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CHANGES IN THE BALANCE OF EXCITATION AND INHIBITION IN
THE HUMAN MOTOR CORTEX WITH VOLUNTARY MOVEMENTS

by

Charlene Reynolds

A thesis submitted in conformity with the requirements
for the degree of Master of Science
Graduate Department of the Institute of Medical Science,
University of Toronto

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The balance between excitation and inhibition of corticospinal neurons in the human motor cortex during voluntary movements was tested by conditioning the motor evoked potentials (MEP) evoked in forearm muscles by transcranial magnetic stimulation, with a preceding subthreshold stimulus through the same coil.

When subjects (n = 9) made a tonic wrist extension, inhibition of the forearm extensor MEP became less. When these subjects made a tonic wrist flexion, inhibition of the forearm flexor MEP diminished.

When subjects (n=10) made a phasic wrist extension, inhibition of the extensor MEP began to decline about 95 ms before the onset of the agonist electromyography (EMG).

The changes in balance of excitation and inhibition of corticospinal neurons precede voluntary movement and are focused on the corticospinal neurons projecting to the agonist motoneurons. These changes in balance may, therefore, help to select the pattern of cortical activity that leads to a discrete voluntary movement.
ACKNOWLEDGEMENTS

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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>CMAP</td>
<td>Compound motor action potential</td>
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<td>D-waves</td>
<td>Direct waves</td>
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<td>ECR</td>
<td>Extensor carpi radialis</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EMG</td>
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<td>EPSP</td>
<td>Excitatory post synaptic potential</td>
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<td>FCU</td>
<td>Flexor carpi ulnaris</td>
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<td>FDI</td>
<td>First dorsal interosseus</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<tr>
<td>IPSP</td>
<td>Inhibitory post synaptic potential</td>
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<td>I-waves</td>
<td>Indirect waves</td>
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<td>MEPs</td>
<td>Motor evoked potentials</td>
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<td>MI</td>
<td>Primary motor cortex</td>
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<td>MII</td>
<td>Supplementary motor cortex</td>
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<td>SE</td>
<td>Standard error</td>
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<td>SMA</td>
<td>Supplementary motor area</td>
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INTRODUCTION

Disorders of the human motor system are a major cause of chronic disability. To successfully manage these disorders, we need to know more about how the human motor system operates. The motor cortex is one of the principal output areas of the motor system. Lesions of the motor cortex interrupt the outflow pathway and result in disturbances of motor performance or even paralysis. The facilitatory components, corticospinal neurons, have been extensively investigated and reviewed (Porter et al., 1993; Rothwell, 1994), however, about 25% of cortical neurons are inhibitory (Benardo et al., 1995; Jones, 1995). Their role in voluntary movement has not been defined. Inhibition in the motor cortex could serve as a nonspecific "down-regulator" of cortical activity or it could be modulated in a focal manner to allow certain corticospinal neurons to be selected for voluntary movements. If changes in inhibition are involved in the performance of discrete voluntary movements, they should be 'focal' and should precede (rather than follow) the activation of motoneurons. I propose to test the hypotheses that changes in the inhibition of the motor evoked potentials (MEPs) that are associated with voluntary movements are 1. focal and 2. precede the movement.

This introduction will review: 1. The neural events that occur before a voluntary contraction, looking at evidence from both primate and human studies. 2. Transcranial magnetic stimulation (TMS), its properties and uses and 3. Cortical inhibition in both the mammalian and human motor cortex.

Neural events that precede a voluntary movement

Many cortical areas are active in the time preceding a voluntary movement. Ghez (1991) categorized these neural events into three complex processes: 1. identification and location in space, 2. plan of action for the movement and 3. the execution of the movement. Distinct regions within the cerebral cortex, mainly the primary motor cortex, premotor cortices and the posterior parietal cortex govern these complex processes and are important in motor preparation and motor control. This section will review the role of the main cortical
areas involved in the preparation for voluntary movement, mainly studied in primates and, as well, discuss the changes of the spinal H-reflex preceding voluntary muscle activity.

*Cortical areas involved in motor preparation*

*Primary motor cortex*

The primary motor cortex (MI) is found within the frontal lobe, in front of the central sulcus, anterior to the somatosensory cortex. The MI is somatotopically arranged, meaning that different regions of the MI bring about movement in different regions of the body. The primary motor cortex contains corticomotoneuronal or Betz cells that project down the spinal cord from the motor cortex and synapse on the motoneurons within the spinal gray matter. These monosynaptic cortical projections to spinal motoneurons represent only a small portion of the total corticospinal projections. There are also indirect pathways that are polysynaptic from the non-primary motor areas (Rothwell, 1994). Studies of the distribution of corticomotoneuronal cells projecting to motoneurons innervating different muscles have shown that, for distal muscles such as the hand and forearm, there are a large number of corticomotoneuronal cells projecting to spinal motoneurons and these cells are concentrated within a specific area of the cortex. Proximal muscles have fewer corticomotoneuronal cells controlling their movement and are distributed over a wider area of the cortex (Phillips et al., 1977; Porter et al., 1993).

The primary motor cortex is important for the *execution* of voluntary movement, but it also has a role in motor preparation. The ability to record the activity of individual cells in the motor cortex using microelectrodes within the brain of conscious animals has allowed for studies of the motor cortical cell activity that is associated with the initiation and control of voluntary movements (see Phillips et al., 1977 for review of technique developed by Evarts). Evarts (1966) measured pyramidal tract neuronal activity within the cortex in relation to voluntary muscle activity. Monkeys were trained to release a telegraph key using wrist extension in response to the onset of a visual stimulus. The discharge pattern of the pyramidal neurons along with the EMG activity from wrist flexor and extensor muscles was
recorded before and during the movement. Evarts (1966) found that the pyramidal neurons, as well as other motor cortical cells, began to fire before the onset of voluntary muscle activity. The pyramidal tract neurons modified their discharge pattern and became active about 80 to 100 ms before the onset of EMG activity (Evarts, 1966; Phillips et al., 1977). Evarts (1966) noted that the response at the retina to a photic stimulus had a latency of 30 ms and that the latency of the activity of the pyramidal neurons was 70 ms greater. He hypothesized that the neuronal events occurring in the intervening 70 ms represent the neuronal mechanisms involved in sensorimotor integration and the preparation for movement production.

Evarts, with Tanji (1976) further investigated the importance of pyramidal neuronal activity in the preparation of movement by having monkeys perform a task where they received instructions indicating the direction of the movement. The monkeys were instructed to either push or pull a handle from the midline hold position in response to a green or red light respectfully. A perturbation of the handle the monkeys were holding signaled the initiation of the movement. Neural cell activity was recorded from neurons in both the precentral and postcentral gyri. Tanji and Evarts (1976) found that in the period between the instruction and the EMG activity, approximately 60% of the pyramidal tract neurons within MI demonstrated changes in their activity with a specific direction. The authors summarized that “an instruction as to a forthcoming movement leads to anticipatory activity in motor cortex neurons” without a corresponding change in muscle activity (Tanji et al., 1976 p. 1066). They postulated that these changes in pyramidal tract neuronal activity could ‘preset’ the spinal cord excitability in the specific movement direction (Tanji et al., 1976). When the timing of the neuronal cells within the precentral gyrus was compared to the cells in the postcentral gyrus, the precentral cells were active before the onset of a voluntary movement, whereas postcentral cells were active after the voluntary movement had occurred (reviewed by Phillips et al., 1977; Porter et al., 1993; Rothwell, 1994).

MI not only demonstrates anticipatory activity, corticomotoneuronal activity is also related to the setting of movement variables such as force, direction and precision of movement. Studies have found that the discharge frequencies of corticomotoneurons encode
the amount of force in relation to movement (Cheney et al., 1980; Humphrey et al., 1991). Cheney and Fetz (1980) examined the activity of corticomotoneuronal cells within the MI cortex of monkeys while they performed two types of ramp-and-hold wrist movement: isometric responses with no displacement and isotonic with wrist displacement. The authors found that the corticomotoneuronal cells response pattern was correlated with torque generation rather than with displacement or velocity. This discharge pattern was variable in its latency, some cells fired before the onset of movement, some after. The neurons that discharged before the initiation of the movement may be facilitating the spinal motoneurons at a subthreshold level and through sensory feedback, the amount of force required and therefore the frequency of discharge of the corticomotoneurons is modulated and regulated during the movement task (Cheney et al., 1980; Picard et al., 1992).

Movement direction is encoded by the activity of the corticomotoneuronal cells within MI. To investigate direction encoding, Georgopoulos and colleagues (1982) studied neuronal activity when monkeys moved a handle to one of several targets arranged around a central starting position. They found that activity of individual neurons did vary with the direction of movement; the neurons fired briskly for movements in the preferred direction and were silent for movements in the opposite direction (Georgopoulos et al., 1982). Georgopoulos and colleagues also reported that movement direction was not encoded by a single cell, but the summation of many cells discharging. "... a given cell participates in movements in various directions, and ... conversely, a movement in a particular direction will engage a whole population of cells" (Georgopoulos, 1996 p. 607).

Contributions of neurons in MI during movement depend on the nature of the task being performed. Muir and Lemon (1983) compared the firing of corticospinal neurons in monkeys during the performance of a 'precision grip', between the index finger and thumb, with that during a 'power grip' when all the digits were used. The authors found that neurons that fire before and during the precision grip task remained silent when the monkey exerted the same force using the 'power' grip. These findings suggest that particular cortical neurons have a specific role in the performance of various voluntary movements as well as a
particular functional role in the fractionation of muscular activity needed to produce fine finger movements compared to gross movement (Muir et al., 1983)

Premotor cortex

The premotor cortex is defined as "the part of the agranular isocortex outside of the precentral motor (MI) and supplementary motor (MII) representations" (Weinrich et al., 1982 p. 1329). It receives input from the deep cerebellar nuclei by way of the thalamus as well as from a number of frontal, visual and auditory areas including the posterior parietal cortex (Wise, 1985; Hopkins et al., 1993). It sends efferent outputs to parts of the red nucleus, the medullary portion of the reticular formation as well as the primary motor cortex; there is little evidence showing it has direct projections to the spinal cord (Wise, 1985). The premotor cortex plays a role in motor preparation, however its exact contribution is poorly understood.

A number of hypotheses of the role of the premotor cortex in movement preparation, based on animal studies, have been put forth. These hypotheses include: the premotor cortex controls posture and orientation movements, mainly of the proximal musculature; the premotor cortex inhibits direct arm projection movements therefore allowing the implementation of more complex motor sequences; the premotor cortex specifies the final target position of the arm, particularly for visually guided movements and the premotor cortex plays a role in sensorily guided movements because of its extensive cortico-cortical connections with the posterior parietal cortex (Weinrich et al., 1982; Wise, 1985; Hopkins et al., 1993).

Based on the efferent projections to the red nucleus and reticular formation, the premotor cortex is believed to play a role in the control of posture, particularly stabilizing the trunk and limbs. However, few studies have been performed that demonstrate direct projections of the premotor cortex to the proximal and axial muscles (Wise, 1985). From primate studies, Wise (1985) believed that proximal fixation of the axial muscles and limbs could be considered an aspect of motor preparation for distal limb movements. It has been hypothesized that specific cells within the premotor cortex adjust the excitability of cortical and brainstem networks so to provide a background or 'postural set' for initiating the
upcoming movement (Hopkins et al., 1993). The premotor cortex couples posture with the perception of movement by providing preparatory ‘tuning’ of the axial and proximal musculature to enable the performance of specific tasks, particularly visuomotor tasks (Hopkins et al., 1993). This aspect of motor planning is thought to be important for the performance of distal movements such as visually guided grasping and reaching for an object (Wise, 1985).

Weinrich and Wise (1982) investigated the premotor cortex in the monkey. Single cell recordings were performed while two monkeys performed different behavioural paradigms of pressing a series of keys in response to visual and auditory cues. The visual or auditory cues were used to instruct the monkey as to the next movement required (the ready signal). The onset of the ‘go’ signal was randomized to allow for a delay period between the ready signal and the ‘go’ signal. The authors classified different cell units by their firing pattern in response to the movement. Movement-related units synchronized their activity bursts with the onset of voluntary movements, however the activity generally began before the movement. It was also found that these units were directionally specific, the cells showed sustained excitation after instruction for movement in one direction and demonstrated unchanged activity after an instruction for movement in the opposite direction (Weinrich et al., 1982). Set-related units demonstrated a change in activity level during the delay period between the ready and go signals. This increase in activity was not associated with any observable change in EMG activity (Weinrich et al., 1982). The authors concluded that the absence of a change in EMG activity precludes generalized co-contraction or muscle tensing during the delay period and therefore the set-related activity reflects movement preparation, specifically motor planning (Weinrich et al., 1982).

The activity of the movement- and set-related units within the premotor cortex in the above study demonstrates the role of motor preparation for sensorily guided movements. However, the authors did not have a behavioural paradigm where the monkeys did not make a motor response. Wise and colleagues (1983) analyzed neuronal activity when monkeys had been given an instruction concerning the next movement when no movement was to be made. The authors recorded neurons within the premotor cortex under two conditions:
movement of the forearm to a new position or maintenance of the current position. Visual cues used for the two conditions were identical. They found that the set-related cells differed markedly in their activity changes during the two conditions. The mean set-related activity during movement trials was greater than the activity in the no movement trials (Wise et al., 1983). The authors concluded that the set-related activity in the premotor cortex was correlated with movement planning and execution rather than the cues that guide the movements (Wise et al., 1983). “Regarding the motor aspects of sensorily guided movements, the vast majority of premotor cortex neurons have discharge modulations temporally correlated with and preceding the onset of movement” (Wise et al., 1983 p. 11).

Similar to the above-mentioned studies, Mauritz and Wise (1986) recorded premovement premotor cortical activity while the monkeys performed a visually guided motor task. The monkeys were first given an instruction stimulus, then a trigger stimulus that indicated the initiation of movement. The authors found that some neurons became active before the instruction stimulus, as in anticipating its location, therefore they concluded that the premotor cortex plays a role in anticipating predictable environmental events (Mauritz et al., 1986). It has been hypothesized that anticipation keeps the spinal motor neurons and the motor relay neurons near firing threshold therefore allowing one to react quicker and more effectively to a given stimuli (Weinrich et al., 1982; Mauritz et al., 1986).

Tanji (1996b) hypothesized that the premotor cortex, along with the supplementary motor cortex, is involved in the earlier stages of neural processing and influence the motor output from the MI. To clarify the differences between the two premotor areas, Mushiake and colleagues (1991) studied the regional differences using single cell recordings in monkeys while they performed sequential motor tasks under two conditions: visually guided or internally generated. The authors found that the premotor cells were more active when the motor task was visually guided and supplementary motor cells were more active when the sequence was self determined or internally driven. Mushiake et al. (1991) hypothesized that while little difference exists in the premovement neuronal activity between the premotor cortices during a simple task, a difference does exist during more complex tasks. When a monkey performs a more complex task, premotor cortical neurons are more active before a
visually guided, externally driven movement compared to supplementary motor cortical neurons, which are more active when the task is internally driven. This is further substantiated when a lesion occurs in the premotor cortex (Wise, 1985). Passingham (1985) reviewed studies of monkeys who had their premotor cortex removed and found the monkeys could not easily relearn or follow visual guided motor tasks. Passingham reported that "the premotor areas are concerned with the conditions for action ... and it appears to play a role in the direction of action based on information provided by the eye and ear about the outside world" (Passingham, 1985 p. 185). Although the exact contribution of the premotor cortex in motor preparation is unclear, the above studies have demonstrated it plays a role in posture-movement coupling, anticipatory activity before a voluntary movement as well as motor preparation preceding more complex, sensorily guided movements which are externally generated.

Supplementary motor cortex

The supplementary motor area (SMA) is located in the superior frontal gyrus, on the medial side of the cerebral hemisphere. In humans, this area is approximately six times larger than the equivalent area in the monkey (Rothwell, 1994). The SMA receives input from the basal ganglia by way of the thalamus as well as sending efferent connections back to the basal ganglia. It also sends efferent fibres to the primary motor cortex and the premotor cortex, as well, it has scant direct projections to the lower cervical segments of the spinal cord (Hopkins et al., 1993). As mentioned above, the SMA is preferentially active during internally generated tasks (Mushiake et al., 1991). Lesions within the SMA disrupt the ability to perform self-initiated movements (Mushiake et al., 1991).

Similar to the premotor cortex, the SMA has a preparatory role in programming sequences of movement. Although active during both simple and complex movements, the SMA is important for the temporal structuring of multiple movements in a predetermined order (Tanji et al., 1994; Tanji et al., 1996a; Tanji et al., 1996b). Tanji and Shima (1994) examined the SMA in two monkeys while they performed a sequence of movements. The monkeys were trained to perform three movements in different orders, and after a training
period were able to perform the sequential movements without any externally guided cues. The authors found a subset of neurons within the SMA that were preferentially active in relation to a particular order of forthcoming movements guided by memory. These cells were not active if the sequential order of the movement was different nor were they active if the movement was visually guided (Tanji et al., 1994). Another group of cells was found to be active during the waiting period before the execution of one of the sequential movements, but only after the performance of the preceding sequential movement (Tanji et al., 1994). The authors concluded that the SMA “is profoundly involved in performing multiple movements in a predetermined order” (Tanji et al., 1994 p. 415). The SMA is involved in preparation for and performing of temporal sequenced multiple movements based on memorization (Tanji et al., 1994; Tanji et al., 1996a; Tanji et al., 1996b).

The SMA is also important in coordinating bilateral hand movements. Brinkman (1984) evaluated the short-term and long-term effects of unilateral SMA lesions in monkeys. Initially she found the monkeys had bilateral clumsiness of the forelimb movements, involving both the proximal and distal muscles. The monkeys were unable to orient hand and fingers appropriately, resulting in awkward hand positions (Brinkman, 1984). In two monkeys studied up to one year post-operatively, the authors found that the monkeys were unable to do tasks requiring both hands. “... the two hands tended to behave in a similar manner instead of sharing the task between them” (Brinkman, 1984 p. 918). The author hypothesized that the SMA may inform the contralateral hemisphere of intended or ongoing movements through the corpus callosum to coordinate bilateral hand behaviour. Therefore, the corpus callosum was sectioned and found the bimanual deficit abolished (Brinkman, 1984). These results support the role of the SMA in the execution of complex movements and its importance in bilateral, coordinated movements.

It has also been hypothesized that the SMA is active during both forelimb and eye movements (Tehovnik, 1995). Through a review of multiple anatomical and electrophysiological studies investigating eye and forelimb movements in monkeys, the author hypothesized that the SMA might be involved in temporally regulated shifts of the eyes and forelimbs (Tehovnik, 1995). This role would be essential for the smooth execution
of visually guided arm and hand movements. Tehovnik (1995) also concluded that the eye field and the forelimb field have overlapping neural space within the SMA and that the SMA contains a map that represents eye position within the orbit, in relation to head position. This map of the SMA and head position organization may be used to specify a desired eye position within the orbit and to maintain gaze (Tehovnik, 1995). The SMA is believed to assist in coordinating binocular eye movements with hand movements during reaching and grasping movements.

Posterior Parietal Cortex

The posterior parietal cortex is not usually associated with motor areas, however lesions within this region result in movement deficits. The posterior parietal cortex is found in the parietal lobe, posterior to the primary sensory area. In the monkey, the posterior parietal cortex is divided up into two areas: 5 and 7. Both have reciprocal connections between each other as well as with the premotor and SMA areas within the frontal cortex (Stein, 1989; Rothwell, 1994) and the visual cortex as well as the primary somatosensory cortex (Hopkins et al., 1993).

The parietal cortex is associated with the visual control of hand movements (Jeannerod et al., 1995). Stein (1989) discussed that in monkeys, a select group of neurons within area 7 were found to be active in association with visually guided movements, such as reaching out to obtain food. Taira and colleagues (1990) studied a class of neurons within area 7 of the parietal cortex using single cell recordings. Neuronal activity was examined while monkeys manipulated objects by hand. The authors found the activity of the neurons within area 7 was related to active movements of the hand depending on the configuration and orientation of the object to be manipulated. They hypothesized that the parietal neurons mediate the matching of the movement pattern to the spatial characteristics of the target object by integrating the motor and visual signals (Taira et al., 1990). Patients with parietal lesions who have deficits in the ability to shape the hand before grasping and errors in hand orientation in space may lack this visuomotor integration in the posterior parietal cortex (Taira et al., 1990).
Area 5 of the posterior parietal cortex is believed to be the location that encodes the spatial parameters of position, direction and displacement associated with arm movements (Kalaska et al., 1990; Hopkins et al., 1993). A study by Kalaska et al. (1990) examined the single cell activity of area 4 and 5 neurons in monkeys while the arm was placed under different load conditions. The authors hypothesized that area 5 single-unit discharges would not be modified by the various loads compared to those found in the primary motor cortex, and that the population activity in area 5 would signal the spatiotemporal trajectory, regardless of the load condition. Their hypotheses were confirmed for they found that the activity in area 5 was only weakly affected by the loads placed on the arm and the overall pattern of population activity was unchanged. Kalaska and colleagues (1990) concluded that area 5 encodes the spatial parameters or kinematics of the movement while the motor cortical cells which demonstrated changes in activity during loading, signal the changes in force, torque or muscle activity. The posterior parietal cortex, as a whole, is important in the coupling of perception and movement to allow for refined goal-directed movement such as reaching and grasping to occur (Hopkins et al., 1993).

In summary, Hopkins and Gramsbergen (1993) suggest the following cortical sequence for the control of voluntary movements: the motor signal for initiating motor preparation is generated in the SMA, the signal is sent to the premotor cortex to be integrated with perceptual information. MI receives the integrated signal and then activates the appropriate musculature as well as controls the various parameters of movement such as force and direction. The posterior parietal cortex controls the kinematic parameters by combining the perceptual information gained by the moving limb with the kinetic parameters driving the musculature. Although oversimplified, this pattern of cerebral activation suggests that motor preparation and motor control is the result of the activation of a cerebral network where different areas work in concert rather than discretely separate.
Changes in the spinal H-reflex before a voluntary movement

The H-reflex reflects presynaptic inhibition of Ia afferents and the excitability of the spinal motoneuronal pool. A number of studies have examined the effect of a voluntary movement on the H-reflex (Gurfinkel' et al., 1965; Kots, 1969; Eichenberger et al., 1984; Burke et al., 1989; Stewart et al., 1993; Nielsen et al., 1996). Initial studies on the lower leg, found that the H-reflex of the agonist muscle increased 60-70 ms before the onset of voluntary muscle activity as recorded by EMG (Gurfinkel' et al., 1965; Kots, 1969). During the first 100 ms before the voluntary movement, little change is noted in the excitability of the motoneurons. The H-reflex begins to increase in amplitude approximately 60 ms before the movement and, just before the start of the voluntary movement, it is almost doubled (Gurfinkel' et al., 1965). Burke and colleagues (1989) examined the effects of a voluntary contraction on the H-reflex in the hand, forearm and lower leg and found that using a voluntary contraction to raise the motoneuron pool to firing threshold made it easier to elicit the H-reflex but had little effect on the reflex latency.

Kots (1969) examined the H-reflex of an antagonist muscle in the lower leg as well, and found that it did not change in the period leading up to the voluntary movement. However, coincident with the onset of the EMG activity of the agonist, the antagonist H-reflex activity sharply decreased. This inhibitory effect was further amplified if the H-reflex was elicited more than 30 ms after the start of the voluntary activity of the agonist muscle (Kots, 1969). The author concluded that that the reciprocal interaction of the motoneurons of the antagonistic muscles was under spinal rather than supraspinal control. If the supraspinal centres were controlling the reciprocal interaction, a simultaneous inhibitory influence of the antagonist motoneurons would have been expected (Kots, 1969). Conversely, Day and colleagues (1983) also examined the H-reflex of an antagonist and found that the H-reflex decreased approximately 30 ms before the onset of movement and therefore descending influences acting presynaptically on the Ia afferent terminals can not be ruled out. Gurfinkel' and Pal’tsev (1965) emphasized that when performing voluntary movements, “the fate of the superspinal influences depends on the state of the segmental apparatus of the spinal cord” (p. 950), therefore both cortical and spinal influences interact to produce voluntary movement.
Stewart and Brooke (1993) explained that the facilitation of the H-reflex prior to and during a movement is partially due to the release of Ia afferent fibres from presynaptic inhibition. The authors wanted to further investigate the presynaptic inhibitory regulation of motoneuronal excitability, particularly prior to a movement. Various trials were performed using a conditioning stimulus that was applied to the common peroneal nerve at varying intervals before the electrical stimuli used to elicit the H-reflex in the soleus muscle. The authors found that facilitation of the H-reflex immediately prior to a movement could be inhibited by stimulation of the antagonist muscles' nerve trunk and this inhibition was mediated entirely through presynaptic inhibition (Stewart et al., 1993). They concluded that before a movement, both peripheral somatosensory induced and centrally induced regulation of presynaptic inhibition could influence the excitability of the motoneuron pool (Stewart et al., 1993).

Tanji and Evarts (1976) hypothesized, based on work analyzing the descending influences of the cortex on the alpha motoneuronal excitability, that changes in the discharge of the pyramidal neurons within the motor cortex prior to an intended movement may give rise to changes in the excitability of the motoneurons of the muscle to be active. The development of transcranial stimulation has allowed for the non-invasive investigation of the role of the cortex in voluntary movement and therefore the cortical influence on the H-reflex will be discussed in the next section.

**Transcranial Magnetic Stimulation**

Transcranial magnetic stimulation, first introduced by Barker and colleagues in 1985 (as referenced in Rothwell et al., 1991) allows for non-invasive stimulation of the human cortex. Magnetic stimulation of the human motor cortex has been widely used to investigate the conduction time of the pathway from the corticomotoneurons in the motor cortex to the spinal motoneurons. This investigation has been performed in normals (Rothwell et al., 1987; Uozumi et al., 1991; Valls-Solé et al., 1995; Wassermann et al., 1996) as well as in patients with neurological conditions such as Parkinson's (Ellaway et al., 1995; Beradelli et
al., 1996; Valzania et al., 1997), multiple sclerosis (Snooks et al., 1985; Caramia et al., 1991), myoclonus (Brown et al., 1996), amyotrophic lateral sclerosis (Awiszus et al., 1995; Kohara et al., 1996; Salerno et al., 1996; Enterzari-Taher et al., 1997; Ziemann et al., 1997b) and dystonia (Ridding et al., 1995b; Rona et al., 1998). TMS combined with electromyography (EMG) allows for detection of prolonged latencies, which can provide earlier diagnosis and treatment for certain neurological conditions (Rothwell et al., 1991). TMS has been used in research for the purpose of mapping motor cortical areas and their functions (Levy et al., 1990; Taylor et al., 1995; Traversa et al., 1997), evaluation of facilitatory and inhibitory intracortical connections (Day et al., 1989; Davey et al., 1994; Ridding et al., 1995c; Ziemann et al., 1996), investigating neural plasticity (Ziemann et al., 1998) and pharmacological effects on the cortical connections (Ziemann et al., 1996; Ziemann et al., 1997a).

A magnetic stimulator consists of an electrical capacitor, which can be suddenly discharged producing a rapidly changing flow of current through a coil, creating a transient but intense magnetic field (Rothwell, 1997). This magnetic field, oriented perpendicular to the coil, induces eddy currents within the brain, exciting the underlying neural tissue (Rothwell, 1997). The magnetic field produced by TMS activates a large volume of neural tissue underneath the stimulus coil (~ 15 cm in diameter when using a round coil), making it difficult to focus the precise site of activation (Rothwell, 1997). A figure-of-eight coil attempts to provide a more focal stimulation by inducing a larger electrical field under the junction region on the coil (Rothwell et al., 1991). The induced current flows parallel to the surface of the brain and it may therefore preferentially activate neural structures that lie horizontally within the neural tissue (Day et al., 1989), such as stellate cells and corticocortical connections (Rothwell, 1997).

TMS differs from electrical stimulation in many ways. TMS can pass through body structures with little attenuation. The currents induced in the scalp by TMS are only a little greater than those induced in the brain below and therefore the procedure is virtually painless (Rothwell et al., 1991). As well, since little attenuation occurs, weaker stimuli can be used compared to that used with electrical stimulation. The current from electrical stimulation
flows between electrodes, with only a small percentage actually flowing into the brain tissue. This can cause local discomfort on the scalp and contraction of the scalp muscles. Magnetic stimulation causes less activation of the scalp and facial muscles resulting in less discomfort for the subjects involved (Rothwell et al., 1991; Rothwell, 1997).

The EMG response measured after TMS over the motor cortex has a longer latency than that produced by electrical stimulation. There is approximately a 2 ms difference in the latency of the motor evoked potential (MEP) in active muscles evoked by TMS compared to electrical stimulation (Rothwell et al., 1987; Hess et al., 1987). Day and colleagues (1989) hypothesized that this difference occurs because TMS activates corticospinal neurons transynaptically whereas electrical stimulation tends to activate the corticospinal axons directly. Direct activation produces ‘direct’ or D-waves where transynaptic activation produces ‘indirect’ or I-waves. The early D-wave produced by electrical stimulation has a rapid conduction velocity of approximately 60 – 70 m/s, followed by I-wave volleys approximately 1.5 ms later (Boyd et al., 1986). Nowak and Bullier (1998) demonstrated that electrical stimulation of the motor cortex in rats activated axons rather than cell bodies. It has also been shown in primates that these sites of activation are some distance below the cortex, at the cerebral peduncle or as far as the medullary pyramid (Edgley et al., 1997). However, Edgley and colleagues (1997) explain that because of the complexity of the cortical architecture, the site of activation of a corticospinal axon is sensitive to the direction of the induced currents and its interaction with the neuron.

Since TMS creates a current that flows parallel to the brain surface, preferentially activating interneurons, this transynaptic activation could be responsible for the preferential recruitment of I-waves by TMS (Hess et al., 1987; Day et al., 1989; Rothwell, 1997). With this being said, Rothwell (1997) does report that TMS can produce D-waves when the intensity of the stimulation is increased sufficiently therefore activating the pyramidal axons directly. This has also been recorded by Fujiki et al. (1996) in unconscious subjects who required a higher intensity to stimulate at threshold EMG activity, as well as by Di Lazzaro and colleagues (1998) in conscious humans who had spinal cord stimulators implanted for treatment of intractable pain.
The 'D- and I-wave' hypothesis has not been universally accepted for explaining the difference in the latencies between transcranial electrical stimulation and TMS. Burke and associates (1993) compared the effects of the magnetic and electrical stimulators in subjects undergoing spinal operations, before and after anaesthesia, but all unconscious. The authors recorded a D-wave in all the subjects in whom magnetic stimulation produced a corticospinal volley, however, the maximal D-wave evoked by TMS was smaller than that evoked by electrical stimulation. The authors concluded that although the corticospinal volley evoked by TMS was more biased towards I-waves than electrical stimulated volleys, their results do not support the 'D- and I-wave' hypothesis (Burke et al., 1993). The authors also noted that anaesthetics altered the thresholds for D- and I-waves to magnetic stimulation and therefore in alert subjects, threshold magnetic stimulation may produce I-waves rather than D-waves.

Influence of a voluntary contraction on motor evoked potentials

Motor evoked potentials are extremely variable in size, even when the force of the contraction, stimulus intensity and coil position are kept constant. MEPs are facilitated for up to 80 ms before the appearance of EMG when stimulated over the contralateral hemisphere (Starr et al., 1988; Rossini et al., 1998). The time course of the motor evoked discharges was found to be between 120 and 50 ms before the onset of the movement and as well, demonstrated an increase in firing rate as the interval to the onset of movement shortened (Starr et al., 1988). Starr and colleagues (1988) also discussed the resemblance of the facilitation of MEPs to the temporal features of the neural changes as recorded by EEG preceding the execution of a voluntary movement. The facilitation of MEPs reported in the study was similar to the negative potential that precedes the movement onset by 50-100 ms and was recorded in the precentral regions, contralateral to the moving limb.

A voluntary contraction of the target muscle during TMS decreases the onset latency by 2-3 ms, lowers the threshold and increases the amplitude of the muscle response (Hess et al., 1987; Rossini et al., 1988; Day et al., 1989; Chiappa, 1994; Ugawa et al., 1995; Kaneko et al., 1996a; Kaneko et al., 1996b; Di Lazzaro et al., 1998; Rossini et al., 1998). This
influence of facilitation is believed to be produced by processes occurring at both the cortical and the spinal level (Chiappa, 1994; Ugawa et al., 1995; Nakamura et al., 1997). In investigating the cortical influence, it was hypothesized that a voluntary contraction facilitates the corticospinal neurons with faster conduction velocities or that it activates the axons deep within the brain (Kaneko et al., 1996b). Kaneko and colleagues (1996b) however demonstrated that the latency shortening of the fastest component was only 0.1-0.2 ms and therefore, they do not believe the existence of a deep intracortical mechanism.

Hess and colleagues (1987) hypothesized that a cortical mechanism must come into action when magnetic stimulation is given during minimal voluntary activity. A sharp rise in twitch force and motor response amplitude was seen when the stimuli were given above threshold during low background muscle activity (1.5% of maximum). This marked facilitation during the background muscle activity was not seen when electrical stimulation was used. The authors hypothesized that when the subject activates the muscle, it focuses his attention on the motor performance, causing a rise in excitability of the pathways to that area and that this process resides in the cortex. The authors also support this hypothesis with the fact that it is not produced with electrical stimulation, since electrical stimulation bypasses the intracortical neuronal elements that regulate the descending volleys and acts directly on the corticospinal axons (Hess et al., 1987).

Ugawa et al. (1995) suggested a tonic voluntary contraction, particularly a large contraction (50% of maximum), lowers the threshold of some cortical elements so that they are more easily recruited by TMS and produce larger MEPs. In studying the facilitatory effect of a tonic voluntary contraction on responses to different types of stimulation over the motor cortex, the authors found that during larger contractions there was considerable enlargement of the MEPs from magnetic stimulation, where there was no effect on responses by electrical or foramen magnum level stimulation (Ugawa et al., 1995). The authors did not hypothesize what the physiological explanation could be for these cortical changes, but suggested that cortical facilitation may play a role in the responses to magnetic stimulation during other types of movements as well.
During a voluntary contraction, it is believed that the summation of descending impulses from cortical areas, afferent impulses from muscle spindles and the descending impulses from the magnetic stimulation activate the spinal motor neurons earlier than seen under resting conditions, bringing them closer to threshold and therefore shorten the onset of the MEP (Izumi et al., 1996; Kaneko et al., 1996a; Kaneko et al., 1996b; Di Lazzaro et al., 1998). Spinal summation during relaxation requires extra time to allow for successive MEPs generated by the cortical stimulation to temporally summate, creating a longer latency in the measured response (Kaneko et al., 1996b). Kaneko et al. (1996a) further explain that spinal summation is the mechanism responsible for shortening the latency of the MEPs during a voluntary contraction, because other studies have found no significant difference between the amplitudes of the MEPs following electrical cervical column stimulation compared to those following TMS.

Another possible explanation for facilitation is the excitation of spinal motoneurons that have a higher threshold and a faster conduction, which are only active during a voluntary contraction (Hess et al., 1987; Izumi et al., 1996). The slower conducting spinal motoneurons discharge at rest, while the faster spinal motoneurons are active during voluntary activity (Izumi et al., 1996). This facilitation of the MEP at the spinal level is similar to the size principle of motor unit recruitment first explained by Henneman and colleagues in 1965 (Rothwell et al., 1987; Hess et al., 1987; Starr et al., 1988; Izumi et al., 1996). The cortical volley first recruits small, slowly conducting motoneurons, which have a lower threshold for activation. As the force increases, the larger, faster units that have higher thresholds are recruited. Therefore, the MEPs resulting from transcranial magnetic stimulation reflect the excitability of cortical as well as spinal motoneurons, and during a voluntary contraction, the exact influence of the cortical and spinal mechanisms is undetermined.

*Inhibitory influence on the H-reflex*

Transcranial stimulation not only produces facilitatory or excitatory effects on the spinal motoneurons, but also inhibitory effects. Cowan et al. (1986) studied the effects of
submotor threshold electrical stimulation of the motor cortex on H-reflex excitability. The authors found that the electrical stimulation initially caused an increase in the H-reflex excitability, which lasted for approximately 1-2 ms and was then followed by an inhibitory effect which lasted a further 1-2 ms. Cowan and colleagues (1986), along with Rothwell et al. (1984), suggested that the inhibition of the H-reflex was due to activation of Ia inhibitory interneurons in the spinal cord by the descending corticospinal volley generated by the electrical stimulation. TMS has been used to further understand the inhibitory mechanisms within the human motor cortex. This will be discussed in the next section.

Cortical Inhibition

The cortical circuitry that makes up the cerebral cortex contains two basic classes of neurons: excitatory and inhibitory (Jones, 1995). The neocortex and various brain regions contain populations of excitatory cells with recurrent connections and strong interactions with local inhibitory interneurons (Tsodyks et al., 1997). A balance between excitatory and inhibitory connections regulates neuronal activity in the cerebral cortex. These inhibitory connections exert a restraining influence on the excitatory synaptic connections affecting cortical neurons. A compromise in this inhibitory influence can result in epileptic-like cortical activity (Benardo et al., 1995; Avoli et al., 1997). "Synaptic inhibition is now rightly and universally accepted as a crucial element in the control of neuronal firing, in the cortex as in other parts of the central nervous system" (Krnjevic, 1997 p. 439). Biochemical, electrophysiological and pharmacological studies have demonstrated that the neurotransmitter responsible for the inhibitory influence is γ-aminobutyric acid (GABA). It has been estimated that GABAergic cells form approximately 25% of the cortical neuronal population (Benardo et al., 1995; Jones, 1995; Gibbs et al., 1996).

There are two general classes of GABA receptors: GABA\textsubscript{A} and GABA\textsubscript{B}. GABA\textsubscript{A} receptors are ionotropic in type; they activate chloride channels and therefore increase the chloride permeability of the cellular membrane when GABA binds to it. This induces a fast inhibitory postsynaptic potential (IPSP). In contrast, GABA\textsubscript{B} receptors are metabotropic; they activate an enzyme by way of an interaction with a G-protein to produce a second
messenger. This metabolic cascade of reactions influences the ion current, hyperpolarizes the cell and produces a slower, later IPSP (Ghosh et al., 1988; Benardo et al., 1995). The receptors for GABA are distributed throughout the cerebral cortex and are evident on the membranes of the cortical cells and interneurons (Gibbs et al., 1996). Between the two receptors, GABA$_A$ receptors are considered of the greatest importance for brain function because the GABAergic synapses are constantly active and changing and therefore can be modulated by the use of pharmacological agonistic agents, whereas these agonists have no effect on GABA$_B$ receptors (Knjivic, 1991).

Two modes of inhibition have been recognized within the cerebral cortex: feedback and feedforward (Benardo et al., 1995). Most evidence of these types of inhibition comes from the hippocampus, but it is believed to apply to the neocortex as well (Benardo et al., 1995). Feedback circuits consist of pyramidal cells that have collateral axons that make contact with an inhibitory interneuron which in turn sends reciprocal connections back to the pyramidal cell causing it to be inhibited. The excitability of the cell is decreased post synaptically. Feedforward circuits consist of inhibitory interneurons that are activated by excitatory afferents (Benardo et al., 1995) and by way of presynaptic inhibition, reduce the amount of excitatory transmitter released. Together, these modes influence pyramidal cells and limit any excessive firing.

GABAergic neurons in the cortex have been identified through various methods: histochemical, autoradiography and immunocytochemistry (Benardo et al., 1995). Different GABAergic neurons within the cerebral cortex have been identified and are located at various levels within the cortical layers. Chandelier cells are present in layers II through to V of the cortex (Matsumura et al., 1992). These cells form synapses on the initial segment of the pyramidal cell. Since the initial segment is known to be the crucial region for initiating action potentials, the chandelier cells exert a very powerful inhibitory influence upon the post-synaptic pyramidal cell (Matsumura et al., 1992; Benardo et al., 1995). Basket cells, so called because they form nests around the cell bodies and the proximal dendrites of the pyramidal cells, are located mainly in layers III and V (Benardo et al., 1995). These cells axons vary in their direction and distance traveled; they can ascend or descend initially and
then travel horizontally for millimeters before forming nests or they can be smaller and localized (Benardo et al., 1995). Vertically oriented cells, also referred to as double bouquet cells, have bipolar dendritic fields and long axons. Their cell bodies are located only within layers II and III of the motor cortex (Benardo et al., 1995). The axon terminals of the double bouquet cells synapse on the side branches of apical dendrites and therefore it has been hypothesized that they “... send a shower of inhibition descending in a blanketing manner through the cortex, rather than being focused on single columns” (Jones, 1995, pg. 115). It is hypothesized that these inhibitory cells within the cortex provide a strong gating control mechanism of the descending output from the pyramidal neurons (Matsumura et al., 1992).

Inhibition in the mammalian cerebral cortex

The above information on cortical inhibition and GABAergic inhibition has been derived from extensive studies performed on animals, mainly rats and cats. Matsumura and colleagues (1991) demonstrated in primates that GABAergic inhibition in the motor cortex was involved in the spatiotemporal patterns of muscle activity. The investigators injected a GABA antagonist, bicuculline, into the motor cortex and found that when the monkeys moved their wrists they demonstrated co-contraction between the agonists and antagonists as well as an overall increased level of muscle activity (Matsumura et al., 1991). This disinhibition caused by the bicuculline, which lead to an increase in total muscle activity and improper timing between activated muscles, indicated the possible loss of reciprocal activity by the pyramidal neurons. The authors suggested that the GABAergic inhibitory mechanisms within the motor cortex might participate in the production and regulation of spatiotemporally organized muscle activity and therefore could be involved in the production of smooth reciprocal or directional movements (Matsumura et al., 1991). They also hypothesized that GABAergic inhibition in the primary motor cortex may be less involved with the control of simple movements and have greater involvement in more complex muscle activities (Matsumura et al., 1991).

Matsumura and colleagues (1992) further investigated the functional role of GABAergic inhibition on neuronal activity of the precentral and premotor cortices in the
primate. The authors recorded single neuron activity while the monkeys pressed and released a lever in response to a visual cue. GABA, its agonist muscimol and its antagonist bicuculline, were applied iontophoretically to the neurons. Matsumura et al. (1992) found that the activity of the movement-related neurons increased after bicuculline was applied and that the activity was enhanced at or near the phase of peak activity. Unidirectional activity was enhanced and in some cases changed to bi-directional activity. There was little change in their background level of activity (Matsumura et al., 1992). The authors concluded that GABAergic inhibition plays a role in both the regulation of the population of task-related neurons as well as the level of activity within the muscles (Matsumura et al., 1992). They also suggested that the GABAergic neural network amplify the small differences in neural activity that accompany different movements, allowing smooth, discrete movements to occur. As well, the effect of GABAergic inhibition may not be tonically constant throughout movement tasks; it was found to be greater during the performance-related phases (Matsumura et al., 1992). Therefore, during voluntary movement, motor neuronal activity within the cortex is not only generated by excitatory inputs, but are also subject to the influence of the GABAergic inhibitory mechanisms, and this influence may subserve the performance of smooth movement (Matsumura et al., 1992).

*Inhibition in the human cerebral cortex*

To investigate inhibition at the cellular level in the human motor cortex, studies have been performed on cortical samples obtained from patients undergoing surgical resection of brain tissue for medically untreatable seizures. Multitudes of in vitro and in vivo studies examining epileptic tissue have demonstrated that inhibition in the human cortex is also governed by GABAergic mechanisms (Avoli et al., 1989; McCormick, 1989; Avoli et al., 1991; Avoli et al., 1994). One must view these studies with caution since the tissue that is being examined is pathological in nature or has been under the influence of surrounding abnormal tissue and therefore might have altered cellular structure and function. Studies have demonstrated that the human cerebral cortex contains a high density of GABA receptors and GABAergic neurotransmission is important in controlling excessive activation of synaptic connections as well as maintaining the balance between excitation and inhibition.
(McCormick, 1989; Avoli et al., 1995; Gibbs et al., 1996). No studies have examined the human motor cortex in terms of its cellular inhibitory properties, however one could assume that the cellular makeup would be similar to that seen in the other areas of the cortex that have been investigated. With invasive examination of the human motor cortex being rare, the cortical inhibitory properties in the human motor cortex have been investigated using non-invasive transcranial magnetic stimulation.

**Testing inhibition in the human motor cortex**

The interneuronal inhibitory circuits in the motor cortex can be studied using a recently developed technique of paired conditioned-test transcranial magnetic stimulation. Ferbert et al. (1992) used a conditioning - test protocol to investigate transcallosal inhibition. The authors applied the weaker conditioning magnetic stimulus over the motor cortex of one hemisphere and evaluated the size of EMG responses evoked in the first dorsal interosseous muscle (FDI) by a stronger test magnetic stimulus given over the opposite hemisphere. They found that a conditioning stimulus over one hemisphere could inhibit EMG responses evoked by stronger magnetic stimulation of the opposite hemisphere given 6 to 30 ms later (Ferbert et al., 1992). Ferbert and colleagues concluded that the inhibition probably occurred at the cortical level because in relaxed subjects, the H-reflexes elicited in the flexor forearm muscles were unaffected by conditioning stimuli to the ipsilateral hemisphere. As well, test responses evoked by an anodal electrical shock in an active FDI were not inhibited by the contralateral magnetic conditioning stimulus (Ferbert et al., 1992). Anodal transcranial stimulation is considered to excite corticospinal axons directly thus bypassing changes in cortical excitability (Rothwell, 1997).

Kujirai and colleagues (1993) used a similar conditioning - test protocol to investigate the intracortical (also referred to as corticocortical) inhibitory system within the ipsilateral hemisphere. The authors applied paired stimuli over the same motor cortex and found that a magnetic conditioning stimulus given at an intensity subthreshold for eliciting EMG responses in relaxed hand muscle (FDI) could suppress the MEP in the same muscle by a suprathreshold magnetic test stimulus. This inhibition occurred at condition-test intervals
from 1 to 6 ms and was followed by facilitation at condition-test intervals 6 to 15 ms (Kujirai et al., 1993). The authors concluded that the suppression of the EMG responses was cortical in nature rather than spinal and that the magnetic conditioning stimulus elicits intracortical inhibitory circuits. It was hypothesized that there exists a relationship between the described inhibitory effects from motor cortical stimulation and the GABAergic mechanisms described in animal studies (Kujirai et al., 1993).

Many other studies have investigated cortical inhibition using TMS in normal subjects (Nakamura et al., 1995; Ridding et al., 1995c; Ziemann et al., 1996; Nakamura et al., 1997). In summary, the inhibition is thought to occur in the cortex because:

1. It can be produced by a conditioning stimulus that does not facilitate the EMG of an active muscle, implying that the stimulus is too weak to generate a descending volley in the corticospinal system (Ferbert et al., 1992; Ridding et al., 1995c).
2. The conditioning stimulus does not inhibit the spinal H-reflex or the MEP evoked by anodal electrical stimulation over the cortex (Ferbert et al., 1992; Kujirai et al., 1993 Figure 2 and Figure 6).
3. Less inhibition results when an anodal conditioning stimulus is used (Kujirai et al., 1993 Figure 4). Again, anodal stimulation is believed to excite corticospinal axons directly (Rothwell, 1997).
4. The conditioning stimulus is subthreshold for producing a descending volley of corticospinal activity, however the later I-waves produced by the test stimulus are reduced (Nakamura et al., 1997).
5. The inhibition is less in disorders such as cortical myoclonus where the pathology lies in the cortex, reflecting alterations in the efficacy of the intracortical circuitry (Ridding et al., 1995c; Brown et al., 1996).
6. The inhibition does not represent refractoriness of corticospinal neurons or their axons, and does not arise from collaterals since both would be activated by anodal stimulation (Kujirai et al., 1993).

To further understand the mechanism of the cortical inhibitory circuitry, TMS has been used in pharmacological studies to examine the drug effects on motor cortical
excitability. Since animal studies have demonstrated that the main inhibitory mechanism in the cerebral cortex is GABAergic in nature, drugs that influence the action and effectiveness of GABA have been used. Ziemann and colleagues (1995) studied the effect of ethanol on human motor system excitability in healthy volunteers. Ethanol was the drug of choice because it is a potentiator of GABA-mediated neurotransmission within the cerebral cortex (Ziemann et al., 1995). Paired TMS was used to evaluate intracortical inhibition and facilitation after the consumption of 0.7 litres of wine. The authors found that ethanol enhanced the intracortical inhibition and reduced the intracortical facilitation and concluded that this induced enhancement of the inhibition was due to the potentiation of GABA-mediated neurotransmission by ethanol (Ziemann et al., 1995). Ziemann and colleagues also hypothesized “that the suppression of intracortical facilitation by ethanol is not merely a consequence of the potentiation of direct GABAergic inhibition of corticospinal neurons, but most likely a potentiation of the inhibitory control of the population of excitatory interneurons which in turn project to the corticospinal cells” (Ziemann et al., 1995 p. 1444).

Ziemann and others have also investigated the effects of antiepileptic drugs as well as dopaminergic drugs on the excitability of the motor cortex (Ziemann et al., 1996; Ziemann et al., 1996a; Ziemann et al., 1996b; Ziemann et al., 1997a). Using the paired pulsed method of TMS the authors investigated the drug-induced changes on motor cortical excitability and inhibition. In support of the suppressive effect demonstrated by the ethanol study above, antiepileptic drugs that reinforce GABAergic action suppressed the intracortical facilitation and enhanced the intracortical inhibition while other antiepileptic drugs whose mode of action is to block sodium and calcium channels had no such effect (Ziemann et al., 1996a; Ziemann et al., 1996b). It was also found that with the administration of dopamine agonist pergolide, whose mode of action potentiates GABA transmission, intracortical inhibition was significantly enhanced (Ziemann et al., 1996). Conversely, antidopaminergic drugs decreased intracortical inhibition and increased intracortical facilitation (Ziemann et al., 1997a). The advent of paired condition-test TMS has allowed for the investigation of the interneuronal inhibitory circuits in the motor cortex, which depend on GABA and the modulatory capabilities of agonistic and antagonistic drugs.
Silent period

When magnetic stimulation is applied over the contralateral primary motor cortex, a silent period can be seen in the EMG of a voluntary, tonically active muscle. It follows the MEP and can last up to 300 ms (Classen et al., 1995). With low amplitude stimuli, there might be a pause without an evident MEP (Fuhr et al., 1991). Its mechanism is complex and a number of different mechanisms contribute to it. A study by Fuhr and colleagues (1991) investigated the spinal contribution of the silent period, working on the hypothesis that the possible mechanism of the silent period could be the inexcitability of the spinal motor neuron pool. The authors measured the H-reflex amplitude of the flexor carpi radialis during the silent period after magnetic stimulation of the contralateral motor cortex. The H-reflex was measured because it affects small motor units, similar to a weak muscle contraction according to the size principle and therefore is believed to involve the same part of the motor neuron pool that is silent after cortical stimulation during a weak tonic contraction (Fuhr et al., 1991; Ziemann et al., 1993). The H-reflex was depressed at the beginning of the silent period but recovered toward the end despite the lack of muscle activation. This recovery of the motoneuronal excitability towards the end of the silent period, despite the absence of muscle activity, led the authors to suggest that this phase was under suprasegmental influence (Fuhr et al., 1991). The authors concluded that segmental inhibitory influences were responsible for the initial portion of the silent period, but were unsure as to the exact mechanisms responsible. They hypothesized that Renshaw cells and Ia interneurons were likely to play a role, but the studies were not conclusive (Fuhr et al., 1991).

To study the possible mechanisms further, Ziemann et al. (1993) examined the time course of soleus motoneuron pool excitability using H-reflex testing after paired TMS. The authors wanted to determine whether corticospinal input renders the motoneurons inaccessible, therefore contributing to the silent period seen on the EMG. In this study, the soleus muscle was both relaxed and active during the testing. Similar to Fuhr and colleagues, the motoneuronal excitability returned to control values prior to the termination of the silent period. The authors concluded that early recovery of the motoneurons during the late part of the silent period was supraspinal in origin. Since the authors examined the silent period in an
active muscle, they could rule out some of the spinal inhibitory mechanisms thought to be responsible for the early portion of the silent period. Unlikely to contribute are: 1. Reciprocal inhibition since the soleus was active, therefore directing the inhibition to the soleus antagonist. 2. Refractoriness of the motoneurons since the refractory period in the human soleus does not exceed 3-4 ms. 3. Golgi tendon organ activation or muscle spindle unloading because they have latencies greater than 40 ms (Ziemann et al., 1993). The authors concluded that Renshaw inhibition and motoneuron afterhyperpolarization were the mechanisms most likely responsible for the early portion of the silent period (Ziemann et al., 1993).

In the above described studies that demonstrated potentiation of the GABAergic action of the inhibitory interneurons, they also measured a prolonged duration of the silent period (Ziemann et al., 1995; Ziemann et al., 1996a; Ziemann et al., 1996b). To further investigate if the lengthening of the silent period was really due to enhancement of inhibition at the cortical level, Ziemann and colleagues (1996b) also examined the peripheral silent period at the same time. The peripheral silent period was elicited by electrical nerve stimulation. After the administration of lorazepam, at the 5 hour period when the cortical silent period was at its maximum, no change of the peripheral silent period was measured therefore concluding that the silent period enhancement was due to cortically induced inhibition.

Alterations of the duration of the silent period have been reported in a number of patients with neurological disorders such as strokes (Braune et al., 1995; Braune et al., 1996; Catano et al., 1997), congenital mirror movements (Concotta et al., 1996), Parkinson's disease (Priori et al., 1994) and amyotrophic lateral sclerosis (Salerno et al., 1996). It has been suggested that before its routine application in clinical neurophysiology, further studies are required to better understand the physiology and the variable factors within the silent period (Ziemann et al., 1996a).
Cortical inhibition during a tonic voluntary contraction

Ridding et al. (1995c) studied cortical inhibition at rest and during a 5% maximum tonic voluntary contraction of the first dorsal interosseus. The authors used a transcranial magnetic conditioning stimulus that was below the threshold for evoking a MEP in a minimally active muscle to inhibit the MEP produced by the stronger stimulus. During voluntary contraction, the inhibition was significantly reduced at the 1, 2 and 3 ms condition-test intervals. The test MEP became larger during voluntary contraction so weaker test stimuli were used to match the original test MEP amplitudes. Overall, there was less inhibition at the shorter interstimulus intervals (1-5 ms) and less facilitation at the longer intervals when the target muscle was active compared to being relaxed (Ridding et al., 1995c). Ridding and colleagues (1995c) postulated that voluntary drive reduces the excitability of inhibitory circuits in cortical areas that project to the active muscle.

OBJECTIVE OF THESIS

The objective of my thesis is to study the importance of inhibition in the human motor cortex on voluntary movement. If the reduction of cortical inhibition is a major component of the normal mechanism of voluntary movement, one might expect a selective reduction in the inhibition of the corticospinal neurons which project to the agonist motoneurons, along with a selective increase in the inhibition of cortical neurons which project to antagonist motoneurons. Further, one would expect that the inhibition would be reduced prior to the onset of a voluntary contraction, before any feedback from the limb could reach the cortex.
HYPOTHESES TESTED

The hypotheses tested include:

1. The inhibition of MEPs in wrist flexor and extensor muscles during tonic wrist extension and flexion is reduced when these muscles act as agonists and increased when they act as antagonists.

2. That the reduction of inhibition occurs in the period leading up to a phasic agonist contraction.
METHODS

Subjects

Nineteen normal subjects were recruited for the study. The study had the approval of the institutional ethical committee (see Appendix). Each subject provided informed consent. Subjects would have been excluded if they had implanted electronic devices, intracortical metal objects, a history of epilepsy or if they had recently used any medications that might affect cortical excitability.

Recording EMG

The subjects were seated comfortably with the left arm resting on a table. Surface electrodes, 1 cm in diameter, were placed 4 cm apart over the extensor carpi radialis (ECR) and flexor carpi ulnaris (FCU) muscles. The EMG signal was amplified 10,000 - 50,000 times with a band pass of 100 Hz to 1 kHz. These filter settings were chosen to reduce movement artifact and to reduce volume conduction from distant muscles. The signal was monitored on an oscilloscope and loud speaker and was digitized at 2000 or 5000 Hz for computer analysis. The EMG signal was also rectified and integrated (time constant 600 ms) and the output was displayed on another oscilloscope. The voltage corresponding to a maximum contraction of the muscle was established and a DC level representing 5% or 30% of this value was displayed on the oscilloscope so that the subject could sustain a constant level of contraction of the active muscle when required. The loud speaker was used to provide feedback of any muscle activity when the subject was required to relax.

Assessing volume conduction across the forearm

In 6 subjects the compound muscle action potentials (CMAP) resulting from supramaximal stimulation of the radial, median and ulnar nerves were recorded using surface electrodes over ECR and FCU. An estimate of volume conduction across the forearm was obtained in the following way:
Suppose that supramaximal stimulation of the radial nerve produced a CMAP at the ECR electrodes of 10 mV (= e) and at the FCU electrodes of 2 mV (= 0.2 * e) and suppose that supramaximal stimulation of the median and ulnar nerves produced a total CMAP at the FCU electrodes of 9 mV (= f) and at the ECR electrodes of 2.5 mV (= 0.28 * f).

If both compartments were activated simultaneously, as might occur with transcranial magnetic stimulation, the total CMAP recorded at the ECR electrodes (E) and at the FCU electrodes (F) would be given by:

\[ E = e + 0.28f \]
\[ F = f + 0.2e \]

From the observed values of E and F, it is possible to deduce the components e and f arising from each compartment using the formulae:

\[ e = (E - 0.28F) / 0.944 \]
\[ f = (F - 0.20E) / 0.944 \]

For example, suppose that magnetic stimulation resulted in a CMAP at the ECR electrodes (E) of 400 μV, and at the FCU electrodes (F) of 80 μV. From the above equations, \( e = 400 \) μV and \( f = 0 \) μV. In other words, all the CMAP recorded over the flexors could be accounted for entirely by volume conduction from the extensors. These arguments assume that CMAPs summate in a more or less linear manner.

**Transcranial magnetic stimulation to elicit the test motor evoked potentials**

A pair of Magstim 200 stimulation units (Magstim, Dyfed, UK), joined by a BiStim module, was used to apply TMS. The 15 cm circular coil was placed on the scalp, over the contralateral motor cortex, at the optimal site where a MEP in the ECR muscle was consistently obtained at the lowest threshold. The stimulus intensity was adjusted to evoke a MEP of about 400 μV with the subject completely at rest. This was used as the ‘test stimulus’ (see Appendix Figure A-1).
Finding 'active threshold' for the conditioning stimulus

The subject was asked to make a steady 30% maximum voluntary contraction of the ECR, which was monitored using the integrated EMG signal on the oscilloscope. Weaker magnetic stimuli were delivered at the previously established optimal site at random intervals (approximately 1Hz) while 50 sweeps of 250 ms epochs of rectified EMG were averaged. A 50 ms pre-stimulus period was used to find the mean level of rectified, averaged EMG plus and minus two standard deviations. Peaks and troughs in the post-stimulus period were identified where they exceeded or fell below these levels. Facilitation of the EMG at latencies between 18-25 ms was assumed to represent the activation of the fast conducting corticospinal system. These runs were repeated and the stimulus intensity was adjusted in steps of 2% of the full scale of the stimulator until the threshold for activation of the 'active' corticospinal system was established. Stimulation at or just below this 'active threshold' was used as the 'conditioning stimulus'.

Paired stimulation with the subject at rest and during tonic wrist extension and flexion

With the subject at rest, either the test stimulus alone (control trial) or the conditioning stimulus followed 2 ms later by the test stimulus (conditioned trial) were delivered at random intervals and in random order until a total of 40 trials had been completed (usually 20 control and 20 conditioned). The maximum peak to peak amplitude of each MEP was measured automatically. The mean peak to peak amplitude of the conditioned MEPs divided by the mean peak to peak amplitude of the control MEPs provided a measure of the inhibition produced by the conditioning stimulus. A further 40 trials were carried out while the subject made a tonic wrist extension (approximately 5% maximum) and a further 40 trials during a tonic wrist flexion (approximately 5% maximum). This sequence: rest, wrist extension, wrist flexion, was repeated three times (a total of 360 trials). The mean values for the control MEP amplitudes and conditioned MEP / control MEP ratios for the rest, extension and flexion conditions were obtained for each subject. The MEPs were corrected for volume conduction using the formulae given above.
Paired stimulation before a phasic wrist extension

The subjects were seated comfortably with the left arm supported and were asked to remain completely relaxed. The computer generated a warning sound. This was followed, at a random interval between 2000-2500 ms, by a ‘Go’ signal. The subject was instructed to make a rapid, phasic extension of the wrist in response to the ‘Go’ signal. At random, either the test stimulus (control trial) or the conditioning stimulus followed 2 ms later by the test stimulus (conditioned trial) were delivered in the interval between the ‘Go’ signal and the onset of the voluntary contraction. The peak to peak amplitude of each MEP was measured automatically and recorded with the corresponding interval from the time of the stimulus to the first burst of voluntary EMG (length B to D in Figure 1). Approximately 80-100 trials were recorded for each subject. Natural variations in reaction time and the imposition of additional delays between the ‘Go’ signal and the stimulus ensured that a substantial range of these stimulus-to-EMG burst intervals (length B to D, from 50-200 ms) were available for analysis. Because of the large variation in the amplitudes of the individual MEPs from trial to trial and the large range of possible intervals (50-200 ms in 1ms increments), the data from all of the subjects were pooled (n = 870) and the mean MEP amplitude was derived for each 10 ms epoch from 50 to 199 ms. The mean amplitude of the conditioned MEPs divided by the mean amplitude of the control MEP provided an estimate of the inhibition in each of the 10 ms epochs from 199 ms to 50 ms prior to a voluntary wrist extension for both the extensor and flexor muscles. The MEPs were corrected for volume conduction using the formulae described above.

Statistical Analysis

The unpaired Student's t-test was used to compare the MEP amplitudes and the degree of inhibition in the relaxed and active conditions. An ANOVA was used to detect changes in the control MEPs in the course of the tonic contraction study. The Pearson Correlation Coefficient determined the relationship between MEP amplitude and degree of
inhibition. Unless otherwise stated, data is given as means ± standard errors (SE). Probabilities of < 0.05 were considered significant.
Figure 1. A schematic drawing of the method for testing inhibition of the MEP in the period before a voluntary contraction. A warning signal was given first (not shown). This was followed, after a random interval between 2000 and 2500 ms, by the 'Go' signal (A). Then, 0 to 70 ms later, either the test stimulus alone (top trace) or the conditioning stimulus followed 2 ms later by the test stimulus (bottom trace) was given (B). The MEP occurred ~18 ms after B, at C and the voluntary EMG at D. The peak to peak amplitudes of the control (top) and conditioned (bottom) MEPs were measured and related to the time BD.
RESULTS

Volume conduction of action potentials across the forearm

In 6 subjects, supramaximal stimulation of the radial nerve produced a mean compound action potential at the FCU electrodes of 20% (± 2.82) of that recorded over ECR. Stimulation of the median and ulnar nerves resulted in a CMAP at the ECR electrodes that was a mean of 28% (± 4.27) of that recorded over FCU. I used these values and the formulae given in the methods to estimate the true CMAP arising in each compartment from transcranial magnetic stimulation.

Paired transcranial stimulation at rest and during tonic wrist extension and flexion

These studies were carried out on 9 subjects aged 27 – 60 years (mean 33.7 years). All were right hand dominant. The conditioning stimuli ranged between 24% and 36% (mean 28%) and the test stimuli ranged from 42% and 60% (mean 53%) of the full scale of the stimulator.

Forearm Extensors

At rest, the mean MEP amplitude recorded over ECR was 418 µV (see Appendix Table A-1 for individual means). This was reduced by the subthreshold conditioning stimulus given 2 ms earlier (Figure 2). The mean conditioned MEP / control MEP ratio was 52% (Table 1). During a tonic wrist extension, the inhibition produced by the conditioning stimulus was now significantly less. The mean conditioned MEP / control MEP ratio was 75% ($F_{(2,24)} = 4.70, p = 0.019$). The MEP increased slightly, but this was not significant ($F_{(2,24)} = 1.06, p > 0.05$). During tonic wrist flexion, neither the MEP nor the degree of inhibition differed significantly from the values at rest (Table 1, Figure 3).
Table 1. Means of the control MEPs and of the MEPs conditioned by a subthreshold stimulus expressed as a percentage of the control MEP (conditioned MEP / control MEP %) for the forearm extensor and flexor muscles obtained at rest, during a tonic wrist extension and during a tonic wrist flexion. Each square contains the grand mean of 120 trials for each of the 9 subjects. The + indicates a significant difference (p<0.05) from the MEP value at rest and * indicates a significant difference (p<0.05) from the conditioned MEP / control MEP ratio (%) at rest. The values in the brackets are standard errors.
Figure 2. An example, from a subject at rest, of the averaged MEPs, recorded with electrodes over the ECR ('Extensor') and FCU ('Flexor') muscles of the forearm, produced by the test stimulus alone (top 2 traces) or the test stimulus preceded 2 ms earlier by a subthreshold conditioning stimulus (bottom 2 traces). Each trace is the average of 20 sweeps, vertical calibration ~ 400 μV. The conditioning stimulus reduces the amplitude of the MEP in both the extensors and flexors.
Figure 3. Means and Standard Errors of the MEP amplitudes and the degree of inhibition (conditioned MEP/control MEP %) in 9 subjects (360 trials per subject) at rest, during tonic extension and flexion of the wrist. During tonic wrist extension, the conditioning stimulus produced significantly less inhibition of the extensor MEP. During tonic wrist flexion, there was less inhibition of the flexor MEP. The control MEP was significantly increased in the flexors during tonic flexion.
Forearm Flexors

The MEPs recorded over the flexors were smaller than those recorded over the extensors. Volume conduction from the extensors completely accounted for the CMAP recorded over the flexors in 2 subjects at rest and in 5 subjects during wrist extension. The following values were calculated from data where MEPs were available. At rest, the mean MEP amplitude recorded with electrodes over FCU was 82 μV. A preceding conditioning stimulus reduced this. The mean of the conditioned MEP / control MEP ratio was 49% (Table 1). During tonic flexion, the mean MEP increased to 310 μV ($F_{(2,17)} = 5.02, p = 0.019$) and the inhibition became less, although this was just not significantly (mean control MEP / conditioned MEP ratio = 76%, $F_{(2,17)} = 3.20, p = 0.066$). During tonic extension, neither the MEP amplitude nor the degree of inhibition differed significantly from the values at rest (Figure 3).

Relationship between the MEP amplitude and the degree of inhibition

Contraction of the agonist was associated with an increase in the amplitude of the MEP of that muscle. Although this was not significant in the case of the extensor muscles, one had to consider the possibility that larger MEPs were harder to inhibit. No significant correlation was found between the degree of inhibition and the MEP amplitude in the data obtained from the 9 subjects at rest either for extensors ($r = 0.20, p > 0.05$) or flexors ($r = 0.22, p > 0.05$). To probe this further, in 6 subjects at rest, the test stimulus intensity was systematically varied to produce a large range of MEP amplitudes while holding the conditioning stimulus constant. This revealed a weak positive correlation between MEP amplitude and conditioned MEP / control MEP ratio for the extensors ($r = 0.47, p < 0.01$) but not for the flexors ($r = 0.04, p > 0.05$). When the mean conditioned MEP / control MEP ratios were plotted against the mean control MEPs with the subjects at rest and during extension (Figure 4), 3 of the 9 subjects demonstrated an increase in the ratio without an increase in the size of the MEP.
Figure 4. Data from 9 subjects showing the mean extensor MEP amplitude (x-axis) and degree of inhibition (y-axis) produced by the conditioning stimulus at rest (open circles) and during tonic wrist extension (closed circles). Each point is derived from 120 trials. The data from each subject is joined by a line. In 3 subjects the inhibition became less (% greater) without an increase in the MEP.
Was there any 'drift' throughout the study?

To determine whether there was any shift in stimulator position or adaptation in the response to magnetic stimulation during the study, I compared the mean MEP amplitude and the mean conditioned MEP / control MEP ratio for each of the three trials with the subjects at rest was compared. There was no significant change in the MEP amplitude during the trials for either the extensors ($F_{(2, 24)} = 0.128, p > 0.05$) or the flexors ($F_{(2,13)} = 0.339, p > 0.05$) and there was no significant change in the conditioned MEP / control MEP ratio over the three trials for either the extensors ($F_{(2, 24)} = 0.85, p > 0.05$) or the flexors ($F_{(2, 13)} = 0.386, p > 0.05$).

Inhibition of the MEP in the period leading up to a voluntary contraction

Studies of the change in the inhibition of MEPs in the period just before a phasic wrist extension were carried out on 10 additional subjects, aged 24-60 years (mean age of 30.8 years). One was left-handed. The conditioning stimuli ranged from 18% to 30% (mean 24%) and the test stimuli ranged from 38% to 56% (mean of 50%) of the full scale of the stimulator. The compound action potentials were corrected for volume conduction as described in the methods.

Inhibition of the MEP by a subthreshold conditioning stimulus with the subject at rest

With the subject at rest, a conditioning stimulus, below 'active threshold', applied 2 ms before the test stimulus, inhibited the MEP of both the forearm extensor and flexor muscles. In this group of subjects the mean extensor MEP amplitude fell from 340 $\mu$V to 229 $\mu$V (67%) ($p < 0.05$) (Figure 6, points on extreme left) and the mean flexor MEP amplitude fell from 74 $\mu$V to 47 $\mu$V (64%) ($p < 0.05$) (Figure 7, points on extreme left).
Figure 5. Data from a single subject showing the amplitude of the control MEPs (open circles) and the MEPs conditioned by a subthreshold stimulus 2 ms earlier (closed circles) plotted against the interval between the stimulus and the onset of voluntary EMG (time BD in Figure 1).
Inhibition of the MEP in the period before a voluntary phasic wrist extension

Subjects were now asked to respond to a 'Go' signal by making a phasic wrist extension. The test stimulus or the conditioning-test stimuli pair was randomly delivered in the interval between the 'Go' signal and the onset of voluntary EMG activity. For each of the 10 subjects, between 80 and 100 trials were recorded. An example is shown in the appendix Figure A-2. The time between the stimulus and the onset of voluntary EMG (length B to D in Figure 1) was plotted against the corresponding MEP amplitude. In every subject there was enormous variation in the MEP amplitude. The results of a typical study on one subject are shown in Figure 5. Because of this variation, the data from all 10 subjects was pooled and the MEP amplitudes in each 10 ms epoch from -199 to -50 ms were averaged.

For the extensors (Figure 6), the inhibition was significant in epochs from -139 ms to -100 ms (shown by the * above the data points). The inhibition became progressively less at epochs closer to the onset of the voluntary contraction. There was no longer any significant difference between the control and conditioned MEPs at bins containing intervals between -99 and -50 ms (five data points on the left in Figure 6 without the *). This occurred without any significant increase in the control MEP amplitude (open circles) compared to the values obtained at rest (extreme left) until the bin containing intervals -59 to -50 ms before the onset of EMG (shown by the + above the data points).

The MEPs in the flexor muscles were rather small and variable (Figure 7). They did not increase in the period before the phasic wrist extension. There was no consistent change in the inhibition produced by the conditioning stimulus (The * in Figure 7 shows were the mean amplitudes of the control and conditioned MEPs were significantly different).
Possible alteration of reaction times by TMS

The possibility that the control and conditioning stimuli differentially altered the reaction time was investigated as follows. Suppose that the control stimulus delayed the onset of EMG when given near to the EMG onset while the conditioning stimuli did not. A larger number of longer reaction times in the control group compared to the conditioned would be expected. The frequency of each reaction time (time BD in Figure 1) was plotted for the control and conditioned trials (Appendix Figure A-3). The overall mean BD times were: control = 98.5 ms, conditioned = 96.7 ms and the difference was not significant (t = 1.57, df = 8, p = 0.155). Even when the frequency distribution of the observations in the shortest three BD epochs was examined separately, the mean BD times were: control = 69.7 ms, conditioned = 68.2 ms, and were not significant. As well, when the frequency distribution of the observations in the longest three BD epochs was examined separately, the mean BD times were: control = 124.2 ms, conditioned = 124.6 ms, again not significant.

Was each subject studied through the whole range of possible reaction times?

To investigate this point, the number of observations at each of the intervals between the stimulus and the onset of voluntary EMG was plotted for each subject (Appendix Figure A-4). Most subjects have data at every interval category. An ANOVA showed no significant difference between the number of observations for each subject at each of the epochs during both the control (F(9,80) = 1.55, p = 0.145) and conditioned (F(9,80) = 0.820, p = 0.599) studies.
Figure 6. Mean amplitudes of the extensor MEPs evoked by the test stimulus either alone (open circles) or conditioned (closed circles), plotted against the time between the stimulus and the onset of voluntary EMG in ECR. The number of observations within each 10 ms bin is shown beside each point. The * indicate a significant difference between the control and conditioned values at that latency (p<0.05), and the + indicate where the amplitude of the control MEP differs from that obtained at rest.
Figure 7. Mean amplitudes of the flexor MEPs evoked by the test stimulus either alone (open circles) or conditioned by a subthreshold stimulus 2 ms earlier (closed circles), plotted against the time between the stimulus and the onset of EMG from FCU. The number of observations within each 10 ms bin is shown beside each point. The * indicate a significant difference between the control and conditioned values at that latency (p<0.05).
DISCUSSION

The MEP and inhibition of the MEP by a conditioning stimulus

These studies demonstrate that a weak transcranial stimulus inhibits the MEPs produced in the forearm flexor and extensor muscles by a stronger test stimulus given 2 ms later. This inhibition was first described for the small hand muscles (Kujirai et al., 1993) and is considered to represent events taking place in the cortex for the reasons given in the introduction.

Inhibition of the MEP by a subthreshold conditioning stimulus is a complex process. The stronger transcranial stimulus that elicits the test MEP is thought to produce a succession of facilitations of corticospinal neurons indirectly, that is, by activating axons or interneurons that excite the corticospinal neuron transynaptically (Rothwell, 1997). One or more of these facilitations may bring a group of corticospinal neurons to threshold, generating descending volleys (‘I-waves’) in corticospinal axons (Day et al., 1989; Burke et al., 1993; Di Lazzaro et al., 1998). The summation of the excitatory postsynaptic potentials (EPSPs) generated in motoneurons by these descending volleys may, in turn, bring a population of motoneurons to threshold, resulting in the MEP (Day et al., 1989; Kaneko et al., 1996a; Nakamura et al., 1997; Di Lazzaro et al., 1998).

The preceding weaker conditioning stimulus, although below the threshold for activating corticospinal neurons, appears to activate inhibitory interneurons within the cortex (Kujirai et al., 1993; Ridding et al., 1995c; Ziemann et al., 1996). The inhibition produced by the conditioning stimulus, the inhibitory postsynaptic potential (IPSP), reduces the effectiveness of the succession of facilitations provided by the test stimulus, resulting in fewer or smaller I-waves (Nakamura et al., 1997) and thus a smaller MEP. A decrease in the inhibition of the MEP could occur because this inhibitory influence is less or because the series of facilitations is greater.
Changes in inhibition of the MEP during a tonic contraction

Ridding and colleagues (1995c) showed that the inhibition of a MEP was less during a tonic voluntary contraction. The present study confirms that this reduction in inhibition is a focal process, affecting the corticospinal neurons projecting to the agonist. Both the flexor and extensor MEPs were less inhibited when the muscles were active in their agonistic role. Voluntary contraction also increased the amplitude of the agonist MEP (see below) but this alone is unlikely to account for the changes in inhibition. A decrease in the inhibition of the agonist MEP could occur without an increase in MEP amplitude and there was no relationship between MEP amplitude and the degree of inhibition for the flexors and only a weak one for the extensors. Ridding et al. (1995c) found no significant difference in the suppression of test MEPs that were less than 1 mV and those that were greater than 2 mV in subjects at rest (their Figure 3) and, in subjects making a voluntary contraction, the inhibition of the agonist MEP was less even when the intensity of the test stimulus was reduced to replicate the size of the original MEP at rest.

The experiments did not demonstrate a corresponding increase in inhibition of the corticospinal neurons projecting to the antagonist muscle. It could be that the cortical inhibition is already maximal at rest and that inhibition of antagonist motoneurons is accomplished through the collateral projections of corticospinal axons to Ia inhibitory interneurons (Jankowska et al., 1976). It could also be that there was co-contraction of the antagonist muscles. Attempts to avoid co-contractions were made by having the subjects make a weak agonist contraction (5% maximum) and instructing them not to use their finger extensors (which might precipitate a stabilizing co-contraction of the wrist flexors). Most subjects were able to make an isolated contraction of the agonist muscles, however, in some subjects, occasional motor unit discharges could be recorded from the antagonist muscles. As well, even those in whom no motor unit activity was seen, there might have been increased activity in the corticospinal system projecting to the antagonists, subthreshold for the activation of motoneurons so some degree of co-contraction can not be excluded.
Changes in the inhibition of the MEP before a phasic contraction

There is evidence that the excitability of a number of spinal and supraspinal neurons is altered in the 100 ms or so before a voluntary movement. The H-reflex of the agonist is increased beginning 50 to 100 ms before the onset of EMG (Kots, 1969; Pierrot-Deseilligny et al., 1973; Day et al., 1983; Eichenberger et al., 1984; Burke et al., 1989; Stewart et al., 1993) and that of the antagonist is decreased 30 to 40 ms before the onset of voluntary EMG (Pierrot-Deseilligny et al., 1973; Day et al., 1983) or at its onset (Kots, 1969). Because of disparities between H-reflex amplitude and motoneuron excitability (judged by the EMG), these changes in the H-reflex have been attributed to alterations in presynaptic inhibition rather than to alterations in alpha motoneuron excitability (Eichenberger et al., 1984). Subsequently, Hultborn et al. (1987) demonstrated a reduction in presynaptic inhibition of afferents projecting to agonist motoneurons at the onset of a voluntary contraction.

Spinal reciprocal inhibition to agonist motoneurons decreases and to antagonist motoneurons increases beginning 50 to 20 ms (Crone et al., 1989) or 40 to 20 ms (Day et al., 1983) before the onset of a voluntary movement. Since these changes precede the movement, they indicate that the spinal Ia interneuronal system can be controlled centrally (Day et al., 1983; Crone et al., 1989). Corticospinal neurons are known to project to Ia inhibitory interneurons in a variety of upper and lower extremity muscles in primates (Jankowska et al., 1976) as well as in the soleus muscle in humans (Iles et al., 1992; Kudina et al., 1993) and the cortical control of Ia inhibitory interneurons may be responsible for these changes in reciprocal inhibition preceding voluntary movements (Porter et al., 1993).

If there were any tonic activity in segmental afferents and spinal interneurons, the excitability of alpha motoneurons would, of necessity, be influenced by these premovement alterations in presynaptic and reciprocal inhibition. This may be one reason why transcranial electrical stimulation, subthreshold for a MEP at rest, results in MEPs of increasing size from about 80 ms prior to a voluntary movement (Starr et al., 1988). Anodal transcranial stimulation is considered to activate corticospinal axons directly (reviewed by Rothwell,
1997). The anodal MEP would therefore be expected to reflect the excitability of spinal not cortical neurons.

Transcranial magnetic stimulation excites corticospinal neurons indirectly (Rothwell, 1997), so the amplitude of the MEP evoked by magnetic stimulation would be expected to reflect the excitability of both cortical and spinal neurons. MEPs evoked by magnetic stimulation increase before a voluntary movement. Hallett et al. (1991) reported that a weak magnetic stimulus, that did not produce a MEP in the abductor pollicis brevis at rest, produced MEPs of increasing size beginning about 97 ms prior to EMG onset. Hoshiyama and colleagues (1996) examined the MEPs recorded with surface electrodes over the ECR and FCU muscles in the period before wrist flexion and extension movements. The MEP evoked by transcranial magnetic stimulation increased in the agonists and decreased in the antagonists from about 60 to 80 ms before the onset of agonist EMG. The authors postulated that the motor program activated corticomotoneurons that projected to the agonist motoneurons and to the associated Ia inhibitory interneurons that inhibit the motoneurons of the antagonist (Hoshiyama et al., 1996).

How much of the increase in the MEP is due to an increase in cortical excitability and how much to an increase in spinal excitability? Most attempts to make the distinction have employed tonic rather than phasic contractions. Nielsen et al. (1993) showed that a magnetic transcranial stimulus, too weak to affect the spinal H-reflex with the subject at rest, did facilitate the H-reflex during a tonic voluntary contraction. This did not occur with anodal stimulation implying that a voluntary contraction does produce a detectable increase in cortical excitability that would presumably contribute to an increased MEP. Mazzocchio and colleagues (1994) reported similar findings. Di Lazzaro et al. (1998) showed that tonic voluntary contraction increased the number and amplitude of I-waves produced by TMS. These studies confirm in humans the increases in cortical excitability, prior to movement that has been demonstrated in recordings from cortical neurons in monkeys (Evarts, 1966; Tanji et al., 1976).
However, Di Lazzaro and colleagues (1998) and (Kaneko et al., 1996a) reported that the increase in the MEP during a voluntary contraction was proportionally greater than the increase in the I-waves. Ugawa and colleagues (1995) found that during weak tonic contractions the MEPs from anodal and magnetic stimulation increased in parallel. Only when contractions reached 50% maximum were the MEPs evoked by magnetic stimulation significantly greater than those evoked by anodal stimulation. Mills and Kimiskidis (1996) reported that the MEP from anodal and magnetic stimuli were equally increased at the onset of a ballistic movement (their Figure 5). These studies seem to indicate that the increase in the magnetically evoked MEP associated with a voluntary contraction is largely due to an increase in spinal excitability. One explanation for this paradox is that a small change in cortical activity results in a large increase in spinal excitability and, for this reason, the increase in MEP appears to be largely spinal because the cortical component is too small to be detected.

The present experiments demonstrate that, in addition to all of these premovement spinal and cortical events, there is an additional change in the balance between excitatory and inhibitory interneurons in the motor cortex. When subjects made a phasic wrist extension, the inhibition of the MEP in the wrist extensor muscles was progressively depressed beginning when the stimuli were given about 95 ms before the onset of EMG. The changes in this balance of excitation and inhibition were independent of the changes in excitability of corticomotoneurons and motoneurons, as the lessened inhibition of the MEP could occur without a significant alteration in the MEP produced by the test stimulus alone (Figure 6). There was no detectable increase in the inhibition of flexor MEPs as was found for tonic wrist extension.
RELEVANCE

The present findings indicate that the changes in the inhibition of the MEP that accompany a voluntary contraction are focal (i.e. directed at the agonist) and that they precede the movement and are therefore not a consequence of it. This change in balance between inhibition and facilitation may therefore be involved in the selection of cortical neurons in the performance of discrete voluntary movements (Ridding et al., 1995c).

The reduction in MEP inhibition associated with voluntary movements could result from a decrease in inhibition produced by the conditioning stimulus (Ridding et al., 1995c) or an increase in the series of facilitations produced by the test stimulus. The present experiments cannot distinguish between these two possibilities. Inhibition in the cortex is likely executed by GABAergic neurons (Benardo et al., 1995). When GABA antagonists are injected directly into the motor cortex of monkeys trained to make wrist movements, there is more co-contraction (Matsumura et al., 1991) and movement-related neurons in the cortex increase their activity and become less directionally specific (Matsumura et al., 1992). This suggests that the inhibitory component is important in selecting the appropriate group of cortical neurons for a given movement.

In certain motor disorders, the balance between cortical inhibition and excitation, tested by paired magnetic stimulation, is abnormal. The inhibition of the MEP is less in cortical myoclonus (Brown et al., 1996). Inhibition of the MEP is also less in focal dystonia (Ridding et al., 1995b) and in parkinsonism but is restored by L-dopa (Ridding et al., 1995a). Pallidotomy increases cortical inhibition in Parkinson's patients, and is associated with improved control of movement (Strafella et al., 1997). Here it has been postulated that the decrease in cortical inhibition is secondary to abnormal output from the basal ganglia (Ridding et al., 1995a).
FUTURE RESEARCH

One of the problems with the interpretation of the present findings is that the reduction in MEP suppression associated with a voluntary contraction could be due to increased inhibition or decreased facilitation. The present study can not distinguish between the two. The effects could be distinguished by pharmacological studies in animals. In humans, GABAergic agonists, such as Lorazepan or Vigabatrin, would be expected to decrease the suppression of inhibition associated with a voluntary contraction but, given the variability of the MEP, this might be difficult to demonstrate.

The other question of interest is the control of cortical inhibition. Is the control of cortical inhibition exerted by other areas of cortex or by deeper structures such as the basal ganglia? Abnormal output from the basal ganglia in Parkinsonism decreases cortical inhibition, and is associated with impairment of voluntary movements. The influence of the basal ganglia on cortical inhibition might be studied in patients with stimulators in the subthalamic nucleus or globus pallidus.
REFERENCES


APPENDIX
Ethics Approval

APPENDIX

APPENDIX I

The Committee for Research on Human Subjects requires a review whenever patients are being subjected to procedures, the purposes of which go beyond the patients' need for conventional therapy. A review is also required for any type of research on healthy subjects.

A review is not required where an unproven form of treatment is used to help a single patient who has not responded to any of the accepted modes of therapy. In this situation the attending physician should assume the responsibility for ensuring that the procedure used is ethical.

BASED ON MRC.

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Wing & Rm. No. 13 Main Pavilion Room 319
THE TORONTO HOSPITAL
COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS
APPLICATION FOR RESEARCH STUDIES INVOLVING HUMAN SUBJECTS

The Committee for Research on Human Subjects meets on the third Monday of each month from September to July. Applications must be submitted 6 weeks prior to the meeting at which the investigator wants the proposal considered. All relevant sections must be completed and proper signatures obtained. A detailed research proposal should be submitted with this application. It is essential to summarize your proposal in lay terms on the form.

ALL SUBMISSIONS MUST BE TYPED and returned to the Research Ethics Committee Office, CCRW 2-814, (416) 340-4557 **INCOMPLETE APPLICATIONS WILL NOT BE ACCEPTED**

1. Principal Investigator(s): Telephone #: Department/Division:
   Peter Ashby 416-603-5017 Medicine

2. Researchers other than TTH who will have patient contact:

3. Project Title:
   Neurophysiological Studies in Man

4. Radiation Committee Approval: x NA YES (Date) NO
5. Biosafety Committee Approval: x NA YES (Date) NO
6. Resource Utilization Approval: x NA YES (Date) NO

PLEASE NOTE THAT HOSPITAL POLICY REQUIRES THE FOLLOWING INFORMATION TO BE PROVIDED BEFORE APPROVAL TO PROCEED MAY BE GIVEN:

7. Source of Funding (Budget, including the amount designated for overhead, MUST be attached).
   7a. Does the Drug Company have liability insurance extending to the investigator? YES NO
   7b. Indicate the cost centre to debit for overhead from contracts and clinical trials

Signature(s):

8. Investigators(s):

   [Signature]

   Department/Division Head: [Signature] 19-12-46

   *Note* This signature will be taken as assurance that the study described in this protocol is scientifically sound. Without such assurance the Ethics Committee CANNOT consider this study for its ethical merits. The department/divisional head also undertakes to ensure that the committee is notified should unexpected results occur or arise.

FOR OFFICE USE ONLY

APPLICATION NO. RE: DATE OF RECEIPT: DATE OF APPROVAL:
**REMEMBER SHEET**

Please provide three suggested reviewers:

REVIEWER (1)

Name & Department: Dr. Arline McLean MD., FRCP(C)  
Institute: Sunnybrook Hospital  
Address: 2075 Bayview Ave., Toronto, Ontario M4N 3M5  
Room: 2024  
City: Toronto  
Province: Ontario  
Postal Code: M4N 3M5  
Phone No: 480-4475  
Fax No: 480-6191  
Specialty: Clinical Neurophysiologist

REVIEWER (2)

Name & Department: Dr. J. Turley MD., FRCP(C)  
Institute: Wellesley Hospital  
Address: 160 Wellesley Street East  
Room: Dept. of Neurophysiology  
City: Toronto  
Province: Ontario  
Postal Code: M4Y 1J3  
Phone No: 926-7074  
Fax No: 926-5172  
Specialty: Clinical Neurophysiologist

REVIEWER (3)

Name & Department: Dr. Henry Berry, MD., FRCP(C)  
Institute: St. Michaels Hospital  
Address: 30 Bond Street  
Room:  
City: Toronto  
Province: Ontario  
Postal Code: M5B 1W8  
Phone No: 416-2640  
Fax No: 416-0320  
Specialty: Clinical Neurophysiologist
Identification: The research will be carried out in the Playfair Neuroscience Unit under the supervision of Dr. Peter Ashby.

Purpose: To explore the circuitry of the human nervous system in normal subjects and in patients with various motor disorders.

Procedure: Reflex activity will be induced by:

1- Non painful electrical stimulation over peripheral nerves using surface electrodes.
2- Brief mechanical taps to a finger.
3- Magnetic stimulation over the cortex to induce cortico-spinal volleys.
4- In a special group of patients, stimulation through electrodes chronically implanted in the basal ganglia for the control of movement disorders.

Reflex output will be recorded by:

1- Surface electrodes over voluntarily or reflexly activated muscles.
2- Standard sterile disposable EMG electrodes when recordings of single motor units are needed.

Risks:

Electrical and magnetic stimuli have no known side effects. There are no complications from the surface electrodes (unless the skin is excessively abraded during application). Needle electrodes cannot transmit infections as they are sterile, used once, and disposed of. It is possible that a haematoma could develop (particularly in patients on anti-coagulants) but such patients will be excluded from the studies.

Risks deep brain stimulation:

Patients with tremor or parkinsonism can be treated by continuous stimulation of the thalamus or basal ganglia. This procedure is replacing thalamotomy and pallidotomy because of the site and intensity of the effect can be varied after the operation. The electrode is implanted stereotactically and for a few days the leads are externalized for testing (stage 1). As a second procedure (stage 2) the wires are buried under the skin and connected to a device like a cardiac pacemaker. In the studies we propose, stimulation through these electrodes in the brain carries no additional risk if the proper procedures are used. When an external stimulator is used (stage 1) it will be a completely isolated, battery powered device incapable of delivering excess current. The recording system is also isolated to avoid any possibility of ground loops. When the internalized stimulator is used (stage 2) there are no additional risks. The overall risks and benefits of this form of treatment and of the surgical procedure are covered by the hospital consent form.

Population:

Normal subjects will be the investigators, lab colleagues and volunteers from the hospital staff. Patients with movement disorders attending the Toronto Western Division will be asked to volunteer after providing informal consent.

Relationship:
Patients will be contacted by the applicant or of the research fellows who will be carrying out the projects under supervision. Patients will not be contacted by the doctor responsible for their care.

Confidentiality of Data:

Patients will be identified only by a study number. The data will be retained in the laboratory. Subsequent publications will not identify patients in any way.

Compensation:

None.

1- Study of cortical inhibition in normal subjects and patients with motor disorders:

Normal Subjects: 20
Patients: 20
Stimulation: Non painful stimulation of motor cortex using magnetic stimulation.
Recordings: Surface electrodes, or EMG needle electrodes to record from voluntarily activated muscles in the forearm.

2- Study of cutaneous reflexes in patients with focal dystonias:

Normal Subjects: 20
Patients: 20
Stimulation: Non painful stimulation of peripheral nerves using surface or near nerve electrodes.
Recordings: Surface electrodes over muscles.

3- Study of neurophysiological effects of deep brain stimulation:

Normal Subjects: 0
Patients: 40
Stimulation: Various combinations of stimulation to peripheral nerves, visual flash, magnetic stimulation and stimulation through the thalamic and basal ganglia electrodes.
Recordings: Surface electrodes over muscles, recording finger position with a goniometer.
9- Description of proposed studies

1- Function of human deep brain nuclei. Electrodes are currently being placed in the thalamus to control tremor and in the globus pallidus internus (GPI) and subthalamic nucleus (STN) for treatment of parkinsonism. These patients provide a unique opportunity to study the function of deep brain nuclei in awake cooperating humans. We will examine patients (n=40) undergoing functional neurosurgery mostly in the few days after the procedure when the electrode wires are still lead out through the scalp and can be connected to an external stimulator controlled by a computer. Visual and somatosensory evoked potentials recorded from the electrodes will be used to identify their "physiological localization". As an initial screen for motor effects, single stimuli will be delivered at 1 Hz while rectified EMG is averaged from voluntary activated muscles. The type of neural element responsible for any observed motor effects will then be identified by establishing the chronaxie and refractory period. Deductions about the efferent fiber system involved will be made from the distribution and latency of effects in various muscles. The projections to motoneurons (monosynaptic/polysynaptic) will be identified by post stimulus time histograms of individual motor units. Effects on the excitability of the cortex and of the spinal cord will be assessed by transcranial magnetic stimulation and by the H-reflex. These physiological findings will be correlated with studies of functional MRI and clinical efficacy. The long term objectives are to understand the function of deep brain nuclei and to derive general principals about the actions of deep brain stimulation.

2- Inhibition of the human motor cortex. We will test the role of inhibition in the cortex in voluntary movements and motor disorders. Weak magnetic stimulation inhibits a voluntary contraction as well as the motor evoked potential produced by a stronger transcranial stimulus. This inhibition has been shown to be cortical. We will establish the time course of the depression of this inhibition which occurs before a voluntary movement and determine whether there is "reciprocal inhibition" in the cortex by measuring the changes in inhibition in antagonist/agonist muscle pairs during voluntary contraction of each. We will identify changes in cortical inhibition produced by stimulation of deep brain nuclei and test whether this inhibition is less in parkinsonian rigidity and whether it is restored by pallidotomy. Inhibition in the cortex may be largely GABA-mediated and we will develop methods to determine whether drugs with GABA-enhancing actions can be tested in this way. The long term objective is to develop further methods for investigating and understanding human motor cortex.
Disorders of the human motor system such as parkinsonism, stroke and ataxia are major causes of chronic disability and human misery. To manage these conditions intelligently we need to know how the human motor system operates.

We have developed non-invasive methods for studying the function of the human motor cortex in awake cooperative subjects and have studied the major out-flow pathways. We will now investigate the inhibitory mechanisms within the cortex which are believed to focus and refine motor activity.

Motor disorders such as tremor and rigidity can be treated by continuous stimulation of precisely selected deep brain centers. It is not known how this stimulation works or how it can be optimized. We have developed methods for investigating the pathways activated by these stimulators and their effects on the motor system. The study of such patients offers a remarkable opportunity to obtain vital information on the function of deep brain nuclei in humans and to improve our understanding of the ways in which this treatment can be used.
UTILIZATION OF DIAGNOSTIC AND NURSING SERVICES

If this study involves services above the normal clinical activity which would apply to the patient please consult with the respective division head and obtain his/her approval signature.

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# GENDER ISSUES RESEARCH

1. (a) Does this study include: (Please Check)
   - (a) men and women subjects [XX]
   - (b) women subjects only
   - (c) men subjects only

(b) If men and women subjects, do you plan to analyze the data for possible gender effects?
   - Yes [XXX] No

(c) If single sex subjects only Please Check reason(s):
   - (a) sex specific condition
   - (b) cost
   - (c) menstrual cycle confounds
   - (d) risks of pregnancy
   - (e) others (please specify)

2. Does this research involve a disease, disorder, process or condition which is unique, more prevalent or more serious in women, or which has different risk factors, course, interventions or outcome in women?
   - Yes [_____] No [XXXXX] Unknown [_____]

STUDIES OF MOTOR FUNCTION IN NORMAL SUBJECTS

This study is being carried out by Dr. P. Ashby, MD., FRCP(C), Neurologist at the Toronto Western Hospital (tel 603-5017), and Charlene Reynolds. The purpose of this study is to examine the motor function of the cortex.

1- Study the Reflex Pathways.
You will be asked to make a steady contraction of a hand muscle while electrical stimuli are given to the finger or wrist. Muscle activity will be recorded with surface electrodes over the muscles or with a sterile disposable needle electrode.

2- Study of the descending motor pathways.
You will be asked to hold a muscle contraction steadily while the motor pathways are activated by placing a magnetic coil over the scalp. This produces an audible click and a sensation like a tap. It is not painful. There are no adverse effects. Your doctor will check that you do not have

1- a cardiac pacemaker
2- an implanted hearing aid
3- epilepsy.

I understand that I can withdraw from the study at any time, simply by requesting to do so. I have had the opportunity to discuss this with Dr. Ashby and my questions have been answered to my satisfaction. Any information about me learned during this study will be confidential and neither my name nor any identifying particulars will be made available to anyone other than the investigators or appear in any publication without prior approval from me.

I agree to participate.

Subjects
signature:_________________Witness:_________________Date:________________
Figure A-1. A schematic drawing illustrating the placement of the magnetic stimulator over the forearm area of the contralateral motor cortex. Surface EMG electrodes were placed over the ECR ('Extensor EMG') and FCU ('Flexor EMG'). An example of a typical set of tracings is given at the bottom. The top two traces show the MEPs of the ECR and FCU during a control trial where only the stronger test magnetic stimulus was given. The bottom two traces show the MEPs where the weaker conditioning magnetic stimulus preceded the test stimulus by 2 ms.
Figure A-2. An example of two recordings from a subject during a brief, phasic wrist extension. The MEPs, recorded with electrodes over the ECR ('Ext') and FCU ('Flex') muscles of the forearm, were produced by the test stimulus alone (top 2 traces) or the test stimulus preceded 2 ms earlier by a subthreshold conditioning stimulus (bottom 2 traces). The peak to peak amplitudes of the MEPs were measured and recorded automatically, as shown by the numbers by the MEPs.
<table>
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<tr>
<th>Name</th>
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<th>ECR Conditioned / Control MEP (%)</th>
<th>FCU MEP (μV)</th>
<th>FCU Conditioned / Control MEP (%)</th>
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Table A-1. Individuals mean MEP and conditioned / control MEP ratio data for both the ECR and FCU at rest and during extension and flexion, corrected for volume conduction.
Figure A-3: Plot of the frequency of various intervals between the stimulus and voluntary EMG. The mean reaction time for the control runs was 98.5 ms and for the conditioned runs 96.7 ms.
Figure A-4. The number of observations at each time interval for each subject, for both the control (top) and conditioned (bottom) experiments. There was no significant difference between the subjects for the number of observations at each time interval.