MEP) recorded in the contralateral hand muscles. Typically, low stimulation frequencies of 1 to 2 Hz induce inhibitory effects (e.g. Chen et al., 1997b; Maeda et al., 2000; Muellbacher et al., 2000; Gerschlager et al., 2001) and high stimulation frequencies between 5 to 20 Hz induce facilitory effects (e.g. Pascual-Leone et al., 1994; Maeda et al., 2000; Peinemann et al., 2000; Romeo et al., 2000). Cortical mechanisms are believed to mediate both inhibitory (Touge et al., 2001) and facilitory effects (Baradelli et al., 1998).

Recent studies demonstrate that low-frequency repetitive TMS applied over the premotor cortex can also induce changes in motor excitability as reflected by: 1) decreases in the amplitude of MEPs elicited by single-pulse stimuli (Gerschlager et al., 2001); 2) increases in intracortical facilitation to paired-pulse stimuli (Münchau et al., 2002); and, 3) reductions in the duration of the silent period (Münchau et al., 2002). These results suggest that repetitive stimulation over the premotor cortex can also

modulate the output of the motor system; mediated perhaps by direct cortico-cortical connections between the premotor and primary motor cortices.

The question we address here is whether repetitive TMS applied over the dorsal premotor cortex, and over the primary motor cortex in a separate experiment, can alter neural activity at distal sites connected synaptically. Previous studies (Fox et al., 1997; Paus et al., 1997, 1998; Siebner et al., 1998, 2000; Bohning et al., 1999) have established the combination of functional brain imaging and TMS as an effective method to measure changes in neural activity induced by repetitive stimulation (reviewed in Paus 2002). Previous positron emission tomography (PET) studies have already described changes in blood-flow and glucose metabolism during repetitive stimulation applied over the primary motor cortex (Fox et al., 1997; Paus et al., 1998, Siebner et al., 2001). These studies reveal focal changes in the primary motor cortex as well as in distant regions known to be connected synaptically in the monkey, including the premotor and supplementary areas. These results suggest that for certain neural networks, connectivity patterns identified in the monkey are similar in the human.

By applying repetitive TMS over two subdivisions of the cortical motor system in the same group of subjects, we can potentially map two networks and the manner in which each is modulated. Because repetitive TMS applied over the dorsal premotor cortex or the primary motor cortex can reduce MEP amplitudes, we used this change in MEP as an index of effectiveness for altering neural activity by repetitive stimulation. To map networks of brain regions in which activity changes reflected modulation of motor excitability, we generated t-statistical maps of correlations between reductions in muscle response and differences in cerebral blood-flow.

IV. Methods

We acquired a total of six 60-second ^{15}O-H_2O PET scans in each of two sessions. In both sessions, we scanned subjects during the following conditions: 1) no TMS before repetitive stimulation (Base); 2) single-pulse TMS before repetitive stimulation (Pre); 3) single-pulse TMS shortly after repetitive stimulation (Post1); 4) single-pulse TMS about 10 minutes after repetitive stimulation (Post2); 5) single-pulse TMS about 20 minutes

after repetitive stimulation (Post3); and, 6) single-pulse TMS about 30 minutes after repetitive stimulation (Post4). We counterbalanced the order of the Base and Pre conditions across subjects. During five of the six PET scans, we applied 12 *supra*threshold single pulses of TMS over the left primary motor cortex while recording MEPs in the right first dorsal interosseous muscle. Between the second and third PET scans, we applied a 15-minute train of 1-Hz *sub*threshold repetitive TMS over the left dorsal premotor cortex in one experiment and over the left primary motor cortex in the other experiment. These experiments were conducted on separate days and in a counterbalanced order; we refer to these as the dorsal premotor and primary motor experiments.

We calculated the amount of MEP reduction induced by repetitive stimulation for every single-pulse condition and used this measure as an index of effectiveness in modulating neural activity. To reveal brain regions modulated by the repetitive stimulation, we performed correlations between reductions in MEP and differences in cerebral blood-flow. In the single-pulse conditions, we applied 20 single-pulses of TMS on average every five seconds (range: 4 to 6 seconds to minimize anticipation), 12 of which occurred during PET scanning.

Subjects

Four female and three male right-handed subjects (19 to 27 years of age, mean=23, SD=3) participated in the study after giving informed written consent. The Research Ethics Board of the Montreal Neurological Institute and Hospital approved all experimental procedures. We pre-selected subjects for their low resting motor thresholds (rMT) to prevent over-heating of the stimulating coil. We determined thresholds for the relaxed right first dorsal interosseus muscle prior to both experiments by first determining the optimal position for activating the muscle and then by reducing the stimulation intensity (in 1% steps) from an initial *supra*threshold level until we found the lowest stimulus intensity sufficient to induce 5 MEPs of at least 50 μV in a series of 10 stimuli applied at least every ~5 seconds. We also pre-selected for right-handed subjects as determined by a handedness questionnaire (Crovitz and Zener, 1965).

Transcranial magnetic stimulation

We carried out TMS using a Cadwell (Cadwell Inc., Kennewick, Washington, USA) high-speed magnetic stimulator and a Cadwell figure-of-eight stimulating coil (Corticoil, 2 tear-shaped coils of ~5-cm diameter each). We chose this coil because it produces a magnetic-field maximum of sufficiently small width to allow stimulation of the dorsal premotor cortex without encroaching on the primary motor cortex. In the scanner, a mechanical arm held the coil over the optimal position for eliciting a muscle twitch in the right index finger. We used a *supra*threshold intensity of 115% rMT for single-pulse TMS and a *sub*threshold intensity of 90% rMT for repetitive TMS. S*ub*threshold intensities allow for more focal stimulation by narrowing the magnetic field produced by the coil, thus enabling better spatial resolution for examining changes between the location of stimulation and more distant cortical structures (Pascual-Leone et al., 1993; Gerschlager et al., 2001; Münchau et al., 2002).

Targeting the stimulation locations

We used a four-step procedure to place the stimulating coil over our stimulation locations. This procedure, developed in our first TMS/PET study (Paus et al., 1997; Paus, 1999), takes advantage of standardized stereotaxic space (Talairach and Tournoux, 1988). First, we acquired magnetic resonance (MR) images (170 contiguous 1-mm-thick sagittal slices) of the subject's brain using a Siemens Vision 1.5-T system and transformed these images into standardized stereotaxic space using an automatic feature-matching algorithm (Collins et al., 1994). Second, we derived locations for the primary motor and dorsal premotor cortices using information gained in previous brain imaging studies. We derived a probabilistic location for the primary motor cortex (X=–31, Y=–22, Z=52; Paus et al., 1998) by averaging the coordinates reported in eight previous studies examining blood-flow activation when subjects moved the fingers of their right hand (Colebatch et al., 1991; Grafton et al., 1993; Matelli et al., 1993: Paus et al., 1993; Jenkins et al., 1994; Schlaug et al., 1994; Dettmers et al., 1995; Jahanshahi et al., 1995). This location served as an estimate as to where we should place the TMS coil relative to the subject's head in

the scanner; subsequent adjustments in coil positioning were made (see below). We defined a location for the dorsal premotor cortex (X=–21, Y=–2, Z=52) as being 10 mm medial and 20 mm anterior to the probabilistic location of the primary motor cortex. This location was estimated by a PET study by Fink et al. (1997) and was used in a previous TMS study of the premotor cortex (Schulter et al., 1998). Third, we transformed these two locations to the subject's brain coordinate space using an inverse version of the native-to-standardized transformation matrix.

The final step required us to position the coil over these locations, now marked on the MR images, which we achieved using frameless stereotaxy. With the subject lying on the couch of the scanner, we first registered the subject's head with their MR images and then placed the coil over the target locations by tracking the position and three-dimensional orientation of the coil with an infrared optical-tracking system (Polaris System, Northern Digital Inc., Waterloo, Ontario, Canada, and *Brainsight* software, Rogue Research Inc., Montreal, Quebec, Canada). We then locked the coil in place after finding these locations. In the case of the primary motor cortex, we made further adjustments in coil positioning to where stimulation resulted in the maximum MEP amplitude. To ensure that we used the same position for subsequent coil placements over the primary motor cortex, we first defined its position in the subject's brain coordinate space and then marked its position on the subject's MR images. We held the coil in different orientations when stimulating the primary motor and dorsal premotor cortices. For the primary motor cortex, we oriented the coil tangentially to the scalp with the short axis of the figure-of-eight coil angled at 45 degrees relative to the interhemispheric fissure and approximately perpendicular to the central sulcus. For the dorsal premotor cortex, we oriented the coil tangentially to the scalp with the short axis of the figure-of-eight coil perpendicular to the interhemispheric fissure. For primary motor and dorsal premotor stimulation, the resulting induced electric current in the brain flowed in posterior-to-anterior and lateral-to-medial directions, respectively.

Verifying final coil positions over the primary motor cortex

Interpretation of results acquired with TMS/PET depends critically on the accuracy of coil positioning. In the present study, this applies specifically to the dorsal premotor experiment where we moved the coil from the primary motor cortex (Pre scan) to the dorsal premotor cortex (between 2nd and 3rd scans) and then back to the primary motor cortex (Post scans). Using a procedure described in detail elsewhere (Paus and Wolforth, 1998), we used 10-minute transmission scans to verify coil positions relative to the acquired PET and MR images. We acquired transmission scans at the beginning of the dorsal premotor and primary motor experiments, and an additional transmission scan at the end of the dorsal premotor experiment. These transmission images showed us the coil's position relative to the subject's head. We then registered an X-ray image of the coil to these images and projected a straight rod orthogonal to the plane of the coil from the coil center. Following PET-to-PET, PET-to-MR, and MR-to-standardized space transformations, we superimposed the locations of the rod on an average anatomical MR image of all subjects. This end product indicates the projected center of the coil in the brain; the figure-of-eight coil used in this study stimulates an estimated volume of 20x20x10 mm (Cohen et al., 1990; Maccabee et al., 1990; Wassermann et al., 1996).

Positron emission tomography

We instructed subjects to relax and keep their eyes closed during PET scanning. Subjects used a bite-bar to maintain a constant head position during the experiments. We measured cerebral blood-flow (CBF) with a CTI/Siemens HR+ 63-slice tomograph scanner operated in 3-D acquisition mode during 60-s scans using the ^{15}O-labeled H_2O bolus method (Raichle et al., 1983). In each scan, we injected 10 mCi of ^{15}O-labeled H_2O into the left antecubital vein. Acquired CBF images were reconstructed with a 14-mm Hanning filter, normalized for differences in global CBF ('normalized CBF'), co-registered with the individual MR images (Woods et al., 1993), and transformed into standardized stereotaxic space (Talairach and Tournoux 1988) by means of an automated feature-matching algorithm (Collins et al., 1994). We placed four 0.5-mm thick sheets of

well-grounded mu-metal to protect the photomultipliers inside the PET scanner from the effects of the coil-generated magnetic field. The mu-metal, however, can attenuate gamma rays and in turn decrease the number of detected coincidence counts (Paus 2002). The transmission data acquired at the beginning of the experiments were also used to correct for the attenuation of gamma rays caused by all objects in the scanner, including the coil, the coil mount, and the metal sheets.

Analyses of muscle evoked potentials

We recorded MEPs from the right first dorsal interosseus muscle using Ag/AgCl surface electrodes fixed on the skin with a belly-tendon montage. We sampled the electromyographic (EMG) signal using an EMG channel of a 60-channel TMS-compatible electroencephalography system (Virtanen et al., 1999) with the amplifier's bandwidth set at 0.1-500 Hz and the sampling rate set at 1.45 kHz. We measured the peak-to-peak amplitudes for each MEP off-line. For practical reasons, we began to deliver single pulses of TMS at the time the radioactive tracer was injected. Acquisition after injection varies from one person to another and there is no way of knowing exactly which of the MEPs occurred during scanning. We therefore calculated the muscle response for a given scan as a percentage of the mean MEP amplitude during the Pre scan based on the 20 trials. We evaluated the effects of repetitive TMS on motor excitability by analysis of variance (ANOVA) using a model of repeated measures with Time as a within-subject factor. We used Tukey's HSD tests, which corrects for multiple comparisons, for all post-hoc pair-wise comparisons. We considered values statistically significant if $p<0.05$. We also performed a Wilcoxon Signed Ranks test to determine whether rMT values were significantly different between the two experiments.

Analyses of cerebral blood-flow

We used a two-step process to generate t-statistical maps. We first subtracted CBF acquired before repetitive TMS from CBF acquired after repetitive TMS. We performed this initial subtraction to obtain CBF differences between before and after repetitive TMS

and to remove confounding intersubject variability. We then correlated these subtractions with the relative amount of MEP reduction, which we calculated in the same way as in a previous TMS/PET study (Strafella and Paus, 2000) that examined the effects of double-pulse stimulation on CBF: [(1 – (MEP amplitude at a given post-rTMS condition / MEP amplitude at the pre-rTMS condition)) X 100]. We carried out calculations for the t-statistical maps for each of the 3-D volume elements (voxels) constituting the entire scanned volume, which tested whether at a given voxel the slope of the regression was significantly different from zero.

After generating our t-statistical maps, we evaluated the presence of a significant peak by a method based on a 3-D Gaussian random-field theory, with correction for the multiple comparisons involved in searching the entire volume (Worsley et al., 1992). Using this method, we performed both an exploratory search of the entire brain and a directed search in specific brain regions. For an exploratory search, we considered values equal to or exceeding a criterion of $t=4.5$ as significant ($p<0.000003$, 2-tailed, uncorrected), yielding a false positive rate of 0.04 (corrected) in 400 resolution elements (each of which has dimensions 14x14x14 mm) for a brain volume of 1,100 cm^3. For a directed search, we considered values equal to or exceeding a criterion of $t=3.5$ as significant ($p<0.0002$, 2-tailed, uncorrected), yielding a false positive rate of 0.01 (corrected) in two resolution elements (each of which has dimensions of 14x14x14 mm) for a brain volume of 5 cm^3. We performed our directed search in the dorsal premotor cortex, in the primary motor cortex, and in brain regions known to be connected with these regions in nonhuman primates (Figure 1). To perform this search in the human brain, we relied on previous functional brain imaging studies that mapped their putative homologues; we describe these later in the discussion. We determined anatomical locations of all significant t-statistic peaks by examining the merged image of our t-statistical maps with the transformed averaged MR image of all subjects in standardized stereotaxic space (Talairach and Tournoux, 1988). We equally performed additional subtractions of the CBF data to examine primarily the local effects at the stimulation sites. The first subtraction examined the possible, but unlikely, local effects of single-pulse TMS and consisted of subtracting CBF in the Base scan from CBF in the Pre scan. The second subtraction examined the presence of local effects of repetitive TMS and

consisted of subtracting CBF in the Pre scan from the average CBF of all Post scans. All brain regions that showed significant CBF changes are reported.

We also provide additional analyses that examine similarities and differences between the effects of repetitive TMS over the dorsal premotor and primary motor cortices. In order to examine similarities, we carried out a conjunction analysis (Price and Friston, 1997). This analysis tests for the presence of correlations in both experiments by revealing the maximum peaks in the two contrasts. We considered values equal to or exceeding a criterion of t=3.5 as significant (Worsley and Friston, 2000). In order to examine differences, we directly tested for differences in the CBF difference / MEP reduction relationship between the dorsal premotor and primary motor experiments. We first extracted CBF values from VOIs centered at the X, Y, and Z coordinates of our correlation peaks (Tables 2 and 3) and then, for each brain region, we used ANOVAs to test for differences in the slope of their correlations between the two experiments. We used Bonferroni corrections to take into account multiple comparisons and considered values statistically significant if p-corrected<0.05.

V. Results

All subjects tolerated the study well without noticeable adverse effects related to TMS and/or the scanning procedures. We excluded data from two subjects in the dorsal premotor experiment from our analyses because of head movement. Figure 2 illustrates the end result of all coil placements over the primary motor cortex for both experiments.

Effects of repetitive TMS on MEP amplitudes

Repeated measures ANOVA revealed a significant effect of Time on the mean MEP amplitude in the dorsal premotor experiment [$F(4,16)=3.48$, $p<0.05$] and in the primary motor experiment [$F(4,24)=3.11$, $p<0.05$]. These results indicate that MEP amplitudes changed during the course of the two experiments. Using Tukey's HSD pair-wise comparison tests, we further examined the pattern of MEP changes (Figure 3). The second Post scan showed significantly smaller MEP amplitudes compared to baseline in

both experiments (both $p<0.05$). No other pair-wise comparisons differed significantly. A Wilcoxon Signed Ranks test revealed that there was no significant difference between rMT values in the two experiments ($W(4)=-1.83$, $p=0.07$; subjects 6 and 7 excluded). Although this was not significant, rMT values tended to be lower in the dorsal premotor experiment compared to the primary motor experiment (Table 1).

Effects of repetitive TMS over the dorsal premotor cortex on CBF

Figure 4 A and Table 2 summarize the findings in the dorsal premotor experiment and show all brain regions that presented significant positive and negative correlations between POST-PRE CBF differences and the amount of MEP reduction. Figure 5 A-B provides plots of CBF differences versus MEP reduction for two of these brain regions: the right anterior parietal and ventral premotor cortices. Motor-related regions with positive correlations include: the left and right ventral premotor areas in the precentral region of the operculi, the left and right cingulate motor areas in the cingulate gyri/sulci, the right premotor area in the precentral sulcus, the right supplementary motor area in the medial frontal gyrus, and the right putamen. Motor-related regions with negative correlations include: the left dorsal premotor area in the precentral gyrus/sulcus (30 mm lateral and 9 mm caudal to the targeted site of repetitive TMS and unlikely to indicate a local effect of stimulation) and the right sensorimotor area in the paracentral lobule.

Parietal brain regions with positive correlations include: the right posterior portion of the superior parietal lobule / intraparietal sulcus (putative medial intraparietal area), the anterior portion of the right inferior parietal lobule / intraparietal sulcus (putative anterior intraparietal area), and the right inferior parietal lobule / postcentral sulcus. Prefrontal brain regions with positive correlations include: the left and right inferior frontal gyrus / sulcus (ventrolateral prefrontal cortex) and the right middle frontal gyrus / sulcus (dorsolateral prefrontal cortex). One medial temporal-lobe region with a positive correlation was found in the right hippocampus. Negative correlations were mostly confined to several areas in the primary and associational visual cortices.

No significant correlations occurred either at the local site of repetitive TMS (i.e. left dorsal premotor cortex) or at the site of single-pulse TMS (i.e. left primary motor

cortex). Further examination using direct subtraction analyses did not reveal significant CBF changes at either of the two sites of stimulation, which equally suggests that no local effects of TMS occurred in the left dorsal premotor cortex or in the left primary motor cortex. A direct subtraction of the Base scan from the Pre scan revealed CBF increases in the left pre-supplementary area on the medial frontal gyrus (X=-5, Y=15, Z=51; t=3.8) and CBF decreases in the right superior parietal lobule / intraparietal sulcus (putative medial intraparietal area; X=31, Z=-64, Z=54; t=-4.1). A direct subtraction of the Pre scan from the average of all Post scans revealed no significant CBF differences anywhere in the brain.

Effects of repetitive TMS over the primary motor cortex on CBF

Figure 4 B and Table 3 summarize the findings in the primary motor experiment and show all brain regions that presented significant positive and negative correlations between POST-PRE CBF differences and the amount of MEP reduction. Figure 5 C provides a plot of CBF differences versus MEP reduction for one of these brain regions, the right primary motor cortex. Motor-related regions with positive correlations include: the left cingulate motor area in the cingulate gyrus/sulcus, the left putamen, the right primary motor area in the precentral gyrus/central sulcus, the right ventral-lateral thalamic nucleus, and the left cerebellum. Negative correlations were mostly confined to several areas in the primary and associational visual cortices.

No significant correlation occurred at the location of single-pulse TMS and repetitive TMS (i.e. left primary motor cortex). A direct subtraction of the Base scan from the Pre scan did not reveal any local changes in CBF. The same subtraction revealed CBF increases in the left primary visual cortex in the calcarine sulcus (X=-4, Y=-86, Z=10, t=5.0) and the right primary visual cortex in the calcarine sulcus (X=7, Y=-71, Z=14, t=4.8). A direct subtraction of the Pre scan from the average of all Post scans revealed, however, a near significant increase of CBF at the stimulated region (X=-35, Y=-26, Z=51; t= 3.2). The same subtraction also revealed CBF increases in the right cingulate motor area in the cingulate gyrus/sulcus (X=1, Y=18, Z=45; t=3.7) and in the left dorsal premotor cortex in the superior frontal sulcus (X=-33, Y=6, Z=52; t=3.6), as

well as CBF decreases in the left primary visual cortex in the calcarine sulcus (X=-5, Y=-85, Z=12; t=4.9). These two subtraction-based results suggest local effects of repetitive TMS but not of single-pulse TMS in the left primary motor cortex.

Conjunction analysis

Table 4 summarizes the findings of our conjunction analysis and lists all brain regions that presented significant correlations between POST-PRE CBF differences and the amount of MEP reduction in both experiments. Brain regions with significant positive correlations include: the right hippocampus and the right mesencephalon, both of which were approximately in the same horizontal plane (Z between –12 and –16). Except for one location in the right cerebellum, brain regions with significant negative correlations were all confined to the primary and associational visual cortices.

Contrast analysis

Table 5 summarizes the findings from our ANOVAs that tested for differences in the CBF difference / MEP reduction relationship between the dorsal premotor and primary motor experiments. We also present in the table Pearson's correlation coefficients between CBF differences and the amount of MEP reduction. Overall, our analysis confirms minimal overlap in the effects of repetitive TMS applied over the dorsal premotor and primary motor cortices on possible fronto-parietal circuits. Similar to the results in the conjunction analysis, the right mesencephalon showed relatively large Pearson's correlation coefficients for both experiments, suggesting strong positive relationships between CBF differences and the amount of MEP reduction—although these still showed significantly different relationships. Contrary to the results in the conjunction analysis, the right hippocampal formation showed a small Pearson's correlation coefficient for the primary motor experiment. This is likely because we extracted VOIs at its correlation peak in the dorsal premotor experiment, which was about 5-mm more medial and 5-mm more dorsal than its activation peak reported in the conjunction analysis.

VI. Discussion

Our results demonstrate that low-frequency repetitive TMS applied over the dorsal premotor and primary motor cortices produced similar inhibitory effects on MEPs but influenced cerebral activity differently. Repetitive stimulation over the dorsal premotor cortex resulted in the modulation of a network encompassing a number of brain regions; these include several regions in the parietal and prefrontal cortices. In contrast, repetitive stimulation over the primary motor cortex resulted in the modulation of a network encompassing a smaller number of brain regions; many of these confined to the cortical and subcortical motor system. In the ensuing discussion we first address methodological issues and then discuss our findings in the light of studies performed by others in the monkey.

Methodological issues

Although we showed significant reductions in motor excitability after both the applications of repetitive stimulation over the dorsal premotor and primary motor cortices, we also noted considerable interindividual differences. Some subjects showed greater reductions in MEP amplitude (1, 3, 5, and 6) compared to others (2 and 7), and one subject (4) showed increases in MEP amplitude. This is consistent with previous findings suggesting that it might be necessary to individualize parameters of repetitive TMS to achieve a consistent change in motor excitability across all subjects (Maeda et al., 2000). It is unlikely that this variability resulted from changes in coil positioning. Verifications of final coil positioning showed that we placed the coil consistently over the primary motor cortex. Most subjects showed minimal head movements as evident from their blood-flow images; we excluded two subjects who had head movements, in the dorsal premotor experiment, from the analyses.

Similar to Gerschlager et al. (2001) and Münchau et al. (2002), we demonstrated changes in motor excitability after applying repetitive TMS over the dorsal premotor cortex. Unlike the aforementioned studies, we held the coil in different orientations when stimulating the dorsal premotor and primary motor cortices. We chose different coil

orientations to reduce the likelihood that stimulation of the dorsal premotor cortex would encroach on the primary motor cortex. Also, unlike the aforementioned studies, we demonstrated a reduction of motor excitability following repetitive TMS over the primary motor cortex. We might have had better access to the primary motor cortex by stimulating at a higher intensity (90% rMT as opposed to 80-90% active MT); as suggested by Gerschlager et al. (2001), it might be easier to stimulate the premotor cortex than relatively deeper structures like the primary motor cortex, located in the anterior bank of the central sulcus. The small figure-of-eight coil used in this study (diameter=5cm) delivers higher intensities while maintaining focality and stimulates an estimated volume of 20x20x10 mm (Cohen et al., 1990; Maccabee et al., 1990; Wassermann et al., 1996). It is unlikely, therefore, that the spread of current to premotor areas induced the effects obtained in the primary motor experiment.

MEPs obtained in the dorsal premotor and primary motor experiments are associated with changes in the size of muscle twitches and, presumably, differential sensory feedback from the hand muscles to the brain. This feedback could conceivably confound the blood-flow response. Two important features of our data argue against this possibility. First of all, we observed no significant blood-flow changes in the contralateral sensory cortices or contralateral sensory thalamus in either experiment, which suggests that the possible effects of the twelve muscle twitches on blood-flow response were negligible. Second of all, our results show that the depression of MEP amplitudes followed a similar time course in both experiments (Figure 3). If our correlations resulted from changes in sensory feedback, we would have obtained more equivalent changes in blood-flow from stimulating the dorsal premotor and primary motor cortices; this was not the case (Table 5).

The lack of blood-flow changes to single-pulse stimulation applied during the scans is not surprising in light of the low number of pulses (12 pulses/scan). On the other hand, we would expect blood-flow changes following the 15-minute train of 1-Hz repetitive stimulation. We found a significant increase in local blood-flow in scans acquired after repetitive stimulation over the primary motor cortex, but no such effects after repetitive stimulation over dorsal premotor cortex. Assuming tight coupling between excitatory synaptic activity and blood-flow (Mathiesen et al. 1998, Logothetis et al. 2001,

for review, see Paus 2002), we hypothesize that the local effects of low-frequency repetitive stimulation on inhibitory and excitatory neurotransmission canceled out while the distal effects remained. The latter might be related to the fact that the majority of cortico-cortical and cortico-subcortical projections are glutamatergic and, hence, their activation is more likely to influence blood-flow in their target regions. As for a lack of a distal effect in the left primary motor cortex after dorsal premotor stimulation, this finding raises the possibility that the observed changes in MEP amplitudes are mediated by cortico-spinal projections originating in the dorsal premotor cortex rather than cortico-cortical connections between the dorsal premotor and primary motor cortices. We also hypothesize that the lateral-to-medial orientation of the short axis of the stimulating coil (virtual anode-cathode), as used in the dorsal premotor experiment, influenced preferentially inter-hemispheric rather than intra-hemispheric cortico-cortical projections. This could explain the general lack of distal effects in the left hemisphere as compared to the right hemisphere following repetitive stimulation over the dorsal premotor cortex.

Before proceeding to the interpretation of the results, we should mention some important aspects related to our correlations. A positive correlation reflects an increase in blood-flow response with the amount of MEP reduction and a negative correlation reflects a decrease in blood-flow response with the amount of MEP reduction. Brain regions that show these correlations were modulated in parallel with MEP reduction, but in some cases, modulation could have resulted from non-specific effects of TMS. Our correlation analyses showed that most negative correlations were located in the primary and associational visual areas. Our conjunction analysis further showed that the majority of these were present in both the dorsal premotor and primary motor experiments. Together, these results suggest that our negative correlations were largely the result of non-specific effects of TMS; one possibility being the result of changes in arousal levels. Several attention studies observed blood-flow fluctuations in similar brain regions and attribute these changes to differences in arousal levels and/or to cross-modal suppression (reviewed in Paus 2000). The rest of this discussion will therefore concentrate on our positive correlations, which were confined predominantly to motor areas and putative fronto-parietal circuits.

Dorsal premotor experiment

Several anatomically distinct areas constitute the premotor cortex, each with a potentially different specialization. In our study, repetitive stimulation likely affected two distinct dorsal premotor areas (reviewed in Picard and Strick, 2001), namely those identified in the monkey as the caudal premotor area F2, which has substantial connections with the primary motor area (Barbas and Pandya, 1987; Dum and Strick, 1991), and the rostral dorsal premotor area F7, which has substantial connections with the prefrontal cortex (Barbas and Pandya, 1987; Lu et al., 1994). Repetitive stimulation over the dorsal premotor cortex might have also affected the frontal eye-field; our stimulation site was in close proximity to the probabilistic location of this area as established by Paus (1996) in a meta-analysis of oculomotor neuroimaging studies.

The dorsal premotor cortex plays a prominent role in coupling arbitrary sensory cues to motor acts (for review, see Freund 1996). Studies in the monkey reveal that lesions to the dorsal premotor cortex disrupt the animal's ability to use such cues to make or withhold particular movements (Halsband and Passingham, 1982, 1985; Petrides, 1985b); the same is true for patients with damage to the dorsal premotor cortex (Halsband and Freund, 1990). The parietal cortex receives somatosensory and visual inputs, and encompasses several subdivisions that have reciprocal connections with motor areas in the frontal cortex, each with a specific target with which it is most densely connected. These circuits provide an anatomical basis for the transformation of sensory information into motor actions (Rizzolatti et al., 1998; Matelli and Luppino, 2000). Anatomical studies in the monkey reveal circuits that include the dorsal premotor area (F2/F7) as their frontal component; one of these is the MIP-F2 circuit (Matelli et al., 1998). A combination of somatosensory and visual information used for the visual guidance of arm movement trajectories is thought to reach F2 from MIP (Colby and Duhamel, 1991; Galletti et al., 1996; Matelli and Luppino, 2000).

In view of these data, we postulate that our findings may show the human homologue of the MIP-F2 circuit. The circuit follows from correlations observed in the right premotor cortex in the precentral sulcus and in the right medial intraparietal cortex along the posterior superior parietal lobule. Stimulation of the left dorsal premotor cortex

might have modulated the right premotor cortex via commissural connections (Pandya and Vignolo, 1971; Marconi et al., 2002). Our MIP coordinates (X=36, Y=–64, Z=54) are similar to those (X=–33, Y=–60, Z=54) established in a previous functional MR imaging study of response switching, which required subjects to switch between two different visuomotor-related intentions (Rushworth et al. 2001). Other functional brain imaging studies show comparable metabolic changes in both the posterior parietal and premotor cortices as subjects selected motor acts based on visual stimuli (Paus et al., 1993; Deiber et al., 1997; Grafton et al., 1998).

Our results also suggest an additional parieto-frontal circuit that connects the right PMv in the precentral operculum with the right AIP in the lateral bank of the intraparietal sulcus along the anterior inferior parietal lobule. Stimulation of the left dorsal premotor cortex might have modulated the right ventral premotor cortex via commissural connections (Marconi et al., 2002). Marconi et al. (2002) recently demonstrated in the monkey that callosal connections exist between the dorsal premotor cortex in one hemisphere and the ventral premotor cortex in the opposite hemisphere. Connections also exist in the monkey between PMv (F5) and the more anterior part of the intraparietal cortex (Luppino et al., 2001). Both F5 and AIP neurons code for selective hand manipulations, grasping movements, and various visual characteristics of 3-D objects (Rizzolatti et al., 1988; Murata et al., 1997). In view of these findings, Jeannerod et al. (1995) suggested that the F5-AIP circuit plays a role in transforming the properties of a 3-D object into the appropriate hand movements required to grasp it. Previous PET data indicate that similar activations occur in the human PMv during the presentation of 3-D objects (Grafton et al., 1997) with coordinates (X=–48, Y=–2, Z=29) that are slightly more dorsal than our PMv coordinates (X=52, Y=–6, Z=12 and X=–43, Y=–6, Z=14).

The prefrontal cortex plays a prominent role in executive functions (reviewed in Fuster, 1993; Petrides, 2000). To select relevant information for action, the prefrontal cortex has access, through its connections with other brain structures, to sensory and spatial aspects of the environment, mnemonic information acquired through experience, and motor control (reviewed in Barbas, 2000). These motor output-related connections mainly arise from the premotor cortices (Barbas and Pandya, 1987; Lu et al., 1994) and might explain our additional correlations in the prefrontal cortices. Anatomical data in the

monkey show reciprocal connections of the prefrontal cortex and the premotor cortex in an orderly pattern along dorsal and ventral axes; interconnections between the two axes are sparse (Barbas 1988; Barbas and Pandya, 1989; Barbas, 1992). One would therefore predict that the blood-flow changes we observed in the ventrolateral prefrontal cortices arose from its connections with the ventral premotor cortices.

Primary motor experiment

A conjunction analysis performed on our data revealed little overlap in the positive correlations obtained between the dorsal premotor and primary motor experiments. Similarly, ANOVAs revealed that most of our brain regions with correlations showed significant differences between the two experiments. These findings suggest that we mapped two separate networks and lends support to the notion that the dorsal premotor and primary motor cortices differ in their functional properties. Unlike the dorsal premotor cortex, the primary motor cortex plays a role mainly in the execution of voluntary movements. Studies in the monkey reveal that lesions to the primary motor cortex disrupt more the execution of skilled movements than lesions to non-primary motor cortices (Passingham et al., 1983; Passingham 1985; Petrides, 1985b). Of all the cortical motor areas, the primary motor cortex contains the highest percentage (31%) of large corticospinal neurons (Dum and Strick, 1991), which directly generate movement in the limbs (for review, see Evarts, 1981).

The primary motor cortex connects predominantly with non-primary motor and non-primary somatosensory cortices; connections between the primary motor cortex and other cortical structures are sparse (Figure 1). Visual and/or auditory information that influence movements must first be processed by associational and/or higher-order sensory cortices, and then be communicated to the non-primary motor cortices (for review, see Ghez et al., 1991). The non-primary motor cortices can in turn use this information to coordinate motor output at the level of both the primary motor cortex and the spinal cord (Dum and Strick, 1991). We propose that our data from the primary motor experiment reflect this pattern of connections: the network mapped in the primary motor

experiment encompasses correlations confined mainly to non-primary motor cortices and subcortical motor structures.

Brain regions with significant correlations in the primary motor experiment include the right cingulate motor area, the left putamen, the right primary motor area, and the right ventral-lateral thalamic nucleus / internal global pallidus. Correlations in this experiment reflect both direct and indirect connections with the stimulation site (i.e. the left primary motor cortex). The cingulate motor area represents most likely the human homologue of CMAr, or the rostral cingulate zone, which is located anterior to the anterior commisure (Paus et al., 1993; Picard and Strick, 1996). The correlation in the left ipsilateral putamen suggests cortico-striatal projections from the primary motor area to the lateral putamen (Takada et al., 1998). The presence of a blood-flow response in the contralateral primary motor area suggests commissural connectivity from the stimulated hemisphere to the unstimulated hemisphere (Jenny, 1979; Rouiller et al., 1994). The right ventral-lateral thalamus and the right cingulate motor area might reflect indirect connections with the site of stimulation, the left primary motor cortex, mediated perhaps via the right primary motor cortex. Both the ventral-lateral thalamus and the internal globus pallidus are components of cortico-basal ganglia-thalamo-cortical loops related to the control of movement (Parent and Hazrati, 1995).

Concluding Remarks

The data presented here suggest that we mapped two separate motor-related networks. Because repetitive TMS over the dorsal premotor and primary motor cortices reduces MEP amplitudes, we used this change as an index of effectiveness for altering neural activity by repetitive stimulation. We then mapped networks of brain regions in which activity changes reflected this modulation. Our data provide complementary insights into the function of the dorsal premotor and primary motor cortices as compared to functional brain imaging studies that measure neural activity during volitional hand movements.

VII. Tables

Table 1. Resting motor thresholds (rMT) for each subject.

Subject	Dorsal Premotor Experiment		Primary Motor Experiment	
	rMT	Average MEP Reduction (%) at POST	rMT	Average MEP Reduction (%) at POST
1	70	83.4	75	55.5
2	74	17.3	74	5.5
3	74	77.6	80	37.7
4	77	14	80	-21.8
5	68	29.5	80	60.7
6*	--	--	78	52.5
7*	--	--	70	39.4
Mean ±SEM	72±2	44.3±15	77±1	32.7±12.4

Values are expressed as a percentage of the maximal stimulation output and the average amount of MEP reductions (%) at POST Conditions in the dorsal premotor and primary motor experiments. *We excluded two subjects from the dorsal premotor experiment due to their head movements in the PET scanner.

Table 2. Effects of repetitive stimulation over the dorsal premotor cortex on cerebral blood-flow.

A. Regions with positive correlations		X	Y	Z	t-value	Ref
right inferior frontal gyrus / sulcus (VL-PFC)		44	39	3	7.7	1
right IPL / postcentral sulcus		55	-26	45	6.4	2
right precentral operculum (PMv)		52	-6	12	6.2	3
right hippocampal formation		26	-18	-12	5.9	4
right anterior IPL / intraparietal sulcus (putative AIP)		46	-47	42	5.9	5
left precentral operculum (PMv)		-43	-6	14	5.8	6
right medial frontal gyrus (SMA)		7	-9	60	5.5	7
left (frontopolar) middle frontal gyrus		-32	61	-6	5.4	8
left inferior frontal gyrus (VL-PFC)		-54	12	15	5.2	9
left anterior cingulate gyrus		-9	24	20	5.2	10
right middle frontal gyrus / sulcus (DL-PFC)		35	34	27	5.0	11
left caudate nucleus (head)		-11	12	8	4.8	12
right anterior IPL / intraparietal sulcus (putative AIP)		54	-40	52	4.8	13
right cingulate gyrus / sulcus (CMA)		15	-4	51	4.7	14
right cingulate gyrus / sulcus (CMA)		13	-6	45	4.7	15
right hypothalamus		4	-2	-15	4.5	16
left cingulate gyrus / sulcus (CMA)		-15	-9	45	4.5	17
right putamen	*	23	17	-6	3.9	18
right precentral sulcus (premotor)	*	35	10	33	3.8	19
right posterior SPL / intraparietal sulcus (putative MIP)	*	36	-64	54	3.7	20

B. Regions with negative correlations		X	Y	Z	t-value
left mesencephalon (~superior colliculus)		-5	-33	-4	5.9
left lingual gyrus / calcarine sulcus (VAA)		-9	-78	-9	5.8
left calcarine sulcus (V1)		-9	-97	8	5.6
left calcarine sulcus (V1)		-7	-68	9	5.5
left lateral occipital cortex (VAA)		-26	-75	22	5.5
right calcarine sulcus (V1)		20	-73	3	5.5
left caudate nucleus (body)		-13	-2	16	5.2
left cerebellum		-5	-50	-8	5.0
left parahippocampal gyrus		-19	-37	-18	4.8
left hippocampal formation		-13	-11	-20	4.7
left middle frontal gyrus / sulcus (DL-PFC)	*	-36	12	30	4.2
right paracentral lobule (SM1)	*	3	-30	54	4.1
left precentral gyrus (PMd)	*	-51	-11	50	4.1

Brain regions in the dorsal premotor experiment with significant positive (A) and negative (B) correlations between differences in CBF and reductions in MEP. *Brain regions with significant correlations after a directed search ($t>3.5$ and $t<-3.5$) but not after an exploratory search ($t>4.5$ and $t<-4.5$). The last column contains numbers for referring to Table 5. Abbreviations: PMd = dorsal premotor area, VL-PFC = ventrolateral prefrontal cortex, S1 = primary sensory area, PMv = ventral premotor area, IPL = inferior parietal lobule, AIP = anterior intraparietal area, SMA = supplementary motor area, DL-PFC = dorsolateral prefrontal cortex, CMA = cingulate motor area, SPL = superior parietal lobule, MIP = medial intraparietal area, VAA = visual association area, V1 = primary visual area, and SM1 = sensorimotor area.

Table 3. Effects of repetitive stimulation over the primary motor cortex on cerebral blood-flow.

A. Regions with positive correlations	X	Y	Z	t-value	Ref
right mesencephalon	7	-11	-15	7.9	21
left putamen	-31	6	0	5.8	22
right ventral-lateral thalamus	17	-9	0	5.5	23
right precentral gyrus / central sulcus (M1)	28	-25	56	5.1	24
left cerebellum	-11	-49	-15	4.8	25
left inferior frontal gyrus (VL-PFC)	-54	18	-5	4.8	26
left basal forebrain nuclei	-16	1	-12	4.6	27
right subgenual gyrus	5	29	-2	4.6	28
right cingulate gyrus (CMA) *	3	10	40	4.4	29

B. Regions with negative correlations	X	Y	Z	t-value
left ventral occipital cortex (VAA)	-17	-76	-6	5.0
right middle temporal gyrus / inferior temporal sulcus	48	-68	12	4.9
right lateral occipital cortex (VAA)	42	-81	3	4.8
left posterior insular cortex	-39	-9	-16	4.8
right calcarine sulcus (V1)	21	-62	-2	4.8
right lateral occipito-temporal gyrus	43	-57	-14	4.6
right anterior insular cortex	31	27	0	4.5

Brain regions in the primary motor experiment with significant positive (A) and negative (B) correlations between differences in CBF and reductions in MEP. *Brain regions with significant correlations after a directed search ($t>3.5$ and $t<-3.5$) but not after an exploratory search ($t>4.5$ and $t<-4.5$). The last column contains numbers for referring to Table 5. Abbreviations: M1 = primary motor area, VL-PFC = ventrolateral prefrontal cortex, CMA = cingulate motor area, VAA = visual association area, and V1 = primary visual area.

Table 4. Similarities between the effects of repetitive stimulation over the dorsal premotor and primary motor cortices on cerebral blood-flow.

A. Regions with positive correlations	X	Y	Z	t-value
right hippocampal formation	20	-21	-16	4.6
right mesencephalon	12	-21	-12	3.8

B. Regions with negative correlations	X	Y	Z	t-value
Left ventral occipital cortex (VAA)	-12	-74	-8	4.5
Left calcarine sulcus (V1)	-4	-93	9	4.1
right calcarine sulcus (V1)	21	-66	0	4.1
Left calcarine sulcus	-7	-92	12	4.1
right cerebellum	9	-37	-21	4.0

Brain regions with positive (A) and negative (B) correlations between differences in CBF and reductions in MEP that were significantly present in both the dorsal premotor and primary motor experiments. Abbreviations: VAA = visual association area and V1 = primary visual area.

Table 5. Differences between the effects of repetitive stimulation over the dorsal premotor and primary motor cortices on cerebral blood-flow.

A. Regions with positive correlations from the dorsal premotor experiment	F	(2, 45)	P (uncorr)	R (PMd)	r (M1)	Ref
right anterior IPL / intraparietal sulcus (putative AIP)	*	14.9	< 0.001	0.74	0.57	5
left caudate nucleus (head)	*	14.1	< 0.001	0.79	-0.26	12
right cingulate gyrus / sulcus (CMA)	*	13.3	< 0.001	0.82	0.49	15
left cingulate gyrus / sulcus (CMA)	*	12.2	< 0.001	0.72	0.28	17
left precentral operculum (PMv)	*	12.0	< 0.001	0.72	0.27	6
right medial frontal gyrus (SMA)	*	11.3	< 0.001	0.78	0.48	7
right cingulate gyrus / sulcus (CMA)	*	11.3	< 0.001	0.84	0.48	14
right IPL / postcentral sulcus	*	11.2	< 0.001	0.80	-0.08	2
right posterior SPL / intraparietal sulcus (putative MIP)	*	10.1	< 0.001	0.72	-0.18	20
right hypothalamus	*	9.6	< 0.001	0.73	0.29	16
right middle frontal gyrus / sulcus (DL-PFC)	*	9.3	< 0.001	0.74	0.54	11
right anterior IPL / intraparietal sulcus (putative AIP)	*	7.2	0.002	0.63	0.15	13
left (frontopolar) middle frontal gyrus		6.6	0.003	0.77	-0.24	8
left anterior cingulate gyrus		5.1	0.010	0.69	0.36	10
right hippocampal formation		4.0	0.026	0.72	0.08	4
right precentral operculum (PMv)		3.4	0.042	0.83	0.18	3
left inferior frontal gyrus (VL-PFC)		3.3	0.045	0.86	0.14	9
right putamen		3.3	0.048	0.51	0.28	18
right inferior frontal gyrus / sulcus (VL-PFC)		3.2	0.050	0.72	-0.07	1
right precentral sulcus (premotor)		2.4	0.100	0.58	0.24	19
B. Regions with positive correlations from the primary motor experiment	**F**	**(2, 45)**	**P (uncorr)**	**R (PMd)**	**r (M1)**	**Ref**
right mesencephalon	*	22.1	< 0.001	0.48	0.76	21
right ventral-lateral thalamus	*	18.5	< 0.001	-0.13	0.71	23
right cingulate gyrus (CMA)	*	15.4	< 0.001	-0.26	0.67	29
left cerebellum	*	13.1	< 0.001	-0.48	0.72	25
right precentral gyrus / central sulcus (M1)	*	12.8	< 0.001	-0.19	0.72	24
left basal forebrain nuclei	*	11.0	< 0.001	-0.46	0.57	27
left inferior frontal gyrus (VL-PFC)	*	10.1	< 0.001	0.34	0.61	26
left putamen	*	7.5	0.002	0.06	0.62	22
right subgenual gyrus		4.6	0.016	-0.10	0.62	28

The table represents differences in the: (A) slope of correlations in brain regions obtained in the dorsal premotor experiment between the two experiments; and, (B) slope of correlations in brain region obtained in the primary motor experiment between the two experiments. *Significant differences between the two experiments after the correction of multiple comparisons. The table also contains Pearson's correlation coefficients between CBF differences and amount of MEP reduction. The last column contains numbers for referring to Tables 2 and 3.

VIII. Figures

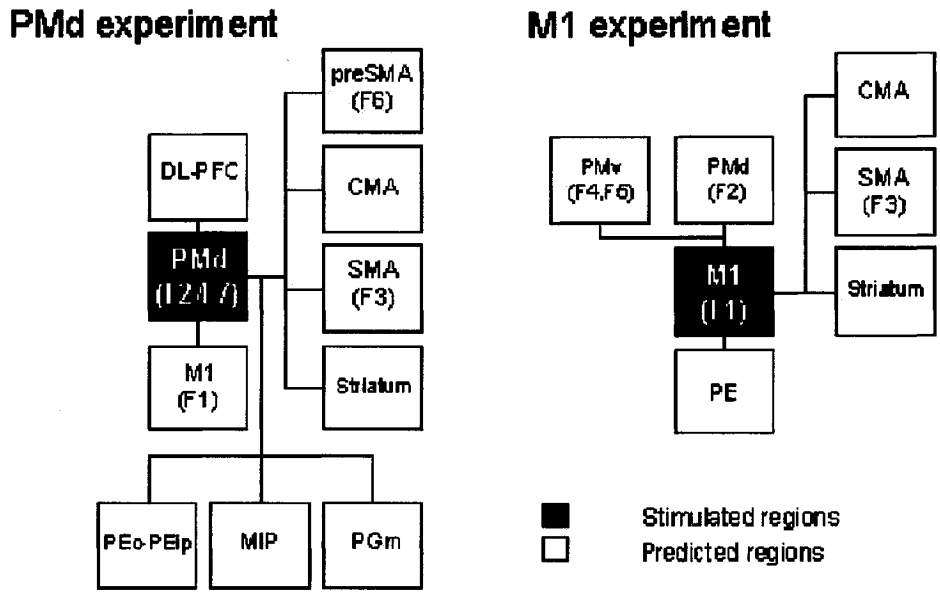

Figure 1. *Overview of possible connections in the human cerebral cortex derived from anatomical studies performed by others in the monkey.* A) Predicted brain regions connected with the dorsal premotor cortex. B) Predicted brain regions connected with the primary motor cortex (reviewed in: Matelli and Luppino, 2000 for parieto-frontal circuits; Matelli and Luppino, 1997 for functional anatomy of human and nonhuman primate motor cortical areas; and, Parent and Hazrati, 1995 for cortical-subcortical connections). Note that we make no distinction between the left and the right hemispheres. Also note that the dorsal premotor cortex in this schematic comprises two anatomical areas (i.e. the caudal (F2) and the rostral (F7), each with distinct interconnections with other brain areas. PE, PEc-PEip, and PGm are cytoarchitectonically defined areas of the parietal cortex. F1 to F7 are subdivisions of the cortical motor system in the frontal lobe. Abbreviations: PMd = dorsal premotor area, M1 = primary motor area, CMA = cingulate motor area, MIP = medial intraparietal area, and SMA = supplementary motor area.

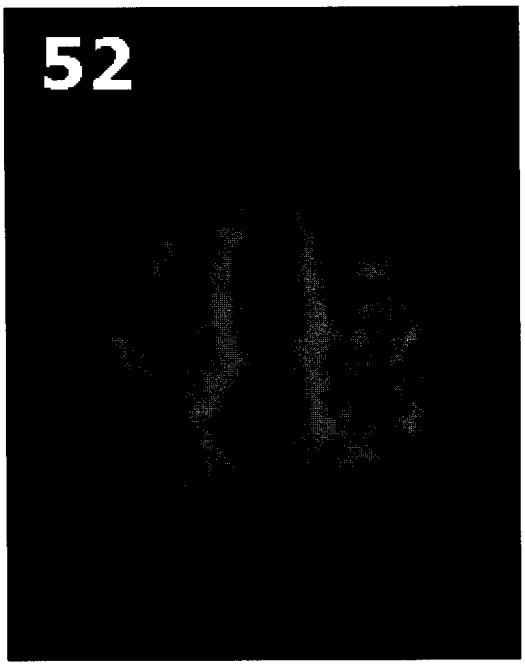

Figure 2. *Verification of coil positioning over the primary motor cortex.* Superimposed in red are virtual rods derived from transmission scans that indicate the end result of all coil placements over the primary motor cortex. Green circles represent the probabilistic locations for the dorsal premotor and primary motor cortices.

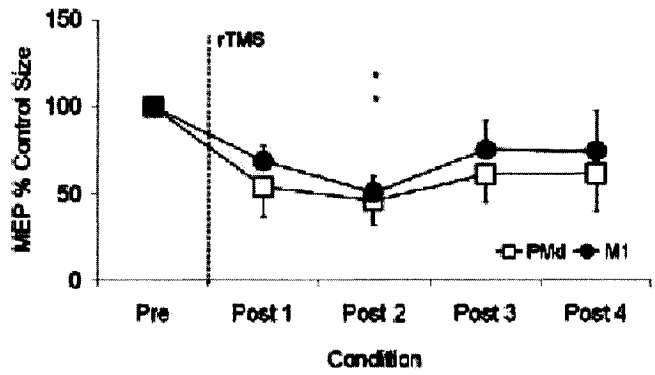

Figure 3. *Effects of repetitive stimulation on motor evoked potentials.* Mean (±SEM) percent MEP amplitude change at Post conditions compared to Pre conditions in both the dorsal premotor and primary motor experiments. Asterisks denote significant differences compared to Pre conditions (*$p<0.05$).

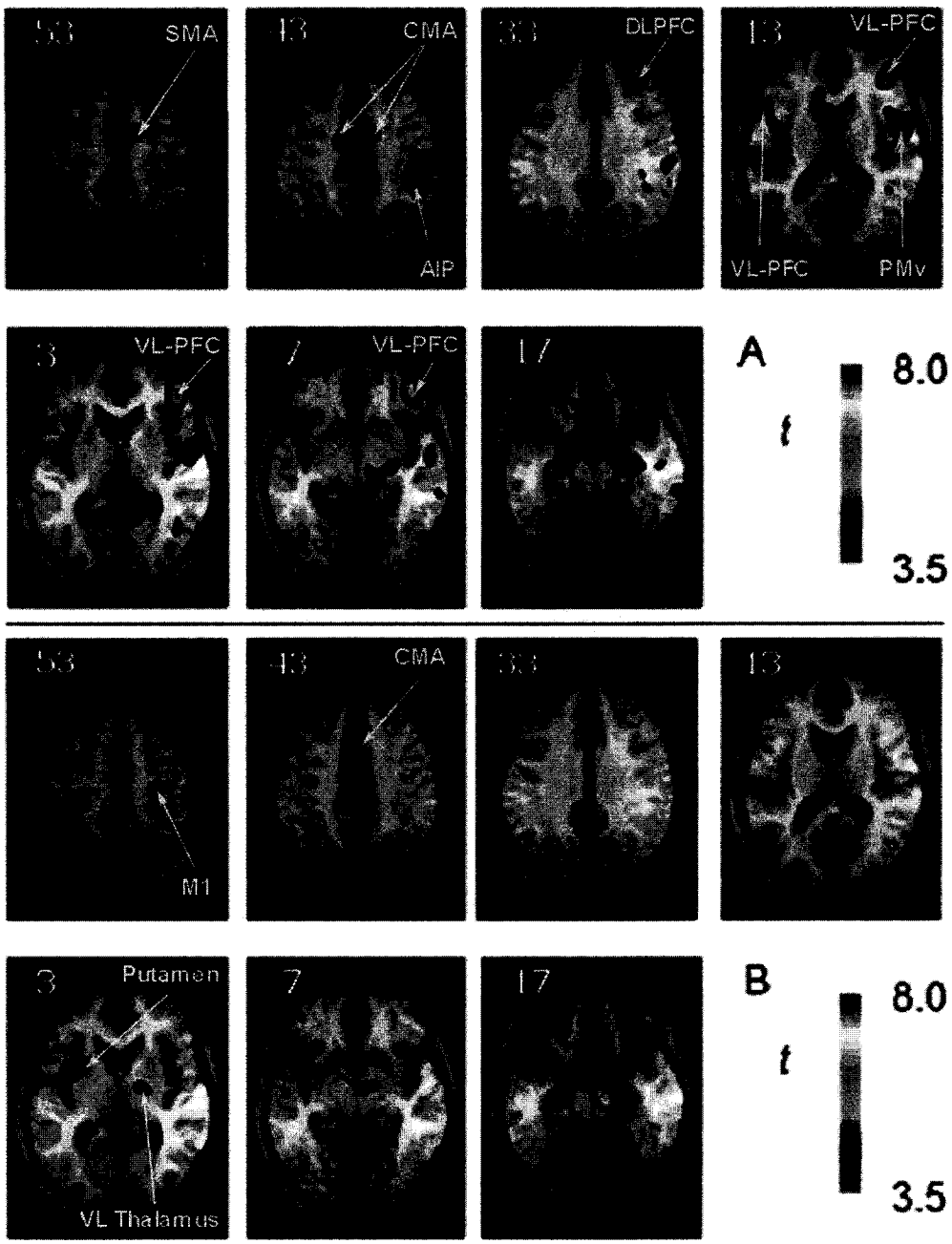

Figure 4. *Effects of repetitive stimulation on cerebral blood-flow.* A) The top half of the page shows horizontal slices of brain regions with positive correlations (t>3.5) that we obtained from the PMd experiment. B) The bottom half of the page shows horizontal slices of brain regions with positive correlations (t<3.5) that we obtained from the M1 experiment.

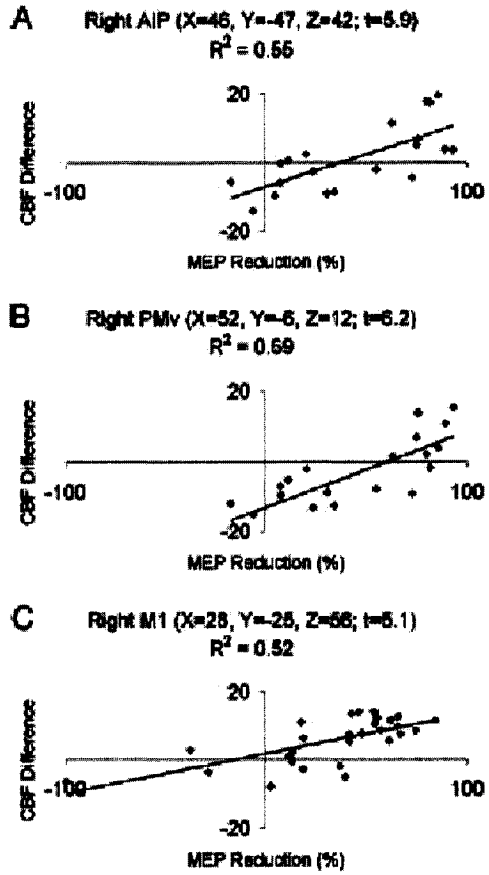

Figure 5. *Cerebral blood-flow differences plotted versus the amount of reduction in motor evoked potentials*. The figure shows extracted CBF values using VOIs centered at the X, Y, and Z coordinates of three correlation peaks. A-B) Extracted CBF values with the VOIs centered at the right anterior intraparietal and ventral premotor cortices in the dorsal premotor experiment. C) Extracted CBF values with the VOI centered at the right primary motor cortex in the primary motor experiment. Abbreviations: AIP = putative anterior intraparietal area, PMv = ventral premotor area, and M1 = primary motor area.

Chapter Three

Role of the primary motor and dorsal premotor cortices in the anticipation of forces during object lifting.[2]

Philippe A. Chouinard, Gabriel Leonard, Tomáš Paus.

I. Prelude

Study 2 examined the role of M1 and PMd during motor control. This was achieved by stimulating the two cortical areas with the same stimulation used in Study 1 and examining subsequent disruptions in the subject's ability to scale forces during object lifting. When lifting small objects, people apply forces that match the expected weight of the object. I hypothesized that repetitive TMS applied over M1 would disrupt the generation of discrete forces when lifting different weights and that repetitive TMS applied over PMd would disrupt the scaling of forces based on arbitrary cues.

II. Abstract

When lifting small objects, people apply forces that match the expected weight of the object. This expectation relies in part on information acquired during a previous lift and on associating a certain weight with a particular object. Our study examined the role of the primary motor and dorsal premotor cortices in predicting weight based either on information acquired during a previous lift (No-Cue Experiment) or on arbitrary color cues associated with a particular weight (Cue Experiment). In the two experiments, subjects used precision grip to lift two different weights in a series of trials both before and after we applied low-frequency repetitive transcranial magnetic stimulation over the primary motor and dorsal premotor cortices. In the No-Cue experiment, subjects did not receive any prior information about which of two weights they would have to lift. In the Cue experiment, a color cue provided information about which of the two weights

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subjects would have to lift. Our results demonstrate a double dissociation in the effects induced by repetitive stimulation. When applied over the primary motor cortex, repetitive stimulation disrupted the scaling of forces based on information acquired during a previous lift. In contrast, when applied over the dorsal premotor cortex, repetitive stimulation disrupted the scaling of forces based on arbitrary color cues. We conclude that the primary motor and dorsal premotor cortices have unique roles during the anticipatory scaling of forces associated with the lifting of different weights.

III. Introduction

The precision grip has been investigated extensively in humans (Johansson, 1996). People typically use the tips of the index finger and thumb when lifting small objects. The lifting of such objects requires fine motor control; too much force can damage the object or result in an excessive lifting movement, and too little force can cause the object to slip away. Throughout life, we build internal representations for the weight of different objects (Gordon et al., 1993; Wolpert and Flanagan, 2001). This provides us with the ability to apply forces for lifting objects using feedforward mechanisms. In cases when the weight is lighter than expected, somatosensory information related to lift-off will generate corrective forces to stabilize the object (Johansson and Westling, 1988). In cases when the weight is heavier than expected, the absence of an expected lift-off will generate corrective forces to overcome gravity on the object (Johansson and Westling, 1988).

Information acquired by a recent lift can influence the anticipatory scaling of forces for a subsequent lift (Johansson and Westling, 1988; Gordon et al., 1993; Fellows et al., 1998). When the weight of an object changes unexpectedly without any changes in appearance, people will generate inappropriate forces on the first lift and quite accurate forces on the subsequent lift. This adaptation indicates that the motor system can update quickly information pertaining to the properties of an object and is thought to involve processes similar to those used to correct for errors made in predicting weight (Johansson and Westling, 1988). Cell recording studies in the monkey demonstrate that a population of primary motor neurons processes information related to a recent experience by altering

their firing properties during motor adaptation (Li et al., 2001). We predict that repetitive transcranial magnetic stimulation (rTMS) applied over the primary motor cortex can disrupt the scaling of forces based on information acquired during a previous lift.

The dorsal premotor cortex selects movements mainly on the basis of learned associations (Wise and Murray, 2000). People generate more accurate forces during the lifting of small objects after they have learned to associate arbitrary color cues with weight (Cole and Rotella, 2002). Semantic identification can also influence forces applied during the lifting of commonly used objects (Gordon et al., 1993). Rapid associative learning is thought to generate internal representations that link object identification with the scaling of forces required to lift them. People can learn new associations as quickly as by the second trial and reproduce forces accurately for up to 24 hours (Gordon et al., 1991b; Flanagan et al., 2001). Lesion studies in the monkey have shown that the removal of the dorsal premotor cortex disrupts the ability to use arbitrary visual cues to make or withhold particular movements (Petrides, 1982, 1985b; Halsband and Passingham, 1982, 1985). We predict that rTMS applied over the dorsal premotor cortex can disrupt the scaling of forces based on arbitrary color cues.

IV. Methods

Overview

Two groups of subjects participated in each of two experiments: the No-Cue and Cue experiments; the individual volunteers were assigned to the two experiments at random. We applied 15 minutes of 1-Hz rTMS over the left primary motor cortex on one day and the left dorsal premotor cortex on another day. We counter-balanced the order in which repetitive stimulation was delivered over the two brain sites in a given subject. Figure 1A illustrates the time course for one session. We acquired motor evoked potentials, or MEPs, by delivering single-pulse TMS over the left primary motor cortex 22 to 20, 12 to 10, and 2 to 0 minutes before the onset of rTMS and 0 to 2, 10 to 12, 20 to 22, and 30 to 32 minutes after the conclusion of rTMS. We also acquired precision grip measurements

20 to 12 and 10 to 2 minutes before the onset of rTMS and 12 to 20 and 22 to 30 minutes after the conclusion of rTMS.

Participants

Subjects in the two experiments matched for sex, age, handedness, pinch strength, and manual dexterity. Four female and four male subjects (19 to 30 yrs of age, mean ± SEM, 24.4 ± 1.4) participated in the No-Cue experiment and another four female and four male subjects (21 to 36 yrs of age, mean ± SEM, 25.6 ± 1.6) participated in the Cue experiment. All subjects had a strong right-hand preference as determined by a handedness questionnaire (Crovitz and Zener, 1965); a paired t-test revealed no significant group difference in handedness (P=0.73). We tested pinch strength for both hands using a JAMAR pinch dynamometer (Sammons Preston, Bolinbrook, Illinois, USA) and manual dexterity for both hands using the grooved pegboard test, model 32025 (Lafayette Instrument Company, Lafayette, Indiana, USA). Paired t-tests revealed no significant group differences in pinch strength for either the right hand (P=0.27) or the left hand (P=0.29) and in performance times in the grooved pegboard test for either the right hand (P=0.80) or the left hand (P=0.78). All subjects provided informed written consent prior to participation. The Research Ethics Board of the Montreal Neurological Institute and Hospital approved all experimental procedures. We selected subjects for whom we had previously acquired anatomical magnetic resonance images, or MRIs (160 to 192 contiguous 1-mm-thick sagittal slices; Siemens Vision 1.5-T system), and who had low resting motor thresholds. We established the latter criterion to prevent over-heating of the stimulating coil.

Apparatus for precision grip

We constructed a manipulandum (Figure 1B) based on the classical apparatus used by Johansson and Westling (1984). The contact surface with which subjects grasped the manipulandum consisted of sandpaper (No 150) attached to both sides of the handle. We measured the grip force using a set of strain-gauge transducers attached to the handle

where subjects grasped with the index finger and the load force using a set of strain gauges attached to the base of the manipulandum. The resulting signals fed continuously to a Grass Model 15A54 quad amplifier (Astro-Med Inc., West Warwick, RI, USA) at a sampling rate of 800 Hz. We saved all data on a laptop computer for off-line analysis. We also attached to the base of the manipulandum an aluminum rod that passed through a hole in the table that held a weight carrier at its bottom end. This allowed us to add or remove from the carrier a 200g weight without the subject seeing us change weights. Subjects wore earphones and listened to white noise at an intensity that they could tolerate comfortably (~60 to ~80 dB). Our pilot experiments revealed that the white noise helped prevent subjects from hearing us add or remove the 200g weight and thus realize when a switch in weight occurred between lifts.

Apparatus for transcranial magnetic stimulation

We carried out TMS using a Cadwell high-speed magnetic stimulator (Cadwell Inc., Kennewick, Washington, USA) and a Cadwell figure-of-eight stimulating coil with a built-in cooling system (Corticoil, 2 tear-shaped coils of ~5-cm diameter each). We chose this coil because it produces a magnetic-field maximum of sufficiently small width to allow stimulation of the dorsal premotor cortex without encroaching on the primary motor cortex. A similar coil was previously found to stimulate an estimated volume of 20x20x10 mm (Cohen et al., 1990; Maccabee et al., 1990; Wassermann et al., 1996). Subjects used a bite-bar during stimulation while a mechanical arm held the coil over the target locations. We determined motor thresholds for the relaxed right first dorsal interosseus muscle prior to each session (see Chouinard et al., 2003).

Apparatus for electromyography

We recorded MEPs from the right first dorsal interosseus muscle using Ag/AgCl surface electrodes fixed on the skin with a belly-tendon montage. We sampled the electromyographic signal using the Grass amplifier with a bandwidth set at 0.1-3000 Hz and the sampling rate set at 2000 Hz. We then saved these data on a laptop computer for

off-line analysis. We measured the peak-to-peak amplitudes for each MEP using the program Matlab (Mathworks Inc., Matick, MA, USA) and then calculated the mean MEP amplitude for each condition based on the 20 trials.

Procedures for precision grip

We performed the two experiments in a quiet room with the lights dimmed where subjects sat comfortably in front of a computer screen. In the No-Cue experiment, we presented a white circle as a neutral stimulus before subjects lifted weights of 325g (light) or 525g (heavy); this circle provided no information about what weight would be lifted. In the Cue experiment, we presented a pink circle before subjects lifted a weight of 325g and a blue circle before subjects lifted a weight of 525g. During task performance, subjects performed 21 lifts in which they fixated their gaze on the computer screen until they saw a cue. Upon cue presentation, they then grasped the manipulandum between the tips of the index finger and thumb and lifted it vertically for a distance of about 10 cm. They maintained the manipulandum in this position until they saw on the computer screen an arrow pointing down.

In the beginning of the first session, we demonstrated how to perform the task properly and then provided subjects with a five-minute training period in which they performed a series of trials with the 325g weight. We instructed subjects to grasp the manipulandum between the tips of the index finger and thumb and lift the manipulandum using appropriate forces. We also instructed subjects to lift vertically for a distance of about 10 cm; the lifting movement of the task required mainly a flexion of the elbow. During the training period, we provided verbal feedback so as to ensure that they grasped the manipulandum with the tips of the index finger and thumb only. We did not provide any further feedback after this five-minute training period. Figure 1A illustrates the time course of each trial. Subjects performed a total of 21 lifts per block; so that after removing the first trial, we obtained five trials for each of the following four conditions: light-after-light, light-after-heavy, heavy-after-heavy, and heavy-after-light. We pre-determined the order of these conditions pseudo-randomly and presented a different order for each of the different blocks.

Procedures for transcranial magnetic stimulation

We reduced excitability by applying rTMS over the left primary motor cortex on one day and the left dorsal premotor cortex on another day (Touge et al., 2001; Münchau et al., 2002). Direct cortico-cortical connections between the dorsal premotor and primary motor cortices are thought to mediate reductions in motor excitability after repetitive stimulation over the dorsal premotor cortex (Münchau et al., 2002). We used single-pulse TMS over the left primary motor cortex to measure MEPs as an index of the effectiveness of rTMS applied over the two sites (Chouinard et al., 2003). We introduced a ~10 minute delay before subjects performed the precision grip task again because we had previously found that it took ~10 minutes after repetitive stimulation of either the primary motor cortex or the dorsal premotor cortex to reduce MEP amplitudes significantly (Chouinard et al., 2003). We expected also to see a gradual return of MEP amplitudes compared with baseline measurements 20 minutes after rTMS (Chouinard et al., 2003).

We used a four-step procedure to place the TMS coil over the primary motor and dorsal premotor cortices (see Paus et al., 1997). First, we transformed the subject's MRI into standardized space (Talairach and Tournoux, 1988; Collins et al., 1994). Second, we derived probabilistic locations for the primary motor (X=-31, Y=-22, Z=52) and dorsal premotor (X=-21, Y=-2, Z=52) cortices using information gained in previous brain imaging studies (see Paus et al., 1998; Chouinard et al, 2003). Third, we transformed the probabilistic locations to the subject's brain coordinate space. Fourth, we used frameless stereotaxy to position the TMS coil over the probabilistic locations marked on the subject's MRI (Brainsight software: Rogue Research Inc., Montreal, Quebec, Canada; Polaris System: Northern Digital Inc., Waterloo, Ontario, Canada). In the case of the primary motor cortex, we made further adjustments in coil positioning to where stimulation resulted in the maximum MEP amplitude.

For single-pulse TMS, we applied 20 single-pulses of stimulation every 5±1 seconds at a *supra*threshold intensity of 120% of the resting motor threshold. For rTMS, we applied 15 minutes of 1-Hz repetitive stimulation at a *sub*threshold intensity of 90% of the resting motor threshold in three 5-minute blocks, each block separated by one

minute, to minimize over-heating of the stimulating coil. S*ub*threshold intensities allow for more focal stimulation by narrowing the magnetic field produced by the coil, thus enabling better spatial resolution for examining changes between different cortical structures (Pascual-Leone et al., 1993). We held the coil in the same orientation when stimulating both the primary motor and dorsal premotor cortices. We oriented the coil tangentially to the scalp with the short axis of the figure-of-eight coil angled 45 degrees relative to the interhemispheric fissure and approximately perpendicular to the central sulcus. For both primary motor and dorsal premotor stimulation, the resulting induced current in the brain flowed in a posterior-to-anterior and lateral-to-medial direction.

Verification of coil positions

We derived projected coil trajectories from the center of the figure-of-eight coil using the Brainsight software (see previous section) as an estimation of where stimulation took place. After placing the coil over the sites of stimulation, we saved the projected coil trajectories in the subject's brain coordinate space. We then marked on the subject's MRI where this trajectory passed in the same perpendicular plane, or parallel plane to the coil, as the site we intended to target. We then transformed these coordinates from voxel space to native space using the software Register (Montreal Neurological Institute) and then to standardized space. Projected coil trajectories for the primary motor cortex revealed minimal overlap with those for the dorsal premotor cortex (Figure 2A&C). Projected coil trajectories for the dorsal premotor cortex generally passed in the rostral dorsal premotor cortex as established by Picard and Strick (2001). Projected coil trajectories for the primary motor cortex showed greater variability. This is likely because we made adjustments in coil positioning to target where stimulation resulted in the maximum MEP amplitude; previous studies have reported that this location can vary among individuals (Classen et al., 1998).

Analyses for motor evoked potentials

For both the No-Cue and Cue experiments, we evaluated the effects of repetitive stimulation on motor excitability by analysis of variance (ANOVA) using Time and Site of Stimulation as within-subject factors. We used Tukey's HSD pair-wise comparison tests, which corrected for multiple comparisons, to examine further significant effects. We also used paired t-tests to compare resting motor thresholds values acquired during sessions with repetitive stimulation over the primary motor cortex with those acquired during sessions with repetitive stimulation over the dorsal premotor cortex.

Analyses for precision grip

Using Matlab, we measured the rates in grip force for each but the first trial and then calculated the means for each of the different four conditions for each block. For measuring the rates in grip force, we divided the magnitude of the peak force by the time difference between the peak grip force and the first increase in grip force signal. For the statistical analyses of rates in grip force, we performed an ANOVA that examined the effects of repetitive stimulation on performance in each of the two experiments. For this ANOVA, we used Switching (No Switch vs. Switch), Weight (Light vs. Heavy), Block (20 to 12 min before rTMS vs. 10 to 2 min before rTMS vs. 12 to 20 min after rTMS vs. 22 to 30 min after rTMS), and Site of Stimulation (Primary Motor vs. Dorsal Premotor) as within-subject factors.

We also performed additional ANOVAs on both the rates in load force and the load force time (time of peak force - time of first increase in signal) in cases when the rates in grip force changed during an experiment. We calculated the rates in load force the same way as we calculated the rates in grip force. For these ANOVAs, we used Switching (No Switch vs. Switch), Weight (Light vs. Heavy), and Block (20 to 12 min before rTMS vs. 10 to 2 min before rTMS vs. 12 to 20 min after rTMS vs. 22 to 30 min after rTMS) as within-subject factors. We performed simple effect tests and Tukey's HSD pair-wise comparison tests, which corrected for multiple comparisons, to examine further significant interactions.

V. Results

Resting motor thresholds

Paired t-tests on the resting motor thresholds revealed no difference between sessions in both the No-Cue [T(7)=0.39, P=0.71] and Cue [T(7)=0.24, P=0.82] experiments.

A) No-Cue experiment

Effects of repetitive stimulation on motor excitability

An ANOVA on the MEP amplitudes revealed an effect of Time [F(6,42)=4.76, P<0.001], no effect of Site of Stimulation [F(1,7)=0.26, P=0.63], and no Time X Site of Stimulation interaction [F(6,42)=1.52, P=0.20]. These results demonstrate that changes in MEP amplitudes did not differ when we applied repetitive stimulation over the primary motor cortex compared with the dorsal premotor cortex (Figure 2B). We performed Tukey's HSD tests to examine further the effect of Time and found reductions in MEP amplitudes 0 to 2 min after rTMS compared with 22 to 20 min before rTMS (P<0.05), 12 to 10 min before rTMS (P<0.01), 2 to 0 min before rTMS (P<0.05), 20 to 22 min after rTMS (P<0.01), and 30 to 32 min after rTMS (P<0.01).

Effects of repetitive stimulation on grip forces

An ANOVA on the rates in grip force revealed a significant Switching X Weight X Block X Site of Stimulation interaction [F(3,21)=3.42, P<0.05].

Before repetitive stimulation, subjects applied rates in force that reflected the scaling of forces for a previous weight (Figure 3A-C). In the No Switch trials, i.e. when subjects lifted the same weight as in the previous lift, subjects applied faster rates in grip force when they lifted the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). When changes in weight occurred between lifts, however, effects of Switching were present. In the Switch trials, i.e. when subjects lifted a different

weight than in the previous lift, the rates in grip force increased after the weight became lighter (light-after-heavy > light-after-light) and decreased after the weight became heavier (heavy-after-light < heavy-after-heavy). These effects of Switching indicate that subjects scaled their grip forces based on the previous weight. Repetitive stimulation over the dorsal premotor cortex had no effect on the rates in grip force (Figure 3B).

Repetitive stimulation over the primary motor cortex disrupted the production of distinct rates in grip force (Figure 3A&D). In the No Switch trials, the rates in grip force at 12 to 20 min after rTMS did not differ when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy ≈ light-after-light). In the Switch trials, the rates in grip force at 12 to 20 min after rTMS did not increase after the weight became lighter (light-after-heavy ≈ light-after-light) and did not decrease after the weight became heavier (heavy-after-light ≈ heavy-after-heavy). A power analysis revealed that a sample size of 37 subjects would be necessary to reject the null hypothesis that no effects of Switching occurred (alpha=0.05). At 22 to 30 min after rTMS, the rates in grip force were similar to those before repetitive stimulation. These results indicate that repetitive stimulation over the primary motor temporarily disrupted the subjects' ability to apply distinct rates in grip force when lifting different weights and to scale forces based on the previous weight.

Effects of repetitive stimulation on load forces

An ANOVA on the rates in load force showed a Switching X Weight X Block interaction in the session with repetitive stimulation over the primary motor cortex [$F(3,21)=7.26$, $P<0.005$]. Further examination of this interaction reveal similar effects as those observed for the rates in grip force (Figure 4A, C-D). An ANOVA performed on the load force times (Figure 4B) also showed a significant Switching X Weight X Block interaction in the session with repetitive stimulation over the primary motor cortex [$F(3,21)=5.98$, $P<0.005$].

Before repetitive stimulation, the load force times did not differ in the No-Switch trials when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy ≈ light-after-light). Effects of Switching, however, were present when changes in

weight occurred between lifts. In the Switch trials, the load force times decreased after the weight became lighter (light-after-heavy < light-after-light) and increased after the weight became heavier (heavy-after-light > heavy-after-heavy).

Repetitive stimulation over the primary motor cortex resulted in distinct load force times for the two different weights (Figure 4B&D). In the No Switch trials, the load force times at 12 to 20 min after rTMS were longer when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). In the Switch trials, the load force times at 12 to 20 min after rTMS did not decrease after the weight became lighter (light-after-heavy ≈ light-after-light) nor did they increase after the weight became heavier (heavy-after-light ≈ heavy-after-heavy). At 22 to 30 min after rTMS, the load force times were similar to those before repetitive stimulation. These results suggest that although subjects at 12 to 20 min after rTMS applied similar rates in force in all four Weight X Switching conditions, the time to scale load forces prolonged for the heavy weight compared with the light weight.

B) The Cue experiment

Effects of repetitive stimulation on motor excitability

An ANOVA on the MEP amplitudes showed an effect of Time [$F(6,42)=3.12$, $P<0.05$], no effect of Site of Stimulation [$F(1,7)=0.10$, $P=0.76$], and no interaction of Time X Site of Stimulation [$F(6,42)=1.85$, $P=0.11$]. These results demonstrate that changes in MEP amplitudes did not differ when we applied repetitive stimulation over the primary motor cortex compared with over the dorsal premotor cortex. We performed Tukey's HSD tests to examine further the effect of Time (Figure 2D) and found significant reductions in MEP amplitudes 0 to 2 min after rTMS compared with 2 to 0 min before rTMS ($P<0.05$) and 30 to 32 min after rTMS ($P<0.01$).

Effects of repetitive stimulation on grip forces

An ANOVA on the rates in grip force showed a significant Switching X Weight X Block X Site of Stimulation interaction [$F(3,21)=5.83$, $P<0.005$].

Before repetitive stimulation, subjects in the Cue experiment could use arbitrary color cues to scale rates in grip force for a current weight (Figure 5A-C). In the No Switch trials, subjects applied faster rates in grip force when they lifted the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). In the Switch trials, unlike the No-Cue experiment, the rates in grip force did not differ after the weight became either lighter (light-after-heavy ≈ light-after-light) or heavier (heavy-after-light ≈ heavy-after-heavy). The lack of any Switching effect indicates that subjects could use arbitrary color cues to scale for forces based on the current weight, even during trials after a *switch* in weight occurred between lifts. Repetitive stimulation over the primary motor cortex had no effect on the rates in grip force (Figure 5A).

Repetitive stimulation over the dorsal premotor cortex resulted in the scaling of rates in grip force for a previous lift (Figure 5B&D). In the Switch trials, the rates in grip force at 12 to 20 min after rTMS increased after the weight became lighter (light-after-heavy > light-after-light) and decreased after the weight became heavier (heavy-after-light < heavy-after-heavy). At 22 to 30 min after rTMS, the rates in grip force were similar to those before repetitive stimulation. Direct comparisons between the light-after-heavy conditions confirm that subjects scaled their forces for a previous weight at 12 to 20 min after rTMS; the rates in grip force were faster when subjects lifted the light weight after the heavy weight at 12 to 20 min after rTMS compared with both before and 22 to 30 min after rTMS.

Effects of repetitive stimulation on load forces

An ANOVA performed on the rates in load force in the session with repetitive stimulation over the dorsal premotor cortex revealed a Switching X Weight X Block interaction [$F(3,21)=3.91$, $P<0.05$]. These results are similar to those observed for the

rates in grip force (Figure 6A, C-D). The same ANOVA on the load force times (Figure 6B) also revealed a Switching X Weight X Block interaction [$F(3,21)=10.93$, $P<0.001$].

Before repetitive stimulation, subjects applied longer load force times for the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). Effects of Switching, however, were not present when changes in weight occurred between lifts. In the Switch trials, the load force times did not differ after the weight became lighter (light-after-heavy ≈ light-after-light) nor did they differ after the weight became heavier (heavy-after-light ≈ heavy-after-heavy).

After repetitive stimulation over the dorsal premotor cortex, subjects applied load force times that reflected the scaling of forces for a previous weight (Figure 6B&D). In the No Switch trials, the load force times at 12 to 20 min after rTMS did not differ when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy ≈ light-after-light). In the Switch trials, the load force times at 12 to 20 min after rTMS decreased after the weight became lighter (light-after-heavy < heavy-after-heavy) and increased after the weight became heavier (heavy-after-light > heavy-after-heavy). At 22 to 30 min after rTMS, the load force times were similar to those before repetitive stimulation.

VI. Discussion

Our results demonstrate that low-frequency repetitive stimulation over the primary motor and dorsal premotor cortices influenced differentially the anticipatory scaling of forces. When applied over the primary motor cortex, repetitive stimulation disrupted the scaling of forces based on information acquired during a previous lift. In contrast, when applied over the dorsal premotor cortex, repetitive stimulation disrupted the scaling of forces based on arbitrary color cues. Together, these findings indicate that during the lifting of different weights, the primary motor cortex scales forces based on information acquired during a previous lift and the dorsal premotor cortex scales forces based on arbitrary visual cues.

Methodological issues

Reductions in motor excitability occurred immediately after repetitive stimulation as compared with a ~10 minute delay observed in our previous TMS/PET study (Chouinard et al., 2003). We speculate that the performance of precision grip prior to repetitive stimulation might have had a 'priming' effect on motor excitability (Iyer et al., 2003). Note that changes in MEPs related modestly to changes in the precision grip. Thus, the MEP data provide only a verification of the effectiveness of rTMS over the two sites (Chouinard et al., 2003).

Our study did not examine the scaling of forces based on information about the 3-D characteristics of objects. Both shape and size can influence the anticipatory scaling of forces that are applied during the lifting of small objects (Gordon et al. 1991ab; Jenmalm et al., 1997; 2000; Goodwin et al, 1998; Flanagan et al., 2001). The dorsal premotor cortex selects movements based mainly on learned associations as opposed to the more pragmatic visual and somatosensory analyses of shape and size (Geyer et al., 2000). In contrast, the ventral premotor cortex receives both visual and somatosensory information about the 3-D characteristics of objects from area AIP in the parietal lobe (Rizzolatti et al., 1988; Murata et al., 1997).

Our study also did not examine the actual learning of associations. Subjects in the Cue experiment learned to associate quickly arbitrary color cues with weight and we found no differences in performance during the two Blocks before repetitive stimulation. Previous studies demonstrate that subjects can learn new associations as quickly as by the second trial in situations when the same weight has changed in physical size from bigger to smaller or smaller to bigger (Gordon et al., 1991b; Flanagan et al., 2001). To examine the actual learning of associations, we would have had to resort to a task that involves more associations and is more taxing than just two arbitrary cues and two corresponding motor outputs. Such a task would have to match in difficulty as the ones used in studies conducted by Petrides (1985a, 1997) in which six or nine different colored lights each cued subjects to perform a different hand gesture.

The primary motor cortex in the anticipatory scaling of forces

As demonstrated in both this study and previous studies, when the weight of an object changes unexpectedly without any visible changes in appearance, people will generate inappropriate forces on the first lift and quite accurate forces on the second lift (Johansson and Westling, 1988; Gordon et al., 1993; Fellows et al., 1998). Measurements acquired in the No-Cue experiment demonstrate that: 1) when *switches* in weight occurred between lifts, subjects scaled rates in force appropriate for a previous weight and not for a current weight, 2) both the production of distinct rates in force and the scaling of forces for a previous weight diminished 12 to 20 minutes after repetitive stimulation over the primary motor cortex and re-emerged 22 to 30 minutes after repetitive stimulation, and 3) repetitive stimulation over the dorsal premotor cortex affected neither the production of distinct rates in force nor the scaling of forces for a previous weight.

In the No-Cue experiment, repetitive stimulation over the primary motor cortex temporarily disrupted the subjects' ability to apply distinct rates in force when lifting different weights and to scale forces based on the previous weight. Further analyses revealed that although subjects applied similar rates in force in all four Weight X Switching conditions, the *load force times* prolonged for the heavy weight compared with the light weight. This additional finding suggests that subjects applied similar rates in load force until a sufficient vertical force was reached to overcome gravity.

It is important to note that in the Cue experiment, repetitive stimulation over the primary motor cortex had no effect on the subjects' ability to scale forces for a current weight. Likely because the arbitrary color cues provided subjects with information about what weight they had to lift and that the subjects were able to use this information to scale for differences in weight. Thus, the observed effects induced by repetitive stimulation over the primary motor cortex do not appear to be at the level of motor execution, but rather at the level of processing motor information associated with a recent experience. Indeed, a number of TMS studies have reported a similar lack of effects on motor execution despite reductions in motor excitability after low-frequency repetitive

stimulation over the primary motor cortex (Chen et al., 1997b; Muellbacher et al., 2000; 2002).

We speculate that the primary motor cortex can form *memory* traces associated with a recent experience. Cell recording studies in the monkey reveal that separate populations of primary motor neurons can process information related to motor function (Georgopoulos et al., 1982; Zhang et al., 1997; Wise et al., 1998; Li et al., 2001), including a population of *memory* neurons that stores information related to an experience beyond its duration. Li et al. (2001) examined activity in primary motor neurons before, during, and after motor adaptation. Their results revealed that a subset of neurons, which they called *memory* neurons, changed their firing properties as monkeys learned to perform forelimb movements in a force field. Once the force-field was turned off, the firing properties of the *memory* neurons remained altered and monkeys in turn produced inappropriate forelimb movements. A recent TMS study also demonstrates that repetitive stimulation over the primary motor cortex can disrupt adaptation in a similar force-field task (Cothros et al., 2004).

The dorsal premotor cortex in the anticipatory scaling of forces

Measurements acquired in the Cue experiment demonstrate that: 1) when *switches* in weight occurred between lifts, subjects could use arbitrary color cues to scale rates in force for a current weight, 2) the ability to use arbitrary color cues to scale rates in force for a current weight diminished 12 to 20 minutes after repetitive stimulation over the dorsal premotor cortex and re-emerged 22 to 30 minutes after repetitive stimulation, and 3) repetitive stimulation over the primary motor cortex did not affect the production of scaling of forces based on the arbitrary color cues.

Contrary to the first observation, Cole and Rotella (2002) found that subjects applied grip forces from a previous lift even in cases when they lifted different colored objects in which color informed them about texture. We speculate that the reason for this discrepancy is that subjects in Cole and Rotella's study had to extract and dissociate color from other visual characteristics (e.g. shape, size) that the brain could have associated

with properties of the object during the previous lift. This differs from our study in which we presented arbitrary color cues on a computer screen.

The scaling of forces for a previous weight is associated with somatosensory information related to errors made during weight prediction (Johansson and Westling, 1988). An alternative explanation for our results could be that repetitive stimulation over the dorsal premotor cortex enhanced the use of somatosensory information from a previous trial in a manner that would drive subjects to ignore the cues and scale forces based on the previous weight. We argue against this possibility for two reasons. First, repetitive stimulation over the dorsal premotor cortex in the No-Cue experiment had no effect in the manner with which subjects scaled forces for a previous weight. Second, cell recording studies in the monkey demonstrate that the dorsal premotor cortex contains only a few neurons that use somatosensory information to control for corrective forces during the precision grip (Boudreau and Smith, 2001).

Thus, the observed effects induced by repetitive stimulation over the dorsal premotor cortex appear to be at the level of coupling arbitrary visual cues and motor output. Indeed, cell recording studies in the monkey reveal that a number of dorsal premotor neurons increase their discharge activity after the presentation of an arbitrary visual cue that represents a learned association for a particular motor response compared with the presentation of a directional cue indicating a particular motor response (Kurata and Wise, 1988; Mitz et al., 1991; Kurata and Hoffman, 1994). GABA-$_A$ agonist muscimol injections in the dorsal premotor cortex diminish the monkey's ability to select a correct response based on an arbitrary visual cue (Kurata and Hoffman, 1994). Petrides (1982; 1985b), as well as Halsband and Passingham (1982; 1985), have shown that the removal of the dorsal premotor cortex disrupts the ability to use arbitrary visual cues to make or withhold particular movements. Our current observations, together with these findings, reinforce the notion that the premotor cortex is critical for implementing associations between visual cues and motor responses.

VI. Figures

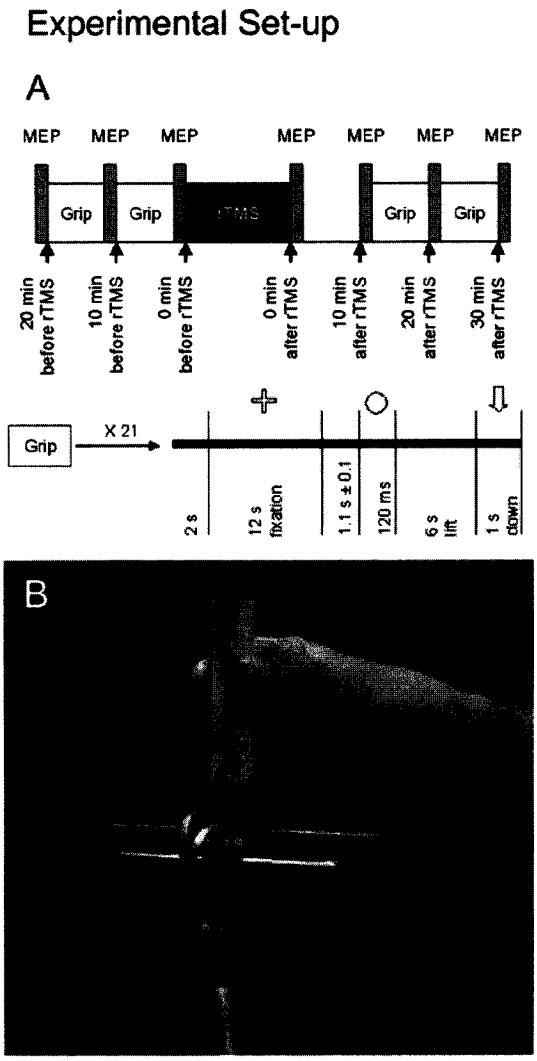

Figure 1. *Experimental Set-up.* A) Illustrates the chronological order of a session. During task performance, subjects performed 21 lifts in which they fixated their gaze on the computer screen until they saw a cue. Upon cue presentation, they then grasped the manipulandum between the tips of the index finger and thumb and lifted it vertically for a distance of about 10 cm. They maintained the manipulandum in this position until they saw on the computer screen an arrow pointing down. B) Illustrates the manipulandum that we used to measure precision grip.

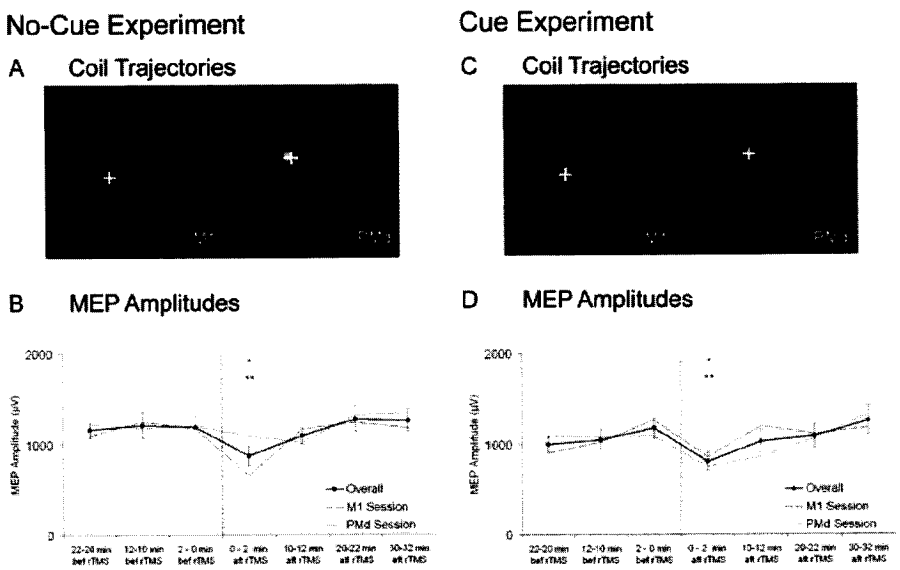

Figure 2. *MEP Amplitudes.* A) and C) Superimposed on magnetic resonance images are projected coil trajectories that indicate estimated locations for induced currents in the brain during repetitive stimulation over the primary motor (M1) and dorsal premotor (PMd) cortices. The brightness of these superimpositions reflects the probability of the coil trajectories. Crosses represent their probabilistic locations. B) and D) Overall mean MEP amplitudes (±SEM) and MEP amplitudes in the primary motor and dorsal premotor sessions. Asterisks denote significant differences for overall MEP amplitudes (No-Cue experiment: *P<0.05 vs. 22 to 20 min before rTMS, 2 to 0 min before rTMS; **P<0.01 vs. 12 to 10 min before rTMS, 20 to 22 min after rTMS, 30 to 32 min after rTMS; Cue experiment: *P<0.05 vs.2 to 0 min before rTMS; **P<0.01 vs. 30 to 32 min after rTMS).

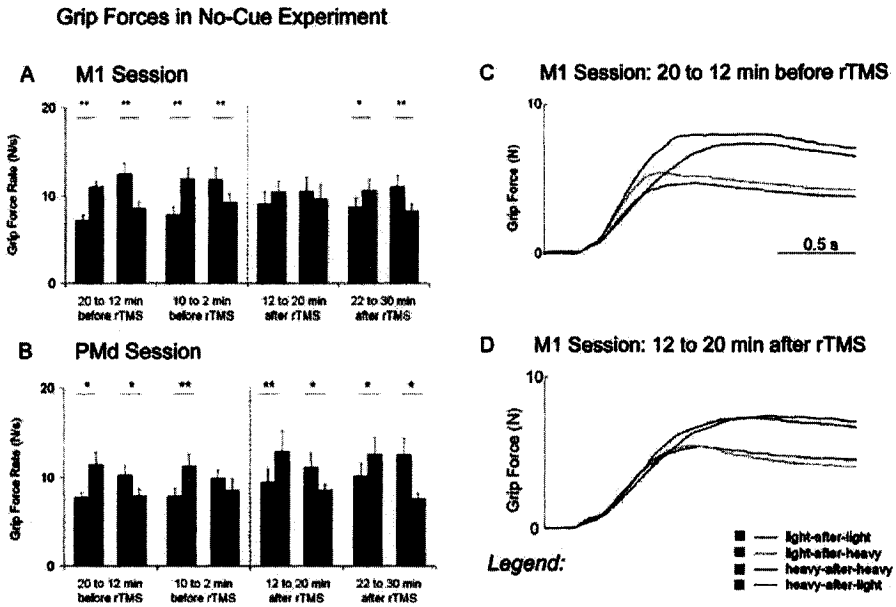

Figure 3. *Grip forces in the No-Cue experiment.* A) Represents means (±SEM) for the rates in grip force before and after repetitive stimulation over the primary motor cortex (M1). B) Represents means (±SEM) for the rates in grip force before and after repetitive stimulation over the dorsal premotor cortex (PMd). C) Represents the overall average traces for grip forces 20 to 12 minutes before repetitive stimulation over M1. D) Represents the overall average traces for grip forces 12 to 20 minutes after repetitive stimulation over M1. Asterisks denote significant differences between Switch conditions (*$P \leq 0.05$, **$P < 0.01$).

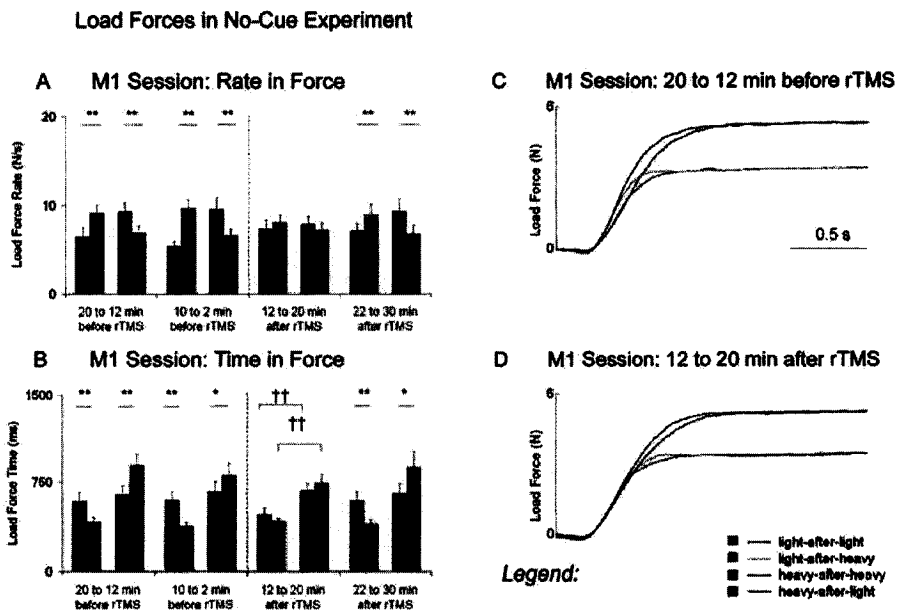

Figure 4. *Load forces in the No-Cue experiment.* A) Represents means (±SEM) for the rates in load force before and after repetitive stimulation over the primary motor cortex (M1). B) Represents means (±SEM) for the load force times before and after repetitive stimulation over M1. C) Represents the overall average traces for load forces 20 to 12 minutes before repetitive stimulation over M1. D) Represents the overall average traces for load forces 12 to 20 minutes after repetitive stimulation over M1. Asterisks denote significant differences between Switch conditions (*P≤0.05, **P<0.01). Daggers denote significant differences between Weight conditions at Post-1 (††P<0.01).

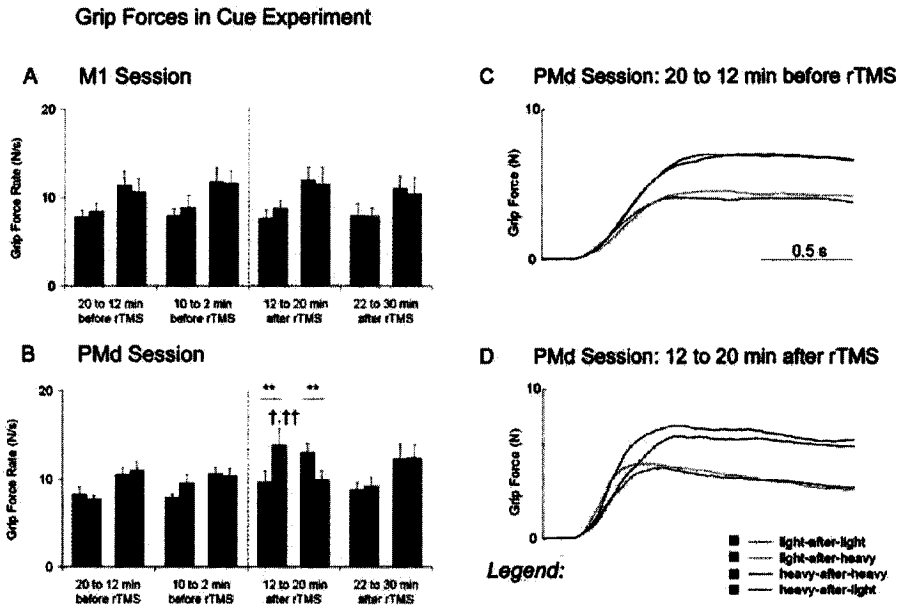

Figure 5. *Grip forces in the Cue experiment.* A) Represents means (±SEM) for the rates in grip force before and after repetitive stimulation over the primary motor cortex (M1). B) Represents means (±SEM) for the rates in grip force before and after repetitive stimulation over the dorsal premotor cortex (PMd). C) Represents the overall average traces for grip forces 20 to 12 minutes before repetitive stimulation over PMd. D) Represents the overall average traces for grip forces 12 to 20 minutes after repetitive stimulation over PMd. Asterisks denote significant differences between Switch conditions (*P≤0.05, **P<0.01). Daggers denote significant differences between Block conditions (†P<0.05 vs. Pre-2, ††P<0.01 vs. Pre-1 and Post-2).

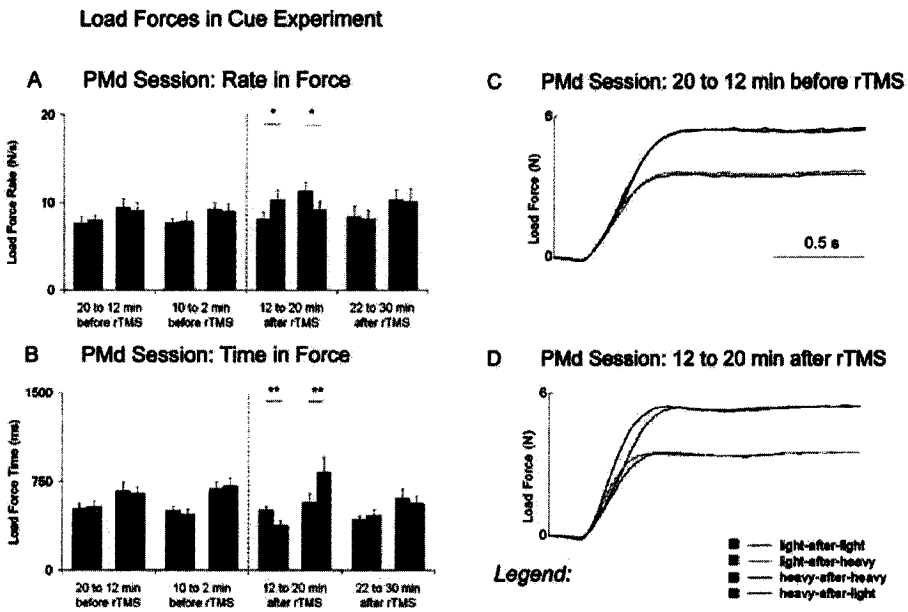

Figure 6. *Load forces in the Cue experiment.* A) Represents means (±SEM) for the rates in load force before and after repetitive stimulation over the dorsal premotor cortex (PMd). B) Represents means (±SEM) for the load force times before and after repetitive stimulation over PMd. C) Represents the overall average traces for load forces 20 to 12 minutes before repetitive stimulation over PMd. D) Represents the overall average traces for load forces 12 to 20 minutes after repetitive stimulation over PMd. Asterisks denote significant differences between Switch conditions (*$P \leq 0.05$, **$P < 0.01$).

Chapter Four

Changes in effective connectivity of the motor cortex in stroke patients after rehabilitative therapy[3]

Philippe A. Chouinard, Gabriel Leonard, Tomáš Paus.

I. Prelude

Study 3 examined changes in the effective connectivity of M1 in stroke patients who underwent CI Therapy more than one year after stroke. During TMS / PET sessions, I applied one-second trains of subthreshold 10-Hz repetitive TMS over the probabilistic hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. I hypothesized that the therapy would lead to a change in the local response of the ipsilesional M1 as well as changes in effective connectivity of the ipsilesional M1 with the non-primary motor areas and the basal ganglia.

II. Abstract

We used a *perturb-and-measure* approach, by combining transcranial magnetic stimulation (TMS) and positron emission tomography (PET), to examine changes in the primary motor cortex (M1) and its effective connectivity in stroke patients with chronic motor deficits (>1-year post-stroke) who underwent 3-weeks of Constraint-Induced Movement Therapy. During the 3-week period, 7 patients spent 4 hours per day performing shaping exercises with the affected arm under our supervision for 14 days and wore a mitt on the unaffected arm at home in situations where safety was not compromised. Anatomical magnetic resonance imaging confirmed that all patients had lesions that encompassed the white matter; no patient had damage in the hand representation of M1. Improvements on various motor tests were observed immediately after therapy and were still present in most tests one-month afterwards. During the TMS /

[3] This paper has been submitted for publication.

PET sessions, we applied trains of subthreshold 10-Hz repetitive TMS over the hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. The results demonstrate changes in the effective connectivity of the ipsilesional M1 with the contralesional M1, the non-primary motor areas in both hemispheres, and the basal ganglia in both hemispheres. We speculate that these results represent a rehabilitation-induced strengthening of a network of brain regions necessary for the development of compensatory skills.

III. Introduction

Functional imaging has provided a wealth of knowledge about brain regions underlying stroke recovery (e.g. Chollet et al., 1991; Weiller et al., 1992; 1993; Cramer et al., 1997; Dettmers et al., 1997; Honda et al., 1997; Cao et al., 1998; Seitz et al., 1998; Nelles et al., 1999b). Three robust findings emerge as recovered patients execute movements with their affected arm: 1) an increase and / or displacement of activity in the ipsilesional M1, 2) a greater involvement of the contralesional M1, and 3) a greater involvement of the non-primary motor areas in both hemispheres.

Yet, we still do not understand the neural mechanisms involved. This is in part because current functional imaging methods have a limited ability to discern how different brain regions influence each other. Several studies have examined connectivity in the brain by correlating the level of activity present in one brain region with the level of activity in remote brain regions (McIntosh and Gozales-Lima, 1994; Friston, 1994; Friston et al., 1996). This type of analysis can be confounded by the engagement of the subject performing a task. Co-activations acquired with functional imaging may reflect relationships between different task components rather than true effective connectivity.

We used instead a *perturb-and-measure* approach (Paus, 2005) in which transcranial magnetic stimulation (TMS) was combined with positron emission tomography (PET). The advantage of combining TMS and PET is that it serves as a behavior-independent assay of connectivity between a cortical area and other structures in the brain. In normal volunteers, Paus et al. (1998) applied subthreshold 10-Hz repetitive TMS over M1 and varied the number of TMS trains delivered during each

scan. The cerebral blood-flow (CBF) response co-varied negatively with the number of stimulus trains delivered both at the site of stimulation and in several distal brain regions known to be connected trans-synaptically in the monkey. The authors proposed that the trains of stimulation resulted in an activation of local inhibitory mechanisms and a subsequent reduction of excitatory synaptic activity in the stimulated region and in the inter-connected network.

In the present study, we applied a similar protocol to examine changes in M1 and its effective connectivity in stroke patients who underwent 3-weeks of Constraint-Induced Movement Therapy (CI Therapy, Taub et al., 1993). Improvements on various motor tests were observed immediately after therapy and were still present in most tests one-month afterwards. During the TMS / PET sessions, we applied trains of subthreshold 10-Hz repetitive TMS over the probabilistic hand representation of the ipsilesional and contralesional M1s and varied the number of TMS trains delivered during each scan. We hypothesized that the therapy would lead to changes in the local response of the ipsilesional M1 as well as in its effective connectivity with the non-primary motor areas and the basal ganglia.

IV. Methods

Patients

One female and six male patients participated in the study (49 to 78 years of age, mean ± SEM, 65.9 ± 1.4 years of age). Table 1 lists their age, sex, overall IQ, months after stroke, type of stroke, side of paresis, and the location of their lesions at the time of the study as determined with magnetic resonance imaging, or MRI [176 to 192 contiguous 1-mm-thick T1-weighted sagittal slices; Siemens (Erlangen, Germany) Vision 1.5-T system]. Six out of the seven patients had a strong right-hand preference before their stroke as determined by a handedness questionnaire (Crovitz and Zener, 1965). The one patient with a left hand preference before stroke suffered an infarct to the right side of his brain, which left him with a paresis of his dominant hand (Patient 1). All patients had received conventional physiotherapy after their stroke, had moderate to good recovery

afterwards, and participated in this study more than one year after their stroke. All experimental procedures were approved by both the Institutional Review Board of McGill University and the Research Ethics Board of the Montreal Neurological Institute and Hospital. Patients gave informed consent in accordance with the Declaration of Helsinki.

We identified potential subjects from a database at the McGill University Health Centre. We screened these patients in a preliminary fashion by telephone and invited potential candidates to meet with us to discuss the project. A neurologist at the Montreal Neurological Hospital then examined each candidate. Once candidates passed their neurological examination, they then had to pass neuropsychological, MRI, and TMS examinations. We used the following inclusion criteria: 1) minimum of 12 months after a stroke, 2) motor weakness on one side of the body, and 3) minimum motor criterion of being able to make a fist and extend their fingers apart. We also used the following exclusion criteria: 1) a personal or family history of epilepsy, 2) serious uncontrolled medical problems, 3) extreme spasticity and / or pain, 4) serious cognitive problems, 5) brainstem lesions or lesions that extended into the probabilistic hand representation of M1, and 6) motor thresholds too high to be detected on both arms.

Movement therapy

Patients underwent a rehabilitation program based on CI Therapy (Taub et al., 1993). The therapy consisted of two components: 1) restricting movement of the unaffected arm, and 2) training the affected arm by a procedure called shaping.

Patients were given a padded safety mitt that enclosed the fingers and the wrist. Each patient agreed to wear this restraint on their unaffected arm for 90% of waking hours. A home treatment contract was made with the patient outlining the agreed-upon activities in which the patient could and could not participate while wearing the mitt. This contract served to encourage patients to wear the restraint except in situations where safety might be compromised (e.g. excretory functions, naps, situations where balance might be compromised).

During the three-week period, all patients, except for one patient, spent four hours at the laboratory where they performed shaping exercises under our supervision for 14 days. For the one patient who did not come to the laboratory, we had agreed instead to come to her apartment for the same amount of time to supervise her shaping exercises. Shaping refers to the substantial practice of the affected arm in which a given movement is approached in small steps of progressively increasing difficulty (Taub et al., 1994). The general principle was to keep extending motor capacity in small increments beyond the level of performance already achieved. Patients were rewarded with enthusiastic approval for improvement but were never blamed, or punished, for failure. Patients performed a total of 10 to 12 different shaping exercises, of which they performed 5 to 6 a day. Each shaping exercise was tailored to the deficit of the patient and targeted movements we thought had the most potential to improve. Task objects were frequently used household objects (e.g. jars, eating utensils, clothespins) and children's toys (e.g. building blocks, marbles). We administered motor tests before, immediately after, and one-month after therapy.

Motor testing

We attempted to make the distinction between motor performance carried out in the laboratory and the use of the affected arm in the real-world setting. We determined motor function in the laboratory with the Wolf Motor Function Test (WMFT, Wolf et al., 1989; Taub et al., 1993) in which patients performed 13 timed tasks in front of a video camera. These tasks ranged in difficulty from placing the affected hand on the table to using precision grip for picking-up a paper clip. The WMFT provided scores for functional ability of the affected arm on a scale from zero to five, with zero indicating that the patient could not perform the task and five indicating normal use. The Actual Amount of Use Test (AAUT, Taub et al., 1998) addressed the issue of amount of use when a task is not requested. We filmed performance on the AAUT in a setting in which patients were unaware that they were being tested by casually prompting them to perform predetermined activities, such as reading a newspaper. The AAUT provided scores for the amount of use of the affected arm on a scale from zero to two, with zero indicating

that the patient did not attempt to use the affected arm and two indicating that the patient used the affected arm and that its use was functional and not rudimentary. An undergraduate student who was blind to the time that testing occurred rated both the WMFT and the AAUT.

We assessed real-world outcome with the Motor Activity Log (MAL, Taub et al., 1993), which consisted of a structured interview with the patient designed to address transfer of acquired motor skills from the laboratory to the home setting. It provided scores on a scale from zero to five for both the quality of movement and the amount of use of the affected arm in specific tasks commonly carried out at home. Zero indicated the lowest possible scores and five indicated the highest possible scores.

We administered additional motor tests to assess coordination, speed, and strength in both arms. Patients used a hand-held stylus to tap on a board that had two circular brass plates, each plate divided into four distinct pie-shaped sectors numbered 1, 2, 3, and 4, respectively (Thurstone, 1944). Using this apparatus, we tested the patients' ability to perform: 1) simple unimanual tapping on one sector, 2) spatially ordered unimanual tapping on all four sectors, and 3) bimanual tapping in which the spatially ordered tapping of the two arms were out-of-phase (Leonard et al., 1988). We also measured pinch and grip strength using dynamometers (Sammons Preston, Bolinbrook, Illinois, USA).

Brain mapping procedures

Overview

We designed the TMS / PET protocol to enable us to compare the effects of stimulating the ipsilesional and contralesional M1s at the Pre and Post sessions (Figure 1A). We stimulated over the probabilistic hand representation of M1 in each of the two hemispheres and measured the cerebral blood-flow (CBF) response with PET. We acquired a total of seven 60-s scans in each of the two sessions using the ^{15}O-labeled H_2O bolus method (Raichle et al., 1983). During the first, second, and third scans, we delivered a different number of TMS trains over the M1 in one hemisphere (1-sec 5, 15

and 30 trains of 10-Hz stimulation). The fourth scan served as a baseline scan without TMS. During the fifth, sixth, and seventh scans, we stimulated over the opposite M1 using the same stimulation parameters as in the first three scans. We applied the same stimulation intensity when stimulating both the ipsilesional and contralesional M1s and during both the Pre and Post sessions. In a given subject, we counter-balanced the orders of the number of TMS trains delivered and the sites of stimulation. We also acquired 10-minute transmission scans after we positioned the TMS coil over the first and second target sites.

Positron emission tomography

We instructed subjects to keep their eyes closed during scanning. We measured CBF with a CTI / Siemens HR+ 63-slice tomograph scanner operated in 3-D acquisition mode. For each scan, we injected 8.5 mCi of ^{15}O-labeled H_2O into the antecubital vein of the unaffected arm. Acquired CBF images were reconstructed with a 14-mm Hanning filter, normalized for differences in global CBF ('normalized CBF'), co-registered with the individual MR images (Woods et al., 1993), and transformed into standardized stereotaxic space (Talairach and Tournoux 1988) by means of an automated feature-matching algorithm (Collins et al., 1994). In addition, we flipped the normalized CBF images in standardized space in Patients 1 and 5 so that the left hemisphere represented the affected hemisphere in all patients. We placed four 0.5-mm thick sheets of well-grounded mu-metal in the scanner gantry to protect the photomultipliers inside the scanner from the effects of the coil-generated magnetic field. The mu-metal, however, attenuates gamma rays and in turn decreases the number of detected coincidence counts (Paus, 2002). We used the transmission scans to correct for the attenuation of gamma rays caused by all objects in the scanner, including the coil, the coil mount, and the metal sheets. The transmission scans also enabled us to verify final coil positioning. This procedure will be described later.

Transcranial magnetic stimulation

We carried out TMS using a Cadwell high-speed magnetic stimulator (Cadwell Inc., Kennewick, Washington, USA) and a Cadwell circular stimulating coil with a cooling system mounted over its outer casing (external diameter, 9 cm). We determined motor thresholds before each PET session by varying the stimulation intensity until a level was reached at which observable hand muscle twitches were reliably induced for at least 50% of stimulations, and lesser levels of stimulation failed to induce consistently these same contractions.

We used a five-step procedure to place the TMS coil over the target sites (Paus et al., 1997). First, we transformed the subject's MRI into standardized space (Talairach and Tournoux, 1988; Collins et al., 1994). Second, we derived a probabilistic location for M1 (X = ±31, Y = –22, Z = 52; Paus et al., 1998) using information gained in previous studies that measured cerebral activity during volitional hand movements. Third, we transformed this probabilistic location to the subject's brain coordinate space for both hemispheres. Fourth, we used frameless stereotaxy to mark with a felt pen the location on the scalp that was closest to the probabilistic location in each of the two hemispheres marked on the subject's MRI (Brainsight software: Rogue Research Inc., Montreal, Quebec, Canada; Polaris System: Northern Digital Inc., Waterloo, Ontario, Canada). The final step required us to position the coil over the marked locations on the scalp. With the patient lying comfortably on the bed of the scanner, we inserted a bite-bar in their mouth, placed them inside the scanner, positioned the TMS coil over one of the marked locations on the scalp, and then used a mechanical arm to lock in place the TMS coil. We positioned the TMS coil so that its lateral rim was placed over the target location with the rest of the coil tilted away from the skull. The anterior tip of the coil was positioned in the anterior direction and the handle of the coil was parallel to the interhemispheric fissure and pointed backwards.

During scanning, we applied 10-Hz trains of one second duration at a *sub*threshold intensity of 95% of the motor threshold of either the affected or unaffected hand, whichever was lowest at the Pre session (Figure 1B). Note that in Patients 4 and 6 we could not detect motor thresholds for one hand. In these cases, we set the stimulation

intensity at 95% of the motor threshold of the opposite hand. To verify that we stimulated below the motor threshold during scanning, we used electromyography to record activity from the first dorsal interosseus muscles using Ag / AgCl surface electrodes fixed on the skin with a belly-tendon montage. We sampled the signals using a Grass Model 15A54 quad amplifier (Astro-Med Inc., West Warwick, RI, USA). The TMS trains applied during scanning did not induce any overt movements nor any significant electromyographic responses.

Verification of coil positions

Using a procedure described in detail elsewhere (Paus and Wolforth, 1998), we used the transmission scans to verify final coil positioning relative to the acquired PET and MR images. These transmission images showed us the coil's position relative to the subject's head. We projected virtual rods orthogonal to the plane of the coil from where the coil touched the scalp. Following PET-to-PET, PET-to-MRI, and MRI-to-standardized space transformations, we superimposed the location of these virtual rods on an average anatomical MRI of all patients. This end product provided us with estimated locations for the TMS-induced currents in the brain during repetitive stimulation (Figure 2). Overall, the projected coil trajectories showed similar consistency as in our previous TMS / PET studies (Chouinard et al., 2003; Barrett et al., 2004).

Analyses

Motor thresholds

We evaluated the effects of therapy on motor thresholds by ANOVA using Session (Pre and Post) and Side of Stimulation (Ipsilesional M1 and Contralesional M1) as within-subject factors. We used Tukey's honestly significant difference (HSD) tests, which corrects for multiple comparisons, for all post-hoc pair-wise comparisons.

Motor performance

For the mean scores obtained in the WMFT, the AAUT, the MAL, and bimanual tapping, we evaluated the effects of therapy on performance by ANOVA using Session (Pre, Post, and Follow-up) as a within-subject factor and Tukey's HSD tests, which corrected for multiple comparisons, for all post-hoc pair-wise comparisons. For the other motor tests in which we obtained measures of performance for both the affected and unaffected arms (i.e. unimanual simple tapping, unimanual sequential tapping, pinch strength, and grip strength), we evaluated the effects of therapy on performance by ANOVA using Session (Pre, Post, and Follow-up) and Arm (Affected and Unaffected) as within-subject factors. We performed simple effect tests and Tukey's HSD pair-wise comparison tests to examine further significant interactions. We also report effect sizes (ε) for the Post and Follow-up sessions; these were calculated by subtracting the Post and Follow-up means from the mean of the Pre session and then dividing this difference by the standard deviation of the post-treatment session (Glass et al., 1981). For the figures, we plotted means ± 95% confidence intervals for within-subject contrasts (Loftus and Masson, 1994).

Cerebral blood-flow

We performed two series of statistical analyses. For the first series, we performed ANOVA using Session (Pre and Post) and TMS Trains (5, 15, and 30 trains) as factors after having removed the effects of Subjects. For the second series, we used a four-step procedure to correlate differences in CBF with the percentage change in motor scores between the Post and Pre sessions. First, we calculated the average CBF response to TMS (i.e. the three TMS Train conditions pooled). Second, we subtracted CBF acquired during the Base scan from the average CBF response to TMS (Average – Base). Third, we calculated the difference of these subtractions at the Pre session from the Post session [(Average – Base) $_{Post}$ – (Average – Base) $_{Pre}$]. The fourth step correlated these CBF differences with the percentage change in motor improvement of the affected arm at the Post session: [((Post / Pre) – 1) × 100].

We carried out calculations for the t-statistical maps for each of the voxels constituting the entire scanned volume. After generating the t-statistical maps, we evaluated the presence of a significant peak by a method based on a 3-D Gaussian random-field theory, with correction for the multiple comparisons involved in searching the entire volume (Worsley et al., 1992). Using this method, we performed both an exploratory search of the entire brain and a directed search in specific brain regions. For an exploratory search, we considered values equal to or exceeding a criterion of T = 4.3 as significant ($P < 0.000008$, 2-tailed, uncorrected), yielding a false positive rate of 0.05 (corrected) in 218 resolution elements (each of which has dimensions 14x14x14 mm) for a gray-matter volume of 600 cm^3. For a directed search, we considered values equal to or exceeding a criterion of T = 3.0 as significant ($P < 0.0002$, 2-tailed, uncorrected), yielding a false positive rate of 0.05 (corrected) in two resolution elements (each of which has dimensions of 14 × 14 × 14 mm) for a tissue volume of 5 cm^3. We performed the directed search in M1 and in brain regions connected to M1 in non-human primates. To perform this search in the human brain, we relied on the results obtained in previous functional imaging studies that mapped their putative homologues. We describe this procedure in detail elsewhere (Chouinard et al., 2003). We determined the anatomical locations of all significant t-statistic peaks by examining the merged image of the t-statistical maps with the transformed averaged MRI of all subjects in standardized space (Talairach and Tournoux, 1988).

V. Results

Motor thresholds

ANOVA did not reveal an effect of Session ($F_{(1,3)} = 0.93$, $P = 0.41$), Side of Stimulation ($F_{(1,3)} = 3.23$, $P = 0.17$), or interaction between Session × Side of Stimulation ($F_{(1,3)} = 4.91$, $P=0.11$).

Motor performance

Effects of therapy on functional ability

We administered the WFMT to assess changes in functional ability of the affected arm (Figure 3A-B). ANOVA on the scores for functional ability revealed an effect of Session ($F_{(2,12)}$ = 10.89, $P < 0.005$). Decreases in the average time to complete the task components of the WMFT almost reached significance after therapy ($F_{(2,12)}$ = 5.43, P = 0.06). Before therapy, patients had a mean score of 3.5, which lies between a score for movements influenced by synergy and / or made with some effort (3) and a score for movements that were not quite as fast or accurate as normal (4). After therapy, patients demonstrated improvements with mean scores of 3.9 at both the Post ($P < 0.01$, $\varepsilon = 0.56$) and Follow-up ($P < 0.01$, $\varepsilon = 0.62$) sessions.

Effects of therapy on amount of use

We administered the AAUT to assess changes in the amount of use of the affected arm (Figure 3C). ANOVA revealed an effect of Session ($F_{(2,12)}$ = 11.15, $P < 0.005$). Before therapy, patients had a mean score of 1.4, which lies between a score for rudimentary use (1) and a score for functional use (2). After therapy, patients demonstrated an improvement at the Post session with a mean score of 1.7 ($P < 0.01$, $\varepsilon = 1.20$), but this improvement was not maintained at Follow-up ($P > 0.05$). In fact, a significant decrease in the amount of use was observed at the Follow-up session compared with the Post session ($P < 0.05$).

Effects of transfer to the home setting

We administered the MAL to assess changes in the quality of movement and the amount of use of the affected arm in tasks commonly carried-out at home (Figure 3D-E). ANOVA revealed effects of Session for both the quality of movement ($F_{(2,12)}$ = 28.38, $P <$ 0.0001) and the amount of use ($F_{(2,12)}$ = 23.88, $P < 0.0001$) scores. Before therapy,

patients had mean scores of 2.6 for both measures. For the quality of movement scores, this lies between a score for 'poor' (2) and a score for 'fair' (3) and for the amount of use scores, this lies between a score for 'rarely' (2) and a score for '1/2 as much as before stroke' (3). After therapy, patients demonstrated improvements in the quality of movement scores with means of 3.9, which is close to a score for 'almost normal' (4), at both the Post ($P < 0.01$, $\varepsilon = 1.41$) and Follow-up ($P < 0.01$, $\varepsilon = 1.10$) sessions. Patients also demonstrated improvements in the amount of use scores with a means of 3.9, which is close to a score for '3/4 as much as before stroke' (4), at both the Post ($P < 0.01$, $\varepsilon = 1.27$) and Follow-up ($P < 0.01$, $\varepsilon = 0.94$) sessions.

Effects of therapy on simple and sequential tapping

We assessed the patients' ability to perform unimanual simple, unimanual sequential, and bimanual sequential tapping movements (Figure 3F-H). For unimanual simple tapping, ANOVA revealed a significant effect of Arm ($F_{(1,6)} = 6.02$, $P < 0.05$), but did not reveal either an effect of Session ($F_{(2,12)} = 1.70$, $P = 0.22$) or a Session × Arm interaction ($F_{(2,12)} = 1.64$, $P = 0.23$). For unimanual sequential tapping, ANOVA revealed an almost significant Session × Arm interaction ($F_{(2,12)} = 3.62$, $P = 0.06$). Further analyses of this interaction revealed that the speed measures for unimanual sequential tapping changed as a consequence of therapy in the affected arm, but not in the unaffected arm. Patients were faster with the affected arm at both the Post ($P < 0.01$, $\varepsilon = 0.32$) and Follow-up ($P < 0.05$, $\varepsilon = 0.30$) sessions compared with the Pre session. ANOVA also revealed an effect of Session on the speed measures for bimanual sequential tapping (Figure 3H, $F_{(2,12)} = 5.91$, $P < 0.05$). Patients could perform faster bimanual sequences at both the Post ($P < 0.05$, $\varepsilon = 0.30$) and Follow-up ($P < 0.05$, $\varepsilon = 0.38$) sessions.

Effects of therapy on pinch and grip strength

We assessed the patients' strength for grip and pinch (Figure 3I-J). For grip strength, ANOVA revealed a Session × Arm interaction ($F_{(1,12)} = 7.36$, $P < 0.01$). Further analyses of this interaction revealed that grip strength changed as a consequence of therapy in the

affected arm, but not the unaffected arm. Patients were stronger with their affected arm at the Post ($P < 0.05$, $\varepsilon = 0.25$) session compared with the Pre session, but not at Follow-up ($P > 0.05$). For pinch strength, ANOVA did not reveal an effect of Session ($F_{(2,12)} = 0.14$, $P = 0.87$), Arm ($F_{(1,6)} = 3.12$, $P = 0.13$), or interaction between Session × Arm ($F_{(2,12)} = 2.18$, $P = 0.16$).

Cerebral blood-flow

Changes in effective connectivity of the ipsilesional M1

Table 2 summarizes the results from the ANOVA performed using Session and TMS Trains as factors on CBF data acquired with TMS applied over the ipsilesional M1. We did not observe any changes with an exploratory search set at $T \geq 4.3$. The following are results obtained from a directed search set at $T \geq 3.0$.

We observed effects of TMS Trains on CBF before and after therapy (Table 2). Before therapy, the CBF response increased with the number of TMS trains in the ipsilesional putamen. After therapy, the CBF response increased with the number of TMS trains in the contralesional M1 on the anterior bank of the central sulcus (Figure 4A). We tested whether these effects differed between sessions using CBF values extracted from the voxel-of-interests centered at the coordinates of the peaks. The effect of TMS Trains on CBF did not differ between sessions in the ipsilesional putamen ($F_{(2,12)} = 1.74$, $P = 0.22$). Conversely, the effect of TMS Trains on CBF differed between sessions in the contralesional M1 ($F_{(2,12)} = 4.57$, $P < 0.05$).

The results also demonstrate changes in the average CBF response to TMS (i.e. the three TMS train conditions pooled). An increase (Post > Pre) in the average CBF response to TMS occurred in the contralesional rostral dorsal premotor cortex in the superior frontal sulcus. A decrease (Post < Pre) in the average CBF response to TMS occurred in the contralesional primary somatosensory cortex on the posterior bank of the central sulcus. Table 2 lists also CBF responses at each of the different number of TMS trains delivered.

There was a trend for the local effects of TMS Trains in the ipsilesional M1 to differ between the two sessions (Table 2, Figure 4B). We observed this trend at a location (X = –46, Y = –14, Z = 58) more lateral to both the focus of stimulation (Figure 2) and our probabilistic location for the M1 hand area (X = ±31, Y = –22, Z = 52). We obtained this trend by generating t-statistics based on the voxel standard deviation as opposed to the standard deviation pooled over the entire brain.

Table 3 summarizes the correlation analyses performed between Post – Pre CBF differences in response to TMS applied over the ipsilesional M1 and the percentage change in arm function. The CBF response as a function of improvement on the WMFT increased in the ipsilesional globus pallidus and decreased in the contralesional VL thalamus. The CBF response as a function of improvement on the AAUT increased in both the ipsilesional globus pallidus and the contralesional caudal dorsal premotor cortex on the precentral gyrus and decreased in both the ipsilesional rostral dorsal premotor cortex in the superior frontal sulcus and the contralesional VL thalamus. The CBF response as a function of improvement in unimanual sequential tapping of the affected arm decreased in the contralesional primary somatosensory cortex on the posterior bank of the central sulcus. The CBF response as a function of improvement in bimanual sequential tapping increased in the contralesional VL thalamus. The CBF response as a function of improvement in grip strength for the affected arm increased in the ipsilesional putamen. We did not observe any changes in the CBF response as a function of improvements on the MAL.

Changes in effective connectivity of the contralesional M1

Table 4 summarizes the results from the ANOVA performed using Session and TMS Trains as factors on CBF data acquired with TMS applied over the contralesional M1. We did not observe any changes with an exploratory search set at $T \geq 4.3$. The following are results obtained from a directed search set at $T \geq 3.0$.

We observed effects of TMS Trains on CBF before and after therapy (Table 4). Before therapy, the CBF response increased with the number of TMS trains in the contralesional putamen. After therapy, the CBF response increased with the number of

TMS trains in the contralesional ventral premotor cortex in the contralesional precentral sulcus. We also tested whether these effects differed between sessions using CBF values extracted from the voxel-of-interests centered at the coordinates of the peaks. The effects of TMS Trains on CBF did not differ between sessions in the contralesional putamen ($F_{(2,12)} = 0.61$, $P = 0.56$) and in the contralesional ventral premotor cortex ($F_{(2,12)} = 0.13$, $P = 0.88$). The results demonstrate also an increase (Post > Pre) in the average CBF response to TMS in the ipsilesional putamen. Table 4 lists also CBF responses at each of the different number of TMS trains delivered. We did not observe any local effects in the contralesional M1.

Table 5 summarizes the correlation analyses performed between Post – Pre CBF differences in response to TMS applied over the contralesional M1 and the percentage change in arm function. The CBF response as a function of improvement on the WMFT decreased in the contralesional VL thalamus. The CBF response as a function of improvement on the AAUT decreased in both the contralesional primary somatosensory cortex on the posterior bank of the central sulcus and the ipsilesional rostral cingulate motor area on the ventral bank of the cingulate sulcus. The CBF response as a function of improvement in unimanual sequential tapping of the affected arm decreased in the contralesional M1 on the anterior bank of the central sulcus. The CBF response as a function of improvement in bimanual sequential tapping increased in both the contralesional VL thalamus and the ipsilesional VPL thalamus and decreased in the contralesional putamen. The CBF response as a function of improvement in both the quality of movement and amount of use scores on the MAL increased in both the contralesional VL thalamus and the contralesional ventral premotor cortex in the precentral operculum. We did not observe any changes in the CBF response as a function of improvement in grip strength of the affected arm.

VI. Discussion

The results confirm previous reports that improvements in coordination and activities of daily living can occur with CI Therapy even when the therapy is conducted one or more years after stroke (Taub et al., 1993; Miltner et al., 1999). The novelty of this study is that

we demonstrated changes in the effective connectivity of the ipsilesional M1 with the contralesional M1, the non-primary motor areas in both hemispheres, and the basal ganglia in both hemispheres.

Motor improvements

The side of hemiparesis would seem important for determining the effect of movement therapy for the arm, with patients having greater motivation to regain use of a pre-morbid dominant arm rather than a pre-morbid non-dominant arm. This was not the case in Patient 4 who was the only participant that received therapy for a pre-morbid non-dominant arm. His percentage change in motor function after therapy was always in the top three patients for all the motor tests that we report improvements. Miltner et al. (1999) demonstrated in a larger sample of patients, all with right-arm dominance before stroke, that patients with a left-sided hemiparesis exerted as large a treatment effect after CI Therapy as those with a right-sided hemiparesis.

Improvements on various motor tests were observed immediately after therapy and were still present in most tests one-month afterwards. The effect sizes reported for the WMFT and the MAL are consistent with previous studies that examined the effects of CI Therapy on motor function after stroke (Taub et al., 1993; Miltner et al., 1999). The patients, however, did not maintain improvements in the amount of use of the affected arm as measured with the AAUT. It is difficult to determine why this would be the case considering that patients reported with the MAL that they maintained improvements in the amount of use of the affected arm in everyday life. We also report improvements with smaller effect sizes in unimanual sequential tapping, bimanual sequential tapping, and grip strength. No improvements were observed in the affected arm for unimanual simple tapping and pinch strength. We speculate that successful performance on these latter tests may dependent more on the integrity of the corticospinal tract and in turn may not allow the development of compensatory skills to enhance performance (Nakayama et al., 1994). Studies in the monkey demonstrate that corticospinal fibers with a direct influence on spinal motor neurons are important for pinch strength and have less of a role during the co-contraction of a number of muscles used in the hand grip (Muir and Lemon, 1983).

Local response of the ipsilesional M1

Paus et al. (1998) demonstrated in normal volunteers that the CBF response to subthreshold 10-Hz repetitive TMS over M1 decreases with the number of TMS trains delivered during each scan. The trains of TMS may have resulted in a preferential activation of local inhibitory circuits and a subsequent reduction of excitatory synaptic activity in the stimulated region. This interpretation is supported by the following evidence. First, the pharmacological administration of γ-aminobuturic acid (GABA) agonists in humans can enhance intra-cortical inhibition in M1 (Ziemann et al., 1996) and decrease CBF in distinct brain regions when used in combination with PET to determine hemispheric dominance (Roland and Friberg, 1988). Second, four 10-pulse trains of repetitive TMS at frequencies from 2 to 15 Hz can prolong the duration of the silent period without changing corticospinal excitability (Romeo et al., 2000).

Although the change in the local effects of TMS applied over the ipsilesional M1 did not reach significance, we observed a trend that may reflect a post-therapy strengthening of local inhibitory neurons. These neuronal pools are important for the fractionation of movements between different distal and proximal muscles (Keller, 1993). The blockade of GABAergic inhibition in the macaque monkey's M1 disrupts the spatiotemporal sequence of movement patterns performed by the forelimb (Matsumura et al., 1991). Interestingly, this trend occurred at a site more lateral to both the focus of stimulation and our probabilistic location for the hand area in M1. This may reflect a possible reorganization of M1. This displacement fits well with previous studies that demonstrated shifts in the hand representation of M1 after rehabilitation of the forelimb following stroke in the squirrel monkey (Nudo et al., 1996b), rehabilitation of the arm following stroke in the human (Liepert et al., 2000), and a period of recovery after stroke in the human (Weiller et al., 1993).

The lack of an increase in local CBF response may reflect both the stimulation intensity and the low number of pulses delivered during scanning (50, 150, and 300 pulses / scan). A small number of pulses of high-frequency (≥5-Hz) repetitive TMS can increase corticospinal excitability only when applied at suprathreshold intensities (Pascual-Leone et al., 1994; Wu et al., 2000). At subthreshold intensities, a greater

number of TMS pulses must be delivered for high-frequency repetitive TMS to increase corticospinal excitability effectively. Maeda et al. (2000) demonstrated that 10-Hz repetitive TMS applied at 90% of the resting motor threshold can increase corticospinal excitability after 1600 pulses and not after 240 pulses. Quartarone et al. (2005) demonstrated similarly that 5-Hz repetitive TMS applied at 90% resting motor threshold can increase corticospinal excitability after 900, 1200, and 1500 pulses and not after 300 and 600 pulses.

Effective connectivity with the contralesional M1

The primary motor cortex influences movement of the ipsilateral arm through its projections to the medial motor nuclei and the intermediate zones of the cervical spinal segments (Armand et al., 1997; Ralston and Ralston, 1985), and thus can not influence directly the lateral motor nuclei which innervate movement in the distal arm muscles (Passingham et al., 1983; Brinkman and Kuypers, 1973). It is therefore unlikely that the contralesional M1 could take over this function.

The change in effective connectivity of the ipsilesional M1 with the contralesional M1 could reflect a strengthening of inter-hemispheric interactions that are important normally for the coordination of hand movements (Ferbert et al., 1992). In normal volunteers, repetitive TMS applied over M1 disrupts finger sequences as subjects play the piano with either hand (Chen et al., 1997a). Cerebral activity also increases in the M1 ipsilateral to the hand that performs a complex task compared with a simpler task (Rao et al., 1993; Shibaski et al., 1993). In patients with good recovery after stroke, similar increases in the contralesional M1 occur during simple hand movements performed by the recovered arm (Chollet et al., 1991; Weiller el al., 1992; 1993; Honda et al., 1997; Cao et al., 1998). The analogy in the level of involvement of the M1 ipsilateral to the arm performing complex hand movements by healthy volunteers and simple hand movements by recovered stroke patients suggests that these patients recruit additional resources in the intact hemisphere to fulfill motor tasks.

Effective connectivity with the non-primary motor areas

The change in effective connectivity of the ipsilesional M1 with the non-primary motor areas could reflect a strengthening of other types of compensatory mechanisms. The cortical motor system is hierarchically organized with M1 executing movements via its direct influence on spinal motor neurons. The non-primary motor areas with a much weaker influence on spinal motor neurons (Maier et al., 2002; Lemon et al., 2002) are responsible normally for the planning, selection, and maintenance of movements (Ashe and Ugurbil, 1994).

Our results revealed changes in the CBF response in the contralesional dorsal premotor cortex when applying TMS over the ipsilesional M1. A meta-analysis of functional imaging studies reveal that the contralesional dorsal premotor cortex is the most frequently reported brain region involved in the execution of recovered movements after stroke (Calautti and Baron, 2003). This brain region is involved normally in the selection of movements (Schluter et al., 1998) and may provide a compensatory mechanism to enable stroke patients to achieve a motor task by selecting motor programs that they can perform. Johansen-Berg et al. (2002a) demonstrated that the contralesional dorsal premotor cortex has an adaptive role. In an fMRI experiment, they demonstrated increased blood oxygen level dependent (BOLD) signal in the contralesional dorsal premotor cortex in stroke patients who performed a simple reaction task as compared with normal controls. In a separate experiment, TMS applied over this brain region not only disrupted task performance, but the degree of disruption correlated with the level of BOLD response.

Our results also revealed changes in the CBF response in the rostral cingulate motor area (Paus et al., 1993; Picard and Strick, 1996) when applying TMS over the ipsilesional M1. The cingulate motor areas are involved normally during motor tasks that require a greater level of voluntary control (Picard and Strick, 1996, Paus, 2001). We speculate that this response may therefore reflect a strengthening of other connections to recruit additional resources to fulfill motor tasks that were previously automatic and / or effortless before stroke.

Effective connectivity with subcortical structures

The change in effective connectivity of M1 with the basal ganglia and thalamus could be related to the role of the cortico-basal ganglia-thalamo-cortical loops in learning motor sequences and the processing of information related to the control of movement (Doyon et al., 2003). Patients in this study demonstrated greater improvements in the functional use of the affected arm in everyday life and in coordinating sequences of movements rather than in making simple repetitive movements. These improvements correlated further with changes in CBF responses in the putamen, globus pallidus, and the motor nuclei of the thalamus.

Our results revealed changes in the CBF response in the ipsilesional putamen when applying TMS over both the ipsilesional and contralesional M1s. This may relate to the fact that both ipsilateral and contralateral representations of different body parts exist in the putamen (Gerardin et al., 2003). Our results reveal further that most CBF changes in the putamen occurred at sites located in its more ventromedial parts. A lateral shift in the local CBF response to TMS in the ipsilesional M1 may explain why this would be the case when we consider that the putamen receives most of the inputs to the basal ganglia from M1 (Parent and Hazrati, 1995). Functional imaging has demonstrated a somatotopic organization of the putamen similar to that of non-human primates (Takada et al., 1998) with a foot-hand-face disposition along a dorsolateral to ventromedial gradient (Gerardin et al., 2003).

Concluding remarks

Functional imaging studies have demonstrated altered cerebral activity in motor regions of the brain in recovered stroke patients who execute movements with their affected arm (e.g. Chollet et al., 1991; Weiller et al., 1992; 1993; Cramer et al., 1997; Dettmers et al., 1997; Honda et al., 1997; Cao et al., 1998; Seitz et al., 1998; Nelles et al., 1999b). A smaller number of studies also describe correlates between motor improvements after rehabilitative therapy and altered fMRI activity (Levy et al., 2001; Johansen-Berg 2002b). Most of these studies, however, do not discern how different brain regions

influence each other. The results presented here provide complementary insight into rehabilitation-mediated recovery by demonstrating changes in the effective connectivity of the ipsilesional M1.

VII. Tables

Table 1. Information on the patients

Patient	Age	Sex	IQ	Months After Stroke	Type of Stroke	Side of Paresis	Location of Lesions
1	68	M	113	34	Ischemic	Left	Dorsal premotor cortex, medullary substance
2	78	F	89	51	Ischemic	Right	Putamen, internal capsule
3	49	M	84	12	Ischemic	Right	Frontal-parietal white matter
4	54	M	74	15	Haemorrhage	Right	Internal capsule
5	70	M	88	12	Ischemic	Left	Multiple subcortical infarcts, white matter hypodensity
6	73	M	76	16	Ischemic	Right	Multiple subcortical infarcts, internal capsule, VL thalamus
7	69	M	127	21	Haemorrhage	Right	Internal capsule, VPL thalamus
MEAN	65.9		93.0	23.0			

Table 2. The effects of Session and TMS Trains applied over the ipsilesional M1 on CBF.

A. Effects of TMS Trains at Pre	X	Y	Z	t-value
Ipsilesional putamen	16	15	−4	3.1

B. Effects of TMS Trains at Post	X	Y	Z	t-value
Contralesional central sulcus, anterior bank, M1	27	−28	64	3.7

C. Effects of Session	X	Y	Z	t-value
Contralesional superior frontal sulcus, PMdr	34	8	60	3.4
Contralesional central sulcus, posterior bank, S1	33	−28	48	−3.0

D. Effects of Session at 5 TMS Trains	X	Y	Z	t-value
Ipsilesional cingulate sulcus, dorsal bank, CMAr	−8	13	42	3.1
Contralesional superior frontal sulcus, PMdr	30	6	63	3.0

E. Effects of Session at 15 TMS Trains	X	Y	Z	t-value
Contralesional superior frontal sulcus, PMdr	36	8	62	3.0
Contralesional putamen	31	−6	−3	−3.7

F. Effects of Session at 30 TMS Trains	X	Y	Z	t-value
Contralesional putamen	20	5	9	3.3
Ipsilesional cingulate sulcus, dorsal bank, CMAr	−7	12	46	3.0

G. Session × TMS Trains *	X	Y	Z	t-value
Contralesional central sulcus, anterior bank, M1	30	−30	64	2.9
Ipsilesional central sulcus, anterior bank, M1	−46	−14	58	−2.8

Abbreviations: M1= primary motor area, PMdr = rostral dorsal premotor area, S1 = primary somatosensory area, and CMAr = rostral cingulate motor area. * T-statistics based on the voxel standard deviation as opposed to the pooled standard deviation.

Table 3. Correlations of CBF differences and the percentage change in motor improvements: repetitive TMS applied over the ipsilesional M1.

A. WMFT	X	Y	Z	t-value
Ipsilesional globus pallidus	−19	−4	−6	3.3
Contralesional thalamus ~VL nucleus	12	−12	6	−3.8

B. AAUT	X	Y	Z	t-value
Ipsilesional globus pallidus	−16	−4	−3	3.6
Contralesional precentral gyrus, PMdc	36	−14	66	3.0
Contralesional superior frontal sulcus, PMdr	−32	−3	52	−3.1
Contralesional thalamus ~VL nucleus	11	−12	8	−3.0

C. Unimanual Sequential Tapping	X	Y	Z	t-value
Contralesional central sulcus, posterior bank, S1	24	−38	58	−3.3

D. Bimanual Sequential Tapping	X	Y	Z	t-value
Contralesional thalamus ~VL nucleus	8	−11	4	3.5
E. Grip Strength	X	Y	Z	t-value
Ipsilesional putamen	−26	−9	2	3.1

Abbreviations: VL = ventral lateral, PMdc = caudal dorsal premotor area, PMdr = rostral dorsal premotor area, and S1 = primary somatosensory area.

Table 4. The effects of Session and TMS Trains applied over the contralesional M1 cortex on CBF.

A. Effects of TMS Trains at Pre	X	Y	Z	t-value
Contralesional putamen	30	−6	9	3.0

B. Effects of TMS Trains at Post	X	Y	Z	t-value
Contralesional precentral sulcus, PMv	56	8	34	3.5

C. Effects of Session	X	Y	Z	t-value
Ipsilesional putamen	−20	6	3	3.1

D. Effects of Session at 5 TMS Trains	X	Y	Z	t-value
None				

E. Effects of Session at 15 TMS Trains	X	Y	Z	t-value
Ipsilesional thalamus ~VPL nucleus	−20	−21	12	3.1
Ipsilesional putamen	−14	8	−4	3.1

F. Effects of Session at 30 TMS Trains	X	Y	Z	t-value
None				

G. Session × TMS Trains	X	Y	Z	t-value
None				

Abbreviations: VPL = ventral posterior lateral and PMv = ventral premotor area.

Table 5. Correlations of CBF differences and the percentage change in motor improvements: repetitive TMS applied over the contralesional M1.

A. WMFT	X	Y	Z	t-value
Contralesional thalamus ~VL nucleus	13	-14	9	-3.8

B. AAUT	X	Y	Z	t-value
Contralesional central sulcus, posterior bank, S1	30	-28	50	-3.4
Ipsilesional cingulate sulcus, ventral bank, CMAr	8	6	36	-3.3

C. Unimanual Sequential Tapping	X	Y	Z	t-value
Contralesional central sulcus, anterior bank, M1	-34	-25	58	-3.2

D. Bimanual Sequential Tapping	X	Y	Z	t-value
Contralesional thalamus ~VL nucleus	9	-16	8	3.6
Ipsilesional thalamus ~VPL nucleus	-16	-18	2	3.0
Contralesional putamen	15	5	-6	-3.7

E. Grip Strength	X	Y	Z	t-value
None				

F. MAL-QOM	X	Y	Z	t-value
Contralesional thalamus ~VPL nucleus	21	-19	9	3.1
Contralesional precentral operculum, PMv	62	0	12	3.0

G. MAL-AOU	X	Y	Z	t-value
Contralesional thalamus ~VL nucleus	21	-19	10	3.5
Contralesional precentral operculum, PMv	62	0	12	3.1

Abbreviations: VL = ventral lateral, S1 = primary somatosensory area, CMAr = rostral cingulate motor area, M1= primary motor area, VPL = ventral posterior lateral, and PMv = ventral premotor area.

VIII. Figures

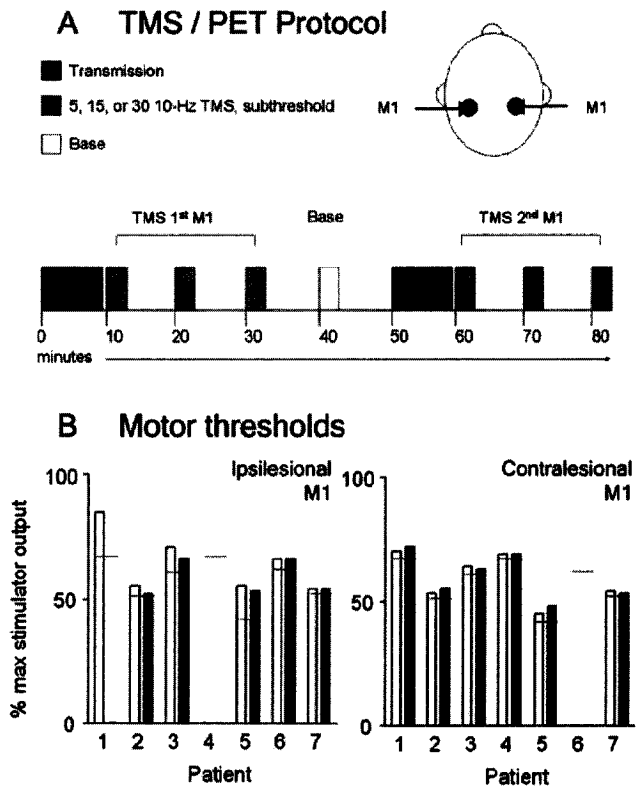

Figure 1. *TMS / PET Protocol.* A) Patients underwent TMS / PET at the Pre and Post sessions. During the first three water-bolus scans, we delivered a different number of TMS trains over one of the two M1s; that is, 5, 15 and 30 trains of 1-second 10 Hz stimulation. During the last three water-bolus scans, we stimulated over the other M1 using the same stimulation as in the previous water-bolus scans. Stimulation intensity was the same for both M1s as well as at the Pre and Post sessions. B) Motor thresholds acquired at the Pre (white) and Post (black) sessions. Note that motor thresholds were undetectable for one hand in Patients 4 and 6. The bars represent the stimulation intensities that we used for each patient. We set the stimulation intensity at 95% of motor threshold of either the affected or unaffected hand as measured at the Pre session, whichever was lowest. In cases when the motor threshold in one hand was undetectable, we set the stimulation intensity at 95% of motor threshold of the other hand as measured at the Pre session.

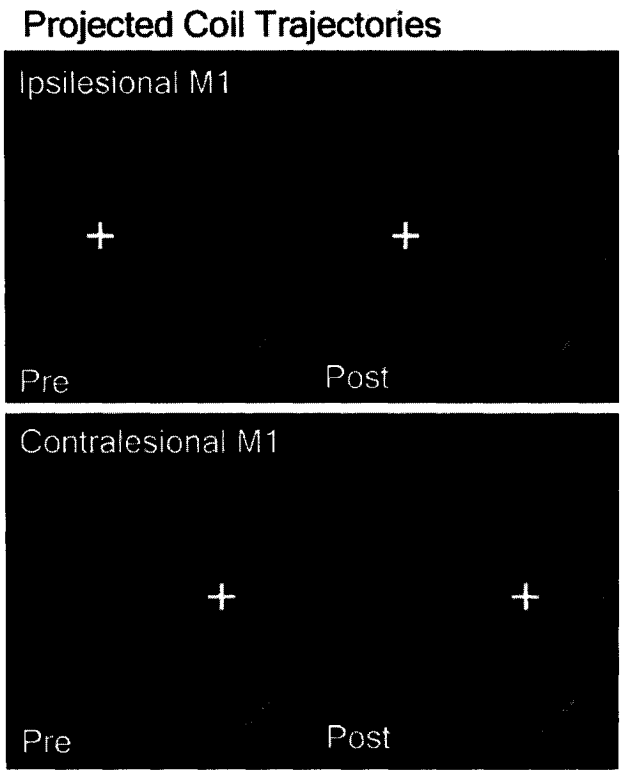

Figure 2. *Projected coil trajectories.* The 10-minute transmission scans acquired during the PET sessions allowed us to see the coil's position relative to the subject's head. We projected virtual rods orthogonal to the plane of the coil from where the coil touched the scalp. Following a series of transformations, we superimposed the location of these virtual rods on an average MRI. The figure illustrates these projected coil trajectories (red circles) to provide estimated locations for TMS-induced currents in the brain during repetitive TMS applied over the ipsilesional M1 (top panel) and contralesional M1 (bottom panel). Crosses represent the intended sites of stimulation.

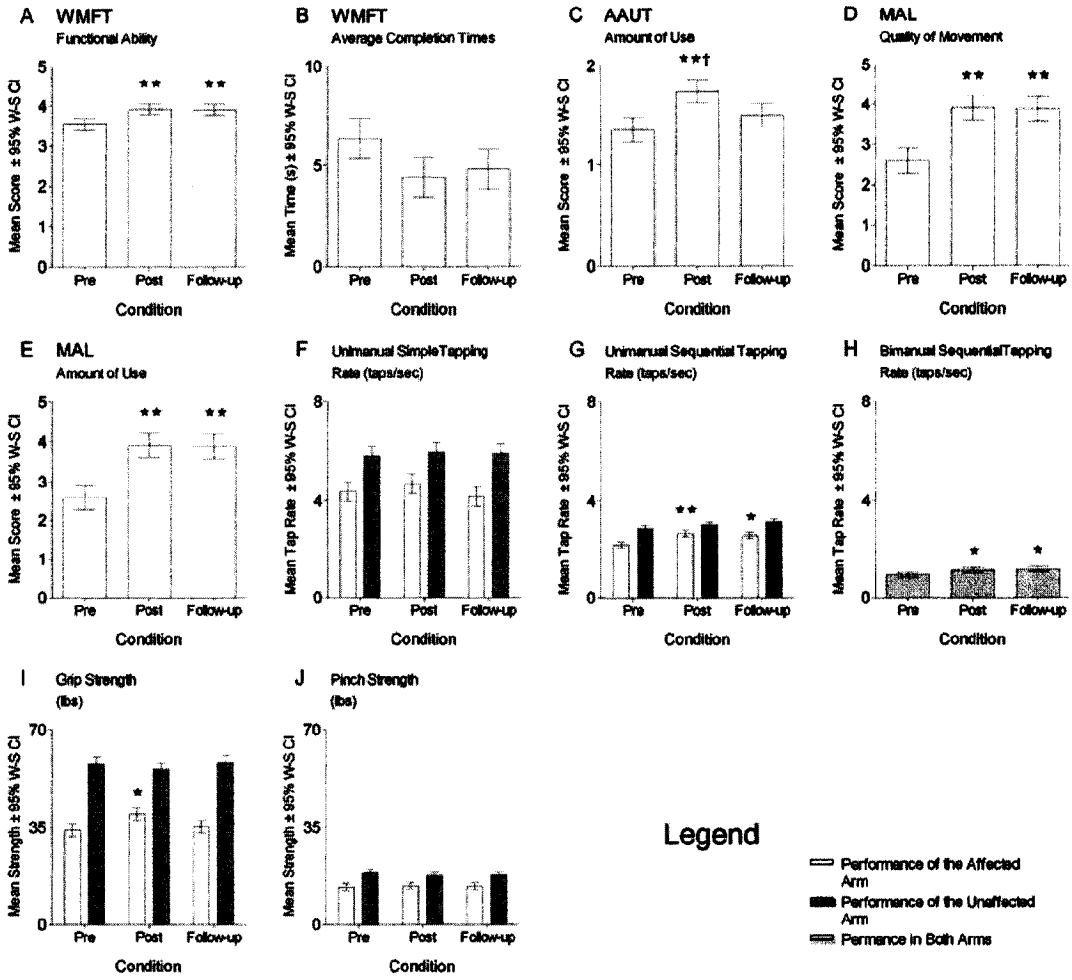

Figure 3. *Motor tests*. The various panels of this figure illustrate mean ± 95% confidence intervals for within-subject contrasts (Loftus and Masson, 1994) at the Pre, Post, and Follow-up sessions for: A) functional ability scores on the WMFT, B) average completion times on the WMFT, C) amount of use scores on the AAUT, D) quality of movement scores on the MAL, and E) amount of use scores on the MAL, F) unimanual simple tapping for both the affected and unaffected arms, G) unimanual sequential tapping for both the affected and unaffected arms, H) bimanual sequential tapping, I) grip strength, and J) pinch strength. Asterisks denote differences compared with the Pre session (*P < 0.05, ** P < 0.01). Daggers denote differences compared with the Follow-up session († P < 0.05).

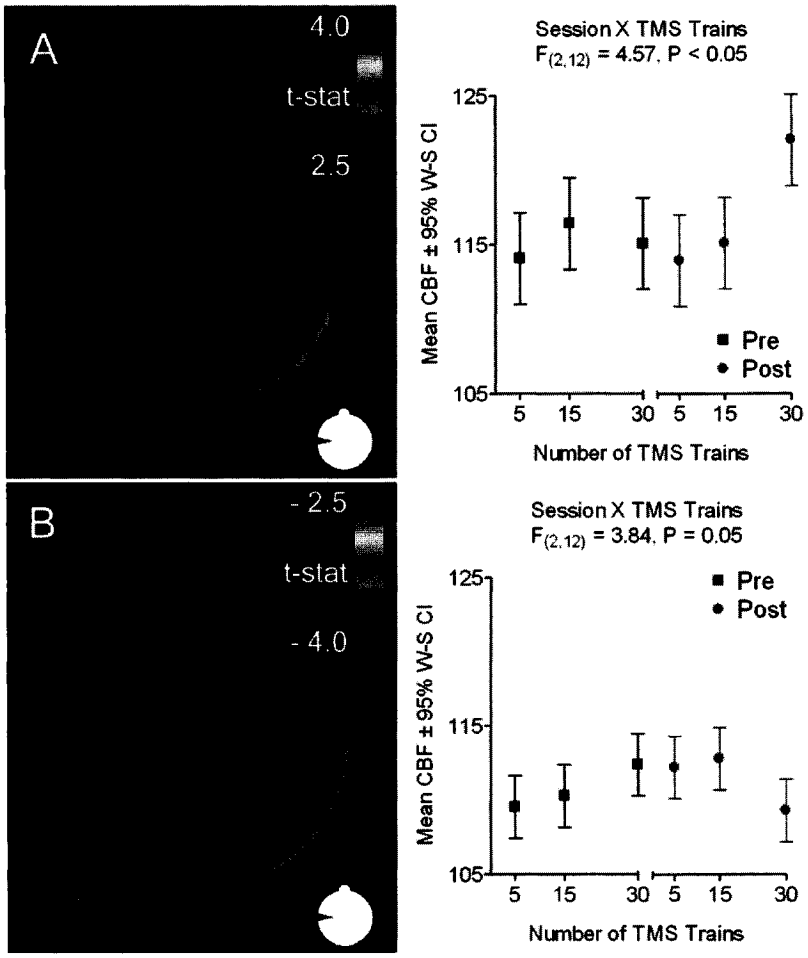

Figure 4. *Effects of Session and TMS Trains applied over the ipsilesional M1.* A) Illustrates a positive Session × TMS Trains interaction in the contralesional M1 (X = 30, Y = –30, Z = 64). B) Illustrates a negative trend for Session × TMS Trains in the ipsilesional M1 at a site (X = –44, Y = –13, Z = 57) located more lateral to both the focus of stimulation and our probabilistic location for the M1 hand area (X = –31, Y = –22, Z = 52; Paus et al., 1998). The corresponding graphs on the side plot mean CBF values ±95% confidence intervals for within-subject contrasts (Loftus and Masson, 1994). We obtained these values by extracting CBF using voxel-of-interests centered at the coordinates of the peaks. Black squares represent normalized CBF values acquired before therapy and red circles represent normalized CBF values acquired after therapy.

Chapter Five

General Discussion

I. Summary

This thesis is composed of three studies. Together, the studies demonstrate that 1) M1 connects predominantly with unimodal areas of the motor system and PMd connects predominantly with associational areas in the prefrontal and parietal cortices 2) M1 processes motor information associated with a previous experience and PMd selects motor programs based on arbitrary visual stimuli, and 3) the effective connectivity of the ipsilesional M1 changes in stroke patients who receive rehabilitative therapy for their affected arm. The results presented in this thesis provide new knowledge about the effective connectivity and function of M1 and PMd, and insight into rehabilitation-mediated recovery. In Chapter 1, I suggested that substitution following any damage to M1 and / or its corticospinal fibers would have to involve pathways that access motor neurons in the spinal cord. I then proposed two mechanisms. The first mechanism was the substitution of spared M1. The second mechanism was the greater involvement of non-primary motor areas for the development of compensatory skills. The following provides a summary about information that I acquired about M1, PMd, and changes in the effective connectivity of the ipsilesional M1 in stroke patients who underwent rehabilitative therapy for their affected arm.

It should be mentioned that there are other TMS / PET studies that demonstrate effects on cerebral activity after 1-Hz repetitive TMS over M1 (Lee et al., 2003) and PMd (Siebner et al., 2003c). These studies differ from Study 1 because they acquired CBF during volitional hand movements. Correlation analyses were used to determine the similarity in regional variations of task-related changes in cerebral activity. Although these studies provide valuable information about connectivity during a behavioral context, the results acquired in these studies should be interpreted cautiously. Co-activations acquired in these studies could reflect relationships between different task components rather than true effective connectivity. In contrast, my studies used TMS /

PET as a behavior-independent assay of connectivity between a cortical area and other structures in the brain.

Primary motor area

In Study 1, repetitive TMS applied over M1 modulated cerebral activity in the non-primary motor areas and in subcortical structures that are part of a cortico-basal ganglia-thalamo-cortical loop. These findings are consistent with the known anatomical connections in the monkey. M1 connects predominantly with non-primary motor areas, non-primary somatosensory areas, and subcortical structures. Most sensory information that influences movement are first processed in associational and / or higher-order sensory cortices and then communicated to the non-primary motor areas (Ghez et al., 1991). M1 is also part of a cortico-basal ganglia-thalamo-cortical loop that plays a role in the learning of movement sequences and the processing of information related to the control of movement (Parent and Hazrati, 1995; Doyon et al., 2003). The putamen receives most inputs to the basal ganglia from M1. The additional CBF responses in the globus pallidus and the ventral-lateral thalamus might reflect indirect connections with the stimulated M1.

In Study 2, repetitive TMS applied over M1 disrupted the subjects' ability to apply distinct forces when lifting different weights. It is important to note that in the Cue experiment, repetitive TMS applied over M1 had no effect on the subjects' ability to scale forces. This is likely because the arbitrary color cues provided subjects with information about what weight they had to lift and that the subjects were able to use this information to scale for differences in weight. Thus, the observed effects induced by repetitive TMS applied over M1 do not appear to be at the level of motor execution, but rather at the level of processing motor information associated with a recent experience. This finding suggests that M1 can form *memory* traces, which is consistent with *memory* neurons present in M1 of the macaque monkey that can store information related to an experience beyond its duration (Li et al., 2001). M1 has traditionally been regarded as an area responsible for the execution of limb movements because of its direct influence on motor neurons in the spinal cord (Lemon et al., 1998). I make the case that M1 can also

process motor information associated with a recent experience. This may relate to the fact that M1 contains intrinsic circuitry devoted to various cognitive-related functions (Rosetti, 1998; Georgopoulos, 2000).

Dorsal premotor area

In Study 1, repetitive TMS applied over PMd resulted in the modulation of a network composed of a number of brain regions. These include several regions in the parietal and prefrontal cortices. The results may have reflected parieto-frontal circuits in the human that are known to provide an anatomical basis in the monkey for the transformation of sensory information into motor actions (Rizzolatti et al., 1998; Matelli and Luppino, 2000). The parietal lobe receives somatosensory and visual inputs, and encompasses several subdivisions that have reciprocal connections with motor areas in the frontal lobe, each with a specific target with which it is most densely connected.

CBF responses observed in the medial bank of the intraparietal sulcus along the posterior superior parietal lobule and PMd may reflect the human homologue of the MIP-F2 circuit. Functional neuroimaging studies show comparable changes in activity in both the posterior parietal cortex and PMd as subjects select motor actions based on visual stimuli (Paus et al., 1993; Deiber et al., 1997; Grafton et al., 1998). CBF responses observed in the lateral bank of the intraparietal sulcus along the anterior inferior parietal lobule and the ventral premotor cortex may reflect the human homologue of the AIP-F5 circuit. Functional neuroimaging studies show comparable changes in activity in both the anterior parietal and ventral premotor cortices during the presentation of both objects (Grafton et al., 1997) and people grasping objects (Buccino et al., 2001). To select relevant information for actions, the prefrontal cortex has access, through its connections with other brain structures, to sensory and spatial aspects of the environment, mnemonic information acquired through experience, and motor control (Barbas, 2000). These motor output-related connections arise mainly from areas in the premotor cortex (Barbas and Pandya, 1987; Lu et al., 1994) and could explain the additional CBF responses in the prefrontal cortex.

In Study 2, repetitive TMS applied over PMd disrupted the subjects' ability to use arbitrary color cues to scale forces for a current weight. Cell recording studies in the monkey reveal that a number of PMd neurons increase their discharge activity after the presentation of an arbitrary visual cue that represents a learned association for a particular motor response compared with the presentation of a directional cue indicating a particular motor response (Kurata and Wise, 1988; Mitz et al., 1991; Kurata and Hoffman, 1994). GABA-$_A$ agonist injections in PMd diminish the monkey's ability to select a correct response based on an arbitrary visual cue (Kurata and Hoffman, 1994). Petrides (1982; 1985b), as well as Halsband and Passingham (1982; 1985), have shown that the removal of PMd disrupts the ability to use arbitrary visual cues to make or withhold particular movements. The results reported in Study 2, together with these findings, reinforce the notion that PMd is critical for implementing associations between visual cues and motor responses.

Changes in the effective connectivity of the ipsilesional M1 after rehabilitative therapy

In Study 3, there was a trend for the relationship between CBF and the number of TMS trains delivered to differ between the two sessions. This trend could reflect a post-therapy strengthening of local inhibitory neurons. These neuronal pools are important for the fractionation of movements between different distal and proximal muscles (Keller, 1993). The blockade of GABAergic inhibition in the macaque monkey's M1 disrupts the spatiotemporal sequence of movement patterns performed by the forelimb (Matsumura et al., 1991). Interestingly, this trend occurred at a site more lateral to both the focus of stimulation and our probabilistic location for the hand area in M1. This displacement fits well with previous studies that demonstrate substitution of adjacent intact cortex in M1 after rehabilitation of the forelimb following stroke in the squirrel monkey (Nudo et al., 1996b), rehabilitation of the arm following stroke in the human (Liepert et al., 2000), and a period of recovery after stroke in the human (Weiller et al., 1993).

Repetitive TMS applied over the ipsilesional M1 resulted in significant changes in distal brain regions. Changes in the effective connectivity of the ipsilesional M1 with other regions in the brain could reflect the strengthening of a network necessary for the

development of compensatory skills. The motor tests revealed that patients improved more in the functional use of the affected arm in everyday life and in coordinating sequences of movements than in making simple repetitive movements. Rehabilitative therapy produced large effect sizes in tests that measured arm function in activities of daily living ($\varepsilon \geq 0.80$; AAUT, MAL) and small to moderate effect sizes in tests that measured arm function at the level of motor execution ($\varepsilon < 0.80$; WMFT, unimanual sequential tapping, bimanual sequential tapping, grip strength). No improvements were observed in the affected arm for unimanual simple tapping and pinch strength.

The change in effective connectivity of the ipsilesional M1 with the contralesional M1 could reflect a strengthening of inter-hemispheric interactions that are important for the coordination of hand movements (Ferbert et al., 1992). Note that Study 1 demonstrated in normal volunteers a CBF response in the right M1 as the result of repetitive TMS applied over the left M1. In normal volunteers, repetitive TMS applied over M1 disrupts finger sequences as subjects play the piano with either hand (Chen et al., 1997a). Cerebral activity also increases in the M1 ipsilateral to the hand that performs a complex task compared with a simpler task (Rao et al., 1993; Shibaski et al., 1993). In patients with good recovery after stroke, similar increases in the contralesional M1 occur during simple hand movements performed by the recovered arm (Chollet et al., 1991; Weiller el al., 1992; 1993; Honda et al., 1997; Cao et al., 1998). The analogy in the level of involvement of the M1 ipsilateral to the arm performing complex hand movements by healthy volunteers and simple hand movements by recovered stroke patients suggests that these patients recruit additional resources in the intact hemisphere to fulfill motor tasks.

The non-primary motor areas with a weak direct influence on spinal motor neurons (Maier et al., 2002; Lemon et al., 2002) are normally responsible for the planning, selection, and maintenance of movements (Ashe and Ugurbil, 1994). The results revealed a change in the CBF response in the contralesional PMd when applying TMS over the ipsilesional M1. This brain region is involved normally in the selection of movements (Schluter et al., 1998) and may provide a compensatory mechanism to enable stroke patients to achieve a motor task by selecting motor programs that they can perform. The results also revealed a change in the CBF response in the rostral cingulate motor area when applying TMS over the ipsilesional M1. I suggested that this response

may have reflected a strengthening of connections to recruit additional resources to fulfill motor tasks that were previously automatic and / or effortless before stroke. The change in effective connectivity of M1 with the basal ganglia and thalamus could relate to the role of the cortico-basal ganglia-thalamo-cortical loops in learning motor sequences and the processing of information related to the control of movement (Doyon et al., 2003). Improvements in the functional use of the affected arm in everyday life and in coordinating sequences of movements correlated further with changes in the CBF response in the putamen, globus pallidus, and the motor nuclei of the thalamus.

II. Suggestions for future research

The combination of TMS and MRI may provide a more accessible and powerful combination to study effective connectivity than the combination of TMS and PET. Although PET can reveal valuable information about the functional organization of the human brain and will continue to be useful for examining specific neurotransmitter systems, the method does have limitations. First, only a limited number of scans can be carried out on a single person because radioactive tracers have to be injected or inhaled. Second, PET is expensive because most types of radioactive tracers have to be created on-site by a cyclotron. As a result, functional MRI has overtaken PET as the main method for examining activity in the human brain. Functional MRI is less expensive and does not always require a substance to be injected. No adverse effects related to MRI are currently known and it is considered safe to scan the same person a number of times.

The combination of TMS and MRI does present certain challenges but can nonetheless be accomplished simultaneously (Siebner et al., 2003b; Bestmann et al., 2004). The first challenge relates to placing the TMS coil securely over the subject's head inside a strong magnetic field. One could overcome this difficulty by using a commercially available non-ferromagnetic coil held in place by a custom-made MRI-compatible coil holder. Such a coil holder would have to be designed to withstand the mechanical perturbations of a high-field MRI environment. The second challenge relates to the possible damage of the MRI head-coil, which one could overcome by placing the TMS coil over the subject's head in a manner so that the TMS pulses do not project onto

the MRI head-coil. The third challenge relates to the radiofrequency emission of the TMS stimulator. One could overcome this difficulty by connecting the TMS coil to the stimulator located inside a radiofrequency-shielded room through a radiofrequency filter tube. The final challenge relates to the interference created by the TMS pulses during acquisition, which one could overcome with a minimum waiting period between the delivery of a TMS pulse and a subsequent image acquisition.

Spectroscopy using MRI can further quantify levels of glutamate and GABA (Sonnewald et al., 2004; Choi et al., 2005). Pharmacological studies in humans have shed light into the effects of paired-pulse TMS on excitatory and inhibitory neurotransmission (Ziemann et al., 1996). Yet, we still do not understand how other variations of TMS can affect neurotransmission. This is because TMS lacks the spatial resolution necessary to study its effect in animals. In Study 1, I proposed that the local effects of low-frequency repetitive TMS canceled out while the distal effects remained. The latter being related to the fact that the majority of cortico-cortical and cortico-subcortical projections are glutamatergic and, hence, their activation was more likely to influence blood-flow in their target regions. In Study 3, I proposed a trend that reflected a post-therapy strengthening of local inhibitory neurons in the stimulated ipsilesional M1. The sensitivity of MR spectroscopy may allow us to verify these interpretations by stimulating M1 and PMd with TMS and quantifying the level of glutamate and GABA release both locally and in distal regions of interest.

Lastly, I had to rely on anatomical studies conducted in non-human primates to interpret the results obtained in Studies 1 and 3. Interpreting functional data in this manner has limitations. Comparative analyses among different species of non-human primates reveal many differences in the anatomy of the motor system (Heffner and Masterton, 1983; Bortoff and Strick, 1993; Nudo et al., 1995). The motor system in the human is thought to have evolved greatly from their phylogenic ancestry (Porter and Lemon, 1993). This problem could be resolved with diffusion-tensor imaging (DTI). DTI is based on the principle that water diffusion is highly directional along the axis of white-matter fiber tracts (Behrens et al., 2003; Ramnani et al., 2004). Image acquisition during MRI can be sensitized to the diffusion of water molecules and the organization of white-matter fiber tracts can in turn be mapped by computing the direction of greatest diffusion

at each voxel. We may one day be able to interpret functional data within the scope of knowledge about anatomical connections in the human brain acquired with DTI.

III. Conclusions

The results demonstrate that the human motor system is hierarchically organized and can undergo adaptive changes in stroke patients who improve function in the affected arm. Study 1 confirmed a hierarchical organization of the motor system in which M1 connects predominantly with unimodal areas of the motor system and PMd connects predominantly with associational areas in the prefrontal and parietal cortices. Study 2 demonstrated that M1 and PMd fulfill different roles during the lifting of different weights. M1 scales forces based on information acquired during a previous lift and PMd scales forces based on arbitrary color cues. Study 3 demonstrated changes in the effective connectivity of the ipsilesional M1 with the contralesional M1, the non-primary motor areas in both hemispheres, and the basal ganglia in both hemispheres. I then proposed that the results from Study 3 represented a rehabilitation-induced strengthening of a network of brain regions necessary for the development of compensatory skills. These findings require further investigation using new advances in neuroimaging. DTI could provide further insight into the results that I obtained in Studies 1 and 3, and the combination of TMS and MR spectroscopy could shed light into the effects of TMS on excitatory and inhibitory neurotransmission.

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RESEARCH ETHICS BOARD APPROVAL FORM

Protocol #: PAUT 2001/3

Protocol title: **Modulation of excitability and connectivity of the primary motor and premotor cortex in healthy subjects and hemiparetic patients (CIHR Operating grant)**

Investigator name: Dr Tomáš Paus, Neuropsychology, MNI

Ethics review board name: Research Ethics Board (REB)

Institution of pertinence: Montreal Neurological Hospital and Institute

REB Approval

The REB reviewed the project on: 2001.05.28 Agenda item: 4.b.

Approval of the following was granted on: 2001.05.28

For protocols with precise ID and version numbers, the following apply:

○	[precise protocol ID and date]	
☑	English consent form - dated	2001.05.17
☑	French consent form - dated	2001.05.17

Remarks: The wording of the advertisement, last sentence of first paragraph, should be rephrased in English to 'Participants will be compensated for time and inconvenience', and the same meaning be reflected in the French.

In Item 9 of the consent forms, "purely scientific reasons" should be replaced with 'any reason'.

In Item 2(c)2 of the consent forms, the phrase "no radioactivity is left" should be replaced with 'insignificant radioactivity is left'.

REB Chairman's signature
for R. Pokrupa

2001/05/29
Date Y/M/D

ETHICS REVIEW BOARD COMPLIANCE STATEMENT

The Research Ethics Boards (REBs) of the McGill University Health Centre are registered REBs working under the published guidelines of the Tri-Council Policy Statement, the "Plan d'action ministériel en éthique de la recherche et en intégrité scientifique" (MSSS, Qc), and in compliance with standards set forth in the (US) Code of Federal Regulations governing human subjects research, and act in conformity with the international standards of Good Clinical Practice: Consolidated Guideline (GCP E6).

Centre universitaire de santé McGill
McGill University Health Centre

18 December 2001

Dr Tomáš Paus
Neuropsychology
MNI

Meeting of 2001.12.17

6.a. PAUT 2001/3 **Modulation of excitability and connectivity of the primary motor and premotor cortex in healthy subjects and hemiparetic patients (CIHR Operating grant)**

Letter of 2001.12.07 explaining Addendum amendment, English and French consent forms of 2001.12.07

Dr Paus abstained from the deliberations.

The board reviewed the foregoing Addendum amendment and consent forms as above and approved them.

This does not alter the existing approval period, which will expire 2002.05.

Yours very truly,

Eugene Bereza, MDCM, Chair
Research Ethics Board
/ve

Centre universitaire de santé McGill
McGill University Health Centre

RESEARCH ETHICS BOARD APPROVAL FORM

Protocol #: PAUT 2002/6

Protocol title: **Constraint-induced rehabilitation therapy in stroke patients: mechanisms of recovery (CIHR MT-14505)**

Investigator name: Dr Tomáš Paus, Neuropsychology, MNI

Ethics review board name: Research Ethics Board (REB)

Institution: Montreal Neurological Hospital and Institute

REB Approval

The REB reviewed the project on: 2002.04.22 Agenda item: **4.k.**

Approval of the following was granted on: 2002.04.22

In 13 copies: New Protocol checklist, letter from Dr A Evans, English and French consent forms of 2002.04.10, 'Home Treatment Contract' of 2002.04.10 in English and French

Remarks: Board members Drs Durcan and Paus abstained from the deliberations.
This approval is valid through April 2003.

REB Chairman's signature

02/04/24
Date Y/M/D

ETHICS REVIEW BOARD COMPLIANCE STATEMENT

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The Faculty of Medicine Institutional Review Board consisting of:

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has examined the research project **A05-M53-02B** entitled **"Constraint-Induced Rehabilitation Therapy in Stroke Patients: Mechanisms of Recovery"**

as proposed by: Dr. Thomáš Paus (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

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I am first author of this paper. Th co-authors Van Der Werf YD as well as Leonard G and Paus T (Ph.D. supervisors) agree to have this paper as a chapter in my Ph.D. thesis.

Yours truly,

Philippe
Philippe Chouinard M.Sc.
Ph.D. Candidate
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Montreal Neurological Institute / McGill University
3801 University Street
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Modulating Neural Networks With Transcranial Magnetic Stimulation Applied Over the Dorsal Premotor and Primary Motor Cortices

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Chouinard, Philippe A., Ysbrand D. Van Der Werf, Gabriel Leonard, and Tomáš Paus. Modulating neural networks with transcranial magnetic stimulation applied over the dorsal premotor and primary motor cortices. *J Neurophysiol* 90: 1071–1083, 2003; 10.1152/jn.01105.2002. Our study uses the combined transcranial magnetic stimulation/positron emission tomography (TMS/PET) method for elucidating neural connectivity of the human motor system. We first altered motor excitability by applying low-frequency repetitive TMS over two cortical motor regions in separate experiments: the dorsal premotor and primary motor cortices. We then assessed the consequences of modulating motor excitability by applying single-pulse TMS over the primary motor cortex and measuring: *1*) muscle responses with electromyography and *2*) cerebral blood flow with PET. Low-frequency repetitive stimulation reduced muscle responses to a similar degree in both experiments. To map networks of brain regions in which activity changes reflected modulation of motor excitability, we generated *t*-statistical maps of correlations between reductions in muscle response and differences in cerebral blood flow. Low-frequency repetitive stimulation altered neural activity differently in both experiments. Neural modulation occurred in multiple brain regions after dorsal premotor cortex stimulation; these included motor regions in the frontal cortex as well as more associational regions in the parietal and prefrontal cortices. In contrast, neural modulation occurred in a smaller number of brain regions after primary motor cortex stimulation, many of these confined to the motor system. These findings are consistent with the known differences between the dorsal premotor and primary motor cortices in the extent of cortico-cortical anatomical connectivity in the monkey.

INTRODUCTION

The cortical motor system can be separated into the primary motor and the nonprimary motor areas. The nonprimary motor areas are defined as all regions in the frontal lobe that have the potential to influence motor output at the level of both the primary motor cortex and the spinal cord (Dum and Strick 1991); these include the premotor, supplementary motor, and cingulate motor areas. Transcranial magnetic stimulation (TMS) applied in trains of pulses can modulate the motor system in a temporary fashion, lasting beyond the duration of stimulation. Studies that have examined these effects generally applied repetitive stimulation over the primary motor cortex and measured the modulation of motor-evoked potentials (MEPs) recorded in the contralateral hand muscles. Typically, low-stimulation frequencies of 1 to 2 Hz induce inhibitory effects (e.g., Chen et al. 1997; Gerschlager et al. 2001; Maeda et al. 2000; Muellbacher et al. 2000) and high-stimulation frequencies between 5 and 20 Hz induce facilitory effects (e.g., Maeda et al. 2000; Pascual-Leone et al. 1994; Peinemann et al. 2000; Romeo et al. 2000). Cortical mechanisms are believed to mediate both inhibitory (Touge et al. 2001) and facilitory (Baradelli et al. 1998) effects.

Recent studies demonstrate that low-frequency repetitive TMS applied over the premotor cortex can also induce changes in motor excitability as reflected by: *1*) decreases in the amplitude of MEPs elicited by single-pulse stimuli (Gerschlager et al. 2001); *2*) increases in intracortical facilitation to paired-pulse stimuli (Münchau et al. 2002); and *3*) reductions in the duration of the silent period (Münchau et al. 2002). These results suggest that repetitive stimulation over the premotor cortex can also modulate the output of the motor system, mediated perhaps by direct cortico-cortical connections between the premotor and primary motor cortices.

The question we address here is whether repetitive TMS applied over the dorsal premotor cortex, and over the primary motor cortex in a separate experiment, can alter neural activity at distal sites connected synaptically. Previous studies (Bohning et al. 1999; Fox et al. 1997; Paus et al. 1997, 1998; Siebner et al. 1998, 2000) have established the combination of functional brain imaging and TMS as an effective method to measure changes in neural activity induced by repetitive stimulation (reviewed in Paus 2002). Previous positron emission tomography (PET) studies have already described changes in blood flow and glucose metabolism during repetitive stimulation applied over the primary motor cortex (Fox et al. 1997; Paus et al. 1998; Siebner et al. 2001). These studies reveal focal changes in the primary motor cortex as well as in distant regions known to be connected synaptically in the monkey, including the premotor and supplementary motor areas. These results suggest that for certain neural networks, connectivity patterns identified in monkeys are similar in humans.

By applying repetitive TMS over two subdivisions of the cortical motor system in the same group of subjects, we can potentially map two networks and the manner in which each is modulated. Because repetitive TMS applied over the dorsal premotor cortex or the primary motor cortex can reduce MEP amplitudes, we used this change in MEP as an index of effectiveness for altering neural activity by repetitive stimulation. To map networks of brain regions in which activity changes reflected modulation of motor excitability, we gener-

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ated *t*-statistical maps of correlations between reductions in muscle response and differences in cerebral blood flow (CBF).

METHODS

We acquired a total of six 60-s ^{15}O-H_2O PET scans in each of two sessions. In both sessions, we scanned subjects during the following conditions: *1*) no TMS before repetitive stimulation (Base); *2*) single-pulse TMS before repetitive stimulation (Pre); *3*) single-pulse TMS shortly after repetitive stimulation (Post1); *4*) single-pulse TMS about 10 min after repetitive stimulation (Post2); *5*) single-pulse TMS about 20 min after repetitive stimulation (Post3); and, *6*) single-pulse TMS about 30 min after repetitive stimulation (Post4). We counterbalanced the order of the Base and Pre conditions across subjects. During 5 of the 6 PET scans, we applied 12 suprathreshold single pulses of TMS over the left primary motor cortex while recording MEPs in the right first dorsal interosseous muscle. Between the second and third PET scans, we applied a 15-min train of 1-Hz subthreshold repetitive TMS over the left dorsal premotor cortex in one experiment and over the left primary motor cortex in the other experiment. These experiments were conducted on separate days and in a counterbalanced order; we refer to these as the dorsal premotor and primary motor experiments.

We calculated the amount of MEP reduction induced by repetitive stimulation for every single-pulse condition and used this measure as an index of effectiveness in modulating neural activity. To reveal brain regions modulated by the repetitive stimulation, we performed correlations between reductions in MEP and differences in CBF. In the single-pulse conditions, we applied 20 single pulses of TMS on average every 5 s (range: 4 to 6 s to minimize anticipation), 12 of which occurred during PET scanning.

Subjects

Four female and three male right-handed subjects (19 to 27 yr of age, mean ± SD, 23±3) participated in the study after giving informed written consent. The Research Ethics Board of the Montreal Neurological Institute and Hospital approved all experimental procedures. We preselected subjects for their low resting motor thresholds (rMT) to prevent overheating of the stimulating coil. We determined thresholds for the relaxed right first dorsal interosseus muscle before both experiments by first determining the optimal position for activating the muscle and then by reducing the stimulation intensity (in 1% steps) from an initial suprathreshold level until we found the lowest stimulus intensity sufficient to induce 5 MEPs of ≥50 μV in a series of 10 stimuli applied at least every approximately 5 s. We also preselected for right-handed subjects as determined by a handedness questionnaire (Crovitz and Zener 1965).

Transcranial magnetic stimulation

We carried out TMS using a Cadwell (Kennewick, WA) high-speed magnetic stimulator and a Cadwell figure-of-eight stimulating coil (Corticoil, 2 tear-shaped coils of approximately 5-cm diameter each). We chose this coil because it produces a magnetic-field maximum of sufficiently small width to allow stimulation of the dorsal premotor cortex without encroaching on the primary motor cortex. In the scanner, a mechanical arm held the coil over the optimal position for eliciting a muscle twitch in the right index finger. We used a suprathreshold intensity of 115% rMT for single-pulse TMS and a subthreshold intensity of 90% rMT for repetitive TMS. Subthreshold intensities allow for more focal stimulation by narrowing the magnetic field produced by the coil, thus enabling better spatial resolution for examining changes between the location of stimulation and more distant cortical structures (Gerschlager et al. 2001; Münchau et al. 2002; Pascual-Leone et al. 1993).

Targeting the stimulation locations

We used a four-step procedure to place the stimulating coil over our stimulation locations. This procedure, developed in our first TMS/PET study (Paus 1999; Paus et al. 1997), takes advantage of standardized stereotaxic space (Talairach and Tournoux 1988). First, we acquired magnetic resonance (MR) images (170 contiguous 1-mm-thick sagittal slices) of the subject's brain using a Siemens Vision 1.5-T system and transformed these images into standardized stereotaxic space using an automatic feature-matching algorithm (Collins et al. 1994). Second, we derived locations for the primary motor and dorsal premotor cortices using information gained in previous brain imaging studies. We derived a probabilistic location for the primary motor cortex (X = −31, Y = −22, Z = 52; Paus et al. 1998) by averaging the coordinates reported in eight previous studies examining blood-flow activation when subjects moved the fingers of their right hand (Colebatch et al. 1991; Dettmers et al. 1995; Grafton et al. 1993; Jahanshahi et al. 1995; Jenkins et al. 1994; Matelli et al. 1993: Paus et al. 1993; Schlaug et al. 1994). This location served as an estimate as to where we should place the TMS coil relative to the subject's head in the scanner; subsequent adjustments in coil positioning were made (see following text). We defined a location for the dorsal premotor cortex (X = −21, Y = −2, Z = 52) as being 10 mm medial and 20 mm anterior to the probabilistic location of the primary motor cortex. This location was estimated by a PET study carried out by Fink et al. (1997) and was used in a previous TMS study of the premotor cortex (Schulter et al. 1998). Third, we transformed these two locations to the subject's brain coordinate space using an inverse version of the native-to-standardized transformation matrix.

The final step required us to position the coil over these locations, now marked on the MR images, which we achieved using frameless stereotaxy. With the subject lying on the couch of the scanner, we first registered the subject's head with the MR images and then placed the coil over the target locations by tracking the position and three-dimensional orientation of the coil with an infrared optical-tracking system (Polaris System, Northern Digital, Waterloo, Ontario, Canada, and *Brainsight* software, Rogue Research, Montreal, Quebec, Canada). We then locked the coil in place after finding these locations. In the case of the primary motor cortex, we made further adjustments in coil positioning to where stimulation resulted in the maximum MEP amplitude. To ensure that we used the same position for subsequent coil placements over the primary motor cortex, we first defined its position in the subject's brain coordinate space and then marked its position on the subject's MR images. We held the coil in different orientations when stimulating the primary motor and dorsal premotor cortices. For the primary motor cortex, we oriented the coil tangentially to the scalp with the short axis of the figure-of-eight coil angled at 45° relative to the interhemispheric fissure and approximately perpendicular to the central sulcus. For the dorsal premotor cortex, we oriented the coil tangentially to the scalp with the short axis of the figure-of-eight coil perpendicular to the interhemispheric fissure. For primary motor and dorsal premotor stimulation, the resulting induced electric current in the brain flowed in posterior-to-anterior and lateral-to-medial directions, respectively.

Verifying final coil positions over the primary motor cortex

Interpretation of results acquired with TMS/PET depends critically on the accuracy of coil positioning. In the present study, this applies specifically to the dorsal premotor experiment where we moved the coil from the primary motor cortex (Pre scan) to the dorsal premotor cortex (between scans 2 and 3) and then back to the primary motor cortex (Post scans). Using a procedure described in detail elsewhere (Paus and Wolforth 1998), we used 10-min transmission scans to verify coil positions relative to the acquired PET and MR images. We acquired transmission scans at the beginning of the dorsal premotor and primary motor experiments, and an additional transmission scan

at the end of the dorsal premotor experiment. These transmission images showed us the coil's position relative to the subject's head. We then registered an X-ray image of the coil to these images and projected a straight rod orthogonal to the plane of the coil from the coil center. After PET-to-PET, PET-to-MR, and MR-to-standardized space transformations, we superimposed the locations of the rod on an average anatomical MR image of all subjects. This indicates the projected center of the coil in the brain; the figure-of-eight coil used in this study stimulates an estimated volume of 20 × 20 × 10 mm (Cohen et al. 1990; Maccabee et al. 1990; Wassermann et al. 1996).

Positron emission tomography

We instructed subjects to relax and keep their eyes closed during PET scanning. Subjects used a bite-bar to maintain a constant head position during the experiments. We measured CBF with a CTI/Siemens HR+ 63-slice tomograph scanner operated in three-dimensional (3D) acquisition mode during 60-s scans using the ^{15}O-labeled H_2O bolus method (Raichle et al. 1983). In each scan, we injected 10 mCi of ^{15}O-labeled H_2O into the left antecubital vein. Acquired CBF images were reconstructed with a 14-mm Hanning filter, normalized for differences in global CBF (normalized CBF), coregistered with the individual MR images (Woods et al. 1993), and transformed into standardized stereotaxic space (Talairach and Tournoux 1988) by means of an automated feature-matching algorithm (Collins et al. 1994). We placed four 0.5-mm-thick sheets of well-grounded mu-metal to protect the photomultipliers inside the PET scanner from the effects of the coil-generated magnetic field. The mu-metal, however, can attenuate gamma rays and in turn decrease the number of detected coincidence counts (Paus 2002). The transmission data acquired at the beginning of the experiments were also used to correct for the attenuation of gamma rays caused by all objects in the scanner, including the coil, the coil mount, and the metal sheets.

Analyses of muscle-evoked potentials

We recorded MEPs from the right first dorsal interosseus muscle using Ag/AgCl surface electrodes fixed on the skin with a belly-tendon montage. We sampled the electromyographic (EMG) signal using an EMG channel of a 60-channel TMS-compatible electroencephalography system (Virtanen et al. 1999) with the amplifier's bandwidth set at 0.1–500 Hz and the sampling rate set at 1.45 kHz. We measured the peak-to-peak amplitudes for each MEP off-line. For practical reasons, we began to deliver single pulses of TMS at the time the radioactive tracer was injected. Acquisition after injection varies from one person to another and there is no way of knowing exactly which of the MEPs occurred during scanning. We therefore calculated the muscle response for a given scan as a percentage of the mean MEP amplitude during the Pre scan based on the 20 trials. We evaluated the effects of repetitive TMS on motor excitability by analysis of variance (ANOVA) using a model of repeated measures with Time as a within-subject factor. We used Tukey's HSD tests, which correct for multiple comparisons, for all post hoc pairwise comparisons. We considered values statistically significant at $P < 0.05$. We also performed a Wilcoxon signed ranks test to determine whether rMT values were significantly different between the two experiments.

Analyses of cerebral blood flow

We used a two-step process to generate *t*-statistical maps. We first subtracted CBF acquired before repetitive TMS from CBF acquired after repetitive TMS. We performed this initial subtraction to obtain CBF differences contrasting scans obtained before and after repetitive TMS, and to remove confounding intersubject variability. We then correlated these subtractions with the relative amount of MEP reduction, which we calculated in the same way as in a previous TMS/PET study (Strafella and Paus 2001) that examined the effects of double-pulse stimulation on CBF: {[1 − (MEP amplitude at a given post-rTMS condition/MEP amplitude at the pre-rTMS condition)] × 100}. We carried out calculations for the *t*-statistical maps for each of the 3D volume elements (voxels) constituting the entire scanned volume, which tested whether at a given voxel the slope of the regression was significantly different from zero.

After generating our *t*-statistical maps, we evaluated the presence of a significant peak by a method based on a 3D Gaussian random-field theory, with correction for the multiple comparisons involved in searching the entire volume (Worsley et al. 1992). Using this method, we performed both an exploratory search of the entire brain and a directed search in specific brain regions. For an exploratory search, we considered values equal to or exceeding a criterion of $t = 4.5$ as significant ($P < 0.000003$, 2-tailed, uncorrected), yielding a false positive rate of 0.04 (corrected) in 400 resolution elements (each of which has dimensions 14 × 14 × 14 mm) for a brain volume of 1,100 cm^3. For a directed search, we considered values equal to or exceeding a criterion of $t = 3.5$ as significant ($P < 0.0002$, 2-tailed, uncorrected), yielding a false positive rate of 0.01 (corrected) in two resolution elements (each of which has dimensions of 14 × 14 × 14 mm) for a volume of 5 cm^3. We performed our directed search in the dorsal premotor cortex, in the primary motor cortex, and in brain regions known to be connected with these regions in nonhuman primates (Fig. 1). To perform this search in the human brain, we relied on previous functional brain imaging studies that mapped their putative homologues; we describe these later in the discussion. We determined anatomical locations of all significant *t*-statistic peaks by examining the merged image of our *t*-statistical maps with the transformed averaged MR image of all subjects in standardized stereotaxic space (Talairach and Tournoux 1988). We performed additional subtractions of the CBF data to examine primarily the local effects at the stimulation sites. The first subtraction examined the possible, but unlikely, local effects of single-pulse TMS and consisted of subtracting CBF in the Base scan from CBF in the Pre scan. The second subtraction examined the presence of local effects of repetitive TMS

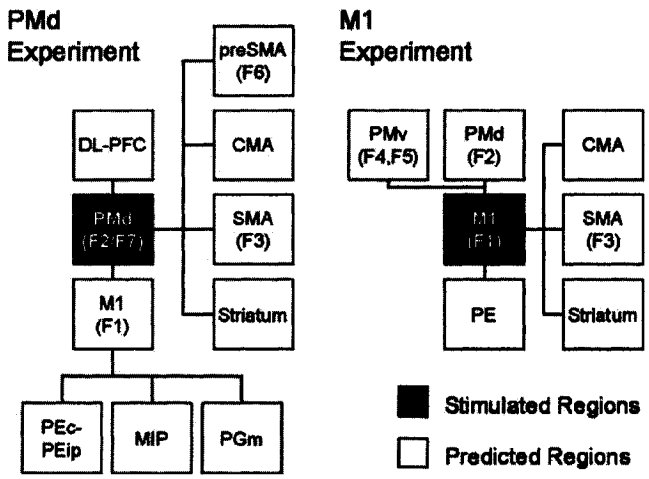

FIG. 1. Overview of possible connections in human cerebral cortex derived from anatomical studies performed by others in monkey. *A*: predicted brain regions connected with dorsal premotor cortex. *B*: predicted brain regions connected with primary motor cortex (reviewed in Matelli and Luppino 2000 for parieto-frontal circuits; Matelli and Luppino 1997 for functional anatomy of human and nonhuman primate motor cortical areas; and Parent and Hazrati 1995 for cortical–subcortical connections). No distinction is made between left and right hemispheres. Dorsal premotor cortex in this schematic consists of two anatomical areas [i.e., caudal (F2) and rostral (F7)], each with distinct interconnections with other brain areas. PE, PEc-PEip, and PGm are cytoarchitectonically defined areas of parietal cortex. F1 to F7 are subdivisions of cortical motor system in frontal lobe. Abbreviations: PMd, dorsal premotor area; M1, primary motor area; CMA, cingulate motor area; MIP, medial intraparietal area; and SMA, supplementary motor area.

TABLE 1. *rMTs for each subject*

	Dorsal Premotor Experiment		Primary Motor Experiment	
Subject	rMT (%)	Average MEP Reduction (%) at Post Conditions	rMT (%)	Average MEP Reduction (%) at Post Conditions
1	70	83.4	75	55.5
2	74	17.3	74	5.5
3	74	77.6	80	37.7
4	77	14	80	−21.8
5	68	29.5	80	60.7
6*	—	—	78	52.5
7*	—	—	70	39.4
Mean ± SE	72 ± 2	44.3 ± 15	77 ± 1	32.7 ± 12.4

Values are expressed as a percentage of the maximal stimulation output and the average amount of MEP reductions (%) at Post conditions in the dorsal premotor and primary motor experiments. * We excluded two subjects from the dorsal premotor experiment because of their head movements in the PET scanner. SE, standard error; MEP, motor evoked potentials; rMTs, resting motor thresholds.

and consisted of subtracting CBF in the Pre scan from the average CBF of all Post scans. All brain regions that showed significant CBF changes are reported.

We also provide additional analyses that examine similarities and differences between the effects of repetitive TMS over the dorsal premotor and primary motor cortices. To examine similarities, we carried out a conjunction analysis (Price and Friston 1997). This analysis tests for the presence of correlations in both experiments by revealing the maximum peaks in the two contrasts. We considered values equal to or exceeding a criterion of $t = 3.5$ as significant (Worsley and Friston 2000). To examine differences, we directly tested for differences in the CBF difference/MEP reduction relationship between the dorsal premotor and primary motor experiments. We first extracted CBF values from volumes of interest (VOIs) centered at the X, Y, and Z coordinates of our correlation peaks (Tables 2 and 3) and then, for each brain region, we used ANOVA to test for differences in the slope of their correlations between the two experiments. We used Bonferroni corrections to take into account multiple comparisons and considered values statistically significant at P (corrected) < 0.05.

TABLE 2. *Effects of repetitive stimulation over the dorsal premotor cortex on cerebral blood flow*

	X	Y	Z	*T*-value	Ref.
A. Regions with positive correlations					
Right inferior frontal gyrus/sulcus (VL-PFC)	44	39	3	7.7	1
Right IPL/postcentral sulcus	55	−26	45	6.4	2
Right precentral operculum (PMv)	52	−6	12	6.2	3
Right hippocampal formation	26	−18	−12	5.9	4
Right anterior IPL/intraparietal sulcus (putative AIP)	46	−47	42	5.9	5
Left precentral operculum (PMv)	−43	−6	14	5.8	6
Right medial frontal gyrus (SMA)	7	−9	60	5.5	7
Left (frontopolar) middle frontal gyrus	−32	61	−6	5.4	8
Left inferior frontal gyrus (VL-PFC)	−54	12	15	5.2	9
Left anterior cingulate gyrus	−9	24	20	5.2	10
Right middle frontal gyrus/sulcus (DL-PFC)	35	34	27	5.0	11
Left caudate nucleus (head)	−11	12	8	4.8	12
Right anterior IPL/intraparietal sulcus (putative AIP)	54	−40	52	4.8	13
Right cingulate gyrus/sulcus (CMA)	15	−4	51	4.7	14
Right cingulate gyrus/sulcus (CMA)	13	−6	45	4.7	15
Right hypothalamus	4	−2	−15	4.5	16
Left cingulate gyrus/sulcus (CMA)	−15	−9	45	4.5	17
Right putamen*	23	17	−6	3.9	18
Right precentral sulcus (premotor)*	35	10	33	3.8	19
Right posterior SPL/intraparietal sulcus (putative MIP)*	36	−64	54	3.7	20
	X	Y	Z	*T*-value	
B. Regions with negative correlations					
Left mesencephalon (~superior colliculus)	−5	−33	−4	5.9	
Left lingual gyrus/calcarine sulcus (VAA)	−9	−78	−9	5.8	
Left calcarine sulcus (V1)	−9	−97	8	5.6	
Left calcarine sulcus (V1)	−7	−68	9	5.5	
Left lateral occipital cortex (VAA)	−26	−75	22	5.5	
Right calcarine sulcus (V1)	20	−73	3	5.5	
Left caudate nucleus (body)	−13	−2	16	5.2	
Left cerebellum	−5	−50	−8	5.0	
Left parahippocampal gyrus	−19	−37	−18	4.8	
Left hippocampal formation	−13	−11	−20	4.7	
Left middle frontal gyrus/sulcus (DL-PFC)*	−36	12	30	4.2	
Right paracentral lobule (SM1)*	3	−30	54	4.1	
Left precentral gyrus (PMd)*	−51	−11	50	4.1	

Brain regions in the dorsal premotor experiment with significant positive (A) and negative (B) correlations between differences in CBF and reductions in MEP. * Brain regions with significant correlations after a directed search ($t > 3.5$ and $t < -3.5$) but not after an exploratory search ($t > 4.5$ and $t < -4.5$). The last column (Ref.) contains numbers for referring to Table 5. Abbreviations: PMd, dorsal premotor area; VL-PFC, ventrolateral prefrontal cortex; S1, primary sensory area; PMv, ventral premotor area; IPL, inferior parietal lobule; AIP, anterior intraparietal area; SMA, supplementary motor area; DL-PFC, dorsolateral prefrontal cortex; CMA, cingulate motor area; SPL, superior parietal lobule; MIP, medial intraparietal area; VAA, visual association area; V1, primary visual area; and SM1, sensorimotor area.

TABLE 3. *Effects of repetitive stimulation over the primary motor cortex on cerebral blood flow*

	X	Y	Z	*T*-value	Ref.
A. Regions with positive correlations					
Right mesencephalon	7	−11	−15	7.9	21
Left putamen	−31	6	0	5.8	22
Right ventral-lateral thalamus	17	−9	0	5.5	23
Right precentral gyrus/central sulcus (M1)	28	−25	56	5.1	24
Left cerebellum	−11	−49	−15	4.8	25
Left inferior frontal gyrus (VL-PFC)	−54	18	−5	4.8	26
Left basal forebrain nuclei	−16	1	−12	4.6	27
Right subgenual gyrus	5	29	−2	4.6	28
Right cingulate gyrus (CMA)*	3	10	40	4.4	29
	X	Y	Z	*t*-value	
B. Regions with negative correlations					
Left ventral occipital cortex (VAA)	−17	−76	−6	5.0	
Right middle temporal gyrus/inferior temporal sulcus	48	−68	12	4.9	
Right lateral occipital cortex (VAA)	42	−81	3	4.8	
Left posterior insular cortex	−39	−9	−16	4.8	
Right calcarine sulcus (V1)	21	−62	−2	4.8	
Right lateral occipito-temporal gyrus	43	−57	−14	4.6	
Right anterior insular cortex	31	27	0	4.5	

Brain regions in the primary motor experiment with significant positive (A) and negative (B) correlations between differences in CBF and reductions in MEP. * Brain regions with significant correlations after a directed search ($t > 3.5$ and $t < -3.5$) but not after an exploratory search ($t > 4.5$ and $t < -4.5$). The last column contains numbers for referring to Table 5. Abbreviations: M1, primary motor area; VL-PFC, ventrolateral prefrontal cortex; CMA, cingulate motor area; VAA, visual association area; and V1, primary visual area.

RESULTS

All subjects tolerated the study well without noticeable adverse effects related to TMS and/or the scanning procedures. We excluded data from two subjects in the dorsal premotor experiment from our analyses because of head movement. Figure 2 illustrates the end result of all coil placements over the primary motor cortex for both experiments.

Effects of repetitive TMS on MEP amplitudes

Repeated-measures ANOVA revealed a significant effect of Time on the mean MEP amplitude in the dorsal premotor experiment [$F(4,16) = 3.48$, $P < 0.05$] and in the primary motor experiment [$F(4,26) = 3.11$, $P < 0.05$]. These results indicate that MEP amplitudes changed during the course of the two experiments. Using Tukey's HSD pairwise comparison tests, we further examined the pattern of MEP changes (Fig. 3). The second Post scan showed significantly smaller MEP amplitudes compared with baseline in both experiments (both $P < 0.05$). No other pairwise comparisons differed significantly. A Wilcoxon signed ranks test revealed that there was no significant difference between rMT values in the two experiments [$W(4) = -1.83$, $P = 0.07$; subjects 6 and 7 excluded). Although this was not significant, rMT values tended to be lower in the dorsal premotor experiment compared with the primary motor experiment (Table 1).

Effects of repetitive TMS over the dorsal premotor cortex on CBF

Figure 4*A* and Table 2 summarize the findings in the dorsal premotor experiment and show all brain regions that presented significant positive and negative correlations between Post-Pre CBF differences and the amount of MEP reduction. Figure 5, *A* and *B* provides plots of CBF differ-

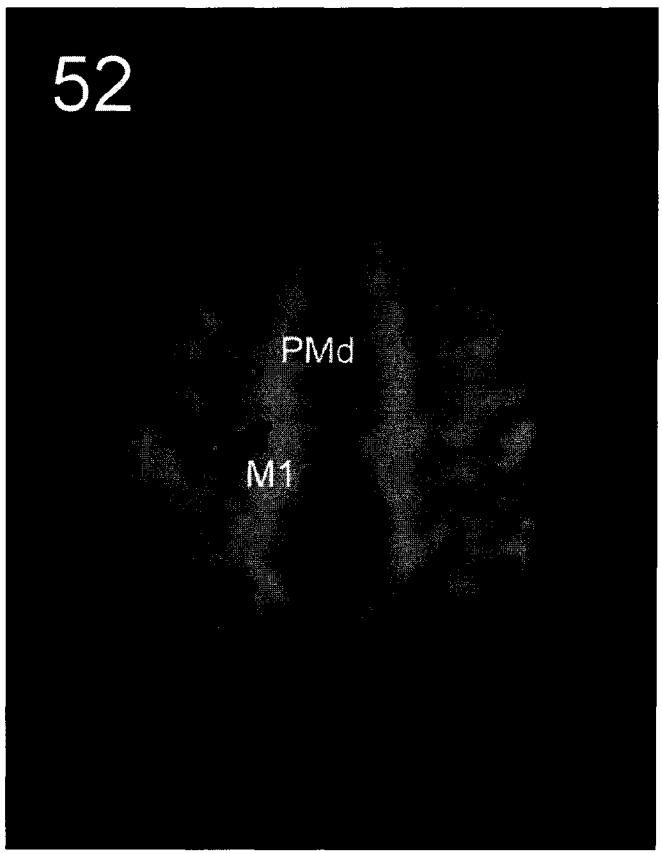

FIG. 2. Verification of coil positioning over primary motor cortex. Superimposed are virtual rods derived from transmission scans that indicate end result of all coil placements over primary motor cortex. Two spheres represent probabilistic locations for dorsal premotor (PMd) and primary motor (MI) cortices.

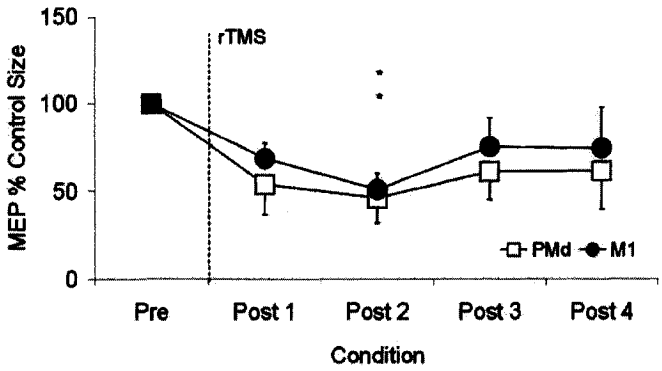

FIG. 3. Effects of repetitive stimulation on motor-evoked potentials. Mean (± SE) percentage motor-evoked potential (MEP) amplitude change at Post conditions compared with Pre conditions in both dorsal premotor and primary motor experiments. Asterisks denote significant differences compared with Pre conditions (*$P < 0.05$).

ences versus MEP reduction for two of these brain regions: the right anterior parietal and ventral premotor cortices. Motor-related regions with positive correlations include: the left and right ventral premotor areas in the precentral region of the operculi, the left and right cingulate motor areas in the cingulate gyri/sulci, the right premotor area in the precentral sulcus, the right supplementary motor area in the medial frontal gyrus, and the right putamen. Motor-related regions with negative correlations include: the left dorsal premotor area in the precentral gyrus/sulcus (30 mm lateral and 9 mm caudal to the targeted site of repetitive TMS and unlikely to indicate a local effect of stimulation) and the right sensorimotor area in the paracentral lobule.

Parietal brain regions with positive correlations include: the right posterior portion of the superior parietal lobule/intraparietal sulcus (putative medial intraparietal area), the anterior portion of the right inferior parietal lobule/intraparietal sulcus (putative anterior intraparietal area), and the right inferior parietal lobule/postcentral sulcus. Prefrontal brain regions with positive correlations include: the left and right inferior frontal gyrus/sulcus (ventrolateral prefrontal cortex) and the right middle frontal gyrus/sulcus (dorsolateral prefrontal cortex). One medial temporal-lobe region with a positive correlation was found in the right hippocampus. Negative correlations were mostly confined to several areas in the primary and associational visual cortices.

No significant correlations occurred either at the local site of repetitive TMS (i.e., left dorsal premotor cortex) or at the site of single-pulse TMS (i.e., left primary motor cortex). Further examination using direct subtraction analyses did not reveal significant CBF changes at either of the two sites of stimulation, which equally suggests that no local effects of TMS occurred in the left dorsal premotor cortex or in the left primary motor cortex. A direct subtraction of the Base scan from the Pre scan revealed CBF increases in the left presupplementary area on the medial frontal gyrus (X = −5, Y = 15, Z = 51; $t = 3.8$) and CBF decreases in the right superior parietal lobule/intraparietal sulcus (putative medial intraparietal area; X = 31, Y = −64, Z = 54; $t = -4.1$). A direct subtraction of the Pre scan from the average of all Post scans revealed no significant CBF differences anywhere in the brain.

Effects of repetitive TMS over the primary motor cortex on CBF

Figure 4*B* and Table 3 summarize the findings in the primary motor experiment and show all brain regions that presented significant positive and negative correlations between Post-Pre CBF differences and the amount of MEP reduction. Figure 5*C* provides a plot of CBF differences versus MEP reduction for one of these brain regions, the right primary motor cortex. Motor-related regions with positive correlations include: the left cingulate motor area in the cingulate gyrus/sulcus, the left putamen, the right primary motor area in the precentral gyrus/central sulcus, the right ventral-lateral thalamic nucleus, and the left cerebellum. Negative correlations were mostly confined to several areas in the primary and associational visual cortices.

No significant correlation occurred at the location of single-pulse TMS and repetitive TMS (i.e., left primary motor cortex). A direct subtraction of the Base scan from the Pre scan did not reveal any local changes in CBF. The same subtraction revealed CBF increases in the left primary visual cortex in the calcarine sulcus (X = −4, Y = −86, Z = 10; $t = 5.0$) and the right primary visual cortex in the calcarine sulcus (X = 7, Y = −71, Z = 14; $t = 4.8$). A direct subtraction of the Pre scan from the average of all Post scans, however, revealed a near significant increase of CBF at the stimulated region (X = −35, Y = −26, Z = 51, $t = 3.2$). The same subtraction also revealed CBF increases in the right cingulate motor area in the cingulate gyrus/sulcus (X = 1, Y = 18, Z = 45; $t = 3.7$) and in the left dorsal premotor cortex in the superior frontal sulcus (X = −33, Y = 6, Z = 52; $t = 3.6$), as well as CBF decreases in the left primary visual cortex in the calcarine sulcus (X = −5, Y = −85, Z = 12; $t = 4.9$). These two subtraction-based results suggest local effects of repetitive TMS but not of single-pulse TMS in the left primary motor cortex.

Conjunction analysis

Table 4 summarizes the findings of our conjunction analysis and lists all brain regions that presented significant correlations between Post-Pre CBF differences and the amount of MEP reduction in both experiments. Brain regions with significant positive correlations include: the right hippocampus and the right mesencephalon, both of which were approximately in the same horizontal plane (Z between −12 and −16). Except for one location in the right cerebellum, brain regions with significant negative correlations were all confined to the primary and associational visual cortices.

Contrast analysis

Table 5 summarizes the findings from our ANOVA that tested for differences in the CBF difference MEP reduction relationship between the dorsal premotor and primary motor experiments. We also present in this table Pearson's correlation coefficients between CBF differences and the amount of MEP reduction. Overall, our analysis confirms minimal overlap in the effects of repetitive TMS applied over the dorsal premotor and primary motor cortices on possible fronto-parietal circuits. Similar to the results in the conjunction analysis, the right mesencephalon showed relatively large Pearson's correlation coefficients for both experiments, suggesting strong positive

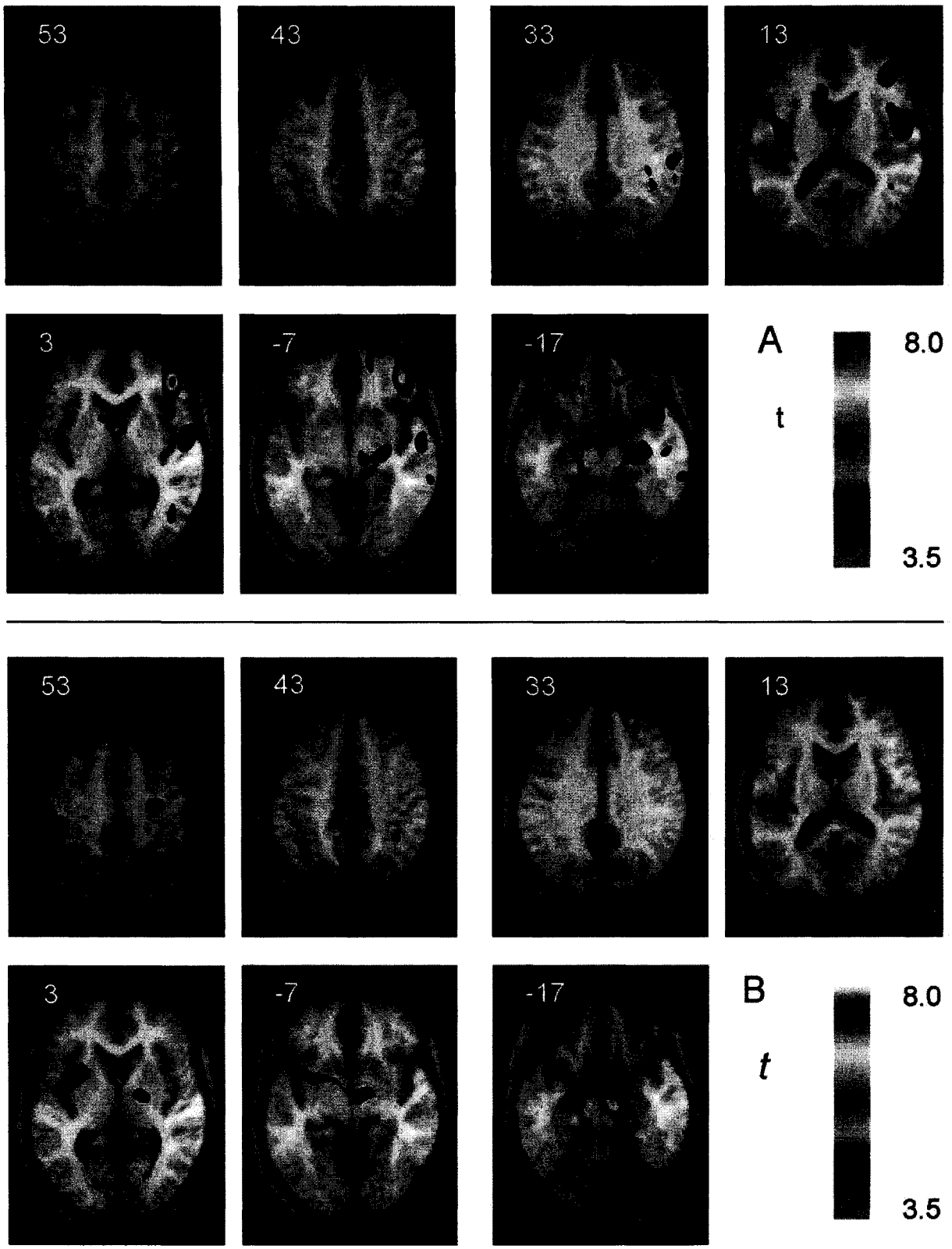

FIG. 4. Effects of repetitive stimulation on cerebral blood flow. *A, top half*: horizontal slices of brain regions with positive correlations ($t > 3.5$) obtained in PMd experiment. *B, bottom half*: horizontal slices of brain regions with positive correlations ($t < 3.5$) obtained in M1 experiment.

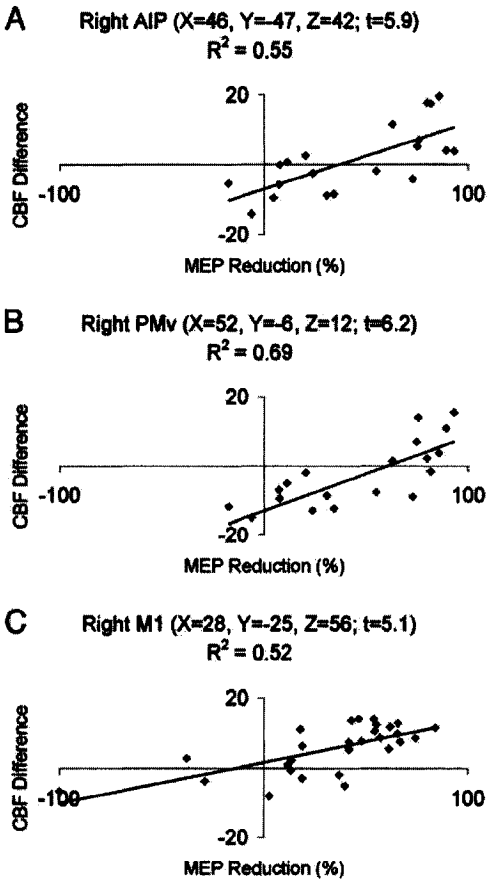

FIG. 5. Cerebral blood flow (CBF) differences plotted vs. the amount of reduction in MEPs. Figure shows extracted CBF values using VOIs centered at X, Y, and Z coordinates of three correlation peaks. *A* and *B*: extracted CBF values with VOIs centered at right anterior intraparietal and ventral premotor cortices in dorsal premotor experiment. *C*: extracted CBF values with VOI centered at right primary motor cortex in primary motor experiment. Abbreviations: AIP, putative anterior intraparietal area; PMv, ventral premotor area; and M1, primary motor area.

relationships between CBF differences and the amount of MEP reduction; although these still showed significantly different relationships. Contrary to the results in the conjunction analysis, the right hippocampal formation showed a small Pearson's correlation coefficient for the primary motor experiment. This is likely because we extracted VOI at its correlation peak in the dorsal premotor experiment, which was about 5 mm more medial and 5 mm more dorsal than its activation peak reported in the conjunction analysis.

DISCUSSION

Our results demonstrate that low-frequency repetitive TMS applied over the dorsal premotor and primary motor cortices produced similar inhibitory effects on MEPs but influenced cerebral activity differently. Repetitive stimulation over the dorsal premotor cortex resulted in the modulation of a network encompassing a number of brain regions; these include several regions in the parietal and prefrontal cortices. In contrast, repetitive stimulation over the primary motor cortex resulted in the modulation of a network encompassing a smaller number of brain regions; many of these were confined to the cortical and subcortical motor system. In the ensuing discussion we first address methodological issues and then discuss our findings in the light of studies performed by others in the monkey.

Methodological issues

Although we showed significant reductions in motor excitability after both applications of repetitive stimulation over the dorsal premotor and primary motor cortices, we also noted considerable interindividual differences. Some subjects showed greater reductions in MEP amplitude (1, 3, 5, and 6) compared with others (2 and 7), and one subject (4) showed increases in MEP amplitude. This is consistent with previous findings suggesting that it might be necessary to individualize parameters of repetitive TMS to achieve a consistent change in motor excitability across all subjects (Maeda et al. 2000). It is unlikely that this variability resulted from changes in coil positioning. Verifications of final coil positioning showed that we placed the coil consistently over the primary motor cortex. Most subjects showed minimal head movements as evident from their blood-flow images; we excluded two subjects who had head movements, in the dorsal premotor experiment, from the analyses.

Similar to Gerschlager et al. (2001) and Münchau et al. (2002), we demonstrated changes in motor excitability after applying repetitive TMS over the dorsal premotor cortex. Unlike the aforementioned studies, we held the coil in different orientations when stimulating the dorsal premotor and primary motor cortices. We chose different coil orientations to reduce the likelihood that stimulation of the dorsal premotor cortex would encroach on the primary motor cortex. Also, unlike the aforementioned studies, we demonstrated a reduction of motor excitability after repetitive TMS over the primary motor cortex. We might have had better access to the primary motor cortex by stimulating at a higher intensity (90% rMT as opposed to 80–90% active MT); as suggested by Gerschlager et al. (2001), it might be easier to stimulate the premotor cortex than relatively deeper structures like the primary motor cortex, located in the anterior bank of the central sulcus. The small figure-of-eight coil used in this study (diameter = 5 cm) delivers higher intensities while maintaining focality and stim-

TABLE 4. *Similarities between the effects of repetitive stimulation over the dorsal premotor and primary motor cortices on cerebral blood flow*

	X	Y	Z	*T*-value
A. Regions with positive correlations				
Right hippocampal formation	20	−21	−16	4.6
Right mesencephalon	12	−21	−12	3.8
B. Regions with negative correlations				
Left ventral occipital cortex (VAA)	−12	−74	−8	4.5
Left calcarine sulcus (V1)	−4	−93	9	4.1
Right calcarine sulcus (V1)	21	−66	0	4.1
Left calcarine sulcus	−7	−92	12	4.1
Right cerebellum	9	−37	−21	4.0

Brain regions with positive (A) and negative (B) correlations between differences in CBF and reductions in MEP that were significantly present in both the dorsal premotor and primary motor experiments. Abbreviations: VAA, visual association area; V1, primary visual area.

TABLE 5. *Differences between the effects of repetitive stimulation over the dorsal premotor and primary motor cortices on cerebral blood flow*

	$F(2, 45)$	P (uncorr)	r (PMd)	r (M1)	Ref.
A. Regions with positive correlations from the dorsal premotor experiment					
Right anterior IPL/intraparietal sulcus (putative AIP)	14.9*	<0.001	0.74	0.57	5
Left caudate nucleus (head)	14.1*	<0.001	0.79	−0.26	12
Right cingulate gyrus/sulcus (CMA)	13.3*	<0.001	0.82	0.49	15
Left cingulate gyrus/sulcus (CMA)	12.2*	<0.001	0.72	0.28	17
Left precentral operculum (PMv)	12.0*	<0.001	0.72	0.27	6
Right medial frontal gyrus (SMA)	11.3*	<0.001	0.78	0.48	7
Right cingulate gyrus/sulcus (CMA)	11.3*	<0.001	0.84	0.48	14
Right IPL/postcentral sulcus	11.2*	<0.001	0.80	−0.08	2
Right posterior SPL/intraparietal sulcus (putative MIP)	10.1*	<0.001	0.72	−0.18	20
Right hypothalamus	9.6*	<0.001	0.73	0.29	16
Right middle frontal gyrus/sulcus (DL-PFC)	9.3*	<0.001	0.74	0.54	11
Right anterior IPL/intraparietal sulcus (putative AIP)	7.2*	0.002	0.63	0.15	13
Left (frontopolar) middle frontal gyrus	6.6	0.003	0.77	−0.24	8
Left anterior cingulate gyrus	5.1	0.010	0.69	0.36	10
Right hippocampal formation	4.0	0.026	0.72	0.08	4
Right precentral operculum (PMv)	3.4	0.042	0.83	0.18	3
Left inferior frontal gyrus (VL-PFC)	3.3	0.045	0.86	0.14	9
Right putamen	3.3	0.048	0.51	0.28	18
Right inferior frontal gyrus/sulcus (VL-PFC)	3.2	0.050	0.72	−0.07	1
Right precentral sulcus (premotor)	2.4	0.100	0.58	0.24	19
B. Regions with positive correlations from the primary motor experiment					
Right mesencephalon	22.1*	<0.001	0.48	0.76	21
Right ventral-lateral thalamus	18.5*	<0.001	−0.13	0.71	23
Right cingulate gyrus (CMA)	15.4*	<0.001	−0.26	0.67	29
Left cerebellum	13.1*	<0.001	−0.48	0.72	25
Right precentral gyrus/central sulcus (M1)	12.8*	<0.001	−0.19	0.72	24
Left basal forebrain nuclei	11.0*	<0.001	−0.46	0.57	27
Left inferior frontal gyrus (VL-PFC)	10.1*	<0.001	0.34	0.61	26
Left putamen	7.5*	0.002	0.06	0.62	22
Right subgenual gyrus	4.6	0.016	−0.10	0.62	28

The table represents differences in the: (A) slope of correlations in brain regions obtained in the dorsal premotor experiment between the two experiments; and (B) slope of correlations in brain region obtained in the primary motor experiment between the two experiments. * Significant differences between the two experiments after the correction of multiple comparisons. The table also contains Pearson's correlation coefficients between CBF differences and amount of MEP reduction. The last column contains numbers for referring to Tables 2 and 3.

ulates an estimated volume of 20 × 20 × 10 mm (Cohen et al. 1990; Maccabee et al. 1990; Wassermann et al. 1996). It is unlikely, therefore, that the spread of current to premotor areas induced the effects obtained in the primary motor experiment.

MEPs obtained in the dorsal premotor and primary motor experiments are associated with changes in the size of muscle twitches and, presumably, differential sensory feedback from the hand muscles to the brain. This feedback could conceivably confound the blood-flow response. Two important features of our data argue against this possibility. First of all, we observed no significant blood-flow changes in the contralateral sensory cortices or contralateral sensory thalamus in either experiment, which suggests that the possible effects of the 12 muscle twitches on blood-flow response were negligible. Second, our results show that the depression of MEP amplitudes followed a similar time course in both experiments (Fig. 3). If our correlations resulted from changes in sensory feedback, we would have obtained more equivalent changes in blood flow from stimulating the dorsal premotor and primary motor cortices; this was not the case (Table 5).

The lack of blood-flow changes to single-pulse stimulation applied during the scans is not surprising in light of the low number of pulses (12 pulses/scan). On the other hand, we would expect blood-flow changes after the 15-min train of 1-Hz repetitive stimulation. We found a significant increase in local blood flow in scans acquired after repetitive stimulation over the primary motor cortex, but no such effects after repetitive stimulation over dorsal premotor cortex. Assuming tight coupling between excitatory synaptic activity and blood flow (Logothetis et al. 2001; Mathiesen et al. 1998; for review, see Paus 2002), we hypothesize that the local effects of low-frequency repetitive stimulation on inhibitory and excitatory neurotransmission canceled out while the distal effects remained. The latter might be related to the fact that the majority of cortico-cortical and cortico-subcortical projections are glutamatergic and, hence, their activation is more likely to influence blood flow in their target regions. As for a lack of a distal effect in the left primary motor cortex after dorsal premotor stimulation, this finding raises the possibility that the observed changes in MEP amplitudes are mediated by cortico-spinal projections originating in the dorsal premotor cortex rather than cortico-cortical connections between the dorsal premotor and primary motor cortices. We also hypothesize that the lateral-to-medial orientation of the short axis of the stimulating coil (virtual anode-cathode), as used in the dorsal premotor experiment, influenced preferentially inter-hemispheric rather than intra-hemispheric cortico-cortical projections. This could explain the general lack of distal effects in the left hemisphere

compared with the right hemisphere after repetitive stimulation over the dorsal premotor cortex.

Before proceeding to the interpretation of the results, we should mention some important aspects related to our correlations. A positive correlation reflects an increase in blood-flow response with the amount of MEP reduction and a negative correlation reflects a decrease in blood-flow response with the amount of MEP reduction. Brain regions that show these correlations were modulated in parallel with MEP reduction but, in some cases, modulation could have resulted from nonspecific effects of TMS. Our correlation analyses showed that most negative correlations were located in the primary and associational visual areas. Our conjunction analysis further showed that the majority of these were present in both the dorsal premotor and primary motor experiments. Together, these results suggest that our negative correlations were largely the result of nonspecific effects of TMS, one possibility of which is the result of changes in arousal levels. Several attention studies observed blood-flow fluctuations in similar brain regions and attribute these changes to differences in arousal levels and/or to cross-modal suppression (reviewed in Paus 2000). The rest of this discussion therefore concentrates on our positive correlations, which were confined predominantly to motor areas and putative fronto-parietal circuits.

Dorsal premotor experiment

Several anatomically distinct areas constitute the premotor cortex, each with a potentially different specialization. In our study, repetitive stimulation likely affected two distinct dorsal premotor areas (reviewed in Piccard and Strick 2000), that is, those identified in the monkey as the caudal premotor area F2, which has substantial connections with the primary motor area (Barbas and Pandya 1987; Dum and Strick 1991), and the rostral dorsal premotor area F7, which has substantial connections with the prefrontal cortex (Barbas and Pandya 1987; Lu et al. 1994). Repetitive stimulation over the dorsal premotor cortex might also have affected the frontal eye field; our stimulation site was in close proximity to the probabilistic location of this area as established by Paus (1996) in a meta-analysis of oculomotor neuroimaging studies.

The dorsal premotor cortex plays a prominent role in coupling arbitrary sensory cues to motor acts (for review, see Freund 1996). Studies in the monkey reveal that lesions to the dorsal premotor cortex disrupt the animal's ability to use such cues to make or withhold particular movements (Halsband and Passingham 1982, 1985; Petrides 1985); the same is true for patients with damage to the dorsal premotor cortex (Halsband and Freund 1990). The parietal cortex receives somatosensory and visual inputs, and encompasses several subdivisions that have reciprocal connections with motor areas in the frontal cortex, each with a specific target with which it is most densely connected. These circuits provide an anatomical basis for the transformation of sensory information into motor actions (Matelli and Luppino 2000; Rizzolatti et al. 1998). Anatomical studies in the monkey reveal circuits that include the dorsal premotor area (F2/F7) as their frontal component; one of these is the MIP-F2 circuit (Matelli et al. 1998). A combination of somatosensory and visual information used for the visual guidance of arm movement trajectories is thought to reach F2 from MIP (Colby and Duhamel 1991; Galletti et al. 1996; Matelli and Luppino 2000).

In view of these data, we postulate that our findings may show the human homologue of the MIP-F2 circuit. The circuit follows from correlations observed in the right premotor cortex in the precentral sulcus and in the right medial intraparietal cortex along the posterior superior parietal lobule. Stimulation of the left dorsal premotor cortex might have modulated the right premotor cortex through commissural connections (Marconi et al. 2002; Pandya and Vignolo 1971). Our MIP coordinates ($X = 36$, $Y = -64$, $Z = 54$) are similar to those ($X = -33$, $Y = -60$, $Z = 54$) established in a previous functional MR imaging study of response switching, which required subjects to switch between two different visuomotor-related intentions (Rushworth et al. 2001). Other functional brain imaging studies show comparable metabolic changes in both the posterior parietal and premotor cortices as subjects selected motor acts based on visual stimuli (Dieber et al. 1997; Grafton et al. 1998; Paus et al. 1993).

Our results also suggest an additional parieto-frontal circuit that connects the right ventral premotor area (PMv) in the precentral operculum with the right putative anterior intraparietal area (AIP) in the lateral bank of the intraparietal sulcus along the anterior inferior parietal lobule. Stimulation of the left dorsal premotor cortex might have modulated the right ventral premotor cortex through commissural connections (Marconi et al. 2002). Marconi et al. (2002) recently demonstrated in the monkey that callosal connections exist between the dorsal premotor cortex in one hemisphere and the ventral premotor cortex in the opposite hemisphere. Connections also exist in the monkey between PMv (F5) and the more anterior part of the intraparietal cortex (Luppino et al. 1999). Both F5 and AIP neurons code for selective hand manipulations, grasping movements, and various visual characteristics of 3D objects (Murata et al. 1997; Rizzolatti et al. 1988). In view of these findings, Jeannerod et al. (1995) suggested that the F5-AIP circuit plays a role in transforming the properties of a 3D object into the appropriate hand movements required to grasp it. Previous PET data indicate that similar activations occur in the human PMv during the presentation of 3D objects (Grafton et al. 1997) with coordinates ($X = -48$, $Y = -2$, $Z = 29$) that are slightly more dorsal than our PMv coordinates ($X = 52$, $Y = -6$, $Z = 12$ and $X = -43$, $Y = -6$, $Z = 14$).

The prefrontal cortex plays a prominent role in executive functions (reviewed in Fuster 1993; Petrides 2000). To select relevant information for action, the prefrontal cortex has access, through its connections with other brain structures, to sensory and spatial aspects of the environment, mnemonic information acquired through experience, and motor control (reviewed in Barbas 2000). These motor output–related connections mainly arise from the premotor cortices (Barbas and Pandya 1987; Lu et al. 1994) and might explain our additional correlations in the prefrontal cortices. Anatomical data in the monkey show reciprocal connections of the prefrontal cortex and the premotor cortex in an orderly pattern along dorsal and ventral axes; interconnections between the two axes are sparse (Barbas 1988, 1992; Barbas and Pandya 1989). One would therefore predict that the blood-flow changes we observed in the ventrolateral prefrontal cortices arose from connections with the ventral premotor cortices.

Primary motor experiment

A conjunction analysis performed on our data revealed little overlap in the positive correlations obtained between the dorsal premotor and primary motor experiments. Similarly, ANOVA revealed that most of the brain regions with correlations showed significant differences between the two experiments. These findings suggest that we mapped two separate networks and lend support to the notion that the dorsal premotor and primary motor cortices differ in their functional properties. Unlike the dorsal premotor cortex, the primary motor cortex plays a role mainly in the execution of voluntary movements. Studies in the monkey reveal that lesions to the primary motor cortex disrupt the execution of skilled movements to a greater extent than lesions to nonprimary motor cortices (Passingham 1985; Passingham et al. 1983; Petrides 1985). Of all the cortical motor areas, the primary motor cortex contains the highest percentage (31%) of large corticospinal neurons (Dum and Strick 1991), which directly generate movement of the limbs (for review, see Evarts 1981).

The primary motor cortex connects predominantly with nonprimary motor and nonprimary somatosensory cortices; connections between the primary motor cortex and other cortical structures are sparse (Fig. 1). Visual and/or auditory information that influence movements must first be processed by associational and/or higher-order sensory cortices, and then be communicated to the nonprimary motor cortices (for review, see Ghez et al. 1991). The nonprimary motor cortices can in turn use this information to coordinate motor output at the level of both the primary motor cortex and the spinal cord (Dum and Strick 1991). We propose that our data from the primary motor experiment reflect this pattern of connections: the network mapped in the primary motor experiment encompasses correlations confined mainly to nonprimary motor cortices and subcortical motor structures.

Brain regions with significant correlations in the primary motor experiment include the right cingulate motor area, the left putamen, the right primary motor area, and the right ventral-lateral thalamic nucleus/internal global pallidus. Correlations in this experiment reflect both direct and indirect connections with the stimulation site (i.e., the left primary motor cortex). The cingulate motor area represents most likely the human homologue of CMAr, or the rostral cingulate zone, which is located anterior to the anterior commisure (Paus et al. 1993; Piccard and Strick 1996). The correlation in the left ipsilateral putamen suggests cortico-striatal projections from the primary motor area to the lateral putamen (Takada et al. 1998). The presence of a blood-flow response in the contralateral primary motor area suggests commissural connectivity from the stimulated hemisphere to the unstimulated hemisphere (Jenny 1979; Rouiller et al. 1994). The right ventral-lateral thalamus and the right cingulate motor area might reflect indirect connections with the site of stimulation, the left primary motor cortex, mediated perhaps through the right primary motor cortex. Both the ventral-lateral thalamus and the internal globus pallidus are components of cortico-basal ganglia-thalamo-cortical loops related to the control of movement (Parent and Hazrati 1995).

In conclusion, the data presented here suggest that we mapped two separate motor-related networks and provide complementary insights into the function of the dorsal premotor and primary motor cortices.

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DISCLOSURES

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Behavioral/Systems/Cognitive

Role of the Primary Motor and Dorsal Premotor Cortices in the Anticipation of Forces during Object Lifting

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When lifting small objects, people apply forces that match the expected weight of the object. This expectation relies in part on information acquired during a previous lift and on associating a certain weight with a particular object. Our study examined the role of the primary motor and dorsal premotor cortices in predicting weight based either on information acquired during a previous lift (no-cue experiment) or on arbitrary color cues associated with a particular weight (cue experiment). In the two experiments, subjects used precision grip to lift two different weights in a series of trials both before and after we applied low-frequency repetitive transcranial magnetic stimulation over the primary motor and dorsal premotor cortices. In the no-cue experiment, subjects did not receive any previous information about which of two weights they would have to lift. In the cue experiment, a color cue provided information about which of the two weights subjects would have to lift. Our results demonstrate a double dissociation in the effects induced by repetitive stimulation. When applied over the primary motor cortex, repetitive stimulation disrupted the scaling of forces based on information acquired during a previous lift. In contrast, when applied over the dorsal premotor cortex, repetitive stimulation disrupted the scaling of forces based on arbitrary color cues. We conclude that the primary motor and dorsal premotor cortices have unique roles during the anticipatory scaling of forces associated with the lifting of different weights.

Key words: repetitive transcranial magnetic stimulation; dorsal premotor area; primary motor area; weight prediction; precision grip; visuomotor association

Introduction

The precision grip has been investigated extensively in humans (Johansson, 1996). People typically use the tips of the index finger and thumb when lifting small objects. The lifting of such objects requires fine motor control; too much force can damage the object or result in an excessive lifting movement, and too little force can cause the object to slip away. Throughout life, we build internal representations for the weight of different objects (Gordon et al., 1993; Wolpert and Flanagan, 2001). This provides us with the ability to apply forces for lifting objects using feedforward mechanisms. In cases when the weight is lighter than expected, somatosensory information related to lift-off will generate corrective forces to stabilize the object (Johansson and Westling, 1988). In cases when the weight is heavier than expected, the absence of an expected lift-off will generate corrective forces to overcome gravity on the object (Johansson and Westling, 1988).

Information acquired by a recent lift can influence the anticipatory scaling of forces for a subsequent lift (Johansson and Westling, 1988; Gordon et al., 1993; Fellows et al., 1998). When the weight of an object changes unexpectedly without any changes in appearance, people will generate inappropriate forces on the first lift and quite accurate forces on the subsequent lift. This adaptation indicates that the motor system can update quickly information pertaining to the properties of an object and is thought to involve processes similar to those used to correct for errors made in predicting weight (Johansson and Westling, 1988). Cell recording studies in the monkey demonstrate that a population of primary motor neurons processes information related to a recent experience by altering their firing properties during motor adaptation (Li et al., 2001). We predict that repetitive transcranial magnetic stimulation (rTMS) applied over the primary motor cortex can disrupt the scaling of forces based on information acquired during a previous lift.

The dorsal premotor cortex selects movements mainly on the basis of learned associations (Wise and Murray, 2000). People generate more accurate forces during the lifting of small objects after they have learned to associate arbitrary color cues with weight (Cole and Rotella, 2002). Semantic identification can also influence forces applied during the lifting of commonly used objects (Gordon et al., 1993). Rapid associative learning is thought to generate internal representations that link object identification with the scaling of forces required to lift them. People can learn new associations as quickly as by the second trial and reproduce forces accurately for up to 24 h (Gordon et al., 1991b; Flanagan et al., 2001). Lesion studies in the monkey have shown that the removal of the dorsal premotor cortex disrupts the ability to use arbitrary visual cues to make or withhold particular movements (Halsband and Passingham, 1982, 1985; Pet-

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rides, 1982, 1985a). We predict that rTMS applied over the dorsal premotor cortex can disrupt the scaling of forces based on arbitrary color cues.

Materials and Methods

Overview. Two groups of subjects participated in each of two experiments, namely the no-cue and cue experiments; the individual volunteers were assigned to the two experiments at random. We applied 15 min of 1 Hz rTMS over the left primary motor cortex on one day and the left dorsal premotor cortex on another day. We counterbalanced the order in which repetitive stimulation was delivered over the two brain sites in a given subject. Figure 1*A* illustrates the time course for one session. We acquired motor-evoked potentials (MEPs) by delivering single-pulse TMS over the left primary motor cortex 22–20, 12–10, and 2–0 min before the onset of rTMS and 0–2, 10–12, 20–22, and 30–32 min after the conclusion of rTMS. We also acquired precision grip measurements 20–12 and 10–2 min before the onset of rTMS and 12–20 and 22–30 min after the conclusion of rTMS.

Participants. Subjects in the two experiments matched for sex, age, handedness, pinch strength, and manual dexterity. Four female and four male subjects (19–30 years of age; mean ± SEM, 24.4 ± 1.4) participated in the no-cue experiment, and another four female and four male subjects (21–36 years of age; mean ± SEM, 25.6 ± 1.6) participated in the cue experiment. All subjects had a strong right-hand preference as determined by a handedness questionnaire (Crovitz and Zener, 1965); a paired *t* test revealed no significant group difference in handedness ($p = 0.73$). We tested pinch strength for both hands using a Jamar pinch dynamometer (Sammons Preston, Bolinbrook, IL) and manual dexterity for both hands using the grooved pegboard test, model 32025 (Lafayette Instrument Company, Lafayette, IN). Paired *t* tests revealed no significant group differences in pinch strength for either the right hand ($p = 0.27$) or the left hand ($p = 0.29$) and in performance times in the grooved pegboard test for either the right hand ($p = 0.80$) or the left hand ($p = 0.78$). All subjects provided informed written consent before participation. The Research Ethics Board of the Montreal Neurological Institute and Hospital approved all experimental procedures. We selected subjects for whom we had previously acquired anatomical magnetic resonance images (MRIs) [160–192 contiguous 1-mm-thick sagittal slices; Siemens AG (Erlangen, Germany) Vision 1.5-T system] and who had low resting motor thresholds. We established the latter criterion to prevent overheating of the stimulating coil.

Apparatus for precision grip. We constructed a manipulandum (Fig. 1*B*) based on the classical apparatus used by Johansson and Westling (1984). The contact surface with which subjects grasped the manipulandum consisted of sandpaper (no. 150) attached to both sides of the handle. We measured the grip force using a set of strain-gauge transducers attached to the handle where subjects grasped with the index finger and the load force using a set of strain gauges attached to the base of the manipulandum. The resulting signals fed continuously to a Grass model 15A54 quad amplifier (Astro-Med, West Warwick, RI) at a sampling rate of 800 Hz. We saved all data on a laptop computer for off-line analysis. We also attached to the base of the manipulandum an aluminum rod that passed through a hole in the table that held a weight carrier at its bottom end. This allowed us to add or remove from the carrier a 200 g weight without the subject seeing us change weights. Subjects wore earphones and listened to white noise at an intensity that they could tolerate comfortably (~60 to ~80 dB). Our pilot experiments revealed that the white noise helped prevent subjects from hearing us add or remove the weight and thus realize when a switch in weight occurred between lifts.

Apparatus for transcranial magnetic stimulation. We performed TMS using a Cadwell (Kennewick, WA) high-speed magnetic stimulator and a Cadwell figure-of-eight stimulating coil with a built-in cooling system (Corticoil; two tear-shaped coils of ~5 cm diameter each). We chose this coil because it produces a magnetic-field maximum of sufficiently small width to allow stimulation of the dorsal premotor cortex without encroaching on the primary motor cortex. A similar coil was previously found to stimulate an estimated volume of 20 × 20 × 10 mm (Cohen et al., 1990; Maccabee et al., 1990; Wassermann et al., 1996). Subjects used a bite bar during stimulation while a mechanical arm held the coil over the target locations. We determined motor thresholds for the relaxed right first dorsal interosseus muscle before each session (Chouinard et al., 2003).

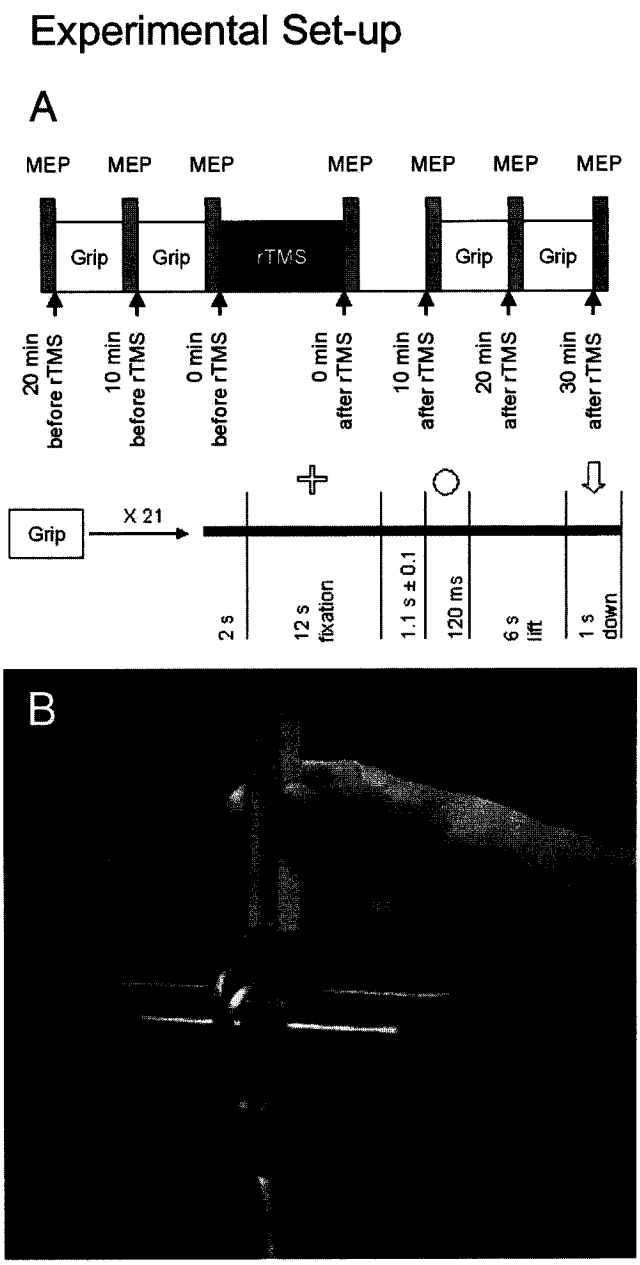

Figure 1. Experimental setup. ***A*** illustrates the chronological order of a session. During task performance, subjects performed 21 lifts in which they fixated their gaze on the computer screen until they saw a cue. After cue presentation, they then grasped the manipulandum between the tips of the index finger and thumb and lifted it vertically for a distance of ~10 cm. They maintained the manipulandum in this position until they saw an arrow pointing down on the computer screen. ***B*** illustrates the manipulandum that we used to measure precision grip.

Apparatus for electromyography. We recorded MEPs from the right first dorsal interosseus muscle using Ag/AgCl surface electrodes fixed on the skin with a belly-tendon montage. We sampled the electromyographic signal using the Grass amplifier with a bandwidth set at 0.1–3000 Hz and the sampling rate set at 2000 Hz. We then saved these data on a laptop computer for off-line analysis. We measured the peak-to-peak amplitudes for each MEP using the program Matlab (MathWorks, Natick, MA) and then calculated the mean MEP amplitude for each condition based on the 20 trials.

Procedures for precision grip. We performed the two experiments in a quiet room with the lights dimmed in which subjects sat comfortably in front of a computer screen. In the no-cue experiment, we presented a white circle as a neutral stimulus before subjects lifted weights of 325 g (light) or 525 g (heavy); this circle provided no information about what weight would be lifted. In the cue experiment, we presented a pink circle before subjects lifted a weight of 325 g and a blue circle before subjects lifted a weight of 525 g. During task performance, subjects performed 21 lifts in which they fixated their gaze on the computer screen until they saw a cue. After cue presentation, they then grasped the manipulandum between the tips of the index finger and thumb and lifted it vertically for a distance of ~10 cm. They maintained the manipulandum in this position until they saw on the computer screen an arrow pointing down.

In the beginning of the first session, we demonstrated how to perform the task properly and then provided subjects with a 5 min training period in which they performed a series of trials with the 325 g weight. We instructed subjects to grasp the manipulandum between the tips of the index finger and thumb and lift the manipulandum using appropriate forces. We also instructed subjects to lift vertically for a distance of ~10 cm; the lifting movement of the task required mainly a flexion of the elbow. During the training period, we provided verbal feedback so as to ensure that they grasped the manipulandum with the tips of the index finger and thumb only. We did not provide any additional feedback after this 5 min training period. Figure 1*A* illustrates the time course of each trial. Subjects performed a total of 21 lifts per block, so that after removing the first trial, we obtained five trials for each of the following four conditions: light-after-light, light-after-heavy, heavy-after-heavy, and heavy-after-light. We predetermined the order of these conditions pseudorandomly and presented a different order for each of the different blocks.

Procedures for transcranial magnetic stimulation. We reduced excitability by applying rTMS over the left primary motor cortex on one day and the left dorsal premotor cortex on another day (Touge et al., 2001; Munchau et al., 2002). Direct corticocortical connections between the dorsal premotor and primary motor cortices are thought to mediate reductions in motor excitability after repetitive stimulation over the dorsal premotor cortex (Munchau et al., 2002). We used single-pulse TMS over the left primary motor cortex to measure MEPs as an index of the effectiveness of rTMS applied over the two sites (Chouinard et al., 2003). We introduced an ~10 min delay before subjects performed the precision grip task again because we had found previously that it took ~10 min after repetitive stimulation of either the primary motor cortex or the dorsal premotor cortex to reduce MEP amplitudes significantly (Chouinard et al., 2003). We expected also to see a gradual return of MEP amplitudes compared with baseline measurements 20 min after rTMS (Chouinard et al., 2003).

We used a four-step procedure to place the TMS coil over the primary motor and dorsal premotor cortices (Paus et al., 1997). First, we transformed the subject's MRI into standardized space (Talairach and Tournoux, 1988; Collins et al., 1994). Second, we derived probabilistic locations for the primary motor ($X = -31$, $Y = -22$, $Z = 52$) and dorsal premotor ($X = -21$, $Y = -2$, $Z = 52$) cortices using information gained in previous brain imaging studies (Paus et al., 1998; Chouinard et al., 2003). Third, we transformed the probabilistic locations to the subject's brain coordinate space. Fourth, we used frameless stereotaxy to position the TMS coil over the probabilistic locations marked on the subject's MRI (Brainsight software, Rogue Research, Montreal, Quebec, Canada; Polaris System, Northern Digital, Waterloo, Ontario, Canada). In the case of the primary motor cortex, we made additional adjustments in coil positioning to where stimulation resulted in the maximum MEP amplitude.

For single-pulse TMS, we applied 20 single pulses of stimulation every 5 ± 1 s at a suprathreshold intensity of 120% of the resting motor threshold. For rTMS, we applied 15 min of 1 Hz repetitive stimulation at a subthreshold intensity of 90% of the resting motor threshold in three 5 min blocks, each block separated by 1 min, to minimize overheating of the stimulating coil. Subthreshold intensities allow for more focal stimulation by narrowing the magnetic field produced by the coil, thus enabling better spatial resolution for examining changes between different cortical structures (Pascual-Leone et al., 1993). We held the coil in the same orientation when stimulating both the primary motor and dorsal premotor cortices. We oriented the coil tangentially to the scalp with the short axis of the figure-of-eight coil angled 45° relative to the interhemispheric fissure and approximately perpendicular to the central sulcus. For both primary motor and dorsal premotor stimulation, the resulting induced current in the brain flowed in a posterior-to-anterior and lateral-to-medial direction.

Verification of coil positions. We derived projected coil trajectories from the center of the figure-of-eight coil using the Brainsight software (see previous section) as an estimation of where stimulation took place. After placing the coil over the sites of stimulation, we saved the projected coil trajectories in the subject's brain coordinate space. We then marked on the subject's MRI where this trajectory passed in the same perpendicular plane, or parallel plane to the coil, as the site we intended to target. We then transformed these coordinates from voxel space to native space using the software Register (Montreal Neurological Institute, Montreal, Quebec, Canada) and then to standardized space. Projected coil trajectories for the primary motor cortex revealed minimal overlap with those for the dorsal premotor cortex (see Fig. 2*A*,*C*). Projected coil trajectories for the dorsal premotor cortex generally passed in the rostral dorsal premotor cortex as established by Piccard and Strick (2000). Projected coil trajectories for the primary motor cortex showed greater variability. This is likely because we made adjustments in coil positioning to target where stimulation resulted in the maximum MEP amplitude; previous studies have reported that this location can vary among individuals (Classen et al., 1998).

Analyses for motor-evoked potentials. For both the no-cue and cue experiments, we evaluated the effects of repetitive stimulation on motor excitability by ANOVA using time and site of stimulation as within-subject factors. We used Tukey's honestly significant difference (HSD) pair-wise comparison tests, which corrected for multiple comparisons, to examine additional significant effects. We also used paired *t* tests to compare resting motor thresholds values acquired during sessions with repetitive stimulation over the primary motor cortex with those acquired during sessions with repetitive stimulation over the dorsal premotor cortex.

Analyses for precision grip. Using Matlab, we measured the rates in grip force for each but the first trial and then calculated the means for each of the different four conditions for each block. For measuring the rates in grip force, we divided the magnitude of the peak force by the time difference between the peak grip force and the first increase in grip-force signal. For the statistical analyses of rates in grip force, we performed an ANOVA that examined the effects of repetitive stimulation on performance in each of the two experiments. For this ANOVA, we used switching (no switch vs switch), weight (light vs heavy), block (20–12 min before rTMS vs 10–2 min before rTMS vs 12–20 min after rTMS vs 22–30 min after rTMS), and site of stimulation (primary motor vs dorsal premotor) as within-subject factors.

We also performed additional ANOVAs on both the rates in load force and the load force time (time of peak force − time of first increase in signal) in cases in which the rates in grip force changed during an experiment. We calculated the rates in load force the same way as we calculated the rates in grip force. For these ANOVAs, we used switching (no switch vs switch), weight (light vs heavy), and block (20–12 min before rTMS vs 10–2 min before rTMS vs 12–20 min after rTMS vs 22–30 min after rTMS) as within-subject factors. We performed simple effect tests and Tukey's HSD pair-wise comparison tests, which corrected for multiple comparisons, to examine additional significant interactions.

Results

Resting motor thresholds

Paired *t* tests on the resting motor thresholds revealed no difference between sessions in both the no-cue ($t_{(7)} = 0.39$; $p = 0.71$) and cue ($t_{(7)} = 0.24$; $p = 0.82$) experiments.

No-cue experiment

Effects of repetitive stimulation on motor excitability

An ANOVA on the MEP amplitudes revealed an effect of time ($F_{(6,42)} = 4.76$; $p < 0.001$), no effect of site of stimulation ($F_{(1,7)} =$

0.26; $p = 0.63$), and no time × site of stimulation interaction ($F_{(6,42)} = 1.52$; $p = 0.20$). These results demonstrate that changes in MEP amplitudes did not differ when we applied repetitive stimulation over the primary motor cortex compared with the dorsal premotor cortex (Fig. 2*B*). We performed Tukey's HSD tests to examine further the effect of time and found reductions in MEP amplitudes 0–2 min after rTMS compared with 22–20 min before rTMS ($p < 0.05$), 12–10 min before rTMS ($p < 0.01$), 2–0 min before rTMS ($p < 0.05$), 20–22 min after rTMS ($p < 0.01$), and 30–32 min after rTMS ($p < 0.01$).

Effects of repetitive stimulation on grip forces

An ANOVA on the rates in grip force revealed a significant switching × weight × block × site of stimulation interaction ($F_{(3,21)} = 3.42$; $p < 0.05$).

Before repetitive stimulation, subjects applied rates in force that reflected the scaling of forces for a previous weight (Fig. 3*A–C*). In the no switch trials (i.e., when subjects lifted the same weight as in the previous lift), subjects applied faster rates in grip force when they lifted the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). When changes in weight occurred between lifts, however, effects of switching were present. In the switch trials (i.e., when subjects lifted a different weight than in the previous lift), the rates in grip force increased after the weight became lighter (light-after-heavy > light-after-light) and decreased after the weight became heavier (heavy-after-light < heavy-after-heavy). These effects of switching indicate that subjects scaled their grip forces based on the previous weight. Repetitive stimulation over the dorsal premotor cortex had no effect on the rates in grip force (Fig. 3*B*).

Repetitive stimulation over the primary motor cortex disrupted the production of distinct rates in grip force (Fig. 3*A*, *D*). In the no switch trials, the rates in grip force at 12–20 min after rTMS did not differ when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy ≈ light-after-light). In the switch trials, the rates in grip force at 12–20 min after rTMS did not increase after the weight became lighter (light-after-heavy ≈ light-after-light) and did not decrease after the weight became heavier (heavy-after-light ≈ heavy-after-heavy). A power analysis revealed that a sample size of 37 subjects would be necessary to reject the null hypothesis that no effects of switching occurred ($\alpha = 0.05$). At 22–30 min after rTMS, the rates in grip force were similar to those before repetitive stimulation. These results indicate that repetitive stimulation over the primary motor cortex temporarily disrupted the subjects' ability to apply distinct rates in grip force when lifting different weights and to scale forces based on the previous weight.

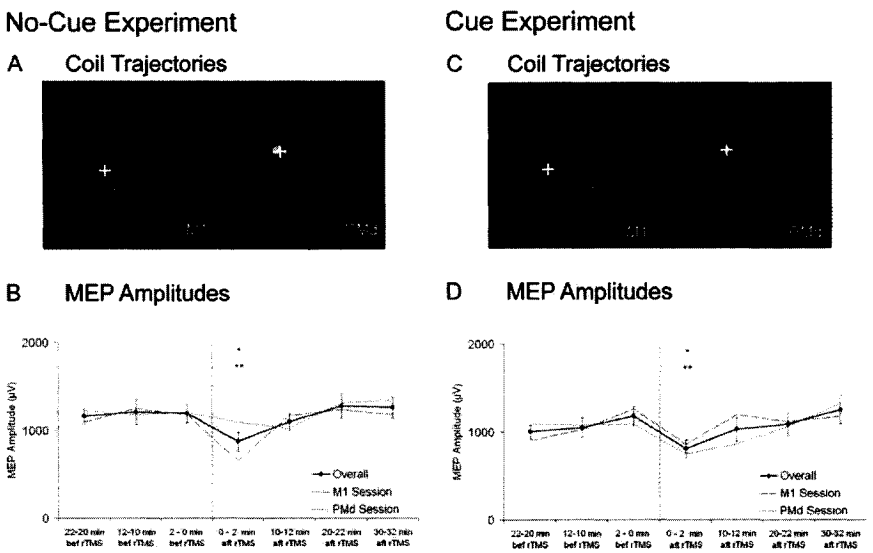

Figure 2. MEP amplitudes. ***A***, ***C***, Superimposed on magnetic resonance images are projected coil trajectories that indicate estimated locations for induced currents in the brain during repetitive stimulation over the primary motor (M1) and dorsal premotor (PMd) cortices. The brightness of these superimpositions reflects the probability of the coil trajectories. Crosses represent their probabilistic locations. ***B***, ***D***, Overall mean ± SEM MEP amplitudes and MEP amplitudes in the primary motor and dorsal premotor sessions are shown. Asterisks denote significant differences for overall MEP amplitudes (no-cue experiment: $*p < 0.05$ vs 22–20 min before rTMS, 2–0 min before rTMS; $**p < 0.01$ vs 12–10 min before rTMS, 20–22 min after rTMS, 30–32 min after rTMS; cue experiment: $*p < 0.05$ vs 2–0 min before rTMS; $**p < 0.01$ vs 30–32 min after rTMS).

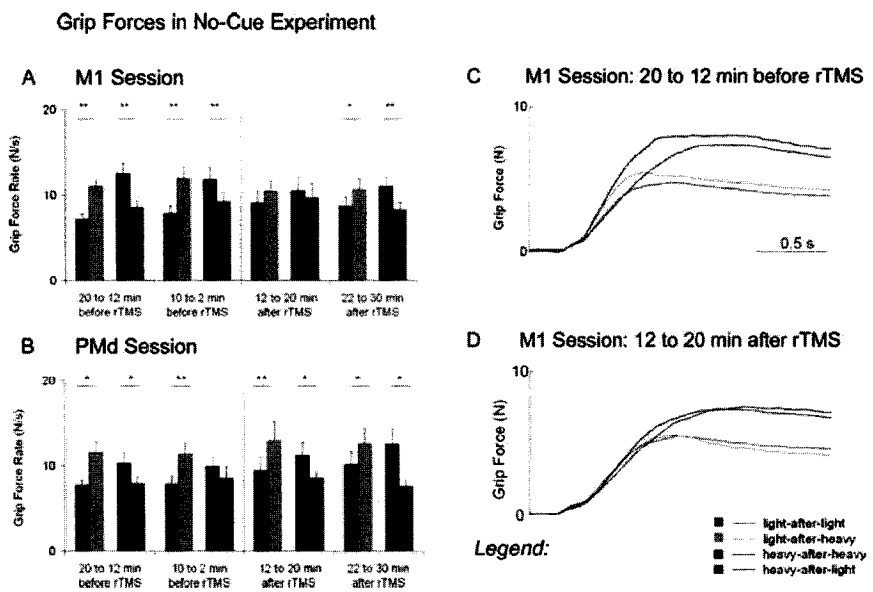

Figure 3. Grip forces in the no-cue experiment. ***A*** represents means ± SEM for the rates in grip force before and after repetitive stimulation over the primary motor cortex (M1). ***B*** represents means ± SEM for the rates in grip force before and after repetitive stimulation over the dorsal premotor cortex (PMd). ***C*** represents the overall average traces for grip forces 20–12 min before repetitive stimulation over M1. ***D*** represents the overall average traces for grip forces 12–20 min after repetitive stimulation over M1. Asterisks denote significant differences between switch conditions ($*p \leq 0.05$; $**p < 0.01$).

Effects of repetitive stimulation on load forces

An ANOVA on the rates in load force showed a switching × weight × block interaction in the session with repetitive stimu-

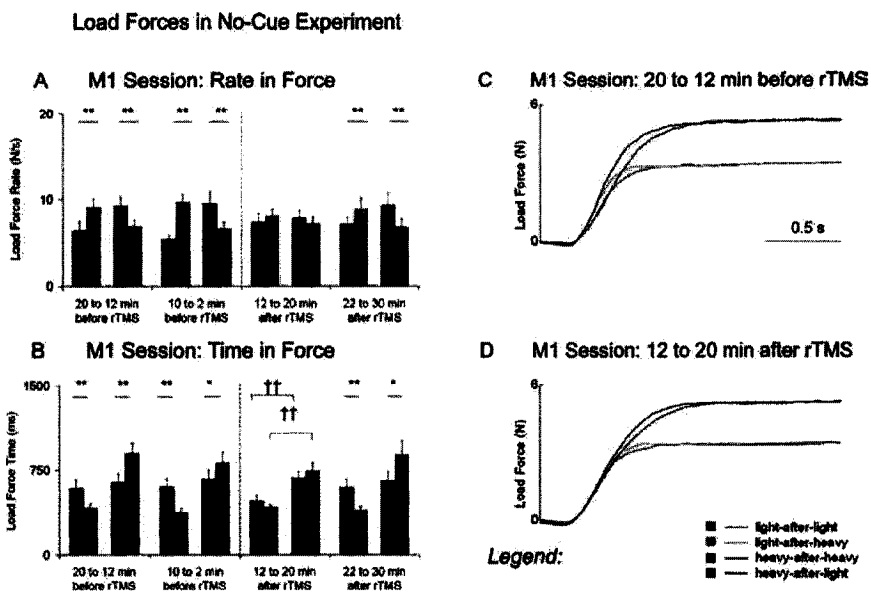

Figure 4. Load forces in the no-cue experiment. ***A*** represents means ± SEM for the rates in load force before and after repetitive stimulation over the primary motor cortex (M1). ***B*** represents means ± SEM for the load-force times before and after repetitive stimulation over M1. ***C*** represents the overall average traces for load forces 20–12 min before repetitive stimulation over M1. ***D*** represents the overall average traces for load forces 12–20 min after repetitive stimulation over M1. Asterisks denote significant differences between switch conditions (*$p \leq 0.05$; **$p < 0.01$). Daggers denote significant differences between weight conditions at post-1 ($^{\dagger\dagger}p < 0.01$).

lation over the primary motor cortex ($F_{(3,21)} = 7.26; p < 0.005$). Additional examination of this interaction reveals similar effects as those observed for the rates in grip force (Fig. 4*A*,*C*,*D*). An ANOVA performed on the load force times (Fig. 4*B*) also showed a significant switching × weight × block interaction in the session with repetitive stimulation over the primary motor cortex ($F_{(3,21)} = 5.98; p < 0.005$).

Before repetitive stimulation, the load-force times did not differ in the no-switch trials when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy ≈ light-after-light). Effects of switching, however, were present when changes in weight occurred between lifts. In the switch trials, the load-force times decreased after the weight became lighter (light-after-heavy < light-after-light) and increased after the weight became heavier (heavy-after-light > heavy-after-heavy).

Repetitive stimulation over the primary motor cortex resulted in distinct load-force times for the two different weights (Fig. 4*B*,*D*). In the no-switch trials, the load-force times at 12–20 min after rTMS were longer when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). In the switch trials, the load-force times at 12–20 min after rTMS did not decrease after the weight became lighter (light-after-heavy ≈ light-after-light) nor did they increase after the weight became heavier (heavy-after-light ≈ heavy-after-heavy). At 22–30 min after rTMS, the load-force times were similar to those before repetitive stimulation. These results suggest that although subjects at 12–20 min after rTMS applied similar rates in force in all four weight × switching conditions, the time to scale load forces prolonged for the heavy weight compared with the light weight.

The cue experiment

Effects of repetitive stimulation on motor excitability

An ANOVA on the MEP amplitudes showed an effect of time ($F_{(6,42)} = 3.12; p < 0.05$), no effect of site of stimulation ($F_{(1,7)} = 0.10; p = 0.76$), and no interaction of time × site of stimulation ($F_{(6,42)} = 1.85; p = 0.11$). These results demonstrate that changes in MEP amplitudes did not differ when we applied repetitive stimulation over the primary motor cortex compared with the dorsal premotor cortex. We performed Tukey's HSD tests to examine further the effect of time (Fig. 2*D*) and found significant reductions in MEP amplitudes 0–2 min after rTMS compared with 2–0 min before rTMS ($p < 0.05$) and 30–32 min after rTMS ($p < 0.01$).

Effects of repetitive stimulation on grip forces

An ANOVA on the rates in grip force showed a significant switching × weight × block × site of stimulation interaction ($F_{(3,21)} = 5.83; p < 0.005$).

Before repetitive stimulation, subjects in the cue experiment could use arbitrary color cues to scale rates in grip force for a current weight (Fig. 5*A*–*C*). In the no-switch trials, subjects applied faster rates in grip force when they lifted the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). In the switch trials, unlike the no-cue experiment, the rates in grip force did not differ after the weight became either lighter (light-after-heavy ≈ light-after-light) or heavier (heavy-after-light ≈ heavy-after-heavy). The lack of any switching effect indicates that subjects could use arbitrary color cues to scale for forces based on the current weight, even during trials after a switch in weight occurred between lifts. Repetitive stimulation over the primary motor cortex had no effect on the rates in grip force (Fig. 5*A*).

Repetitive stimulation over the dorsal premotor cortex resulted in the scaling of rates in grip force for a previous lift (Fig. 5*B*,*D*). In the switch trials, the rates in grip force at 12–20 min after rTMS increased after the weight became lighter (light-after-heavy > light-after-light) and decreased after the weight became heavier (heavy-after-light < heavy-after-heavy). At 22–30 min after rTMS, the rates in grip force were similar to those before repetitive stimulation. Direct comparisons between the light-after-heavy conditions confirm that subjects scaled their forces for a previous weight at 12–20 min after rTMS; the rates in grip force were faster when subjects lifted the light weight after the heavy weight at 12–20 min after rTMS compared with both before and 22–30 min after rTMS.

Effects of repetitive stimulation on load forces

An ANOVA performed on the rates in load force in the session with repetitive stimulation over the dorsal premotor cortex revealed a switching × weight × block interaction ($F_{(3,21)} = 3.91; p < 0.05$). These results are similar to those observed for the rates in grip force (Fig. 6*A*,*C*,*D*). The same ANOVA on the load-force times (Fig. 6*B*) also revealed a switching × weight × block interaction ($F_{(3,21)} = 10.93; p < 0.001$).

Before repetitive stimulation, subjects applied longer load-force times for the heavy weight compared with the light weight (heavy-after-heavy > light-after-light). Effects of switching, however, were not present when changes in weight occurred between lifts. In the switch trials, the load-force times did not differ

after the weight became lighter (light-after-heavy ≈ light-after-light) nor did they differ after the weight became heavier (heavy-after-light ≈ heavy-after-heavy).

After repetitive stimulation over the dorsal premotor cortex, subjects applied load-force times that reflected the scaling of forces for a previous weight (Fig. 6*B*,*D*). In the no-switch trials, the load-force times at 12–20 min after rTMS did not differ when subjects lifted the heavy weight compared with the light weight (heavy-after-heavy ≈ light-after-light). In the switch trials, the load-force times at 12–20 min after rTMS decreased after the weight became lighter (light-after-heavy < heavy-after-heavy) and increased after the weight became heavier (heavy-after-light > heavy-after-heavy). At 22–30 min after rTMS, the load-force times were similar to those before repetitive stimulation.

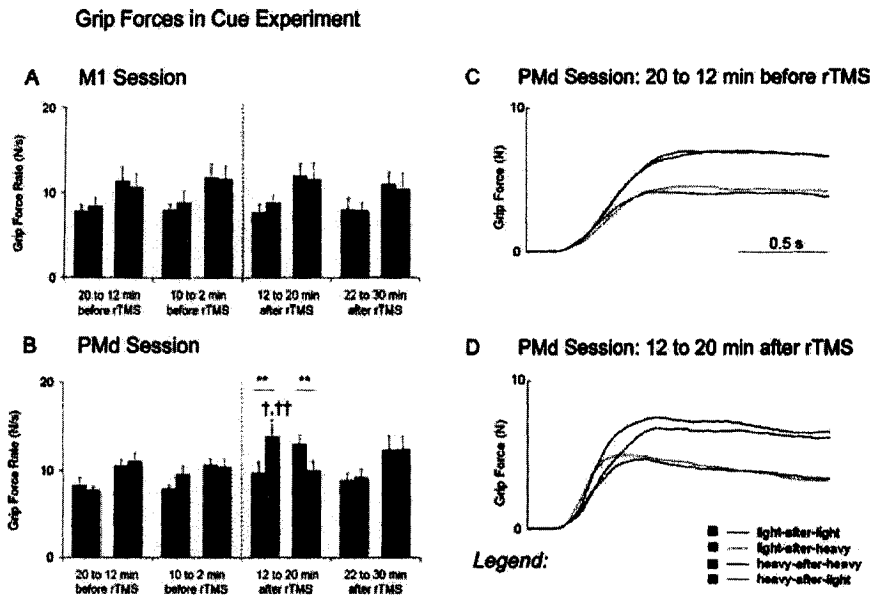

Figure 5. Grip forces in the cue experiment. ***A*** represents means ± SEM for the rates in grip force before and after repetitive stimulation over the primary motor cortex (M1). ***B*** represents means ± SEM for the rates in grip force before and after repetitive stimulation over the dorsal premotor cortex (PMd). ***C*** represents the overall average traces for grip forces 20–12 min before repetitive stimulation over PMd. ***D*** represents the overall average traces for grip forces 12–20 min after repetitive stimulation over PMd. Asterisks denote significant differences between switch conditions (*$p \leq 0.05$; **$p < 0.01$). Daggers denote significant differences between block conditions ($^{\dagger}p < 0.05$ vs pre-2; $^{\dagger\dagger}p < 0.01$ vs pre-1 and post-2).

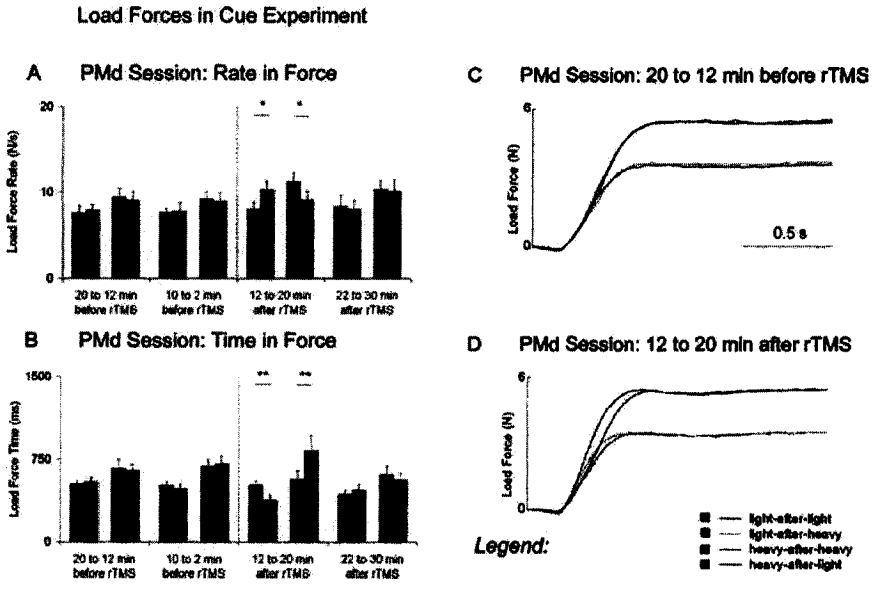

Figure 6. Load forces in the cue experiment. ***A*** represents means ± SEM for the rates in load force before and after repetitive stimulation over the dorsal premotor cortex (PMd). ***B*** represents means SEM for the load-force times before and after repetitive stimulation over PMd. ***C*** represents the overall average traces for load forces 20–12 min before repetitive stimulation over PMd. ***D*** represents the overall average traces for load forces 12–20 min after repetitive stimulation over PMd. Asterisks denote significant differences between switch conditions (*$p \leq 0.05$; **$p < 0.01$).

Discussion

Our results demonstrate that low-frequency repetitive stimulation applied over the primary motor and dorsal premotor cortices influenced differentially the anticipatory scaling of forces. When applied over the primary motor cortex, repetitive stimulation disrupted the scaling of forces based on information acquired during a previous lift. In contrast, when applied over the dorsal premotor cortex, repetitive stimulation disrupted the scaling of forces based on arbitrary color cues. Together, these findings indicate that during the lifting of different weights, the primary motor cortex scales forces based on information acquired during a previous lift and the dorsal premotor cortex scales forces based on arbitrary visual cues.

Methodological issues

Reductions in motor excitability occurred immediately after repetitive stimulation compared with a ~10 min delay observed in our previous TMS/positron emission tomography study (Chouinard et al., 2003). We speculate that the performance of precision grip before repetitive stimulation might have had a "priming" effect on motor excitability (Iyer et al., 2003). Note that changes in MEPs related modestly to changes in the precision grip. Thus, the MEP data provide only a verification of the effectiveness of rTMS over the two sites (Chouinard et al., 2003).

Our study did not examine the scaling of forces based on information about the three-dimensional (3-D) characteristics of objects. Both shape and size can influence the anticipatory scaling of forces that are applied during the lifting of small objects (Gordon et al., 1991a,b; Jenmalm and Johansson, 1997, 2000; Goodwin et al., 1998; Flanagan et al., 2001). The dorsal premotor cortex selects movements based mainly on learned associations as opposed to the more pragmatic visual and somatosensory analyses of shape and size (Geyer et al., 2000). In contrast, the ventral premotor cortex receives both visual and somatosensory information about the 3-D characteristics of objects from the anterior intraparietal area (Murata et al., 1997; Rizzolatti et al., 1998).

Our study also did not examine the actual learning of associations. Subjects in the cue experiment learned to associate quickly arbitrary color cues with weight and we found no differences in

performance during the two blocks before repetitive stimulation. Previous studies demonstrate that subjects can learn new associations as quickly as by the second trial in situations when the same weight has changed in physical size from bigger to smaller or smaller to bigger (Gordon et al., 1991b; Flanagan et al., 2001). To examine the actual learning of associations, we would have had to resort to a task that involves more associations and is more taxing than just two arbitrary cues and two corresponding motor outputs. Such a task would have to match in difficulty as the ones used in studies conducted by Petrides (1985b, 1997) in which six or nine different colored lights each cued subjects to perform a different hand gesture.

Primary motor cortex and anticipatory scaling of forces

As demonstrated in this study and in previous studies, when the weight of an object changes unexpectedly without any visible changes in appearance, people will generate inappropriate forces on the first lift and quite accurate forces on the second lift (Johansson and Westling, 1988; Gordon et al., 1993; Fellows et al., 1998). Measurements acquired in the no-cue experiment demonstrate that: (1) when switches in weight occurred between lifts, subjects scaled rates in force appropriate for a previous weight and not for a current weight, (2) both the production of distinct rates in force and the scaling of forces for a previous weight diminished 12–20 min after repetitive stimulation applied over the primary motor cortex and reemerged 22–30 min after repetitive stimulation, and (3) repetitive stimulation applied over the dorsal premotor cortex affected neither the production of distinct rates in force nor the scaling of forces for a previous weight.

In the no-cue experiment, repetitive stimulation applied over the primary motor cortex temporarily disrupted the subjects' ability to apply distinct rates in force when lifting different weights and to scale forces based on the previous weight. Additional analyses revealed that although subjects applied similar rates in force in all four weight × switching conditions, the load-force times prolonged for the heavy weight compared with the light weight. This additional finding suggests that subjects applied similar rates in load force until a sufficient vertical force was reached to overcome gravity.

It is important to note that in the cue experiment, repetitive stimulation over the primary motor cortex had no effect on the subjects' ability to scale forces for a current weight. This is likely because the arbitrary color cues provided subjects with information about what weight they had to lift, and the subjects were able to use this information to scale for differences in weight. Thus, the observed effects induced by repetitive stimulation over the primary motor cortex do not appear to be at the level of motor execution, but rather at the level of processing motor information associated with a recent experience. Indeed, a number of TMS studies have reported a similar lack of effects on motor execution despite reductions in motor excitability after low-frequency repetitive stimulation over the primary motor cortex (Chen et al., 1997; Muellbacher et al., 2000, 2002).

We speculate that the primary motor cortex can form memory traces associated with a recent experience. Cell recording studies in the monkey reveal that separate populations of primary motor neurons can process information related to motor function (Georgopoulos et al., 1982; Zhang et al., 1997; Wise et al., 1998; Li et al., 2001), including a population of memory neurons that stores information related to an experience beyond its duration. Li et al. (2001) examined activity in primary motor neurons before, during, and after motor adaptation. Their results revealed that a subset of neurons, which they called memory neurons, changed their firing properties as monkeys learned to perform forelimb movements in a force field. Once the force field was turned off, the firing properties of the memory neurons remained altered, and monkeys in turn produced inappropriate forelimb movements. A recent TMS study also demonstrated that repetitive stimulation over the primary motor cortex can disrupt adaptation in a similar force-field task (Cothros et al., 2004).

Dorsal premotor cortex and anticipatory scaling of forces

Measurements acquired in the cue experiment demonstrate that: (1) when switches in weight occurred between lifts, subjects could use arbitrary color cues to scale rates in force for a current weight, (2) the ability to use arbitrary color cues to scale rates in force for a current weight diminished 12–20 min after repetitive stimulation applied over the dorsal premotor cortex and reemerged 22–30 min repetitive stimulation, and (3) repetitive stimulation applied over the primary motor cortex did not affect the production of scaling of forces based on the arbitrary color cues.

Contrary to the first observation, Cole and Rotella (2002) found that subjects applied grip forces from a previous lift even in cases when they lifted different colored objects in which color informed them about texture. We speculate that the reason for this discrepancy is that subjects in Cole and Rotella's study had to extract and dissociate color from other visual characteristics (e.g., shape, size) that the brain could have associated with properties of the object during the previous lift. This differs from our study in which we presented arbitrary color cues on a computer screen.

The scaling of forces for a previous weight is associated with somatosensory information related to errors made during weight prediction (Johansson and Westling, 1988). An alternative explanation for our results could be that repetitive stimulation applied over the dorsal premotor cortex enhanced the use of somatosensory information from a previous trial in a manner that would drive subjects to ignore the cues and scale forces based on the previous weight. We argue against this possibility for two reasons. First, repetitive stimulation over the dorsal premotor cortex in the no-cue experiment had no effect on the manner with which subjects scaled forces for a previous weight. Second, cell recording studies in the monkey demonstrate that the dorsal premotor cortex contains only a few neurons that use somatosensory information to control for corrective forces during the precision grip (Boudreau et al., 2001).

Thus, the observed effects induced by repetitive stimulation over the dorsal premotor cortex appear to be at the level of coupling arbitrary visual cues and motor output. Indeed, cell recording studies in the monkey reveal that a number of dorsal premotor neurons increase their discharge activity after the presentation of an arbitrary visual cue that represents a learned association for a particular motor response compared with the presentation of a directional cue indicating a particular motor response (Kurata and Wise, 1988; Mitz et al., 1991; Kurata and Hoffman, 1994). $GABA_A$-agonist muscimol injections in the dorsal premotor cortex diminish the monkey's ability to select a correct response based on an arbitrary visual cue (Kurata and Hoffman, 1994). Petrides (1982, 1985a), as well as Halsband and Passingham (1982, 1985), have shown that the removal of the dorsal premotor cortex disrupts the ability to use arbitrary visual cues to make or withhold particular movements. Our current observations, together with these findings, reinforce the notion that the premotor cortex is critical for implementing associations between visual cues and motor responses.

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