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Neurophysiology Of Spared Motor Tracts In Spinal Cord Injury

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Neurophysiology of Spared Motor Tracts in Spinal Cord Injury

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Abstract

Recent experimental and therapeutic initiatives have been directed towards enhancing the survival and function of preserved central axons following spinal cord injury (SCI). The continued development of these initiatives depends largely on the sensitivity of techniques to detect the presence of residual innervation in descending motor tracts. Detection of preserved innervation in SCI patients provided the focus of the present thesis.

Preserved motor innervation was investigated in patients with established SCI using transcranial magnetic stimulation of the motor cortex to elicit motor evoked potentials (MEPs) in muscles innervated below the level of the lesion. In particular, a set of experiments was designed to enhance the probability of eliciting MEPs or detecting subliminal innervation in patients with SCI.

Experiment 1 tested the hypothesis that cutaneous afferent stimulation facilitates MEPs in lower limb muscles. This was demonstrated and therefore may be used to reveal latent but preserved innervation in SCI patients.

Experiment 2 tested the hypothesis that induced whole body hypothermia would enhance the detection of MEPs in control subjects and patients with SCI. While MEP amplitudes were significantly ($p < .05$) enhanced in control subjects and some high functioning SCI patients, hypothermia was not helpful in revealing latent innervation in patients with severe SCI.

Experiments 3 and 4 used subthreshold and suprathreshold cortical conditioning of lower limb H-reflexes to reveal preserved short and long latency facilitation of lumbosacral

motor neurons in control subjects and SCI patients. The principal finding was that residual subthreshold descending influences in patients with SCI, which were previously undetected by clinical assessment or cortical stimulation, were detected by cortical conditioning of H-reflexes in some patients with severe SCI.

A second important finding was the detection of late facilitation (60 - 150 ms) following subthreshold cortical stimulation. This result establishes descending supraspinal innervation as a potential source of the late excitatory synaptic inputs. Cortical conditioning of H-reflexes provides a viable new means to detect preserved innervation in descending motor tracts.

Collectively, these results provide support for the emerging concept that patients with SCI may possess intact but latent innervation despite the absence of useful sensory or motor function.

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Chapter 1 - Introduction

1.1 - Background

Society has long been aware of the severe consequences of spinal cord injury (SCI). The various signs and symptoms of SCI, and the bleak prognosis associated with trauma to the cervical vertebral column, were first described in the Edwin Smith Surgical Papyrus over 4000 years ago (Breasted, 1930). It has only been over the last century, however, that the survival rate and quality of life of patients with SCI has been appreciably improved. This has been due largely to the efforts of pioneers such as Riddoch and Guttmann who established specialized rehabilitation centers resulting in improved management of the myriad of complications typically encountered by patients with SCI (Donovan et al., 1984; Frankel, 1987; Geisler et al., 1983; Guttmann, 1976)

The importance of the proper initial handling of an individual with suspected SCI has recently been realized (Collins et al., 1986; Kakulas, 1987; Meyer, 1989) resulting in advances in the training of paramedics and others who provide first aid. As well, major initiatives have been directed towards the development of treatment techniques to be administered immediately after the accident. These include the application of local hypothermia (Albin et al., 1968; Ducker & Hamit, 1969) and various pharmaceutical approaches (Faden et al., 1981; Flamm et al., 1982; Geisler et al., 1991) of which the most promising appears to be high-dose steroid therapy (Bracken et al., 1990, 1992; Ducker & Zeidman, 1994; Hall & Braughler, 1982). These approaches seek to minimize the damage caused by secondary injury response mechanisms which contribute to the loss of function

(Collins et al , 1986; Ducker & Assenmacher, 1969; Freeman & Wright, 1953; Young, 1987). The aim of these initiatives has been to enhance neuronal survival and thereby minimize the loss of function following SCI. Methods for enhancing neuronal survival become especially important when one considers that only a small proportion of fibers (i.e , 5-10%) are necessary to subserve useful sensory and motor function (Eidelberg, Story, et al., 1981; Eidelberg, Walden, et al., 1981; Kaelan et al., 1989; Noordenbos & Wall, 1976; Windle et al., 1958).

In addition to these approaches which serve to enhance neuronal survival, there have been recent developments in the emerging field of restorative neurology whose aim is to improve function within existing neural structures (Dimitrijevic, 1985). For example, evidence from experiments conducted on various compressive and contusive animal models of SCI (Blight & Decrescito, 1986; Gledhill et al., 1973; Griffiths & McCulloch, 1983) as well as from neuropathological examination of human cords (Bunge et al., 1993, 1995; Byrne & Waxman, 1990; Holmes, 1906) has revealed that demyelination of central axons appears to comprise a significant component of the pathology in some patients with SCI (Waxman, 1989). This revelation has led to recent trials using the potassium channel blocking drug, 4-aminopyridine, in an attempt to ameliorate the conduction deficits due to demyelination in these patients (Hansebout et al., 1993; Hayes, 1994 ; Hayes, Blight, et al., 1993; Hayes et al., 1994).

Emerging neuropathological and neurophysiological evidence provides further support for these clinical and experimental initiatives which seek to enhance the survival and function of axons following SCI. For example, there has been mounting pathological

evidence describing the surprising extent of intact axons following trauma to the spinal cord even in rather serious injuries (Bunge et al., 1993; Kakulus, 1987; Kakulas & Taylor, 1992). In addition, Dimitrijevic and his colleagues have demonstrated that there are a significant number of patients with SCI who possess intact descending axons, as determined by electrophysiological tests, despite being assessed as having clinically complete spinal injuries upon neurologic examination (Dimitrijevic, Hsu, et al., 1992, Sherwood et al., 1992).

Electrophysiological techniques which allow the sensitive detection of preserved axonal continuity in patients with SCI are critical to the development and evaluation of therapeutic approaches which seek to enhance neuronal survival and improve function in surviving neurons. One new technique being used for this purpose is transcranial magnetic stimulation of the motor cortex wherein a pulsed magnetic field is employed to induce a depolarizing current in pyramidal tract neurons (Barker et al., 1985). The muscular responses to cortical stimulation, termed ***motor evoked potentials*** (MEPs), are recorded as compound muscle action potentials and are thought to be mediated through fast conducting corticospinal pathways (Amassian et al., 1990; Brouwer & Ashby, 1990; Edgley et al., 1990; Rothwell et al., 1991). Detection of MEPs in patients with SCI confirms the continuity of the corticospinal pathway. However, MEPs are often difficult to elicit and have several characteristics which limit their detection in these patients (Dimitrijevic et al., 1988; Hayes et al., 1991). For example, MEPs evoked in patients with SCI are often of low amplitude, temporally dispersed, long latency, and labile. This has prompted several investigators (Hayes et al., 1991; Lissens et al., 1992; Dimitrijevic, Hsu,

et al., 1992) to explore methods by which neurological reinforcement may increase the sensitivity of cortical stimulation to detect preserved innervation in patients with SCI.

The sensitive detection of residual innervation is beneficial for a variety of reasons. It is helpful in assessing the subtle effects of therapeutic and experimental manipulations. This may lead to more appropriately guided rehabilitation and aid in the development of experimental interventions. There may also be a potential role for these techniques in providing prognostic information. Presently, the value of MEPs in predicting future recovery is promising but has yet to be established (Levy et al., 1987). Therefore, methods which enhance the sensitivity of transcranial magnetic stimulation in detecting residual innervation have widespread application in the management of patients with SCI.

1.2 - Research Objectives and Plan

The primary objective of the present thesis was to examine the presence and nature of preserved motor pathways in patients with SCI. Three techniques were employed to enhance the detection of residual influences in these patients using transcranial magnetic stimulation of the motor cortex.

The three procedures were:

a) conditioning of MEPs using preceding cutaneous afferent stimulation,

b) enhancement of MEPs using induced whole body hypothermia, and

c) cortical conditioning of H-reflexes in the lower limb. The H-reflex is a predominately monosynaptic reflex elicited by electrical stimulation of large diameter afferents which allows determination of changes in motor neuron pool excitability (Magladery, 1955; Taborikova, 1973).

A set of studies was designed, using these procedures, to determine the nature and properties of preserved innervation in patients with SCI. These studies were focused on obtaining new information relating to the following theoretical issues:

a) the source of late arriving inputs to the motor neuron pool following cortical stimulation. These inputs are thought to reflect conduction in descending pathways and are separate and distinct from the short latency inputs which reflect conduction in the fast-conducting corticospinal pathways.

b) the presence and detection of subliminal corticospinal influences on the segmental interneuronal or motor neuron pool in control subjects and patients with SCI.

c) the correspondence between the electrophysiological evidence of preserved innervation and the clinical status of patients with SCI.

The results of these studies are viewed in the context of the emergent concept of the ***discomplete*** injury. This term is defined as the presence of preserved intact central axons in patients with SCI, established by neuropathological (Bunge et al., 1993, Kakulus, 1987; Kakulas & Taylor, 1992) or neurophysiological (Dimitrijevic, 1987; Dimitrijevic, Hsu, et al., 1992; Sherwood et al., 1992) examination, despite the clinical designation of ***complete*** SCI.

Chapter 2 - Review of Literature

2.1 - Preserved Innervation in Patients with Spinal Cord Injury

There are a variety of approaches for assessing the severity of injury and the residual motor, sensory and autonomic function of patients with SCI. The type of data obtained depends largely on the purpose for which it is collected. Radiographic analysis may be sufficient for planning management of any fractures, while computerized axial tomography (CAT) or magnetic resonance imaging (MRI) may be required for other surgical intervention, and frequent neurological assessments are appropriate for establishing therapeutic criteria (Young & Mayer, 1988). Neurophysiological testing is used to augment the information gained from clinical assessments and to provide more objective and reliable measures for the evaluation of experimental or clinical treatments. Neuropathological information is used to guide experimental and clinical treatments and the rational development of appropriate animal models of SCI. From many of these sources there emerges evidence of preserved innervation in patients with SCI.

2.1.1 - Clinical Neurological Assessment

The clinical assessment of patients with SCI is based on a careful and systematic evaluation of the motor, sensory and autonomic function of the patient. Early investigations and clinical experience suggested that the primary distinction was whether there was a ***complete*** or ***incomplete*** injury based on functional examination (Kuhn, 1950; Riddoch, 1917). This simple distinction has been found inadequate when considering the large variability encountered in patients with incomplete lesions (Guttmann, 1976;

Michaelis, 1969) as well as the occasional occurrence of recovery seen in patients initially designated as having complete injuries (Ducker et al., 1983; Meinecke, 1985; Young, 1989).

Over the past decade, consensus has been reached internationally with respect to a uniform measure of SCI severity and the definitions used to describe various aspects of SCI. Injuries are described as ***incomplete*** "if partial preservation of sensory and/or motor functions is found below the neurological level and includes the lowest sacral segment" or ***complete*** if there "is an absence of sensory and motor function in the lowest sacral segment" (Ditunno et al., 1994, p. 72). Greater resolution with respect to the degree of impairment is achieved with the American Spinal Injury Association (ASIA) Impairment Scale (Ditunno et al., 1994). This classification scheme employs the grades of A to E and denotes complete patients as A and incomplete patients as B to E depending on their degree of residual motor and sensory function as outlined in Table 1. This scale was employed in the present thesis in order to aid in the classification of patients.

The degree of motor and sensory impairments are further assessed by examining individual myotomes and dermatomes respectively to derive overall sensory or motor scores and obtain sensory and motor levels of impairment (Ditunno et al., 1994). The degree of motor impairment is assessed by the method of grading 10 key muscle groups reflecting 10 different myotomes (Lucas & Ducker, 1979). Each muscle is graded according to the widely used system first defined by the Nerve Injuries Committee (1943)

Table 1 - ASIA Impairment Scale

Grade	Completeness	Definition
A	Complete	No sensory or motor function is preserved in the sacral segments S4-S5.
B	Incomplete	Sensory but not motor function is preserved below the neurological level and extends through the sacral segments S4-S5.
C	Incomplete	Motor function is preserved below the neurological level, and the majority of key muscles below the neurological level have a muscle grade less than 3.
D	Incomplete	Motor function is preserved below the neurological level, and the majority of key muscles below the neurological level have a muscle grade greater than or equal to 3.
E	Incomplete	Sensory and motor function is normal.

Note: From Ditunno et al., 1994, p. 80.

of the Medical Research Council (U.K.) so that a score from 0 (total paralysis) to 5 (normal active movement) is obtained. Results are obtained bilaterally and are summarized as an overall motor score out of 100. These tests also establish the neurological motor level of injury which is defined by the lowest key muscle that has a grade of at least 3. A similar rationale is employed in the assessment of sensory function (Ditunno et al., 1994).

2.1.2 - Neuropathological Observations of Preserved Innervation

Neuropathological studies involving post-mortem examinations of injured human spinal cords have demonstrated that there is a surprisingly high incidence of preserved axonal continuity even in relatively severe traumatic injuries (Bunge et al , 1993; Kakulas, 1987; Kakulas & Taylor, 1992). Several observations have been made utilizing the extensive clinicopathological data base established in the Department of Neuropathology at the Royal Perth Hospital (Perth, Australia) (Kakulas, 1984, 1987; Kakulas & Taylor, 1992; Woods et al., 1991). Of 203 spinally injured patients who survived for less than 24 hours post-injury, the majority of whom possessed quite severe vertebral fractures, 61 (30%) showed an intact spinal cord without morphological, or even microscopic evidence of injury (Kakulas & Taylor, 1992). It is not clear how the pathology would have progressed had these patients survived for longer periods of time.

Evidence of the surprising extent of preserved innervation has prompted neuropathologists to adopt the term, ***discomplete lesion*** (Dimitrijevic, 1987). In the neuropathological sense, this distinction applies to those patients with pathological evidence of anatomical continuity of cord parenchyma through the level of the lesion yet

possess no clinically detectable function while alive (Kakulas & Taylor, 1992). Of 130 SCI patients whose clinical status was known just before death and who had survived at least 24 hours, 37 (28%) showed anatomic evidence of discompleteness (Kakulas & Taylor, 1992). Unfortunately, it was not reported how many of these 130 patients were clinically complete or incomplete if indeed such assessments had been made. More rigorous axonal counts of preserved corticospinal fibers were conducted at T4 on 18 complete and 3 incomplete patients (Kaelan et al., 1989). Of the 18 complete cases, 7 demonstrated central axonal fibers (i.e., between 27 and 2619 fibers) which survived the injury. Examination of the incomplete patients revealed that an estimated minimal percentage of 3.5-10% of corticospinal fibers (i.e., ~ 5000) was required in order to retain motor function of at least grade 2 of 5.

Recently, a similar approach utilizing detailed pathological examinations has been initiated at the Center for Paralysis Research (University of Miami School of Medicine, Miami, USA) which attempts to correlate pathological observations with the neurological status of the patient just before death (Bunge et al., 1993; Quencer et al., 1992). Initial reports showed that of 21 patients examined, 13 (62%) demonstrated preserved neural connectivity (Bunge et al., 1993). As well, of 16 patients deemed clinically complete, 8 (50%) demonstrated evidence of preserved parenchymal continuity through the lesion site and therefore could be judged as being neuropathologically discomplete. Of the remaining 8 patients with no evidence of continuity, 5 of these had lacerations of the spinal cord due to gun shot wounds. Similar observations have followed in more recent reports (Bunge et al., 1995).

In general, there is greater potential for preserved axonal continuity when the injury is a contusive or compressive type as opposed to trauma involving a laceration or a maceration of the cord resulting from an injury which breaches the pial surface of the cord (Bunge et al., 1993; Jellinger 1976). Typically, in injuries of the former type there is damage to the central grey area with the eventual evolution of a central fluid-filled cyst and a surviving population of axons in the circumferential pial rim. Microscopic examinations of experimental contusive spinal cord injury in monkeys (White et al., 1969, Ducker et al., 1971), dogs (Allen, 1914), and cats (Goodkin & Campbell, 1969) have revealed initial central hemorrhages evident within 15-20 minutes post-injury, which progress in size and number in a radially outward fashion. The extent of this progression is related to the severity of the trauma and may remain confined to the central grey matter in mild injuries or may engulf the entire spinal cord with more severe blows (Ducker et al., 1971).

In addition to centrally located hemorrhages, there often exists a central zone of damaged nervous tissue with early disruption of cell membranes which undergo liquefaction necrosis with eventual dissolution and disintegration. Neurons in the adjacent grey matter are affected over the first few hours after injury as this central necrosis progresses and this progression is accompanied by edema (Jellinger, 1976). The typical centrifugal progression of central hemorrhage, edema and parenchymal damage seen transversely is even more evident in a longitudinal direction within the grey matter. This is probably due to the greater vascularity and looser structure of the grey matter relative to the long, tightly arranged fiber tracts of the white matter. Therefore hemorrhagic necrosis

and edema often extend over several segments both below and above the epicenter of a lesion (Jellinger, 1976). Alternatively, contusive or compressive injuries may result in a ***solid cord injury*** (Bunge et al., 1993) in which the lesion is primarily localized to the white matter regions of the cord and may involve preferential focal damage to the larger diameter axons of the lateral columns.

While there continues to be much debate about the appropriateness of various animal models of SCI (Beattie et al., 1986), due in part to the variability seen with experimentally induced trauma, there are several important observations that relate to the survival of myelinated axons following experimental SCI. By employing a systematic line-sampling method to count and map myelinated axons in cats with chronic contusive lesions of the thoracic spinal cord, Blight (1983) was able to demonstrate the survival of significant numbers (40,000-110,000) of axons passing through the site of the lesion even in cats with chronic hindlimb paralysis. Although this represented only 5-10% of the original axonal population, a few of the injured cats recovered to the extent of being able to perform effective locomotion. Axonal survival was more probable closer to the pial surface of the cord and in smaller diameter myelinated axons.

Evidence from neuropathological examination of human cords (Bunge et al., 1993, 1995; Byrne & Waxman, 1990; Holmes, 1906) has implicated demyelination as a significant component of the pathology in some patients with SCI (Waxman, 1989). These observations parallel those from experiments conducted on various compressive and contusive animal models of SCI in which extensive demyelination was noted particularly in those axons surviving in the pial rim surrounding the central core of hemorrhagic necrosis

(Blight & Decrescito, 1986; Gledhill et al., 1973; Griffiths & McCulloch, 1983). Focal demyelination severely impairs axonal conduction. This is manifest by slowing of conduction velocity and inability to sustain repetitive discharges or may be sufficient to produce conduction failure (McDonald & Sears, 1970; Smith & Hall, 1980; Waxman, 1977). These effects of demyelination on axonal conduction are more severe in large diameter than small diameter axons as a result of the higher input resistance in smaller axons (Hille, 1970; Waxman, 1989). Therefore, action potential propagation is more easily maintained following demyelination in smaller axons despite the reduced density of Na^+ channels in the internodal region exposed with demyelination (i.e., less shunting of current away from adjacent axonal membrane with increased axonal resistance).

2.1.3 - Neurophysiological Evidence of Preserved Motor Function

Despite the prevalence of axonal continuity seen with pathological observations in both humans (Bunge et al., 1993; Kakulas, 1987; Kakulas & Taylor, 1992) and in animal models of spinal cord injury (Blight, 1983; Blight & Decrescito, 1986), patients often present with complete or near complete functional losses. With this apparent incongruency, it is not surprising that electrophysiological approaches have been advocated as a means to more sensitively assess the existence and potential function of preserved innervation after spinal cord injury.

In particular, Dimitrijevic and his colleagues have employed various neurophysiological tests in order to reveal minimal, or latent, preserved innervation even in patients designated as clinically complete. These techniques have included examination of the effect of supraspinal influences on spinal reflex activity (Cioni et al., 1986;

Dimitrijevic et al., 1983), assessment of the initiation of voluntary muscle activity using polyelectromyographic recording techniques (Dimitrijevic, 1984; Dimitrijevic et al., 1984) and evaluation of the potential for functionally appropriate muscle activity with respect to gait (Dimitrijevic & Lenman, 1980). These investigations resulted in development of the distinction of the ***discomplete*** patient as one who is judged to be clinically complete but who possesses neurophysiological evidence of residual brain influence on the function of the spinal cord below the level of the lesion (Dimitrijevic, 1987).

More recently, these investigators have employed a comprehensive test battery, in order to evaluate more systematically the prevalence and features of preserved motor control in patients with SCI (Dimitrijevic, Hsu, et al., 1992; Sherwood et al., 1992). This procedure included the suppression and enhancement of tonic vibratory responses (Sherwood et al., 1993), plantar withdrawal reflexes and voluntary muscle activation using a variety of reinforcement maneuvers (i.e., deep inspiration, neck flexion, remote muscle contraction). In testing a series of 581 SCI patients with lesions above T-10, Dimitrijevic, Hsu, et al. (1992) reported 284 (49%) patients as having an incomplete lesion; 211 (36%), a discomplete lesion; and only 86 (15%), a complete lesion. This means that of 297 SCI patients deemed to have neurologically complete injuries, 211 (71%) possessed at least some evidence of suprasegmental descending influences on the alpha motor neurons. In a separate study, an even greater proportion of patients with SCI were deemed discomplete when considering only patients referred for the evaluation and management of spasticity (Sherwood et al., 1992). Of 88 patients referred for spasticity and also deemed to have neurologically complete injuries, 74 (84%) were distinguished as having discomplete

injuries.

The prevalence of discomplete injuries may be slightly overestimated in these studies (Dimitrijevic, Hsu, et al., 1992; Sherwood et al., 1992) as the protocol used does not attempt to limit increases in intrathoracic or intraabdominal pressure during the various reinforcement procedures. Therefore, electromyographic (EMG) activity could be triggered by afferent activity below the level of the lesion (Dobkin et al., 1994). These considerations have prompted the suggestion that other methods be included to assess conduction in specific descending motor pathways. For example, the use of auditory inputs to condition upper and lower limb H-reflexes has been advocated as a means to assess the completeness of a SCI with respect to conduction in the reticulospinal system (Dobkin et al., 1994). Pilot studies using this technique have demonstrated preserved innervation in this pathway in 3 (1 complete; 2 incomplete) of a total of 7 (5 complete, 2 incomplete) patients with SCI.

2.2 - Transcranial Magnetic Stimulation of the Motor Cortex

The technique of electrical cortical stimulation has long been used to aid in the description of numerous features of the nervous system. In 1809 Rolando demonstrated movement elicited with electrical stimulation of mammalian cortex, but it was not until the pioneering work of Fritsch and Hitzig (1870), and subsequent experiments by Ferrier (1873) and Luciani and Tamburini (1878-1879), that Flouren's (1824) assertion of an unexcitable, homogeneous cortex was ultimately dismissed (as cited in Walker, 1957) Later work by Sherrington and his colleagues who examined anthropoid cerebral cortex (Leyton & Sherrington, 1917), and Penfield and his colleagues who stimulated exposed

human cortex during neurosurgical procedures (Penfield & Boldrey, 1937), provided a wealth of information related to the localization and organization of function within the cortex. More recently, investigators have employed various methods such as microstimulation, intracellular recording techniques, and computer averaging to elucidate many features of the motor cortex and the function of pyramidal cells (Asanuma & Rosen, 1972; Jankowska et al., 1975; Landgren et al., 1962; Phillips, 1956). However, it was not until the development of the technique of high voltage, brief, anodal stimulation applied percutaneously to the motor cortex (Merton & Morton, 1980) and the relatively painless technique of transcranial magnetic stimulation (Barker et al., 1985) that stimulation of the motor cortex in intact, alert human subjects has become a viable tool for investigative and clinical purposes.

2.2.1 - Physiological Basis of MEPs Elicited With Transcranial Magnetic Stimulation

Transcranial stimulation of the motor cortex, by an electrical current or a pulsed magnetic field, causes a volley of discharges in pyramidal tract neurons (Amassian et al., 1987; Day, Rothwell, et al., 1987; Day et al., 1989; Edgley et al., 1990). The initial volley, or ***D-wave***, results from direct activation of pyramidal cells as was demonstrated by electrical stimulation of exposed cortex in cats and monkeys (Patton & Amassian, 1954). Later volleys, termed ***I-waves***, reflect indirect activation of these neurons transsynaptically via interneurons (Kernell & Wu, 1967; Patton & Amassian, 1954). The temporal and spatial summation of these D and I-wave excitatory synaptic inputs to the target motor neuron pool causes activation of one or more motor units and the appearance of the

relatively synchronous compound muscle action potential usually termed the ***motor evoked potential*** (MEP). There is some debate as to the precise mechanism of the activation of corticospinal neurons following transcranial magnetic stimulation. The shape, size, and orientation of the stimulating coil and the intensity of stimulation all appear to affect whether these cells are excited more easily indirectly via other neurons or directly either at the axon hillock (i.e., initial segment) or at more distal nodes along the course of the axon (Amassian et al., 1990; Day, Thompson, et al., 1987; Day et al , 1989). Regardless, evidence from studies of the stimulation of the motor cortex in monkeys as well as the short latency of the MEPs recorded from various muscle groups has been interpreted to indicate that these responses are mediated through fast conducting corticospinal pathways (Amassian et al., 1990; Brouwer & Ashby, 1990; Edgley et al., 1990; Rothwell et al., 1991).

Many thousands of individuals have undergone transcranial magnetic stimulation (Jalinous, 1992) and individual investigators have reported receiving as many as 2000 stimulations (Bridgers, 1991) without undue effects since the introduction of this technique in 1985. Numerous studies examining such measures as EEG activity, serum prolactin or adrenocorticotropic hormone levels and various features of cognitive function have demonstrated that there are no persistent deleterious effects of transcranial magnetic stimulation although transient effects may occur (Agnew & McCreery, 1987; Bridgers, 1991; Bridgers & Delaney, 1989; Chokroverty et al., 1995; Krain et al., 1990; Levy et al., 1990). There have been two reports of eliciting seizures with transcranial magnetic stimulation and this remains the primary safety concern of most investigators (Homberg &

Netz, 1989; Hufnagel et al., 1990). In both instances seizures were elicited in individuals who were prone to seizure activity. One additional safety consideration relates to the observation of hearing loss in rabbits due to the acoustic artifact emitted by the magnetic coil (Counter et al., 1990). Subsequent investigations in humans have suggested that this risk is minimal (Pascual-Leone et al., 1992).

2.2.2 - Long Latency Responses to Cortical Stimulation

Recently, evidence has been presented of long latency excitatory inputs to the target motor neuron pool in addition to the early corticospinal inputs following cortical stimulation (Calancie et al., 1987). These responses have been termed ***secondary peaks*** in the peristimulus time histogram of single motor unit discharges (Mills et al., 1991). In the lower limbs two distinct later periods of excitability have been identified although there appears to be some variability as to the precise timing of these responses as well as in the extent to which they appear. These lower limb late responses have been characterized with respect to their latencies by the designations MEP_{70} and MEP_{120} (Dimitrijevic, Kofler, et al., 1992) or ***S100*** and ***S>150*** (Holmgren et al., 1990, 1992). Early responses elicited in the lower limb typically possess latencies of 25-30 ms (Rothwell et al., 1991).

The neural structures which mediate these late responses are as yet unresolved. Initially it was suggested that afferent inputs associated with the early MEP were responsible for these responses (Calancie et al., 1987). This was disputed by Dimitrijevic, Kofler, et al. (1992) who used recordings of the evoked twitch contraction to show that the timing of the late responses actually preceded the expected time of the afferent input during twitch relaxation. Sammut et al. (1995) noted that transcranial magnetic

stimulation of the lower limb representation of motor cortex results in relatively larger amplitude early MEPs in tibialis anterior (i.e., dorsiflexor) compared to those elicited in the soleus and gastrocnemius muscles (i.e., plantarflexors). The net effect was overall ankle dorsiflexion resulting in stretch of the soleus. Late responses elicited in soleus could be reduced or enhanced by manipulations designed to alter the stretch reflex response. Therefore, soleus late responses were thought to be due to segmental influences mediated by excitation of Ia afferents (Sammut et al., 1995). Other investigators have suggested that late MEPs may be mediated, at least in part, by central descending pathways (Dimitrijevic, Kofler, et al., 1992; Holmgren et al., 1992; Mills et al., 1991). Putative mechanisms include corticospinal pathways involving more synapses and/or more slowly conducting fibers than those known to mediate the short latency response (Dimitrijevic, Kofler, et al., 1992), bulbospinal pathways activated by either corticobulbar fibers or startle mechanisms (Holmgren et al., 1992), or cortical activation of γ-motoneurons with subsequent Ia afferent mediated α-motoneuron discharge following cortical stimulation (Mills et al., 1991). Recent experiments examining the properties of late responses elicited during the preparatory period of a simple or choice reaction time task have demonstrated an agonist-antagonist organization between the short latency MEP of a muscle and the late MEP elicited in its antagonist (Tarkka et al., 1995). It remains to be elucidated whether this reflects the nature of descending inputs or a purely segmental organization.

This issue of whether central mechanisms mediate long latency MEPs is one of the questions addressed in the present thesis. There is some evidence that these later responses are more prevalent in patients with SCI (Dimitrijevic et al., 1988; Segura et al ,

1992). If these responses are mediated by central structures their presence in patients with SCI would indicate residual descending innervation. Moreover, if these responses are mediated through ventromedial pathways they assume considerable importance because of the function of these pathways in stance and gait and because they are readily disrupted by spinal cord trauma. These pathways have not previously been directly accessible to electrodiagnostic assessment.

2.2.3 - Transcranial Magnetic Stimulation of the Motor Cortex in Patients With SCI

Transcranial magnetic stimulation has been used as a tool for the assessment of motor conduction following spinal cord injury (Caramia et al., 1988; Dimitrijevic, Hsu, et al., 1992; Dvorak et al., 1990; Hayes et al., 1991, 1992; Lissens et al., 1992; Tegenthoff, 1992). Although there are some reports that later MEPs are evident in patients with SCI (Dimitrijevic et al., 1988; Segura et al., 1992), most researchers have confined their analysis to short latency MEPs and the significance of these later responses remains to be elucidated. The presence or absence of short latency MEPs is generally consistent with the clinical and functional status of the patient. For example, Dimitrijevic and his colleagues examined a group of 32 patients with chronic SCI for the existence of MEPs in order to assess the value of including transcranial magnetic stimulation in their routine neurophysiological test battery (Dimitrijevic, Hsu, et al., 1992; Lissens et al., 1992). MEPs were not elicited in any of the 9 complete and 13 discomplete subjects but were present with prolonged latencies in all 10 subjects with incomplete SCI. The properties of these MEPs were correlated with the results of an assessment of motor control and functional performance; the better the motor control and functional performance, the

shorter the MEP latency and greater the number of muscles exhibiting MEPs. These results demonstrate the usefulness of transcranial stimulation of the motor cortex as part of a neurophysiological assessment to detect residual brain influences.

Typically, in patients with SCI, MEPs are either absent or possess some of the following characteristics: a) high response threshold, b) prolonged latency, c) reduced amplitude, d) more polyphasic, e) prolonged duration, and g) increased lability (Dimitrijevic et al., 1988; Hayes et al., 1991). Given the potential for many of these features to result in a high probability for false negative interpretations (i.e., patients with nonexistent MEPs but with residual corticospinal function), several authors have advocated the use of various reinforcement procedures in order to enhance the probability of eliciting MEPs (Dimitrijevic, Hsu, et al., 1992; Hayes et al., 1991). These procedures include attempted target muscle contraction, remote muscle contraction, Jendrassik maneuver (i.e., hand grip), vibration and cutaneous stimulation. In particular, Hayes et al (1991) demonstrated the importance of several of these techniques in enhancing MEPs in patients with clinically complete injuries. This suggests that the technique of transcranial magnetic stimulation with reinforcement may be especially useful for determining the degree of preserved motor function.

2.2.4 - Facilitation of MEPs using Conditioning Afferent Stimulation

The utility of peripheral afferent stimulation in facilitating MEPs has been examined by numerous investigators (Deletis et al., 1992; Kasai et al., 1992; Komori et al , 1992; Maertens de Noordhout et al., 1992; Troni et al., 1988). Generally, periods of facilitation and inhibition of MEPs have been noted that are consistent with previously

observed modulation of motor neuron excitability with the specific peripheral stimulation employed. There is some controversy, however, as to the ability of cutaneous afferent stimulation, as distinct from proprioceptive input, to provide facilitation thereby enhancing MEPs (Komori et al., 1992; Maertens de Noordhout et al., 1992; Troni et al., 1988). While some investigators question whether or not the stimulation of cutaneous afferents alone yields facilitation (Komori et al., 1992; Troni et al., 1988), others have presented evidence, at least for the upper limb, that cutaneous afferent stimulation does facilitate MEPs (Maertens de Noordhout et al., 1992), although the mechanisms by which this is accomplished remain contentious.

Variations of these afferent conditioning techniques have also been used to enable detection of low amplitude responses and preserved corticospinal innervation in patients with SCI (Hayes et al., 1991, 1992). In particular, appropriately timed transcranial magnetic stimulation has been demonstrated to lower the stimulation intensity required to elicit flexion reflexes in 4 patients (1 complete; 3 incomplete) with SCI (Hayes et al., 1992). This suggests the presence of preserved descending innervation despite the finding that MEPs were not able to be elicited in 3 of these patients.

2.3 - Neurophysiologic Assessment of Peripheral Nerve Conduction

Although the assessment of conduction in central pathways is most important in patients with SCI, MEPs involve conduction in both central (i.e., corticospinal) and peripheral (i.e., α-motor neuron) pathways. Therefore, the peripheral motor conduction time (PMCT) is often subtracted from the overall MEP latency to estimate the central motor conduction time (CMCT) according to the following formula (Robinson et al.,

1988):

$$CMCT = MEP\ Latency - PMCT$$

PMCT is calculated with tests of peripheral nerve conduction including M-waves and F-waves. These electrodiagnostic tests and the calculation of PMCT are employed in the present thesis to determine the effects of cooling on central and peripheral conduction and are elaborated below. In addition, experiments of the present thesis seek to examine excitability changes in the motor neuron pool following a) cooling or b) cortical stimulation. These changes in motor neuron excitability are assessed with the H-reflex.

M-waves, F-waves, and H-reflexes are muscular responses (i.e., compound muscle action potentials) resulting from the percutaneous electrical stimulation of peripheral nerves. A brief description of each of these procedures may be found below.

2.3.1 - M-waves

M-waves are the compound action potentials elicited in a muscle following a single electric pulse applied to the motor nerve. These responses are used extensively in assessing peripheral motor nerve conduction in a wide variety of disorders of the nervous system (Kimura, 1989). M-waves are obtained with the cathode overlying a standardized location accessible to the motor nerve under investigation and the anode proximal. Stimulation intensity is increased until the maximal amplitude of the response is attained and this or a slightly greater intensity of stimulation is employed for eliciting the M-wave. Supramaximal stimulation ensures activation of all motor nerve fibers innervating the muscle being tested (Kimura, 1989). Stimulus pulse duration is commonly 0.1 ms as motor nerve fibers have been demonstrated to be optimally excited with this stimulus

setting (Veale et al., 1973). Nerve conduction velocities are often calculated using the M-wave by eliciting responses at 2 distinct points along the anatomical course of the nerve.

2.3.2 - F-waves

F-waves, so named because of the relative ease of eliciting these responses in muscles of the foot (Magladery & McDougal, 1950), are elicited with supramaximal (i.e., for the maximal M-wave) stimulation of the motor nerve. Stimulation parameters are similar to that employed with M-waves other than a proximal positioning of the cathode relative to the anode. However, unlike the M-wave, the F-wave is elicited by antidromic volleys in the motor fibers resulting in activation of the motor neuron (Eccles, 1955; Fox & Hitchcock, 1987; Mayer & Feldman, 1967). Typically, only a few motor units comprise the F-wave and the precise motor neurons activated vary with successive stimuli as motor neurons are not easily excited following antidromic conduction (Feasby & Brown, 1974; Schiller & Stalberg, 1978; Yates & Brown, 1979). For this reason, F-waves are not always elicited and are of variable waveform and low amplitude. Therefore, clinical practice has been to select the earliest reproducible potential following a series of stimuli (Kimura et al., 1994). The total conduction time of the M-wave and F-wave represents conduction in the α-motor neuron (i.e., 2 x the course of this pathway). The delay of the central synapse has been estimated at 0.5 ms which allows calculation of an overall estimate of the time for peripheral motor conduction time (PMCT) according to the following formula (Robinson et al., 1988):

$$\text{PMCT (in ms)} = (\text{M latency} + \text{F latency} - 1)/2$$

2.3.3 - H-reflexes

The H-reflex, named for Hoffmann (1922), is the muscular response (i.e., compound muscle action potential) recorded following electrical stimulation of large diameter Ia afferent fibers. It is thought to reflect conduction in a monosynaptic reflex arc consisting of these afferents and α-motor neurons (Lloyd, 1943; Magladery et al., 1951) There has been some suggestion that potential oligosynaptic afferent connections (Burke et al., 1984) may also contribute to this reflex. The H-reflex has been employed extensively in experiments to assess motor neuron pool excitability (Brown et al., 1978; Magladery, 1955; Schieppatti, 1987; Taborikova, 1973). Typically, H-reflexes are elicited in muscles innervated by the tibial nerve in the lower limb and in muscles innervated by the median nerve in the upper limb (Kimura et al., 1994). Optimal stimulation parameters consist of a single, long duration (i.e., 0.5-1.0 ms) pulse applied to the nerve (Panizza et al., 1989) with the stimulating cathode positioned proximal to the anode. Ideally, stimulation intensity is subthreshold for exciting motor fibers (Kimura, 1989)

2.4 - The Effect of Temperature in Clinical Neurophysiology

Temperature has long been recognized as a critical factor affecting neurophysiological events (Denys, 1980, 1991). Of particular interest to the present thesis are effects on nerve conduction velocity and the amplitude of evoked potentials

Reductions in nerve conduction velocity with cooling have been well documented (Buchthal & Rosenfalck, 1966; de Jesus et al., 1973; de Jong et al , 1966; Halar et al , 1981) and various temperature correction factors have been calculated to compensate for temperature reductions. These factors have estimated the reduction in conduction velocity

between 1.1-2.1 m/s/°C following cooling (Buchthal & Rosenfalck, 1966; de Jong et al., 1966; Gassel & Trojaborg, 1964; Halar et al., 1983). The actual value calculated with each experiment varied depending on the particular nerve under investigation, the precise temperature range tested (i.e., ranging from 21 - 38 °C), and the location of temperature measurement (i.e., skin, subcutaneous, or intramuscular). Although these linear approximations of the temperature effect on nerve conduction velocity are sufficient for most clinical purposes, the relationship between temperature and conduction velocity may be more accurately described by a semilogarithmic function thereby allowing calculation of a temperature coefficient, or Q_{10} (de Jesus et al., 1973). The Q_{10} represents the ratio of the highest to lowest conduction velocity over a 10 °C range. Theoretical values of 1.75 were calculated for Q_{10} for the squid giant axon (Huxley, 1959) while experimental values of 1.51-1.6 have been obtained for motor and sensory nerve fibers in man (de Jesus et al., 1973; de Jong et al., 1966).

Experiments employing the local application of cooling agents have demonstrated increased amplitudes of evoked nerve and muscle compound action potentials in man following peripheral nerve stimulation (Bolton et al., 1981; Louis & Hotson, 1986). These amplitude increases have been explained on the basis of increased single nerve fiber action current due to delayed Na^+ inactivation (Frankenhaeuser & Moore, 1963; Hodgkin & Katz, 1949; Louis & Hotson, 1986). This results in a large increase in duration and a lesser increase in amplitude of individual action potentials. Wider individual action potentials produce an increased compound action potential amplitude given the net effect of a population of action potentials traveling at different velocities as seen with surface

recording electrodes (i.e., increased spatial summation). In addition, limb cooling has been demonstrated to increase motor neuron pool excitability as shown by an increased H-reflex amplitude (Urbscheit & Bishop, 1970). These changes are dependent on a variety of factors and the time course of these excitability changes remain to be established (Bell & Lehmann, 1987; Knutsson & Mattsson, 1969).

2.5 - Relationship of Prior Research to the Present Thesis

Evidence from a variety of sources confirms the existence of a surprising degree of preserved innervation in patients with SCI even in patients with clinically complete or near complete SCI. Elicitation of MEPs by cortical stimulation in these patients may be useful for detecting preserved innervation not detectable by other means. This is especially so when techniques, such as those used in the present thesis, are employed to enhance the probability of detecting responses to cortical stimulation. The importance of detecting even minimal residual innervation remains to be fully realized but the increased sensitivity afforded by these techniques should enhance the assessment of effects of therapeutic and experimental interventions and may provide prognostic information.

The present thesis initially builds on the foundation of previous work from our laboratory of employing conditioning afferent stimulation in order to enhance MEPs (Hayes et al., 1991, 1992; Kasai et al., 1992). In particular, the issue of whether cutaneous afferents (i.e., sural nerve stimulation) may contribute to the facilitation of MEPs seen previously with mixed nerve (i.e., medial plantar nerve) stimulation in control subjects has now been examined. Following this, two other techniques to enhance the detection of preserved innervation were examined in patients with SCI and control

subjects. The first technique involved the use of induced hypothermia to enhance the probability of eliciting MEPs. Cooling has been long established clinically as a means of increasing the amplitude of evoked responses (Bolton et al., 1981; Louis & Hotson, 1986) but has not previously been examined as to its effectiveness in enhancing low amplitude MEPs in patients with SCI. The second technique used a procedure of cortical conditioning of H-reflexes to show the time course of inputs to the motor neuron pool following cortical stimulation. By employing both subthreshold and suprathreshold conditioning stimuli, it was possible to dissociate any afferent consequences of the early evoked response (i.e., short latency MEP), from the late arriving descending inputs. Of practical interest was the determination of the relative effectiveness of these various techniques in enhancing the detection of preserved innervation in patients with SCI and the correspondence between the evidence of preserved corticospinal innervation and functional status.

Chapter 3 - The Experiments

3.1 - Experiment 1 - Conditioning Effects of Sural Nerve Stimulation on Lower Limb MEPs in Control Subjects

3.1.1 - Introduction

Percutaneous electrical stimulation of the medial plantar or tibial nerves produces a stimulus intensity dependent modulation of MEPs elicited in muscles of the lower limbs in normal subjects (Deletis et al., 1992; Kasai et al. 1992). This type of modulation is useful in understanding basic neurophysiological processes as well as enhancing the detection of low amplitude MEPs in patients with compromised central motor conduction (Hayes et al., 1991, 1992). There remains, however, some dispute as to the physiological mechanisms involved in this modulation.

MEP modulation is evident with conditioning stimuli that are just subthreshold for eliciting the flexion reflex (Kasai et al., 1992). The time course of MEP facilitation corresponds to the time course of the flexion reflex, elicited by cutaneous afferent input, and is similar to that observed with cutaneous afferent conditioning (i.e., stimulation of the sural nerve) of H-reflexes (Delwaide & Crenna, 1983). This implies that cutaneous afferents mediate the facilitation. However, because the medial plantar and tibial nerves are mixed nerves containing both cutaneous and muscle afferents, the role of the cutaneous afferents, per se, in modulating MEP amplitudes has not been definitively established. In the upper limb, evidence from studies employing digital nerve stimulation has suggested that cutaneous afferent stimulation is ineffective in enhancing MEPs elicited

in the thenar muscles (Komori et al., 1992, Troni et al. 1988). These authors conclude that it is muscle afferents that lead to MEP facilitation.

3.1.1.1 - Research objectives and plan.

This study was designed to provide a direct test of the hypothesis that conditioning cutaneous afferent stimulation leads to facilitation of MEPs elicited in lower limb muscles. This was accomplished by applying a conditioning percutaneous electrical stimulation to the sural nerve at various times preceding transcranial magnetic stimulation of the motor cortex. Conditioned and control MEPs were elicited bilaterally in the tibialis anterior (TA) and lateral gastrocnemius (LG) muscles. In accordance with the original classification of peripheral nerves (Erlanger & Gasser, 1937) and subsequent proposals (Lloyd, 1943) the normal sural nerve has been shown to contain cutaneous afferent fibers of group II (Aβ) and group III (Aδ) and unmyelinated fibers of group IV (C) but no muscle afferents (Behse, 1990; Jacobs & Love, 1985; O'Sullivan & Swallow, 1968) and it was for this reason that the sural nerve was employed in the present study. The TA and LG were selected because of their clinical applicability in patients with SCI due in part to the relative ease of eliciting and recording MEPs in these muscles. In addition, sural nerve stimulation has previously been shown to enhance motor neuron excitability in the TA although the pathways by which this occurs have not been fully established (Delwaide & Crenna, 1983).

This study also provided an opportunity to examine cutaneous afferent conditioning effects on late responses evoked by cortical stimulation. These late responses have been attributed to slow conducting and/or oligosynaptic corticospinal or cortico-

bulbospinal pathways (Dimitrijevic, Kofler, et al., 1992), although brainstem or peripheral inputs may also be involved (Holmgren et al., 1992; Mills et al., 1991). Therefore, the purpose of this study was to test the hypotheses (a) that stimulation of cutaneous afferents, in the absence of muscle afferent input, facilitates short latency MEPs elicited in lower limb muscles and (b) that cutaneous afferent stimulation facilitates late responses to cortical stimulation.

3.1.2 - Methods

3.1.2.1 - Subjects.

Eleven healthy adults (4 males; 7 females; age = 30.7 ± 7.2 years), with no history of neurological deficits, provided informed consent to participate in this study. Subjects were recruited from the staff of Parkwood Hospital and were screened for history of epilepsy or cardiovascular disease that would be contraindications for the use of cortical stimulation.

3.1.2.2 - Procedures.

Conditioning (C) percutaneous electrical stimuli were applied to the sural nerve at various intervals prior to the test (T) transcranial magnetic stimulation of the motor cortex. The intensity of the conditioning sural nerve stimulation was adjusted to the maximum that was non-painful and this occurred at a mean level of 3.6 x sensory threshold (ST) across subjects. As well, 2 subjects were retested at a higher stimulation voltage (5 x ST) which was above the pain threshold for each of these subjects The limb which demonstrated the lowest threshold for the TA to cortical stimulation was designated as ***ipsilateral*** with sural nerve stimulation being applied to this leg Cortical stimulation

intensities were set at 1.1 x motor threshold . The mean stimulus intensity was 76.6% ± 10.6% of maximal stimulator output. Conditioning to test (C-T) intervals from 0 to 150 ms were investigated at 10 ms increments. Two additional early intervals of 5 and 15 ms were examined to determine if there was any early, short lasting, direct segmental convergence that may have been missed with the relatively long 10 ms increment. The procedure was limited to two stimulations at each C-T interval to avoid an excessive number of cortical stimulations within an experimental session. The order of C-T intervals was randomized within and across subjects and six control trials involving cortical stimulation only were randomly inserted among these trials. In addition, control trials of the afferent conditioning stimulus only were administered at the beginning and end of the experimental session. A minimum interval of 30 s was allowed between successive trials to minimize response habituation.

3.1.2.3 - Cortical stimulation.

Transcranial magnetic stimulation was delivered by a Cadwell MES-10 stimulator (Cadwell Labs, Kennewick, WA) through a 9 cm focal point stimulating coil electrode. The coil was positioned tangential to the scalp, with the edge of the coil over C_z (vertex) of the 10-20 international system for EEG recording (Jasper, 1958) and with the initial direction of current flow clockwise. This location is optimal for eliciting MEPs in the lower limbs as the maximal field strength occurs under the coil edge and this placement is over the lower limb representation of primary motor cortex. The waveform of each stimulus of the Cadwell stimulator is that of a damped 3560 Hz, 4-cycle sine wave. Peak voltage is 187 V and peak magnetic induction is nominally 2.2 tesla at 100% stimulator

output. All stimuli, including the cortical stimulation, were delivered with the subjects lying supine on a bed with the head of the bed inclined so that the subjects' hips were flexed by approximately 45°. These stimulations were triggered by a Digitimer D4030 timing pulse generator (Medical Systems Corp., Greenvale, NY) which also served to trigger display and data collection.

Stimulation intensity was initially set at a level that was barely perceptible and gradually increased in 5% increments. Motor thresholds were defined as the stimulus intensity at which MEPs were present in at least 50% of trials.

3.1.2.4 - Conditioning afferent stimulation.

The conditioning afferent stimulation consisted of a 20 ms train of 10 pulses applied percutaneously to the sural nerve just posterior to the lateral malleolus Each pulse was 0.1 ms in duration and the inter-pulse interval was 2 ms (i.e., 500 Hz). The stimulation was applied to the leg having the lowest threshold for eliciting MEPs in the TA. This leg was therefore ***ipsilateral*** to the cutaneous stimulation. The stimuli were delivered from a Devices Type 3072 stimulator (Devices Sales Ltd., Hertfordshire, England) and applied through stimulating electrodes positioned along the course of the nerve The anode and cathode were separated by 3 cm with the anode positioned distally.

3.1.2.5 - MEP recordings.

Bilateral recordings of MEPs were obtained from TECA 1 cm metal disk surface electrodes in a bipolar configuration positioned 4 cm apart over the motor point of the TA and LG muscles. A 2 cm metal disk electrode placed over the patella served as a ground The myoelectric signals were amplified and filtered (3 dB down at 10 Hz-1 kHz) with Disa

Type 15C01 (Disa Dantec Elektronik, Skovlunde, Denmark) amplifiers. Continuous recordings of the myoelectric signals were stored on video tape using a PCM VCR adapter (Medical Systems Corp., NY) with a frequency response of 0-3.5 kHz for off line analysis.

3.1.2.6 - Data Analysis.

After the experimental session, the data were analog to digital converted (MDAS 7200, Kaye Instruments) at 2000 Hz and then stored and analyzed for latency, duration, and peak to peak amplitude. The conditioned peak to peak MEP amplitude values (i.e., using largest negative to positive peak) were averaged and normalized with respect to the mean unconditioned control MEP amplitude for trials with similar C-T intervals. As the cortical stimulation intensity was selected to elicit responses in the ipsilateral TA (i.e., lowest threshold) , not all subjects displayed responses in the contralateral TA or the ipsilateral and contralateral LG. Data for these muscles were not included in the overall results if there was not a consistent short latency MEP elicited in at least 50% of the control trials. Of the 11 subjects, this resulted in 7 subjects demonstrating consistent responses in the contralateral TA and 8 in the ipsilateral LG. There were insufficient responses in the contralateral LG (n=2) to warrant reporting.

The short latency (~ 30 ms) MEPs evoked after conditioning with the cutaneous afferent stimulation were polyphasic and of variable configuration. This contrasted with the normally stable bi- or triphasic waveform recorded in the control conditions. It was deemed appropriate therefore to document the effect of conditioning on both the peak to peak amplitude and the time integral of the responses. Based on the *a priori* expectation

(Kasai et al., 1992) that there would be a facilitation of the conditioned response at C-T: 70 ms, a single tailed, paired sample t-test was conducted to determine the significance of the conditioning effect ($\alpha = 0.05$) at this interval.

3.1.3 - Results

3.1.3.1 - Short latency MEPs in ipsilateral TA.

The conditioning sural nerve stimulation yielded a well defined pattern of short latency MEP peak to peak amplitude modulation in the ipsilateral TA. Ten of 11 subjects displayed a period of MEP facilitation within the interval C-T: 60-100 ms. Seven subjects also exhibited a lesser and more variable facilitation at intervals C-T: 0-40 ms. Data from a representative subject are shown in Figure 1. Evident in this figure, are longer latency responses (~ 125 ms) time locked to the cortical stimulation, and appearing when the conditioning afferent stimulation was delivered at C-T: 60-110 ms.

Averaged data for the group ($n = 11$) at the various C-T intervals are shown in Figure 2. The mean control MEP peak to peak amplitude across all subjects was 0.32 ± 0.36 mV. The greatest facilitation of MEPs occurred at C-T: 70 ms ($t = 3.71$; $p < 0.05$) reaching a mean value of 258.1% of the normalized control MEP peak to peak amplitude. The earlier period of facilitation, evident to some degree in 7 of the subjects within C-T: 0-40 ms, was not apparent when the data were averaged across subjects

Analysis of the effects of cutaneous afferent conditioning using the time integral of the evoked responses revealed virtually identical results. There was a significant increase in the integrated EMG value at C-T: 70 ms ($t = 3.21$; $p < 0.05$). Pearson product moment correlation analysis of the temporal similarity in the peak to peak and integrated EMG

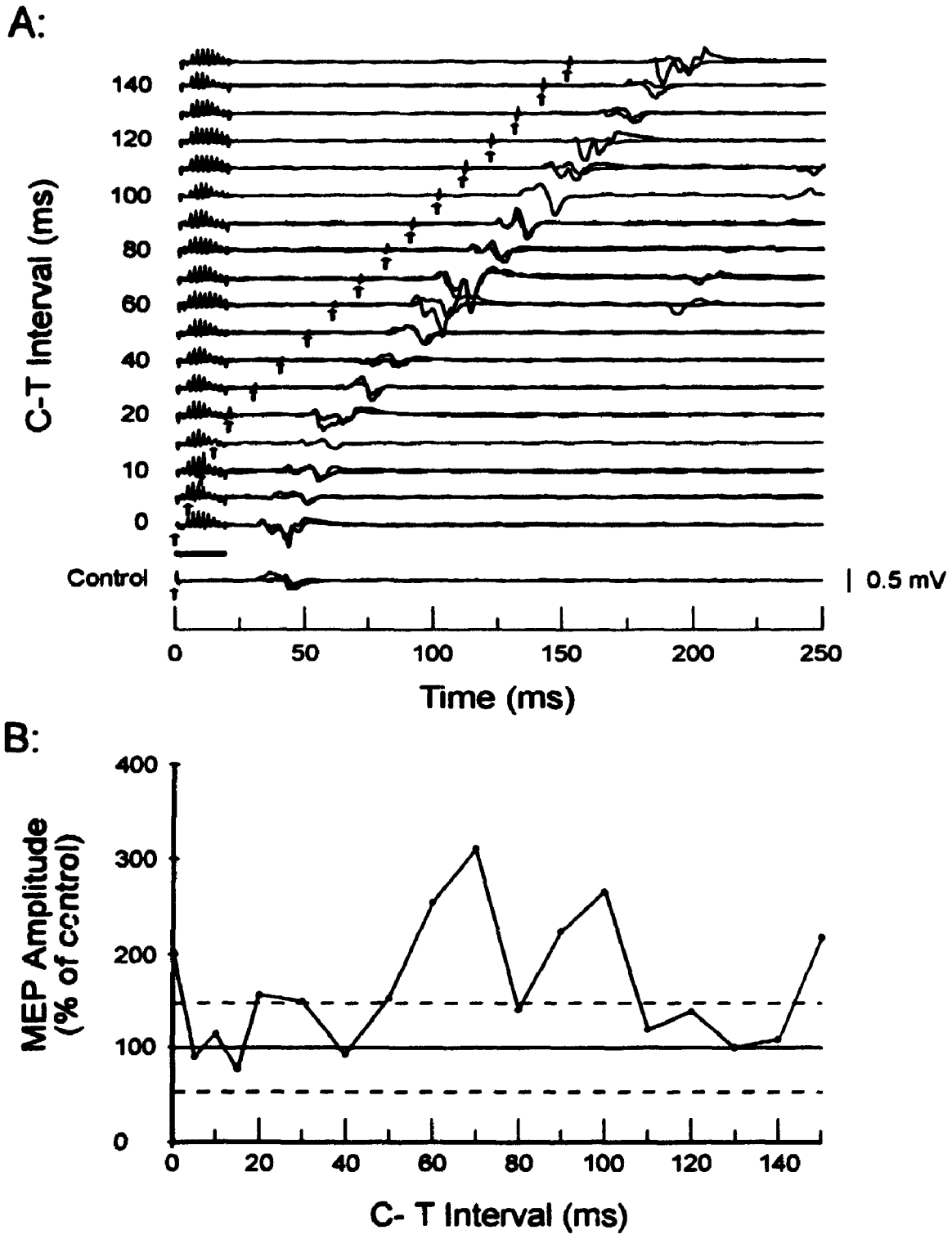

Figure 1. A: EMG traces for one subject showing MEPs elicited in the ipsilateral tibialis anterior following cutaneous conditioning. Two trials are superimposed at each C-T interval. Late responses are seen in some trials at C-T: 60-110 ms. Cutaneous stimulation occurred at the beginning of the displayed trace for 20 ms as indicated by the horizontal bar. Arrows indicate cortical stimulation. B: Summary of mean MEP amplitudes normalized to the control value for this subject. Dashed lines represent 2 SE values about mean control amplitude. Note that marked facilitation of MEPs was present at C-T: 0, 60, 70, 90, 100, and 150 ms.

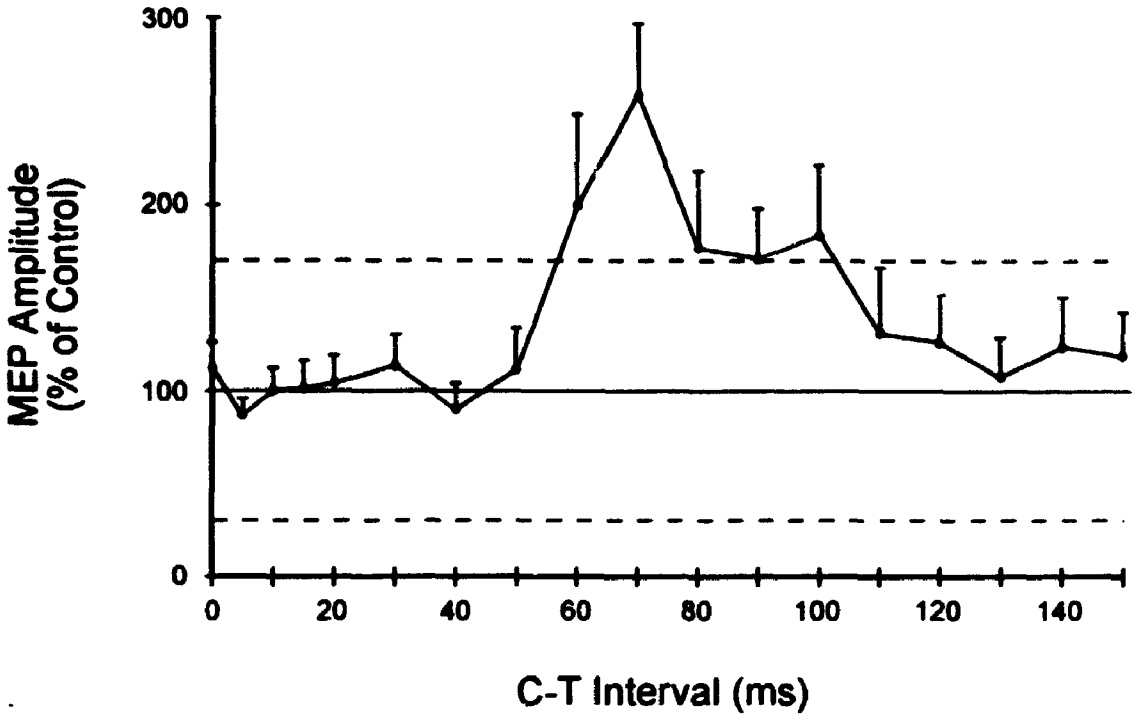

Figure 2: **Mean MEP amplitudes conditioned with cutaneous afferent stimulation for the ipsilateral tibialis anterior normalized to the control value at each C-T interval (n=11). Dashed lines represent 2 SE values about the mean control amplitude. Vertical bars denote 1 SE of normalized conditioned values at each C-T interval.**

conditioning profiles yielded r = 0.97. Similarly, an analysis conducted on the time duration of the evoked responses revealed a peak conditioning effect at C-T: 60-70 ms and a correlation of r = 0.82 between the duration and the peak-to-peak amplitude. It appears therefore that the overall facilitatory effect was manifest as both an increase in the peak to peak amplitude of the evoked response, and its duration, and consequently in the EMG integral. The covariance among the profiles for each of these variables suggests that the variables were mutually dependent. These analyses further attest to the robustness of the cutaneous afferent conditioning effects. Further evidence of the reproducibility of the group results was demonstrated by a Pearson product moment correlation of r = 0.85 between the profiles obtained from the first trial and the second trial respectively.

3.1.3.2 - Short latency MEPs in contralateral TA.

Short latency MEPs were elicited in the contralateral TA in 7 of 11 subjects at the same cortical stimulation intensity of 1.1 x the threshold for evoking responses in the ipsilateral TA. Facilitation of the MEPs in the contralateral TA was evident in all 7 subjects following electrical stimulation of the sural nerve in at least one or more intervals within C-T: 60-100 ms. The group averaged data at the various C-T intervals are shown in Figure 3. The mean control MEP amplitude in these subjects was 0.31 ± 0.51 mV. An increased MEP amplitude was apparent at C-T: 70 and 90 ms. The greatest facilitation occurred at C-T: 70 ms with a mean value of 313.0% of the mean normalized control MEP amplitude. Although this period of facilitation was present in all subjects there was more variability in the time course of MEP modulation than in the ipsilateral limb. This may have been due in some part to the variability across subjects of the control MEP

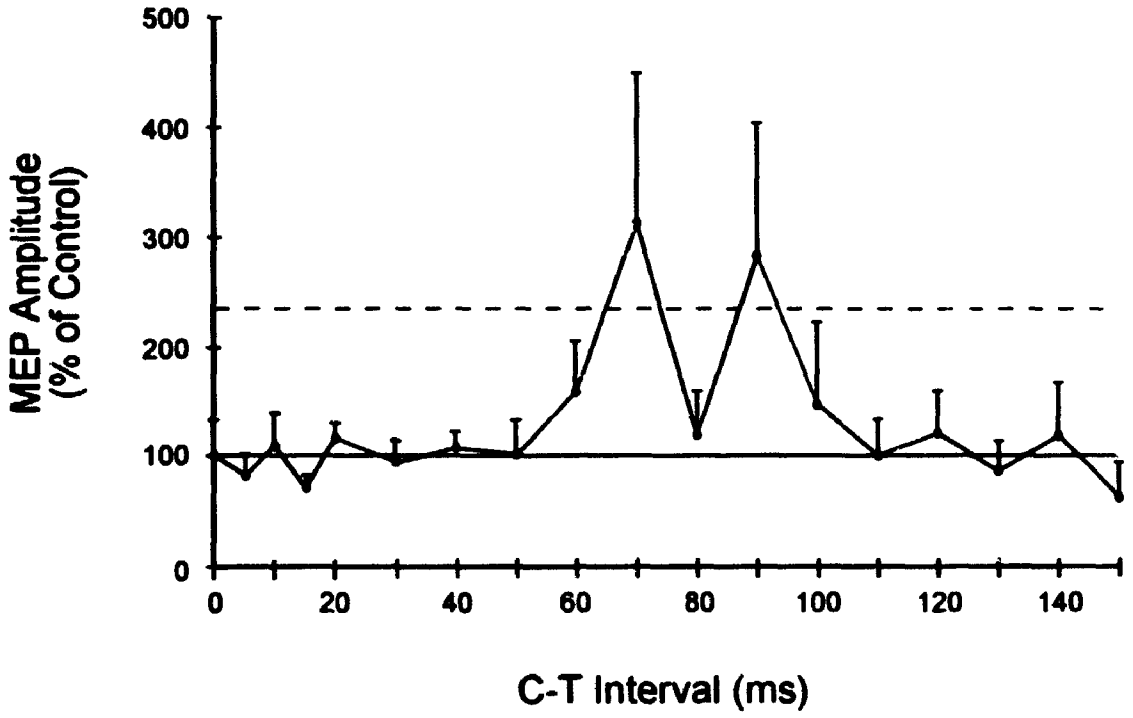

Figure 3: Mean MEP amplitudes following cutaneous afferent stimulation for the contralateral tibialis anterior normalized to the control value at each C-T interval (n=7). Dashed lines represent 2 SE values about the mean control amplitude. Vertical bars denote 1 SE of normalized conditioned values at each C-T interval.

amplitude given that the cortical stimulation intensity was set to be consistent for the ipsilateral and not the contralateral TA

3.1.3.3 - Short latency MEPs in ipsilateral LG.

The pattern of short latency MEP amplitude modulation in the ipsilateral LG was very similar to that seen in the ipsilateral TA. Of the 8 subjects demonstrating MEPs in the ipsilateral LG, 7 displayed a period of MEP facilitation within the interval C-T: 60-100 ms. Averaged data for the group (n = 8) at the various C-T intervals are shown in Figure 4. The mean control MEP amplitude across all subjects was 0.26 ± 0.39 mV. Facilitation was evident at C-T intervals of 60-70 ms. The largest facilitation of MEPs occurred at C-T: 70 ms reaching a peak mean value of 275.5% of the normalized control MEP amplitude. As well, 5 subjects demonstrated facilitated MEPs at various C-T intervals within the period of C-T: 0-40 ms. However, as with the ipsilateral TA, this period of facilitation was weak and variable between subjects and was not reflected in the averaged data.

3.1.3.4 - Late responses.

In addition to the short latency test MEPs recorded from the ipsilateral TA, two types of late responses were evoked reproducibly in some subjects. The first type of late response occurred at a latency of 70-95 ms following cortical stimulation, when the cortical stimulation was preceded by high intensity (5 x ST) conditioning afferent stimulation at C-T: 0-15 ms. This type of late response was evident in both subjects tested with high intensity conditioning inputs. The late response appeared as a relatively synchronous compound action potential with amplitude independent of the short latency

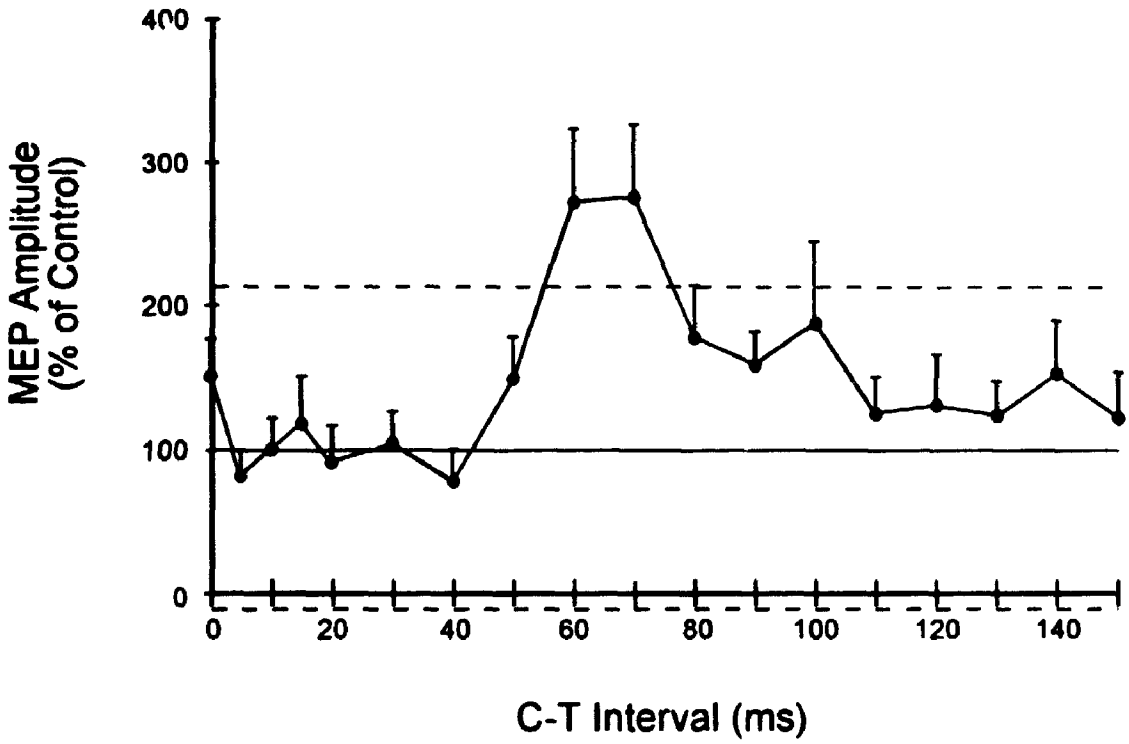

Figure 4: Mean MEP amplitudes following cutaneous stimulation for the ipsilateral lateral gastrocnemius normalized to the control value at each C-T interval (n=8). Dashed lines represent 2 SE values about the mean control amplitude. Vertical bars denote 1 SE of normalized conditioned values at each C-T interval.

MEP amplitude. This type of late response was not evoked at other C-T intervals nor under control conditions (i.e., cortical stimulation alone). This late response also appeared in the ipsilateral antagonist muscle (LG) even though the cortical stimulation on its own was subthreshold for eliciting a short latency MEP in this muscle and the cutaneous stimulation alone was subthreshold for eliciting a cutaneous reflex. The response was thus independent of the short latency MEP. Figure 5 illustrates these late responses.

The second type of late response recorded following cortical stimulation occurred at latencies of 120-140 ms when the cortical stimulation was conditioned by cutaneous afferent input at C-T: 60-120 ms (see Figure 1). The response was generally an asynchronous burst of motor unit potentials and its amplitude tended to covary with that of the short latency MEP. Two subjects exhibited this response in the ipsilateral TA. This response is most likely mediated by afferent consequences of the muscle twitch evoked following the short latency MEP (Dimitrijevic, Kofler, et al., 1992) and resembles similar responses associated with muscle twitch induced afferent activity (Szumski et al., 1974; Hayes & Clarke, 1978).

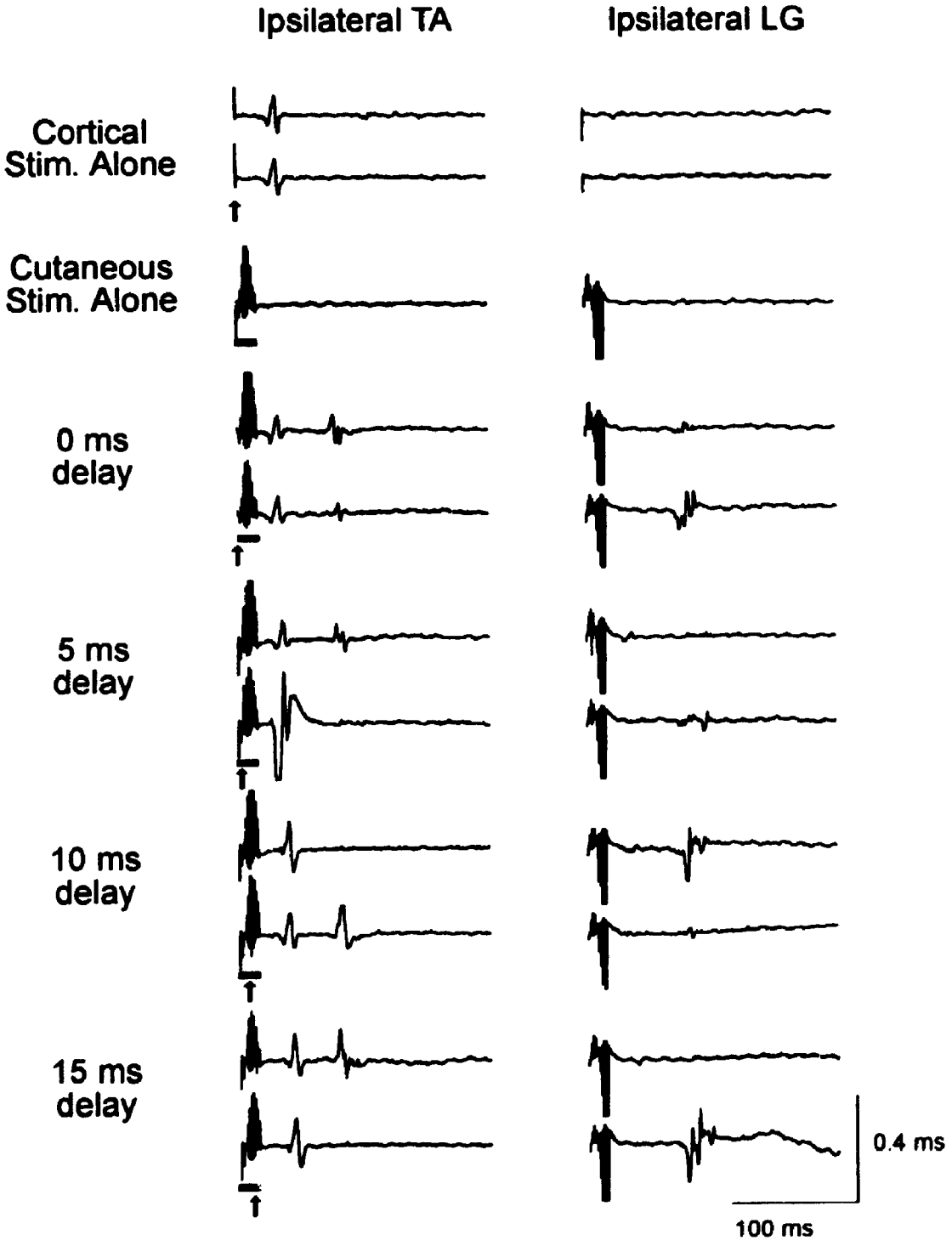

Figure 5: EMG traces from tibialis anterior (TA) and lateral gastrocnemius (LG) displaying late responses at latencies of 70-95 ms. Horizontal bars reflect the timing of the cutaneous stimulation while the vertical arrows show the time of cortical stimulation. Note that late responses were evident only when cortical stimulation was combined with high intensity cutaneous stimulation at C-T: 0-15 ms but not in the control trials. Note also that late responses were evoked in the ipsilateral LG in the absence of an early, short latency MEP in this muscle.

3.1.4 - Discussion

This study was designed primarily to characterize the influence of non-painful conditioning sural nerve stimulation on MEPs elicited in the ipsilateral and contralateral TA and LG muscles. The purpose was to test the hypothesis that stimulation of cutaneous afferents results in a facilitation of MEPs elicited in the lower limb. The results of the present study clearly identify a period of facilitation of MEPs elicited in the ipsilateral TA muscle when the sural nerve stimulation preceded cortical stimulation by 60-100 ms. A similar facilitation was evident in the contralateral TA and ipsilateral LG with the time course in the ipsilateral LG more closely paralleling the ipsilateral TA results. These results establish that stimulation of cutaneous afferents does result in a facilitation of MEPs with a consistent and definable temporal pattern.

Facilitation of short latency MEPs has been demonstrated previously in the lower limb following conditioning of peripheral nerves comprised of both muscle and cutaneous afferent fibers. Percutaneous stimulation of the medial plantar nerve on the sole of the foot produced facilitation of magnetically elicited MEPs in TA at C-T: 50-85 ms (Kasai et al., 1992). Stimulation of the posterior tibial nerve at the popliteal fossa facilitated electrically elicited MEPs in flexor digitorum longus at C-T: 55-85 ms (Deletis et al., 1987) while stimulation of the same nerve at the ankle resulted in facilitation of electrically and magnetically elicited MEPs at C-T: 50-65 ms in several lower limb muscles (Deletis et al., 1992). These intervals were slightly shorter than those found in the present study (C-T: 60-100 ms) with a purely cutaneous nerve stimulation and may reflect conduction

differences in the types of afferents mediating the effects as well as the fact that a longer (20 ms) train was used in the present study as a conditioning stimulus. Although muscle and cutaneous afferents have similar conduction velocities in peripheral nerves (Macefield et al., 1989), there appears to be differences in conduction in central pathways. This is reflected in the longer latency of the cortical somatosensory evoked potential following sural nerve stimulation when compared with tibial nerve stimulation (Amantini et al., 1992; Vogel et al., 1986).

The appearance of late responses following high intensity (5 x ST) cutaneous afferent conditioning of cortical stimulation at C-T: 0-15 ms (Figure 5) is also a new observation. The responses occurring 70-95 ms after cortical stimulation resemble the MEP_{70} identified by Dimitrijevic, Kofler, et al., (1992) or the S100 response described by Holmgren et al., (1992). These are thought to originate from slow conducting and/or oligosynaptic corticospinal or cortico-bulbospinal pathways (Dimitrijevic, Kofler, et al., 1992) or reflect brain stem (startle reaction) or peripheral influences (Holmgren et al., 1992; Mills et al., 1991). These responses never appeared when either cortical or cutaneous stimulation were administered alone and only in the two subjects that were retested with more intense, painful (5 x ST) cutaneous stimulation. This suggests that these responses reflect a convergence of late descending input with excitatory synaptic potentials conducted by peripheral cutaneous afferent fibers. The site of such convergence is not clear. The independence of the amplitude of the late response from the early MEP amplitude, together with the appearance of the late response in the antagonist (LG) muscle, in the absence of the short latency MEP, supports the view that the late response

is derived from descending inputs, as opposed to afferent consequences of the early response. The timing of the MEP_{70} has previously been shown to be inconsistent with the time course of twitch evoked spindle afferent input (Dimitrijevic, Kofler, et al., 1992) that would follow an early MEP. Together, these observations suggest that appropriately timed high intensity cutaneous afferent conditioning inputs may be used to reveal otherwise undetectable late descending inputs to the motor neuron pool.

The site of convergence of the cutaneous afferent input and the corticospinal output giving rise to the potentiated short latency MEPs at C-T: 60-100 ms is not known. Some insight can be gained from the present data by comparison with known conduction times. Cortical sensory evoked potentials elicited with sural nerve stimulation at the ankle occur at latencies of 45-50 ms (Vogel et al., 1986). Allowing a brief delay (< 10 ms) for intracortical conduction from sensory to motor cortex, one would expect that cortical stimulation would yield convergence of the 20 ms train of afferent input with motor cortical excitation commencing at C-T: 60 ms. The present data indicated a profound facilitation commencing at C-T: 60 ms (see Figure 2) consistent with a cortical site of convergence.

The period of MEP facilitation in the present study is similar to the later phase of MEP facilitation noted in various lower limb muscles following tibial nerve stimulation (Deletis et al., 1992). Deletis et al. (1992) noted latency differences between the afferent conditioning of MEPs in upper vs. lower limb muscles suggestive of the afferent and cortical input being mediated, at least in part, by a transcortical component. The results of experiments examining cutaneous reflex responses in the lower limbs in normal subjects

and in patients with various central and peripheral neurological lesions are also in accordance with these observations. These reflex responses consisted of an early spinal component and a later supraspinal component (Jenner & Stephens, 1982) compatible with the timing of the period of MEP facilitation in the present study. Elsewhere, evidence has also been presented that sural nerve conditioning of H-reflexes in TA yielded motor neuron facilitation at C-T: 60-100 (Delwaide & Crenna, 1983). This was also thought to be mediated by a supraspinal circuit.

The present results establish clear facilitatory influences of cutaneous afferent stimulation of short latency MEPs in lower limb muscles. A corollary of this is that muscle afferent input is not necessary to produce conditioning afferent facilitation of lower limb MEPs. These results confirm observations made in the upper limbs (Maertens de Noordhout et al., 1992), in which digital nerve stimulation yielded time varying inhibitory and excitatory effects on MEPs, but they differ with earlier reports that failed to detect any cutaneous afferent facilitation of upper limb MEPs (Troni et al., 1988; Komori et al., 1992). The task and context dependent nature of cutaneomuscular reflex organization in both upper and lower limbs (Datta et al., 1989; Evans et al., 1989; Yang & Stein, 1990) is well recognized and undoubtedly contributes to the complexity of analysis of cutaneous afferent influences on corticospinal output. A polysynaptic segmental reflex overlaid with one or more supraspinal pathways would provide an appropriate anatomical substrate enabling such functional diversity in cutaneous afferent - corticospinal interactions.

The clinical implications of the present results are threefold. First, they reinforce the position that peripheral afferent stimulation may be used to enhance low amplitude

short latency MEPs in patients with compromised corticospinal conduction (Hayes et al., 1991). This is especially useful as these patients are often unable to produce voluntary efforts which would otherwise result in the facilitation and detection of low amplitude MEPs. Secondly, they provide preliminary observations to suggest that high intensity cutaneous afferent conditioning may be used to reveal previously latent long latency descending inputs to the motor neuron pool. Such a procedure may assist in detecting preserved innervation in patients with SCI. Presently, these long latency responses are most easily identified using peri-stimulus time histogram techniques (Mills et al., 1991). This technique requires some degree of voluntary motor unit control which may not be possible in these patients. Thirdly, the present results offer an alternative interpretation to previous reports that the time course of MEP modulation (following mixed nerve conditioning) in SCI patients is altered. Transcranial magnetic stimulation was demonstrated to bring flexion reflexes above threshold at short C-T intervals (20-40 ms) but not at C-T intervals later than 50 ms in these patients (Hayes et al., 1992). This finding may in fact reflect the preservation of late arriving corticospinal input, through pathways other than those mediating the short latency MEP.

In summary, the present study tested the hypothesis that a conditioning cutaneous afferent input results in a facilitation of short latency MEPs with a well defined time course. The results clearly supported this hypothesis, confirming that sural nerve conditioning leads to a facilitation present in both the ipsilateral and contralateral TA muscle, as well as the ipsilateral LG muscle. In addition, with higher intensity cutaneous afferent stimulation, long latency responses (70-95 ms) were evoked following cortical

stimulation suggesting that the conditioning paradigm may be helpful in revealing later descending inputs to the motor neuron pools of lower limb muscles.

3.2 - Experiment 2 - Enhancement of Lower Limb MEPs With Induced Hypothermia in Control Subjects and Patients with SCI

3.2.1 - Introduction

Temperature has long been recognized as having a significant influence on neurophysiologic events (Denys, 1980, 1991). In particular, local cooling results in increased amplitudes of evoked potentials in nerve and muscle (Bolton et al., 1981; Louis & Hotson, 1986). These amplitude increases have been explained on the basis of increased action current due to delayed Na^+ inactivation (Frankenhaeuser & Moore, 1963; Hodgkin & Katz, 1949; Louis & Hotson, 1986) resulting in increased spatial summation in the compound action potential. Evidence also suggests that there is an increase in motor neuron pool excitability with limb cooling as shown by an increase in the amplitude of the H-reflex (Urbscheit & Bishop, 1970). These changes are dependent on a variety of factors and the time course of these excitability changes remain to be established (Bell & Lehmann, 1987; Knutsson & Mattsson, 1969). The present study was devised to examine these two potential effects of cooling as a means of enhancing MEPs in order to detect preserved innervation in patients with SCI more sensitively.

Cooling has also been shown to overcome conduction block in demyelinated nerves (Davis & Jacobson, 1971; Rasminsky, 1973) and, as such, has useful therapeutic properties for patients with multiple sclerosis (Symington et al., 1977; van Dieman et al., 1992; Watson, 1959). Since it appears that demyelination may contribute to the reduced function evident in some patients with SCI (Bunge et al., 1993, 1995; Holmes, 1906; Waxman, 1989), it seems reasonable to hypothesize that cooling may have a similar effect

in some patients with SCI. Indeed, total body cooling has been used successfully to enhance sensory conduction (i.e., increased amplitude of SEPs) in some patients with SCI (Hayes, Hsieh, et al., 1993).

The objectives of this study were therefore a) to test the hypothesis that cooling enhances MEP amplitudes elicited in lower limb muscles in control subjects and patients with SCI, b) to determine whether or not MEPs are elicited in more muscles of SCI patients following cooling, and c) to determine the relative effectiveness of enhancing MEPs using cooling versus target muscle contraction in demonstrating preserved innervation in patients with SCI.

3.2.2 - Methods

3.2.2.1 - Subjects.

Eleven adults (6 males; 5 females; age = 29.1 ± 5.0 years) with no known neurological deficits and 19 adults with established SCI provided informed consent to participate in the study. The majority of patients with SCI had traumatic SCI; one patient had transverse myelitis. Thirteen patients were injured in motor vehicle accidents, 2 in diving accidents, 2 after falls, and 1 from a sport-related accident. The level of injury was in the cervical spine between C4 and C8 in 14 of these patients while 5 had injuries in the lower thoracic spine between T8 and T12. Patients were classified by injury severity using the ASIA Impairment Scale (see Table 1). This information was obtained from the hospital chart or following neurologic testing by a physiatrist. A summary of the number, age, and months post-injury of male and female subjects within each ASIA Impairment Scale level is reported in Table 2.

Table 2 - Patient Information Grouped by ASIA Impairment Level

ASIA Impairment Scale	Number of Subjects	Number of Males	Number of Females	Age (Years)	Months Post-Injury
A	5	4	1	33.2 (13.4)	127 (160)
B	2	2	0	29.0 (8.5)	14 (9)
C	8	6	2	34.6 (8.4)	112 (78)
D	4	3	1	35.8 (2.8)	o4 (99)
All Subjects	19	15	4	33.3 (8.7)	94 (105)

Note. *SD* in parentheses.

3.2.2.2 - Procedures.

Transcranial magnetic stimulation of motor cortex was employed in order to elicit MEPs from the lower limb muscles for both control subjects and patients with SCI. The cortical stimulation procedures were the same as reported for Experiment 1 other than the fact that stimulation intensity was maintained at 100% of the maximal stimulator output. This was done to maximize the possibility of eliciting responses in patients with SCI and this stimulation intensity was comfortably tolerated by all subjects. A minimum of 2 trials were performed before and after cooling (i.e., the cooling period was terminated when the oral temperature was reduced by approximately 1 °C). MEPs were recorded bilaterally from the TA and LG in control and SCI patients and, additionally, from the extensor digitorum brevis (EDB) in 5 control subjects. The effect of attempted voluntary contraction on MEPs was also assessed in the patients as 3 trials of cortical stimulation were performed during attempted bilateral ankle dorsiflexion before and after cooling.

Peripheral nerve conduction tests, including M-waves, H-reflexes and F-waves were conducted before and after cooling in a small group of control subjects. The determination of H-reflex amplitude allowed an estimate of motor neuron excitability while M-wave amplitude reflected changes in the evoked muscle potential. Therefore, these measures permitted a means of inferring the location (i.e., motor neuron pool or muscle) of changes in MEP amplitude following cooling. The effect of cooling on peripheral and central nerve conduction time was established by the latencies of the various muscular responses. In particular, the determination of M and F-wave latencies combined with the MEP latency permitted the estimation of peripheral and central motor

conduction times (PMCT and CMCT respectively) for the right and left EDB by employing the method of Robinson et al. (1988) according to the equations below.

PMCT (in ms) = (M latency + F latency -1)/2 (1)

CMCT (in ms) = MEP latency - PMCT (2)

(To account for central delay, 1 ms is subtracted in equation 1.)

F-waves are most reliably obtained from the distal muscles of the foot and for this reason the EDB was employed for the determination of peripheral conduction times.

3.2.2.3 - M-waves.

Maximal M-waves were elicited in the LG, TA, and EDB by applying a 0.1 ms square pulse percutaneously to the appropriate nerve. This was the posterior tibial nerve at the popliteal crease for the LG and the peroneal nerve at the popliteal crease and the anterior aspect of the distal shin for the TA and EDB respectively. The stimuli were delivered from a Devices Type 3072 stimulator (Devices Sales Ltd., Hertfordshire, England) and applied through stimulating electrodes positioned along the course of the nerve. The anode and cathode were separated by 3 cm with the cathode positioned distally.

3.2.2.4 - F-waves.

F-waves were elicited in the EDB by applying a 0.1 ms square pulse percutaneously to the peroneal nerve at the anterior aspect of the distal shin with the cathode positioned proximally. The same stimulator and surface stimulating electrodes were employed as used for M-waves. The stimulation intensity was set at a level between 10-25% greater than that required for eliciting the maximum M-wave. A minimum of 10 trials were conducted with the trial with the shortest latency used for subsequent analysis.

3.2.2.5 - H-reflexes.

Maximal H-Reflexes were elicited in the LG by applying a 0.5 ms square pulse percutaneously to the posterior tibial nerve with the cathode positioned proximally overlying the popliteal crease. The same stimulator and surface stimulating electrodes were employed as those noted above.

3.2.2.6 - Induction of hypothermia.

Total body hypothermia was induced by controlled circulation of a coolant (propylene glycol) through a microclimate head-vest garment (Life Support Systems Inc Mark VII) worn over light underclothing. A fan was used to aid heat loss through convection. Cooling was continued until oral temperature was reduced by approximately 1 °C which normally required about 2 hours. Temperatures were monitored every 15 minutes with oral temperatures being taken by a Questemp II data logging thermometer (Quest Electronics) and auricular temperatures by a Thermoscan infrared ear thermometer (Thermoscan Inc.). Peripheral skin temperature was monitored with a Model 49TA digital thermometer (Yellow Springs Inst. Co.) using a probe placed over the dorsum of the foot. After maximum cooling subjects were rewarmed to their baseline temperature with an electric heating blanket. The methodology is illustrated in Figure 6.

Most subjects easily tolerated the cooling procedure. However, one patient demonstrated signs of autonomic dysreflexia associated with the cooling procedure. This patient was managed by an attendant physician and discontinued involvement in the study. There were no residual complications.

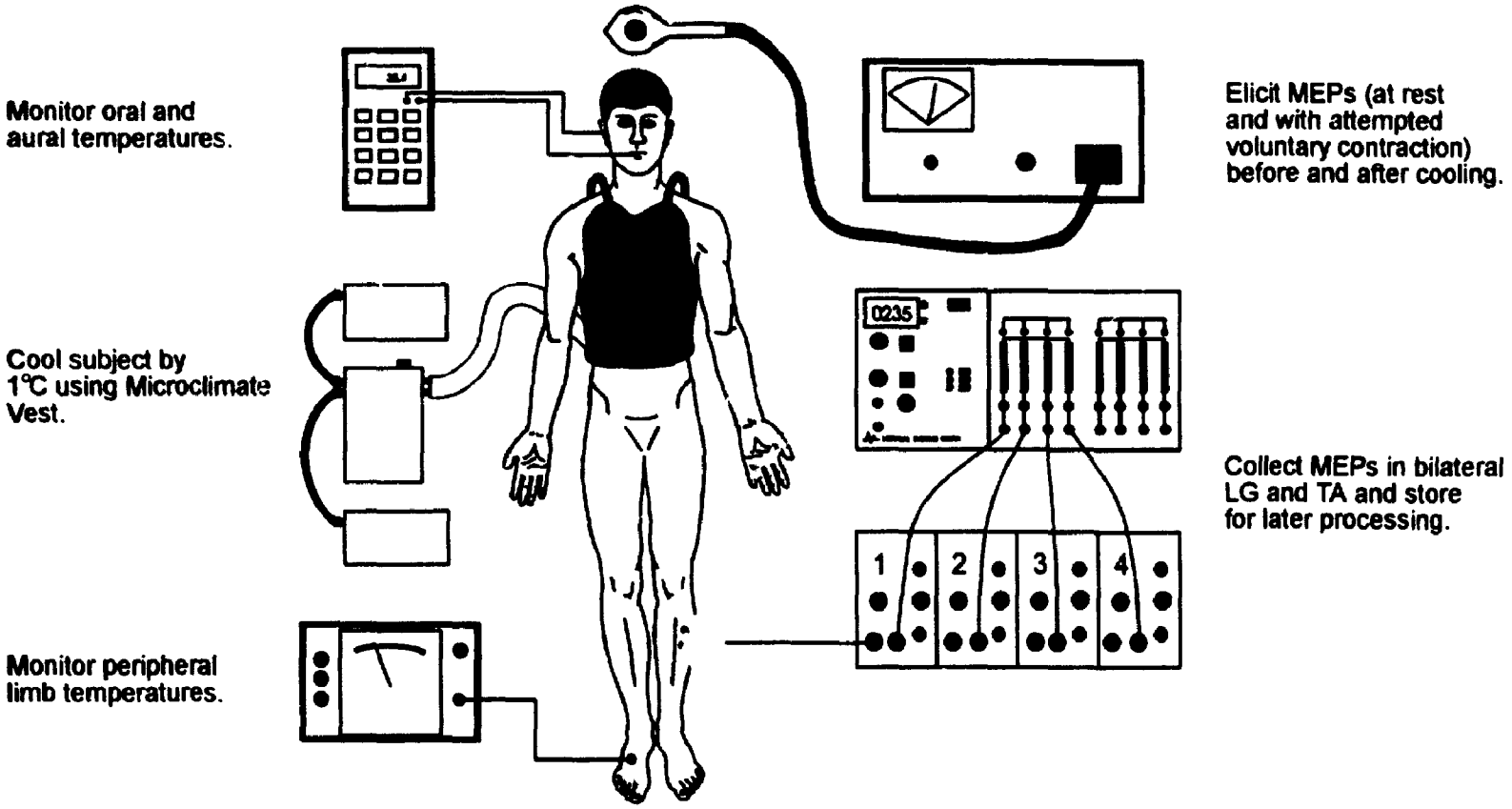

Figure 6: Methods for determining the effects of induced hypothermia on MEPs.

3.2.2.7 - Data analysis.

Following analog to digital conversion and calibration procedures, mean latencies and peak to peak amplitudes (i.e., using largest negative to positive peak) were calculated for all EMG responses (i.e., MEPs, M-waves, H-reflexes and F-waves) for each control subject using a custom software program. Means were calculated for each measure across subjects. Although the peak to peak MEP amplitude was the principal outcome measure, the significance of all mean latency and amplitude differences was tested using one-tailed paired sample *t*-tests.

Given the greater variability and difficulty in eliciting MEPs in patients with SCI, a more objective method than visual inspection was employed to aid in the detection of potential responses. This analysis was conducted following signal rectification and ensemble averaging of the trials for each condition (i.e., rest or attempted dorsiflexion) MEPs were defined as present if the signal was found to rise above a predetermined threshold level for a minimum of 10 ms. The threshold level was determined by calculating the mean plus 1.5 x *SD* of the averaged signal over the time period of 2.5 to 22.5 ms following the cortical stimulation. In this manner, any significant deviations from the average EMG activity were identified objectively allowing a rigorous evaluation of the incidence as well as the amplitude of MEPs in patients. This procedure resulted in the use of average EMG (AEMG) values computed for a period of 20 ms to describe the MEP amplitude rather than the peak to peak values used with control subjects. Statistical analyses were not conducted on the patient data due to the limited number of responses

3.2.3 - Results

3.2.3.1 - Temperature changes following cooling in control subjects and patients with SCI.

The mean (*M*) initial oral temperature of the control subjects was $M = 36.8$ °C and this was reduced to $M = 35.9$ °C after cooling. It took $M = 124.5$ minutes to reach this state The initial oral temperature of the SCI patients was $M = 36.5$ °C and this reduced to $M = 35.5$ °C after $M = 108.7$ minutes of cooling. Auricular temperatures were reduced by $M = 1.0$ and 1.2 °C and peripheral temperatures were reduced by $M = 4.4$ and 3.9 °C for control subjects and patients with SCI respectively. These results are summarized in Table 3.

3.2.3.2 - MEPs in control subjects.

The most striking result of the present study was that total body cooling resulted in increased peak to peak MEP amplitudes in all 6 muscles tested. These increases ranged from 156.9 - 200.9% of the pre-cooled MEP amplitude. Mean MEP amplitudes and latencies and the results of *t*-tests are shown in Table 4. The increases in MEP amplitudes with cooling were significant for the right and left TA and LG ($p < 0.01$) and the left EDB ($p < 0.05$). These are illustrated in Figure 7.

MEP latencies showed no significant changes with cooling in any of the muscles tested ($p > 0.05$). However, there was a tendency for prolonged latencies with cooling in the EDB as mean differences of 1.5 ms and 2.2 ms were found for the right and left EDB respectively.

Table 3 - Mean Temperature Changes with Cooling

Subject Group	Cooling Time (min)	Initial Oral Temp. (°C)	Oral Temp. Change (°C)	Initial Aural Temp. (°C)	Aural Temp. Change (°C)	Initial Periph. Temp. (°C)	Periph. Temp Change (°C)
Controls	124.5 (50.5)	36.8 (0.2)	-0.9 (0.4)	36.9 (0.5)	-1.0 (0.9)	30.4 (2.2)	-4.4 (2.0)
Patients	108.7 (30.5)	36.5 (0.4)	-1.0 (0.3)	36.4 (0.4)	-1.2 (0.5)	29.8 (1.7)	-3.9 (1.2)

Note. SD in parentheses.

Table 4 - Mean Peak to Peak MEP Amplitudes and Latencies in Control Subjects

Muscle	*n*	MEP Amplitude (mV)			MEP Latency (ms)		
		Pre-cooled	Cooled	*t* Statistic	Pre-cooled	Cooled	*t* Statistic
Right TA	11	0.61 (0.45)	1.17 (0.80)	4.66 **	29.07 (2.95)	29.46 (1.85)	0.78
Left TA	11	0.74 (0.64)	1.43 (0.91)	5.32 **	29.18 (2.37)	29.33 (2.55)	0.52
Right LG	9	0 35 (0.34)	0.55 (0.36)	3.13 **	29.03 (0.97)	29.13 (0.84)	0.34
Left LG	9	0.32 (0.29)	0.64 (0.52)	3.49 **	29.76 (2.34)	29.80 (2.15)	0.09
Right EDB	5	1.41 (1.41)	2.49 (1.60)	2.11	40.75 (1.78)	42.24 (3.26)	1.71
Left EDB	5	1.52 (1.55)	2.79 (2.63)	2.55 *	40.29 (3.21)	42.48 (4.31)	1.50

Note. SD in parentheses. * $p < 0.05$. ** $p < 0.01$.

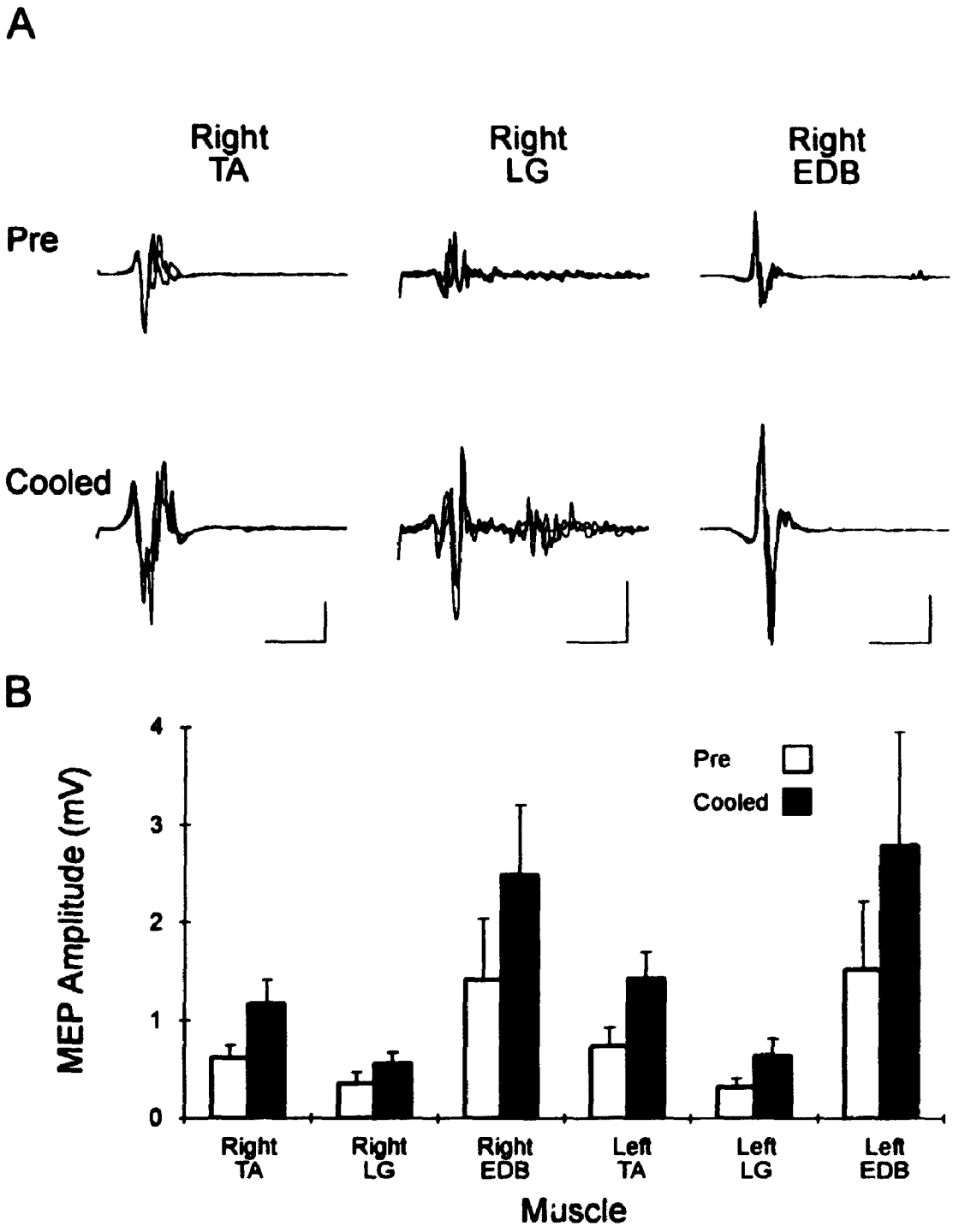

Figure 7: Effect of cooling on MEP amplitudes in control subjects. A. Raw data traces (3 trials superimposed) for right tibialis anterior (TA), lateral gastrocnemius (LG), and extensor digitorum brevis (EDB) for a representative control subject. Note the enhanced MEPs with cooling and the presence of late MEPs in the LG in this subject. Vertical calibration bars = 0.5, 0.1, and 1.0 mV for the TA, LG, and EDB respectively. Horizontal calibration bars = 50 ms. B. Overall, mean MEP amplitudes. Note enhanced amplitudes with cooling in all muscles. Vertical bars represent 1 SE.

3.2.3.3 - M-waves, H-reflexes, and F-waves in control subjects.

Mean M-wave amplitudes increased slightly with cooling in 5 of the 6 muscles tested. These changes were much less than the increases in MEP amplitude as the post-cooled M-wave amplitudes ranged from 95.2% - 113.5% of the pre-cooled M-wave amplitudes. These changes were mostly not significant ($p > 0.05$) other than in the right TA which showed a significant ($p < 0.05$) increase in M-wave amplitude with cooling. M-wave latencies were prolonged with cooling in all muscles other than the right LG. These changes were significant in the right TA and EDB ($p < 0.01$) and the left TA, LG, and EDB ($p < 0.05$). These M-wave results are summarized in Table 5.

H-reflex amplitudes increased in a similar manner to the MEP amplitudes following cooling. Mean post-cooled H-reflex amplitudes were 160.1% and 153.0% of the mean pre-cooled H-reflex amplitude in the right and left LG respectively. These changes were significant ($p < 0.05$) despite the small number of subjects tested for each muscle ($n = 4$ and 5). H-reflex latencies were prolonged by ~ 0.5 ms with these changes being significant ($p < 0.05$) for the left LG.

Significant changes were also seen in the F-wave latencies elicited in the right and left EDB following cooling despite the limited number of subjects for which F-waves were recorded ($n = 4$ and 3). A prolongation of the F-wave latencies in both the right and left EDB was seen with cooling ($p < 0.05$). Mean H-reflex and F-wave data are presented in Table 6.

Table 5 - Mean M-wave Amplitudes and Latencies in Control Subjects

Muscle	*n*	M-wave Amplitude (mV)			M-wave Latency (ms)		
		Pre-Cooled	Cooled	*t* Statistic	Pre-Cooled	Cooled	*t* Statistic
Right TA	9	6.29 (2.07)	6.62 (1.78)	2.08 *	2.52 (0.24)	2.98 (0.32)	4.90 **
Left TA	6	5.59 (0.74)	5.70 (1.35)	1.13	2.80 (0.25)	3.14 (0.22)	2.98 *
Right LG	5	14.97 (4.54)	15.95 (2.95)	0.82	3.77 (0.72)	3.76 (0.36)	0.00
Left LG	6	9.71 (4.45)	10.19 (4.46)	1.13	3.71 (0.95)	3.97 (0.81)	2.23 *
Right EDB	5	7.22 (2.91)	8.19 (2.50)	1.06	5.07 (0.95)	7.13 (1.65)	4.54 **
Left EDB	5	7.94 (2.82)	7.55 (3.45)	0.54	5.29 (0.79)	7.12 (1.15)	3.63 *

Note. SD in parentheses. * $p < 0.05$. ** $p < 0.01$.

Table 6 - H-reflexes and F-waves following Cooling in Control Subjects

Muscle	*n*	Amplitude (mV)			Latency (ms)		
		Pre-Cooled	Cooled	*t* Statistic	Pre-Cooled	Cooled	*t* Statistic
				H-reflex			
Right LG	4	1.96 (0.76)	3.14 (1.34)	3.10 *	28.03 (1.61)	28.45 (2.15)	1.30
Left LG	5	1.64 (1.56)	2.50 (2.18)	2.52 *	28.12 (1.48)	28.64 (1.89)	2.48*
				F-wave			
Right EDB	4	-	-	-	46.88 (3.27)	49.60 (3.33)	2.73 *
Left EDB	3	-	-	-	45.29 (6.18)	49.46 (5.92)	3.85 *

Note. F-wave amplitude was not calculated as H-reflex amplitude was considered the more reliable estimate of motor neuron excitability. *SD* in parentheses. * $p < 0.05$.

3.2.3.4 - Peripheral and central motor nerve conduction times in control subjects.

Mean PMCT was prolonged with cooling by 1.50 and 2.96 ms for the right and left EDB respectively. This prolongation was significant ($p < 0.05$) for the left EDB despite the small number of subjects who displayed responses for each of MEPs, M-waves and F-waves ($n = 3$). Conversely, mean CMCT was reduced (nonsignificantly) with cooling by 0.59 and 2.55 ms for the right and left EDB respectively. Mean PMCT and CMCT data are summarized in Table 7.

3.2.3.5 - MEPs in patients with SCI.

Prior to cooling, MEPs were not elicited from ASIA A or B patients with or without reinforcement from attempted target muscle contraction. MEPs were recorded from various muscle groups in ASIA C and D patients although these were often low amplitude, asynchronous (temporally dispersed) responses with prolonged latencies The incidence (i.e., number of recorded MEPs in all muscles across all subjects) increased from 8 to 24 of a total of 60 muscles when target muscle contraction was employed to facilitate the responses. The responses were also of higher amplitude and shorter latency. Late responses were also evident after reinforcement. Figure 8 illustrates the facilitatory effect of target muscle contraction in one subject and Figures 9 and 10 illustrate the ensemble averaged, rectified, group EMG data showing reinforcement of both early and late responses.

After cooling, it was still not possible to evoke MEPs in any of the ASIA A or B patients. Similarly, ASIA C patients did not reveal additional MEPs with cooling. However, in ASIA D patients MEPs were evoked in some resting muscles ($n=3$) which prior to cooling yielded no response. These responses were low amplitude, asynchronous

Table 7 - Mean Peripheral and Central Motor Conduction Times in Control Subjects

Muscle	*n*	PMCT (ms)			CMCT (ms)		
		Pre-Cooled	Cooled	*t* Statistic	Pre-Cooled	Cooled	*t* Statistic
Right EDB	4	25.05 (1.43)	26.55 (2.76)	1.42	15.34 (0.50)	14.75 (1.30)	1.03
Left EDB	3	24.03 (3.32)	26.99 (2.69)	6.41 *	16.69 (0.83)	14.14 (2.76)	1.24

Note. *SD* in parentheses. * $p < 0.05$.

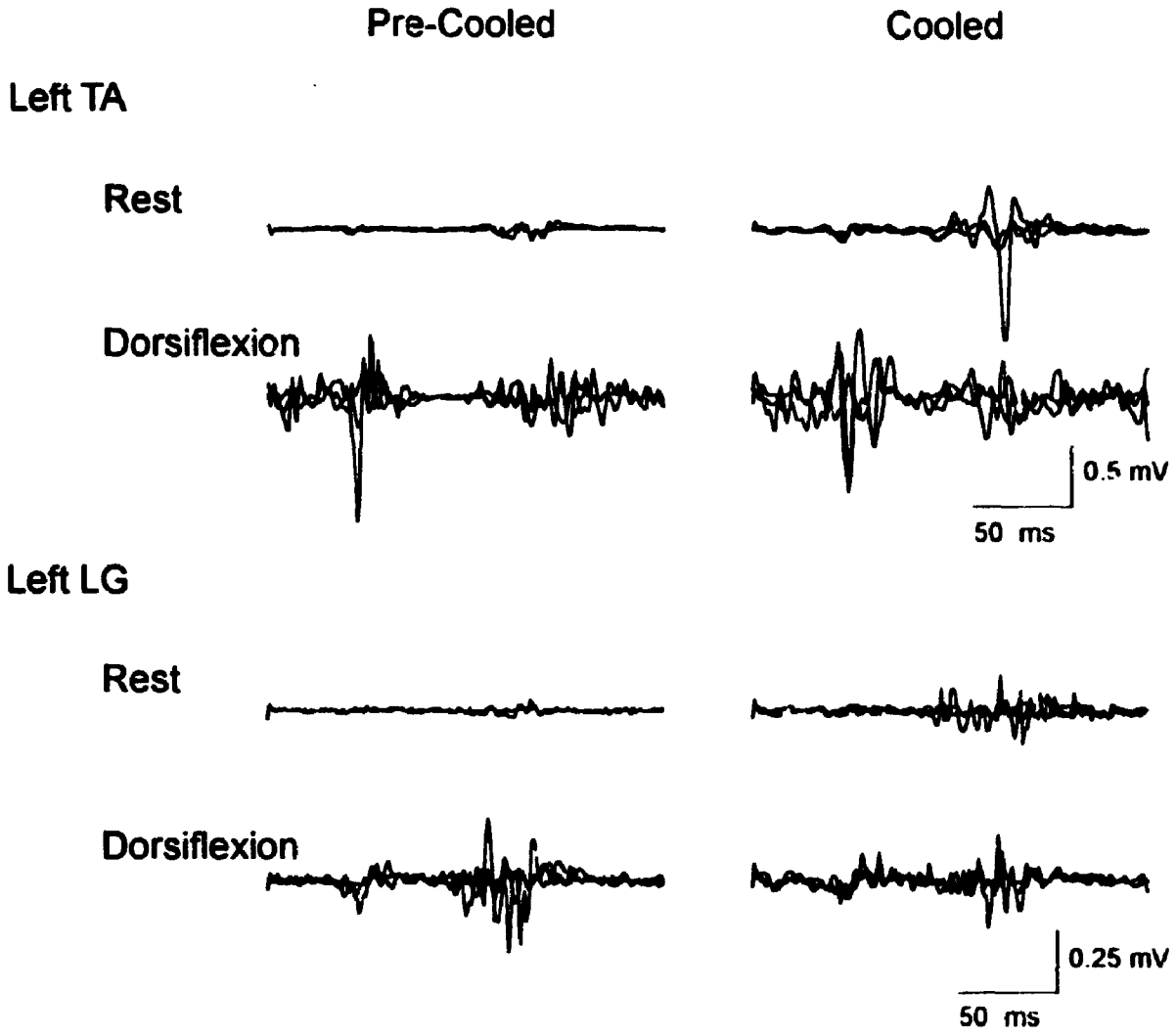

Figure 8: MEPs elicited in the tibialis anterior (TA) and the lateral gastrocnemius (LG) before and after cooling in a representative patient (ASIA D). Three traces are superimposed for each condition. Note the marked enhancement of early and late MEPs in this patient with dorsiflexion This patient also demonstrated a slight enhancement of MEPs elicited at rest following cooling (particularly late MEPs).

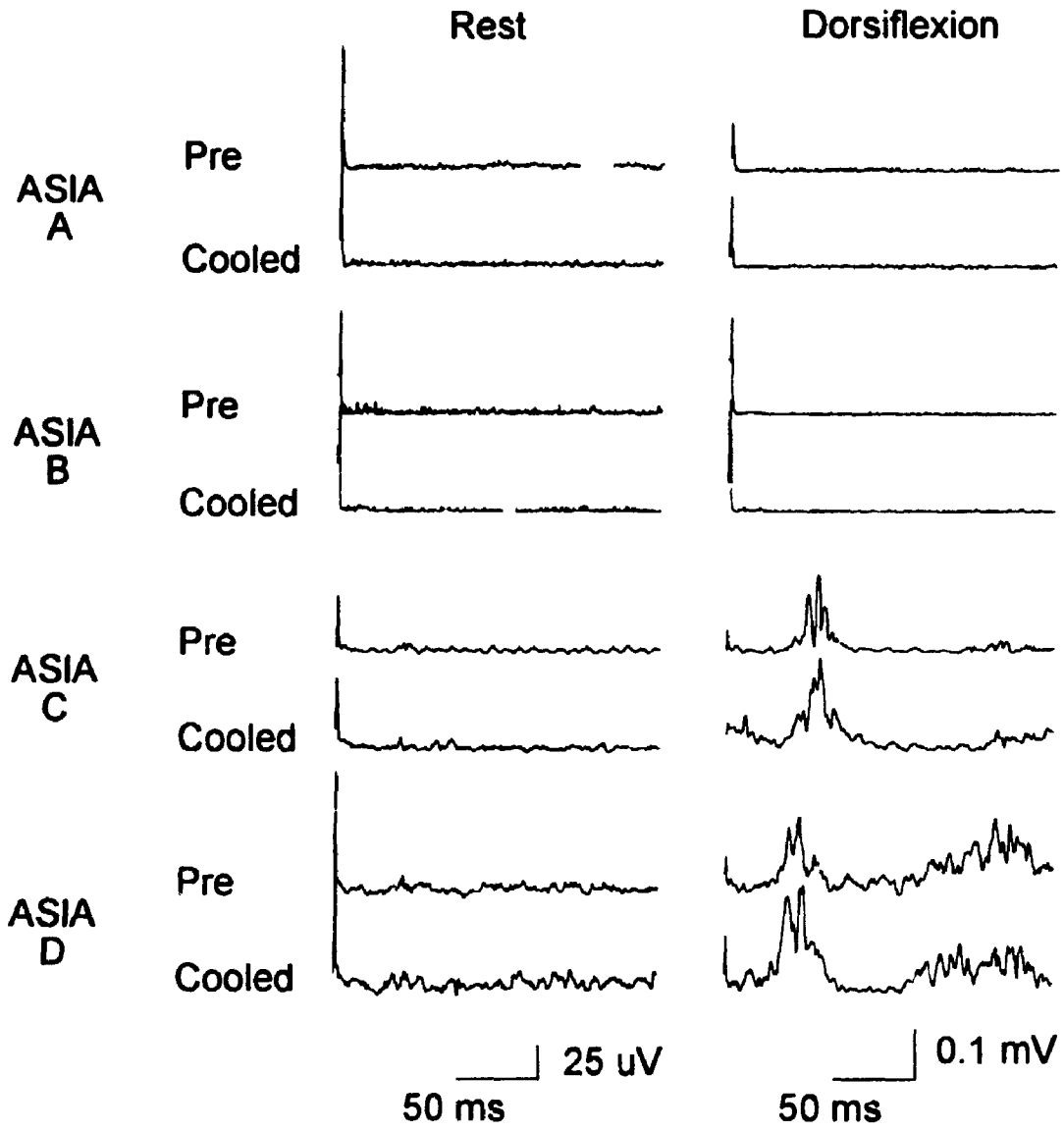

Figure 9: Mean, ensemble averaged EMG activity in the right tibialis anterior (TA) following cortical stimulation in patients with SCI before and after cooling. Note the marked enhancement of early and late EMG acitvity when MEPs were reinforced with attempted ankle dorsiflexion. Cooling had very little effect on the responses.

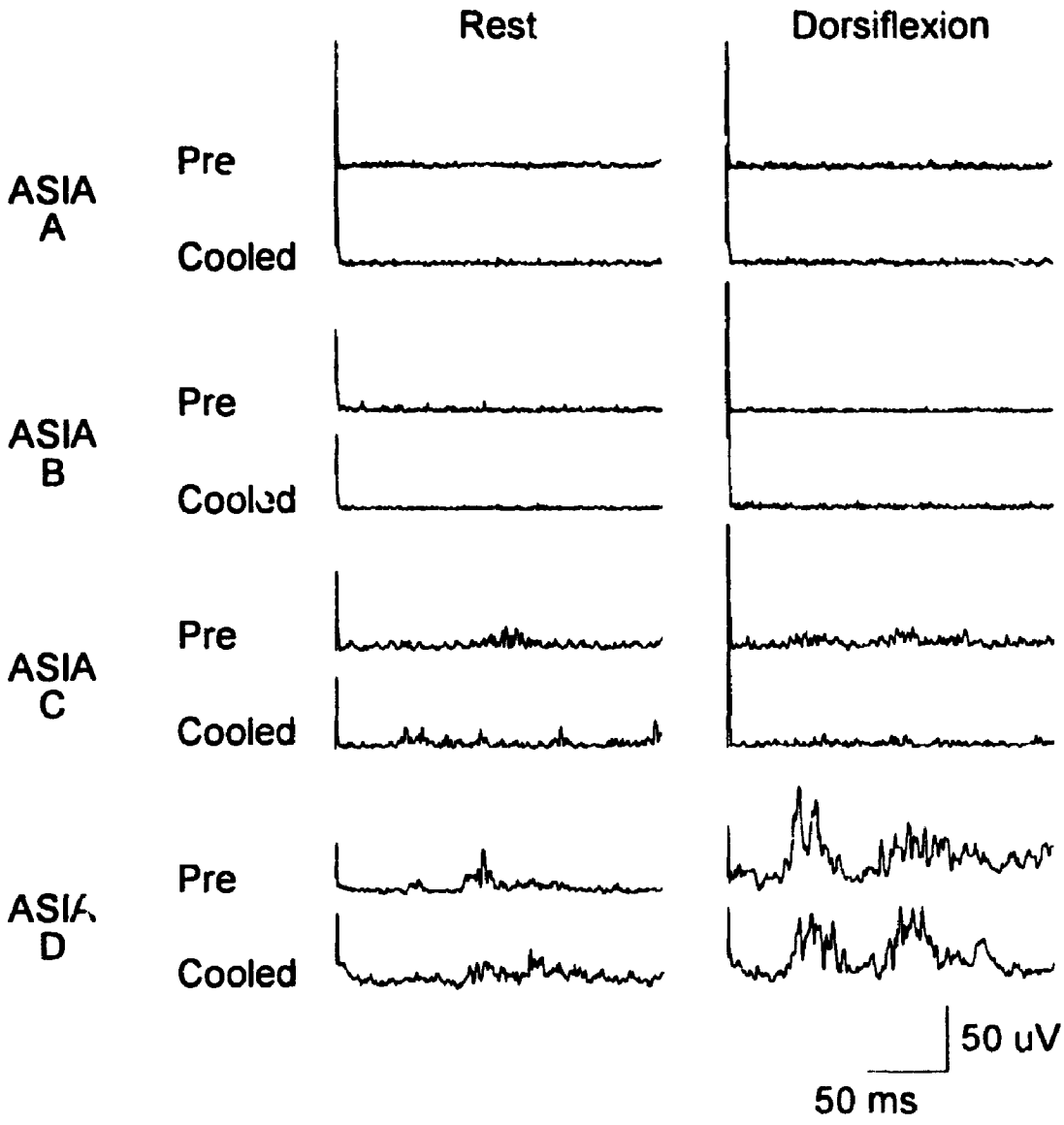

Figure 10: Mean, ensemble averaged EMG activity in the left lateral gastrocnemius (LG) following cortical stimulation in patients with SCI before and after cooling. As with the tibialis anterior (TA), cooling had ver, little effect on the responses but attempted dorsiflexion greatly facilitated the MEPs.

and long latency. Table 8 provides a summary of the incidence of MEPs, before and after cooling, according to the patients' ASIA classification. Although newly recorded MEPs were apparent, this did not appreciably change the detection of preserved corticospinal innervation because in each case MEPs were apparent in these muscles when target muscle contractions were used to facilitate the response.

Inspection of Figures 9 and 10 and Table 8 shows that, overall, the MEP amplitudes in SCI patients were not increased by cooling. This is in contrast to what was observed in the control subjects. Further analysis (Table 9) reveals, however, that the effects of cooling on MEP amplitude appear to differ according to patients' ASIA classification; thus patients at Level C showed no change, or even reduced MEP amplitudes, whereas patients at Level D (i.e., least severely injured) behaved more like controls and showed enhanced MEPs (e.g., Figure 8). This was apparent in both resting muscles and when target muscle contraction was employed. The small number of subjects within each classification precludes statistical treatment of this interaction.

The latency of the MEPs tended to be shorter in ASIA D patients relative to ASIA C patients. This is apparent in both the onset latency and time to peak deflection in Figure 9. Latencies were determined with the objective method of MEP detection for the trials involving attempted ankle dorsiflexion. By grouping responses across all of the muscles (i.e., TA and LG), overall onset latencies of $M = 36.7 \pm 5.8$ and $M = 39.2 \pm 2.0$ ms were obtained for ASIA D and C patients respectively. Onset latencies were prolonged with cooling with mean latencies of $M = 39.2 \pm 6.4$ and $M = 41.4 \pm 4.9$ ms obtained for ASIA D and C patients respectively.

Table 8 - Incidence of MEPs in Patients Before and After Cooling

ASIA Impairment Scale	*n*	Total # of Muscles Monitored (Over all Patients)	# of Muscles with MEPs			
			Pre-cooling at Rest	Cooling at Rest	Pre-cooling with Dorsiflexion	Cooling with Dorsiflexion
A	5	16	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
B	2	7	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
C	8	22	4 (18.2%)	4 (18.2%)	10 (45.5%)	9 (40.9%)
D	4	16	4 (25.0%)	5[a] (31.3%)	14 (87.5%)	14 (87.5%)
All Patients	19	60	8 (13.3%)	9 (15.0%)	24 (40.0%)	23 (38.3%)

Note. Percentage of total number of muscles monitored with MEPs present in parentheses. [a]With cooling, MEPs were elicited at rest in three muscles in which MEPs were absent prior to cooling. However, MEPs were absent with cooling in two other muscles in which MEPs were previously present.

Table 9 - Mean MEP Amplitudes in Patients Before and After Cooling

ASIA Level	# of Muscles	MEP Amplitude (AEMG)		
		Pre-cooling (uV)	Cooling (uV)	Mean Change (%)
		At Rest		
C	4	20.3 (9.0)	15.5 (7.4)	76.8
D	2	10.3 (1.0)	20.8 10.6)	200.8
Total	6	17.0 (8.7)	17.3 (7.9)	102.0
		With Attempted Ankle Dorsiflexion		
C	9	75.1 (133.4)	44.6 (42.0)	59.4
D	13	115.4 (111.9)	125.6 (97.8)	108.9
Total	22	98.9 (119 8)	92.4 (88.3)	93.5

Note. MEP amplitudes were included in means only if responses were present before and after cooling. *SD* in parentheses.

3.2.3.6 - Late responses in patients with SCI.

Several patients (ASIA C and D only) also exhibited late responses to transcranial magnetic stimulation in the present experiment. An illustrative case is shown in Figure 8 and the grouped data appear in Figures 9 and 10. Late responses were defined as any period of increased motor unit activity from 60 - 150 ms following cortical stimulation as detected by the algorithm described earlier and verified by visual inspection These responses were more labile and variable with respect to latency than the early responses and ranged in onset latency from 73 to 148 ms. Late responses were more pronounced in the LG than the TA when the muscles were at rest. This was especially evident with ASIA D patients as can be seen in Figures 9 and 10. In addition, late periods of motor unit activity were more pronounced in ASIA D patients compared to ASIA C patients when ankle dorsiflexion was attempted. This was particularly the case for the TA

Cooling did not have any consistent or interpretable effect on late responses The group data reported in Figure 10 for the LG shows an apparent reduction of amplitude for ASIA C patients, and an increase in amplitude for ASIA D patients, in accord with the early MEP changes. However, this was not the case in the TA (Figure 9). The small number of responses recorded fails to provide robust evidence of the effects of cooling on late responses.

3.2.4 - Discussion

The principal objective of the present study was to test the hypothesis that cooling enhances MEP amplitudes elicited in lower limb muscles in control subjects and patients with SCI. The results demonstrate clearly that lower limb MEP amplitudes were significantly enhanced following cooling in neurologically normal individuals thereby confirming part of the *a priori* hypothesis. However, cooling did not have a similar effect on the incidence or amplitude of MEPs elicited in patients with SCI. There was a tendency for increased amplitudes with cooling in the less severely impaired patients (i.e., ASIA D). Patients with lower levels of function (ASIA C and B) did not demonstrate any systematic increase in MEP amplitudes with cooling. Thus the second part of the hypothesis was partly confirmed but partly rejected.

In control subjects, and high functioning SCI patients, the enhanced amplitude of MEPs is potentially attributable to both peripheral and central mechanisms. Local cooling delays Na^+ inactivation and thereby prolongs the duration of local action currents (Frankenhaeuser & Moore, 1963; Hodgkin & Katz, 1949; Louis & Hotson, 1986) which results in increased amplitude of the muscle action potential. Peripheral (limb) cooling would be expected to have similar effects on the amplitude of the MEP, H-reflex, and M-wave. In fact, the M-wave amplitude was only marginally increased in the present experiment (albeit consistently increased with cooling) when compared to the larger increases seen with the H-reflex and MEP. It seems likely, therefore, that some central factors contributed to the additional increases seen in the H-reflex and MEP amplitude. The profound limb and core cooling would have induced an involuntary increase in motor

neuron excitability, as part of a homeostatic shivering mechanism (Johnson, 1992) This increase in central excitability would lead to increased motor unit recruitment in response to corticospinal (i.e., MEP) or afferent (i.e., H-reflex) inputs. The cumulative effect of these small peripheral and large central mechanisms would account for the consistently observed cooling induced increase in MEP and H-reflex amplitude in the control subjects and to a lesser extent in patients at ASIA level D.

The differential effect of cooling between controls and the more severely impaired patients was not predicted. Patients with more severe levels of impairment (i e , ASIA C and B) typically exhibit dysfunctional thermoregulatory c ontrol. On exposure to cooling, shivering is absent, the patients more rapidly lose body heat, and are at risk from severe hypothermia. This is a consequence of disrupted autonomic control (Johnson, 1992) The patients, therefore, do not exhibit increased motor neuron excitability It seems likely that the cumulative effect of these pathological mechanisms outweighed any peripheral facilitatory effects introduced by cooling.

Cooling-induced changes in central and peripheral conduction times (Table 7) were evident and largely predictable from prior work (Buchthal & Rosenfalck, 1966, de Jesus et al., 1973; de Jong et al., 1966; Halar et al., 1981) Since this issue was not integral to the hypothesis under examination, discussion of these changes has been deferred to Appendix 1 and 2.

The use of the cooling vest in the present study was adopted in an attempt to achieve total body cooling thereby reducing central core temperatures in addition to peripheral skin and intramuscular temperatures In this manner, it was hoped to determine

if central conduction was enhanced in patients with SCI which would aid in the detection of previously latent innervation. In addition, this would lend support to the hypothesis that demyelination is a significant component of the pathophysiology in some patients with SCI (Bunge et al., 1993; Holmes, 1906; Waxman, 1989). Conduction deficits due to demyelination have been demonstrated to be improved or ameliorated with cooling (Davis & Jacobson, 1971; Rasminsky, 1973). However, little support was provided for this concept in the present study. None of the SCI patients exhibited any evidence of enhanced central conduction as would have been indicated by marked reductions in MEP latency. If anything, the opposite results were obtained as TA and LG MEP latencies were prolonged by ~ 2 ms in patients with SCI as compared to lesser latency prolongation (i.e., < 0.5 ms) with control subjects.

An unexpected finding in the present study was the rather high incidence of late responses elicited with cortical stimulation in patients with SCI. These responses have been noted to occur in these patients (Dimitrijevic et al., 1988, Segura et al., 1992), but there is little information as to their frequency of occurrence or the pathways by which they are mediated. These responses exhibited more trial to trial variability than the early responses and it was often difficult to determine precise response onsets and durations for these responses. Qualitatively, several important observations were apparent from the overall responses averaged across subjects (see Figures 9 and 10). When the muscles were at rest, late responses elicited in some ASIA C and most ASIA D patients were present most often in the LG and less often in the TA. In some patients, these late responses were even more prominent than the early responses. When patients attempted

bilateral ankle dorsiflexion, early responses were greatly facilitated, especially in the TA, in both ASIA C and D patients. However, late responses were only facilitated to a similar extent in ASIA D patients. As with the short latency MEPs, there was little, if any, effect of cooling on any of these late responses.

In summary, while cooling did yield marked enhancement of MEP amplitudes in control subjects, and to a lesser extent in high functioning SCI patients, cooling did not prove to be an effective procedure to enhance the elicitation of MEPs for the detection of preserved innervation in patients with more severe SCI. Target muscle contraction was more effective in demonstrating preserved innervation in these patients In particular, MEPs were able to be elicited with attempted target muscle contraction in some ASIA C patients and all ASIA D patients.

3.3 - Experiment 3 - Detection of Short and Long Latency Inputs to Lower Limb Motor Neuron Pools by Conditioning H-reflexes with Transcranial Magnetic Stimulation in Normal Subjects

3.3.1 - Introduction

Experiments employing electrical stimulation of the motor cortex to condition H-reflexes in humans (Brown et al., 1978; Cowan et al., 1986; Milner-Brown et al., 1975; van der Linden & Bruggeman, 1993) and monosynaptic reflexes in primates (Preston & Whitlock, 1960) have demonstrated that a single cortical stimulus produces a brief period of altered motor neuron excitability. The precise pattern of facilitation and inhibition varies between different muscles due to differences between the specific excitatory and inhibitory inputs to each motor neuron pool (Cowan et al., 1986). Similar techniques have also been shown to reveal later (i.e., > 50 ms following cortical stimulus) effects of magnetic cortical stimulation on the excitability of human soleus and anterior tibial motor neuron pools (Holmgren et al., 1992).

The method of conditioning H-reflexes with transcranial magnetic stimulation complements the approaches used in the first two experiments of the present thesis. In the previous experiments, reinforcement procedures were used to enhance the probability of eliciting MEPs thereby allowing detection of short and long latency influences on the motor neuron pool. By employing cortical conditioning of H-reflexes, these same inputs can be detected and, more importantly, the time course of these influences can be examined even if they are subthreshold for eliciting an observable response.

3.3.1.1 - Research objectives and plan.

This study was designed to maximize the opportunity for revealing descending influences on the LG motor neuron pool following cortical stimulation in normal subjects In addition, it examined the issue of whether late periods of excitability following cortical stimulation are mediated by descending pathways or are due to the afferent consequences of the short latency MEP. These objectives were achieved by the administration of either supra- or subthreshold cortical stimulation followed by a segmental, electrically evoked H-reflex acting as a test response to detect changes in the excitability of the motor neuron pool.

The employment of subthreshold cortical stimulation was critical for addressing the second objective relating to the origin of any late periods of excitability following cortical stimulation. Demonstration of any late period of H-reflex modulation occurring in the absence of an early MEP would support the hypothesis that late periods of excitability in the motor neuron pool following cortical stimulation are mediated by central descending tracts (i.e., they are not attributable to afferent consequences of the twitch from the early MEP).

Therefore, the purposes of this study were a) to describe the pattern of excitability changes in the LG motor neuron pool following cortical stimulation in normal subjects and b) to test the hypothesis that late periods of excitability demonstrated by the cortical conditioning of H-reflexes are the result of excitatory synaptic inputs from descending tracts.

3.3.2 - Methods

3.3.2.1 - Subjects.

Ten healthy adults (5 males; 5 females; age = 32.2 ± 6.8 years) recruited from the staff of Parkwood Hospital provided informed consent to participate in separate studies examining the effect of supra- and subthreshold transcranial magnetic stimulation on the H-reflex. Subjects were screened for history of epilepsy or cardiovascular disease that would be contraindications for the use of cortical stimulation.

3.3.2.2 - Procedures.

A condition-test (C-T) paradigm was employed to examine the pattern of excitability changes in the motor neuron pool following transcranial magnetic stimulation. In this experiment, cortical stimulation was used to condition (i.e., modulate) test H-reflexes elicited in the LG muscle. The conditioning stimulus was therefore the cortical stimulation and the test response was the H-reflex. Cortical stimulation intensity was set to either 10% above or 10% below the threshold for eliciting a short latency (~ 30 ms) MEP in the LG muscle in the leg that exhibited the lowest response threshold. Subjects were examined under either of these conditions (i.e., supra- or subthreshold stimulation) on separate days. The C-T intervals examined were 0 to 150 ms in 10 ms increments and C-T 200 and 300 ms. Trials involving control H-reflexes (i.e., no cortical stimulation) were paired with conditioned trials with the order of these pairings being randomized. Each C-T interval was examined twice with a minimum of 30 s between successive trials.

3.3.2.3 - Conditioning cortical stimulation.

Transcranial magnetic stimulation was delivered by a Cadwell MES-10 stimulator.

Stimulation intensity was initially set at a level that was barely perceptible and gradually increased in 5% increments until motor threshold was obtained. Motor thresholds were defined as the stimulus intensity at which MEPs were present in at least 50% of trials. For the rest of each testing session, cortical stimulation intensity was set to either 10% above or 10% below the motor threshold. The mean cortical stimulation intensity was 76.8 ± 17.2% and 58.5 ± 12.1% of maximal stimulator output for the supra- and subthreshold stimulation respectively. All stimuli were delivered with the subjects lying supine on a bed with the head of the bed inclined so that the subjects' hips were flexed by approximately 45°.

3.3.2.4 - Test H-reflexes.

H-Reflexes were elicited in the LG by applying a 0.5 ms square pulse percutaneously to the posterior tibial nerve with the cathode overlying the popliteal crease. The stimuli were delivered from a Devices Type 3072 stimulator as noted in Experiment 2 and applied through stimulating electrodes positioned along the course of the nerve. The anode and cathode were separated by 3 cm with the cathode positioned proximally. The intensity of stimulation was adjusted to produce a response of ~ 33% maximum H-reflex amplitude in order to allow detection of either facilitation or inhibition. The actual recorded mean control H-reflex amplitude was 32.9 ± 8.5% and 35.4 ± 6.2% of the maximum H-reflex amplitude for the supra- and subthreshold stimulation experiments respectively.

3.3.2.5 - Data analysis.

Following analog to digital conversion and calibration procedures, mean peak to

peak H-reflex amplitudes (i e., using largest negative to positive peak) were calculated for each C-T interval. These amplitudes were normalized with respect to the overall mean control H-reflex amplitude. Mean normalized H-reflex amplitudes were calculated across subjects for both control and conditioned H-reflexes. In addition, each conditioned H-reflex amplitude was normalized relative to its respective paired control H-reflex amplitude and then means were calculated for each C-T interval. Overall results for the mean amplitudes normalized in this manner were highly correlated with those normalized with respect to the overall mean control H-reflex amplitude ($r = 0.88$ and $r = 0.97$ for supra- and subthreshold stimulation respectively) demonstrating the robustness of the results independent of the method of analysis.

3.3.3 - Results

3.3.3.1 - Suprathreshold cortical conditioning of H-reflexes.

All subjects demonstrated a common pattern of reflex modulation when H-reflexes were conditioned by suprathreshold cortical stimulation. Individual raw data traces and mean H-reflex amplitudes for each C-T interval for a representative subject are presented in Figure 11. Mean H-reflex amplitudes averaged across all subjects were facilitated at all C-T intervals by at least 2 *SE* values greater than the mean control H-reflex amplitude. Conditioned H-reflex amplitudes normalized with respect to the mean control H-reflex amplitude and averaged across subjects are presented in Figure 12. Two distinct periods of H-reflex facilitation with cortical stimulation were evident consisting of an early, relatively short lasting period at C-T: 10-30 ms and a late, longer lasting period of facilitation at C-T 60-130 ms. The early period was of slightly larger magnitude with a maximum value of 299.7 ± 46.5% ($M \pm SE$) of the control H-reflex amplitude at C-T 20

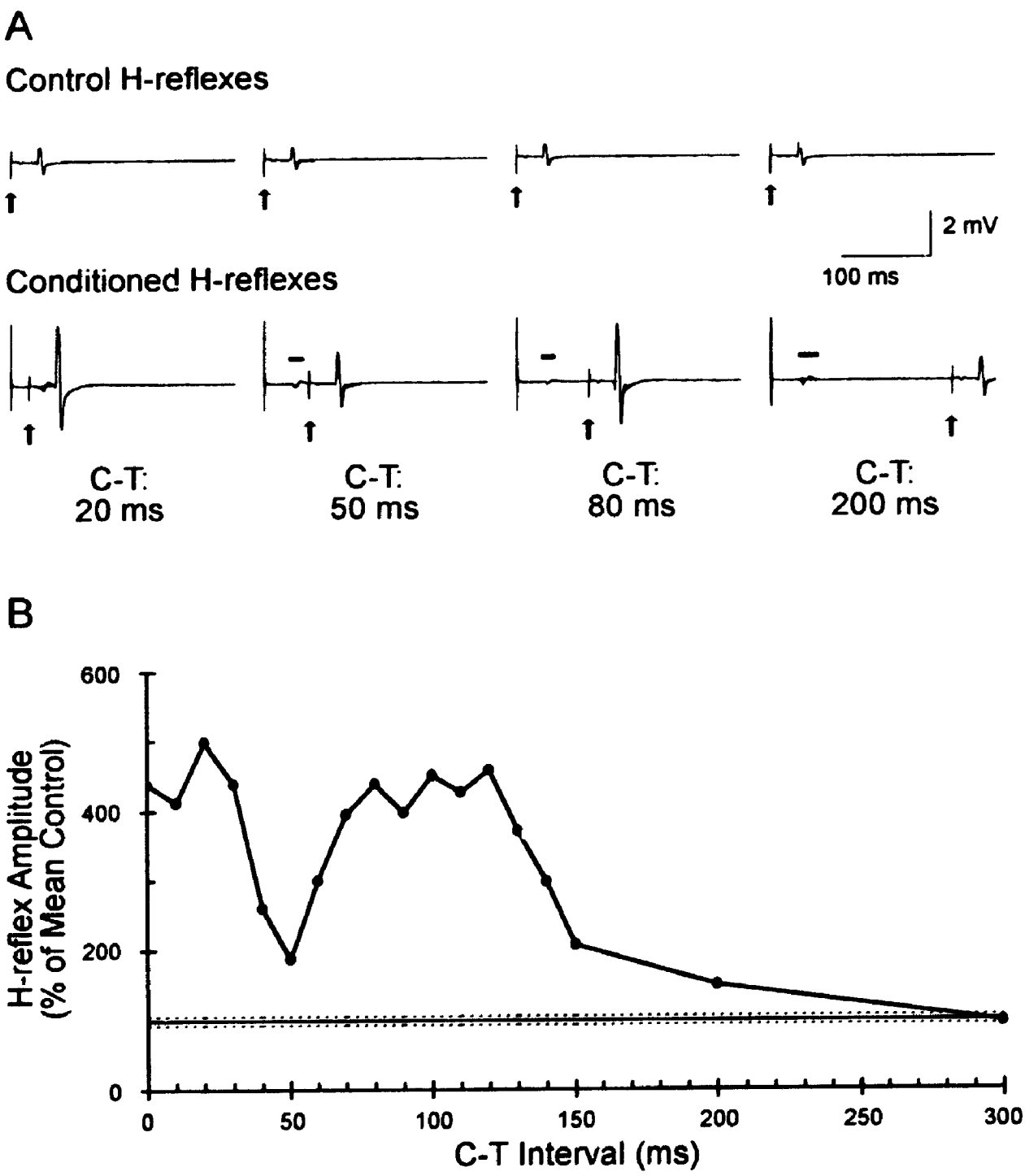

Figure 11: Effect of suprathreshold cortical stimulation on H-reflexes in a representative subject. A. Raw data traces showing control and conditioned H-reflexes at selected C-T intervals. Two trials are superimposed. Note the enhanced H-reflexes at C:T 20 and 80 ms and the presence of a small MEP designated by horizontal bar above trace in conditioned trials. Conditioned traces begin with cortical stimulation stimulus artifact while arrows denote stimulation artifact for H-reflex. B. Mean, normalized H-reflex amplitudes demonstrating the early and late periods of enhanced motor neuron excitability. Dashed horizontal lines represent 2 SE about the mean control H-Reflex amplitude.

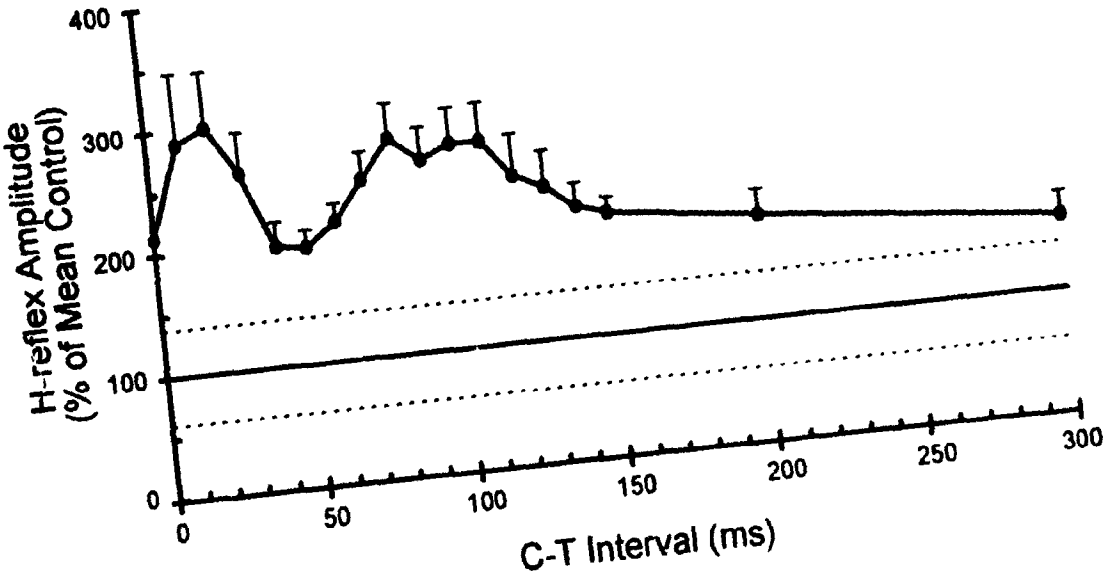

Figure 12: Effect of suprathreshold cortical conditioning on H-reflex amplitude averaged across subjects. Mean amplitudes normalized to the mean control amplitude. Note the two periods of enhanced motor neuron excitability at C-T: 10-30 and 60-130 ms. Dashed horizontal lines represent 2 SE about the mean control amplitude and vertical lines represent 1 SE of the mean conditioned H-reflex amplitudes.

ms while the late period had a maximum value of 276.8 ± 29.2% ($M \pm SE$) of the control amplitude at C-T. 80 ms.

3.3.3.2 - Subthreshold cortical conditioning of H-reflexes.

The same patterns of enhanced motor neuron excitability were demonstrated with subthreshold cortical stimulation, however, the overall levels of facilitation were less pronounced. Raw data traces and mean amplitudes of H-reflexes conditioned with subthreshold stimulation are presented in Figure 13 for the same subject as portrayed in Figure 11. Group mean normalized H-reflex amplitudes are shown in Figure 14. The same two periods of facilitation as that noted with suprathreshold cortical stimulation were evident with peak facilitation of 180.4 ± 20.3% and 192.3 ± 17.4% ($M \pm SE$) of the control H-reflex amplitude at C-T: 10 ms and C-T: 90 ms respectively.

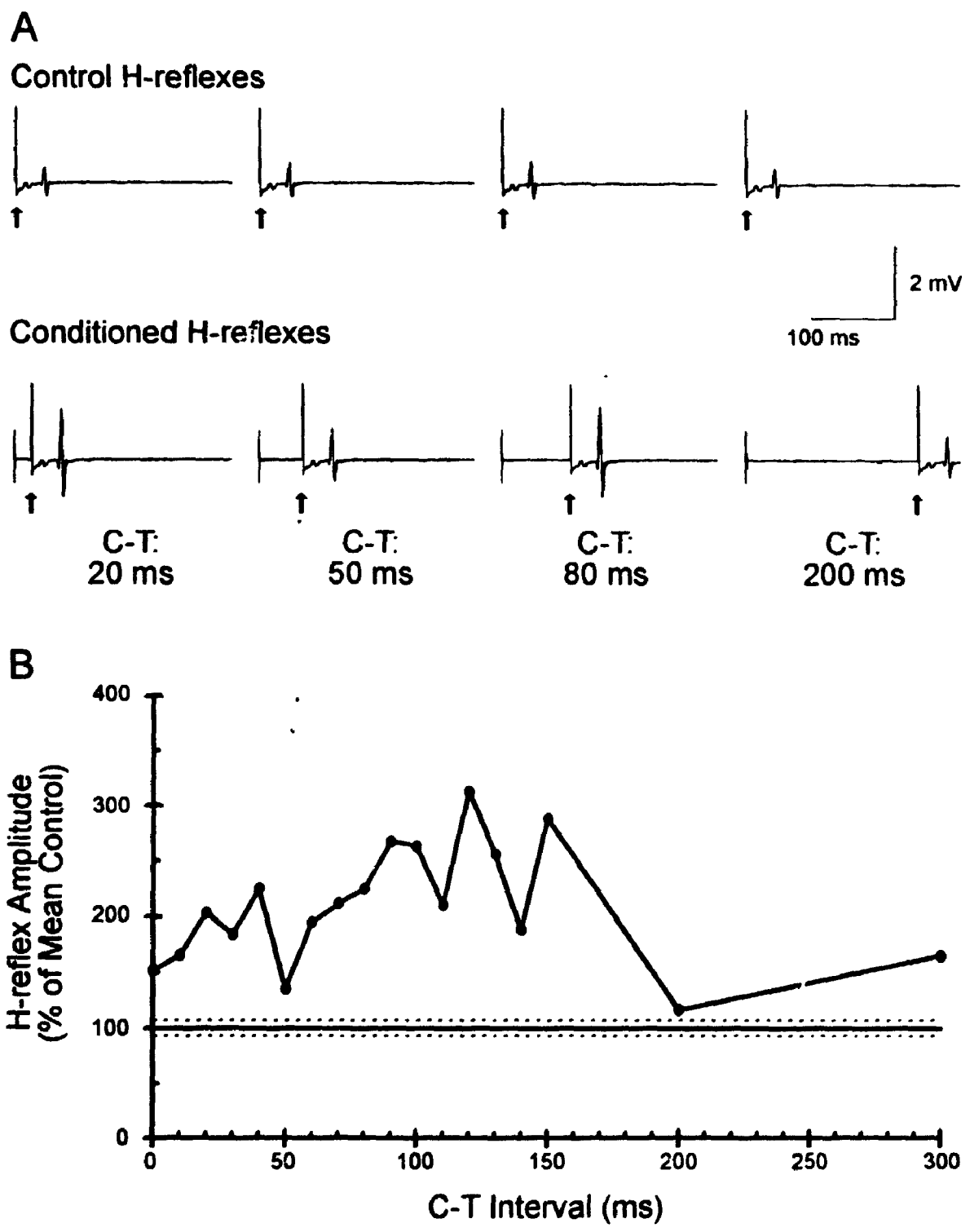

Figure 13: Effect of subthreshold cortical stimulation on H-reflexes in a control subject. A. Raw data traces showing control and conditioned H-reflexes at selected C-T intervals. Two trials are superimposed. Arrows denote H-reflex stimulus artifact while cortical stimulation artifact occurs at onset of conditioned H-reflex traces. Note enhanced H-reflexes at C-T: 20 and 80 ms despite absence of MEPs following cortical stimulation. B. Mean, normalized H-reflex amplitudes demonstrating the early and late periods of ennanced motor neuron excitability. Dashed horizontal lines represent 2 SE about the mean control H-reflex amplitude.

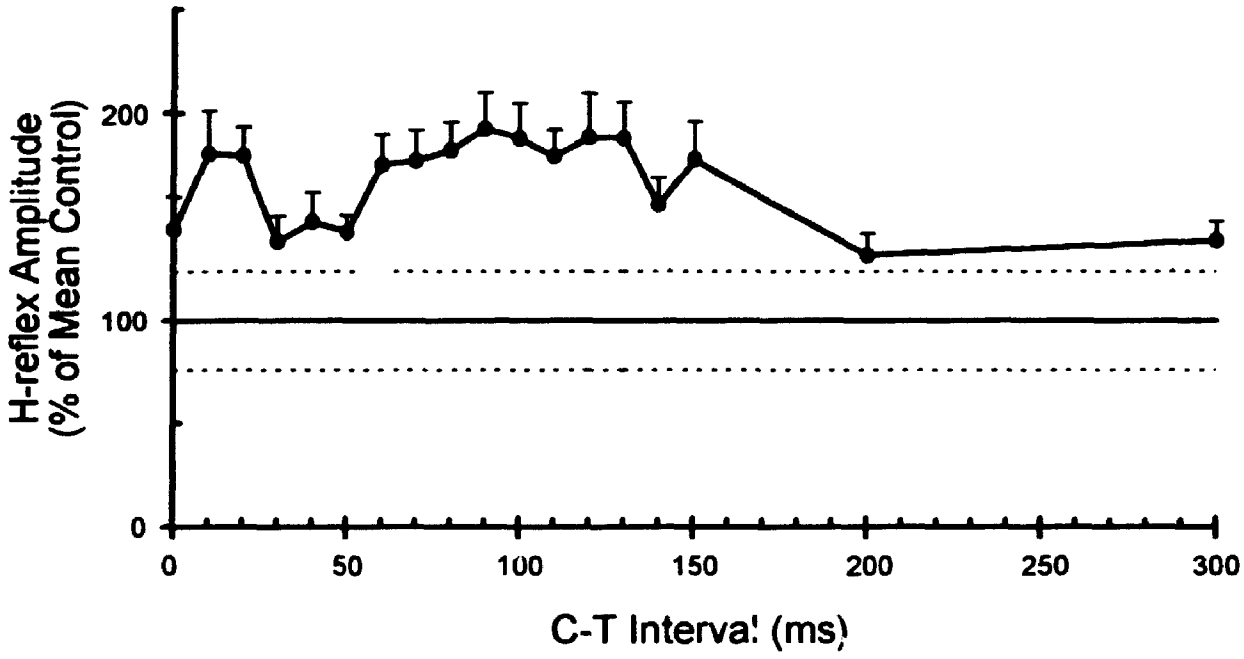

Figure 14: Effect of subthreshold cortical conditioning on H-reflex amplitude averaged across subjects. Mean amplitudes normalized to the mean control amplitude. Note the two periods of enhanced motor neuron excitability at C-T: 10-20 and 60-150 ms. Dashed horizontal lines represent 2 SE about the mean control amplitude and vertical lines represent 1 SE of the mean conditioned amplitude.

3.3.4 - Discussion

The subjects exhibited a well defined temporal pattern of H-reflex amplitude modulation with facilitation evident as two distinct phases at C-T: 10-30 and C-T: 60-130 ms. This profile was most evident following suprathreshold cortical stimulation, but was also clearly present with cortical stimulation that was subthreshold for evoking short latency MEPs. The early phase of facilitation is consistent with inputs mediated through the fast corticospinal tract (Amassian et al, 1990; Brouwer & Ashby, 1990, 1992, Edgley et al., 1990; Rothwell et al., 1991) while the origin of the late facilitation is less clear

The early period of facilitation is most likely attributed to the cortical stimulation having excited large diameter corticospinal neurons. The fastest of these neurons conduct at ~ 60 m/s (Lloyd, 1941) which give rise to a central motor conduction time (i.e., time from cortex to lumbosacral motor neuron pool) of ~ 15 ms (Tomita et al., 1989). The afferent limb of the H-reflex also has a conduction time of ~ 15 ms (Magladery, 1955). Thus, if both cortical stimulation and H-reflex stimulation are delivered at C-T: 0 ms, convergence of excitatory synaptic inputs at the lumbosacral motor neuron pool would be expected. The present results yielded facilitation commencing in some subjects at C-T: 0 ms (n = 3) and others at C-T: 10 ms (n = 7)

A short cut to determining the exact C-T interval at which convergence would be expected is to subtract the H-reflex latency from the MEP latency (see Figure 15). The mean MEP latency was 31.1 ms and the mean H-reflex latency was 29.2 ms. This leads to the prediction that, on average, the earliest C-T interval at which convergence would be expected was C-T: 2 ms. C-T intervals of this resolution were not investigated in the

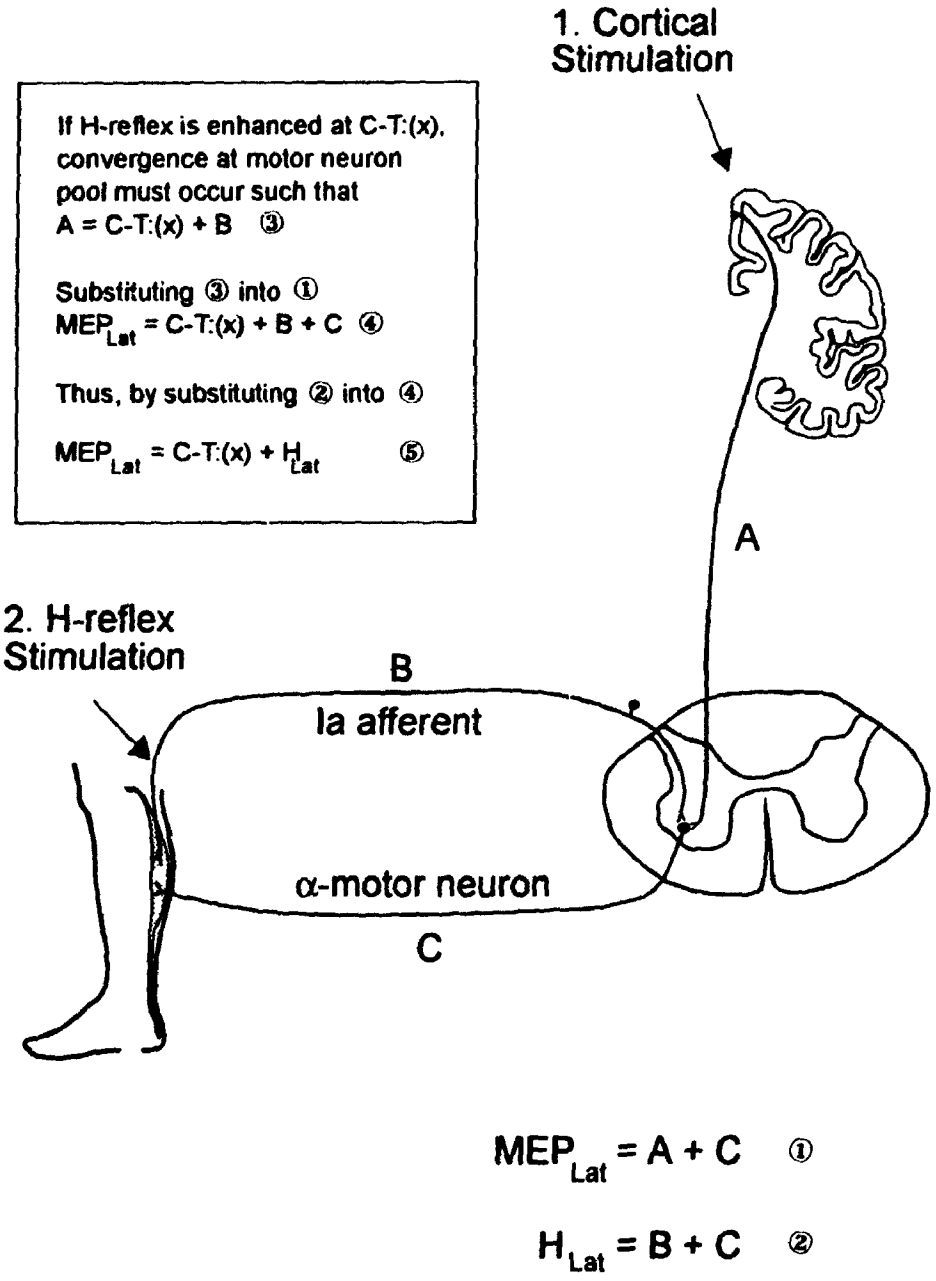

Figure 15: Schematic representation of neurons mediating enhanced motor neuron excitability following cortical conditioning of H-reflexes. A = central motor conduction time. B = Ia afferent conduction time from stimulation site. C = peripheral motor conduction time of α-motor neuron. MEP$_{lat}$ = MEP latency. H$_{lat}$ = H-reflex latency. C-T:(x) = C-T interval. Equations in upper left demonstrate that enhanced motor neuron excitability at a particular C-T interval corresponds to a muscular response occuring with a latency of the C-T interval plus the H-reflex latency. Note: In the case of later periods of enhanced motor neuron excitability, A reflects conduction in alternate pathways as discussed in text.

present study as the primary focus was on the detection of the overall pattern of excitability over an extended time period. Thus, the majority of subjects (i.e., 7 of 10) did not exhibit enhanced H-reflexes until C-T: 10 ms even though it is likely that cortical stimulation resulted in earlier periods of enhanced motor neuron excitability in these subjects.

The long lasting nature of the early period of facilitation (i.e., lasting until C-T: 30 ms) was similar to that seen with electrical stimulation of the motor cortex in humans (Brown et al., 1978; Cowan et al., 1986; Milner-Brown et al., 1975, van der Linden & Bruggeman, 1993). This was also evident in primates in which the entire brainstem except for the pyramidal tracts were destroyed (Preston & Whitlock, 1960, 1961). This was interpreted as evidence that corticospinal fibers of various diameters and a range of conduction velocities contribute to the duration of the facilitation. In addition, the repetitive discharges elicited in pyramidal tract neurons with single pulses of cortical stimulation (Amassian et al., 1987; Day, Rothwell, et al., 1987; Day et al., 1989, Edgley et al., 1990) also contribute to this duration.

The H-reflex facilitation commencing at C-T: 60 ms would be generally equivalent to a muscular response occurring with a latency of ~ 90 ms following cortical stimulation (see Figure 15). Long latency responses (onset 70-100 ms) to transcranial stimulation of motor cortex have been reported previously and variously termed MEP_{70} (Dimitrijevic, Kofler, et al., 1992) or S100 (Holmgren et al., 1990, 1992). Examination of the peristimulus time histogram (PSTH) of motor unit discharge has revealed late periods of increased activation termed E_2 (Calancie et al., 1987) or the secondary peak (Mills et al.,

1991). They have been attributed to slow conducting and/or oligosynaptic corticospinal, or corticobulbospinal projections (Dimitrijevic, Kofler, et al., 1992), peripheral afferent inputs (Calancie et al., 1987; Sammut et al., 1995; Wilson et al., 1995), or a summation of cort' 'ospinal, brainstem (startle reaction) and peripheral influences (Holmgren et al., 1990, 1992; Mills et al., 1991). The possible contribution of peripheral afferent (Ia) discharge, in the declining twitch phase from the short latency MEP, has been previously disputed based on (i) the observed temporal relations between the observed EMG response and the twitch-time course (Dimitrijevic, Kofler, et al., 1992), (ii) the latency and amplitude differences between distal and proximal muscle responses (Dimitrijevic, Kofler, et al., 1992), and (iii) late responses occurring in the absence of short latency MEPs (Wolfe & Hayes, 1995).

The present results, showing long latency H-reflex amplitude modulation at C-T: 60-130 following subthreshold cortical conditioning is new evidence consistent with the view that this late facilitation is mediated by descending inputs rather than peripheral afferent inputs. For this argument to hold, one must be confident that short latency MEPs were not evoked in other lower limb muscles. Some assurance that this was satisfied came from the fact that the cortical stimulation was subthreshold for evoking MEPs in TA and LG bilaterally (i.e., facilitation was present in the absence of any short latency MEPs). However, this does not preclude the possibility of MEPs being evoked in other, non-monitored, muscles of the lower limb, that have afferent projections to the LG motor neuron pool.

As noted by previous investigators, long latency inputs elicited by transcranial

magnetic stimulation may be mediated by a variety of descending tracts including slow conducting and/or oligosynaptic corticospinal neurons. If a single pyramidal tract cell were involved, long latency responses of 90 ms would reflect a central conduction time of ~ 75 ms and a mean conduction velocity of ~ 9 m/s (as calculated with estimated conduction distance of 0.7 m). Conduction velocities of this magnitude are typical for smaller diameter (i.e., ~ 2 μm) corticospinal fibers. The vast majority (i.e., 90%) of fibers in the human pyramidal tract have diameters of 1-4 μm (Verhaart, 1970). These would yield conduction times consistent with the timing of the late period of enhanced motor neuron excitability. With this reasoning, it is unclear why 2 distinct periods of facilitation are present (i.e., early and late) and there is not a more uniform profile more closely matching the fiber spectrum of pyramidal tract cells. This may be due to the interposition of an inhibitory effect such as has been demonstrated in the medial gastrocnemius following transcranial magnetic stimulation (Brouwer & Qiao, 1995).

Conditioning effects of distant somatosensory, cutaneous, or acoustic stimuli have previously been shown to modulate the activity of the lumbosacral motor neuron pool (i.e., H-reflexes) with a time course generally similar to that observed for the late facilitation (Delwaide & Crenna, 1983; Delwaide et al., 1993; Dobkin et al , 1994; Piesiur-Strehlow & Meinck, 1980; Rossignol & Melvill Jones, 1976; Rudell & Eberle, 1985) This raises the possibility that the late facilitation at C-T: 60-130 ms was not a result of a pyramidal tract neuron activation per se, but was from vicarious effects of the transcranial magnetic stimulation such as scalp cutaneous afferent stimulation, scalp muscle proprioception, or acoustic inputs. Magnetic stimulation leads to an audible click and

acoustic stimuli lead to auditory-spinal evoked responses of a latency commensurate with these reported here. Auditory (startle) spinal responses are mediated through reticulospinal pathways (Davis et al., 1982; Shimamura & Livingston, 1963; Wu et al., 1988) and the brain stem nuclei giving rise to these pathways also receive corticobulbar projections. Thus, although the exact afferent and central pathways mediating the late facilitation cannot be definitively identified with the present protocol, the balance of evidence supports the view that descending ventromedial or slow conducting corticospinal tracts are involved. Subthreshold cortical conditioning of H-reflexes thus represents a new electrophysiological method of investigating the continuity and conduction in spared tracts, other than the fast corticospinal pathway, in cords where conduction has been compromised by trauma or other forms of extramedullary compression.

3.4 - Experiment 4 - Detection of Preserved Motor Pathways to Lower Limb Motor Neuron Pools by Conditioning H-reflexes with Transcranial Magnetic Stimulation in Patients with SCI

3.4.1 - Introduction

Experiment 4 employed the technique of cortical conditioning of H-reflexes to enhance the probability of detecting preserved descending motor influences on the spinal structures in patients with SCI. As noted in the previous experiment in control subjects there is a significant, long lasting, late period of enhanced motor neuron excitability following subthreshold cortical stimulation which may reflect conduction in descending pathways other than the fast conducting corticospinal tract. There is some evidence that late responses following cortical stimulation are prevalent in patients with SCI (see results of Experiment 2 and Dimitrijevic et al., 1988; Segura et al., 1992) although it is not clear what structures mediate these responses. Similar approaches of conditioning H-reflexes have been used to detect changes following auditory (Dobkin et al., 1994) or caloric (Raffensperger & York, 1984) stimulation as a means of assessing function in motor pathways of the anterior spinal cord in patients with SCI

3.4.1.1 - Research objectives and plan.

This study was designed to maximize the opportunity for detecting preserved innervation in patients with SCI using the technique of transcranial magnetic stimulation of motor cortex. In particular, attempted target muscle contraction was utilized to maximize the probability of eliciting MEPs and the cortical conditioning of H-reflexes was used to detect descending short and long latency influences of cortical stimulation which may have

been subthreshold for eliciting MEPs. The objectives of this study were a) to reveal preserved descending innervation in patients with SCI by examining descending influences on the LG motor neuron pool following cortical stimulation, b) to determine the relative effectiveness of the techniques of cortical conditioning of H-reflexes versus reinforcing MEPs with target muscle contraction in demonstrating preserved innervation, and c) to examine the short and long latency periods of enhanced motor neuron excitability and their relationship to the clinical features and severity of injury in patients with SCI.

3.4.2 - Methods

3.4.2.1 - Subjects.

Eleven adults with established SCI (9 males; 2 females; age = 36.1 ± 9.3 years) provided informed consent to participate in this study. Subjects were only admitted into the study after a minimum of 3 months had elapsed following their injury. Seven of these subjects had incomplete SCI (i.e., ASIA B, C, or D) while the remaining 4 had complete SCI (i.e., ASIA A). Subjects were drawn from the population of inpatients or outpatients of the Regional Spinal Cord Rehabilitation Program, Parkwood Hospital, on referral from the Physiatrist-in-Charge, or were volunteers who requested admission to the research program. Demographic data and information about the severity and mechanism of injury is outlined for each subject in Table 10.

Table 10 - Severity of Injury and Demographic Information for Patients

Subject	Sex	Age (Years)	Injury Mechanism	Months Post-Injury	Neurological Level (Motor)	ASIA Impairment Scale
1	M	25	MVA	3	C7	A
2	M	30	MVA	4	C7	A
3	M	53	MVA	381	T8	A
4	M	22	Diving	32	C7	A
5	M	33	MVA	5	C6	B
6	M	29	MVA	82	C8	C
7	M	43	MVA	69	T1[a], C8[b]	C
8	F	46	MVA	42	C6	C
9	F	40	MVA	24	T1	D
10	M	38	Diving	28	C6[b]	D
11	M	38	Transverse Myelitis	53	L2	D

Note: MVA = motor vehicle accident. [a] For right side only. [b] For left side only

3.4.2.2 - Procedures.

A minimum of 2 trials of transcranial magnetic stimulation of the motor cortex were administered to each subject under each of the following conditions: a) with target muscles at rest, b) with attempted ankle dorsiflexion, and c) with attempted ankle plantarflexion. EMG recordings were obtained bilaterally from LG and TA muscles. The stimulation intensity for these and subsequent trials was adjusted to the maximum level comfortably tolerated by the subjects which in all cases was the maximal output of the Cadwell MES-10 stimulator.

Following these preliminary trials, subjects were presented with a series of trials designed to examine the effect of cortical stimulation on H-reflexes employing procedures similar to those in Experiment 3. Unlike the experiments in normal subjects, however, a minimum of 4 blocks of trials (and as many as 6) were administered to each subject. Within a trial block, the C-T intervals tested were 20, 50, 80 and 150 ms presented in random order. A control H-reflex trial was inserted randomly within each trial block with the magnetic coil maintained in position so that the subject remained unaware of the specific trial order. In a few instances subjects experienced muscle spasms within a trial block. In these cases the experiment was continued from the onset of the interrupted trial block after reestablishing a stable control H-reflex of ~33% maximum amplitude.

ASIA Impairment Scale scores (See Table 1 for description of this scale) were obtained from clinical neurologic examinations involving manual muscle testing performed by the attendant physiatrist.

3.4.2.3 - Data analysis.

Following analog to digital conversion and calibration procedures, tests were conducted to determine if MEPs were present in the LG and TA muscles bilaterally In some cases, identification of MEP latencies or amplitudes was difficult because of background muscle activation or low response amplitude relative to signal noise, yet it was apparent that MEPs were still present. Therefore, the same signal detection algorithm as described in Experiment 2 (Data Analysis) was used to identify any significant deviations from the background activity. This procedure was conducted on the ensemble averaged EMG activity following signal rectification and was verified by visual inspection Following this analysis, MEP amplitudes were determined by calculating the AEMG value from the ensemble averaged signal for a 20 ms window beginning with the onset latency. Short latency responses were defined as any response with an onset latency of less than 60 ms. Long latency responses were defined as occurring between 60-200 ms.

In the trials involving the conditioning of H-reflexes, mean peak to peak H-reflex amplitudes were calculated for each C-T interval. These amplitudes were normalized relative to the overall mean control H-reflex amplitude. Mean normalized H-reflex amplitudes were calculated across subjects for both control and conditioned H-reflexes Conditioned H-reflex amplitudes were deemed to be enhanced if they exceeded the control H-reflex amplitude by at least 2 *SE* (i.e., 95% confidence interval). A repeated measures ANOVA, with independent variables of completeness (complete SCI vs. incomplete SCI) and C-T interval (Control, 20 ms, 50 ms, 80 ms, 150 ms) was conducted for the raw H-reflex amplitude data. Dunnett's multiple comparison procedure allowing pairwise

comparisons of treatment means with a control mean was used to determine significance for specific C-T intervals.

3.4.3 - Results

3.4.3.1 - MEPs at rest and with attempted voluntary contraction.

Raw data traces showing EMG activity in the LG following cortical stimulation are displayed in Figure 16 for all patients (i.e., all trials superimposed). As well, ensemble averages calculated across patients for each ASIA level are shown in this figure. The data displayed were from the same LG (i.e., right or left) that was employed in the cortical conditioning of H-reflexes. Short latency MEPs were not able to be reproducibly elicited in this muscle at rest in any patient. With background contraction, short latency responses were evident in the tested LG in all ASIA D patients and in 2 of 3 ASIA C patients.

Short latency MEPs were not able to be elicited in any muscles while subjects were at rest except in 1 of the 3 patients with the least severe injuries - ASIA Impairment score of D. MEPs were elicited in all 4 muscles in all ASIA D patients when ankle dorsiflexion or plantarflexion was attempted. In addition, MEPs were elicited in all of the ASIA C patients when dorsiflexion or plantarflexion was attempted although in 2 of these patients MEPs were only evident in 1 of 4 muscles tested. In each of these cases, the MEP was of low amplitude relative to the background contraction and the detection of an MEP was equivocal. In all other patients (i.e., ASIA A and B) MEPs were absent. These findings are summarized in Table 11 and the actual MEP latency and amplitude values are displayed in Appendix 3 to 6 for each subject.

Late MEPs with onset latencies ranging from 82.5 - 156.5 ms were evident in 2 of

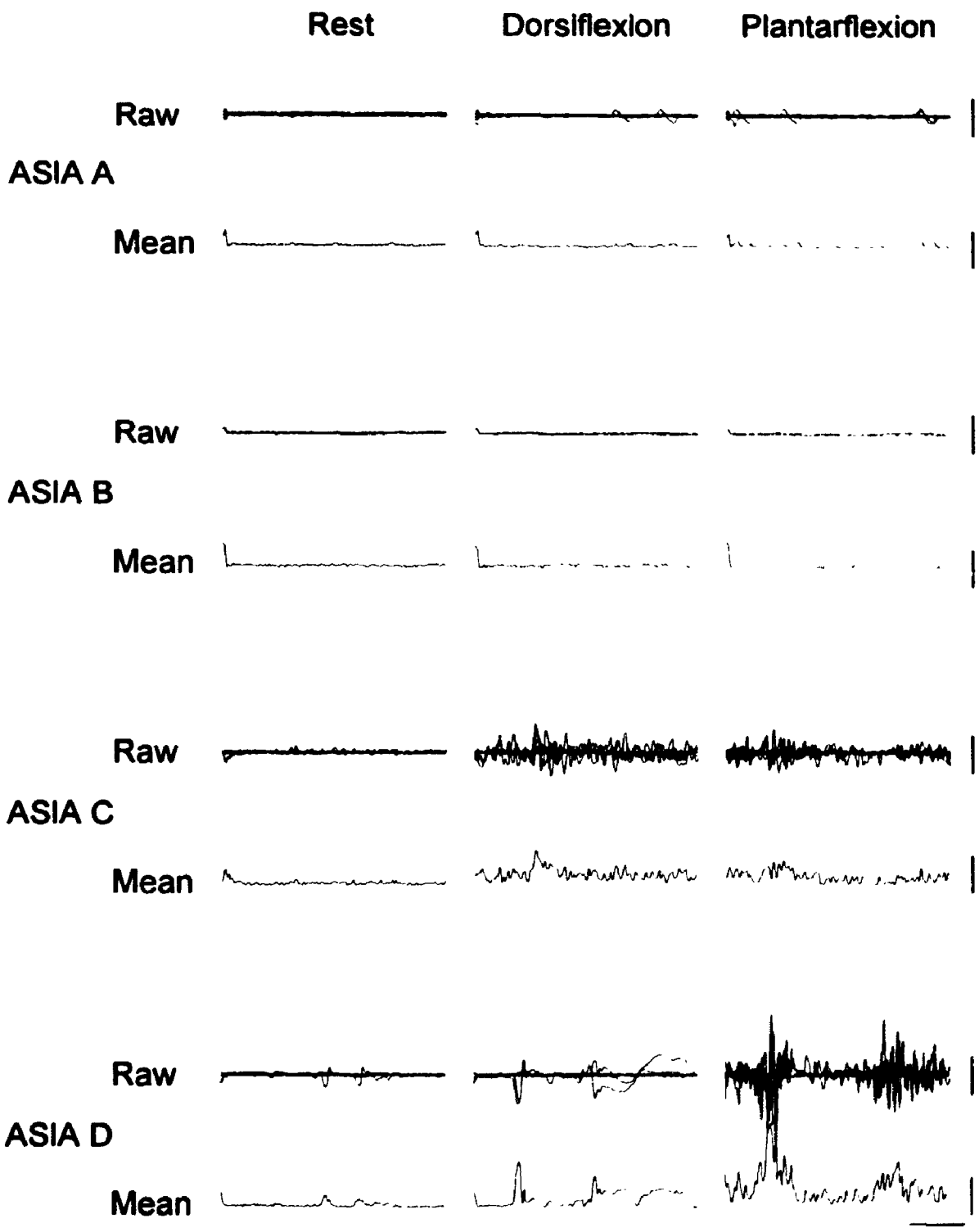

Figure 16: EMG activity in the LG following cortical stimulation for patients at each ASIA level. Raw data traces are superimposed in upper trace for each ASIA level while lower trace contains rectified, ensemble averaged EMG activity. Note that target muscle contraction was required for the consistent elicitation of short latency MEPs although 1 patient (ASIA D) displayed late MEPs at rest in the absence of short latency MEPs. Spontaneous motor unit discharges unrelated to the cortical stimulation were noted in 1 ASIA A patient. Vertical calibration bars represent 0.5 mV (raw) and 0.1 mV (ensemble average) and horizontal calibration bar represents 50 ms.

Table 11 - Presence of MEPs at Rest and with Target Muscle Contraction in Patients

Subject	ASIA Impairment Scale	Rest	Attempted Dorsiflexion	Attempted Plantarflexion
1	A	NR	NR	NR
2	A	NR	NR	NR
3	A	NR	NR	NR
4	A	NR	NR	NR
5	B	NR	NR	NR
6	C	NR	RLG[a]	NR
7	C	NR	RTA	NR
8	C	NR	RTA, RLG LTA, LLG	RTA, RLG LTA, LLG
9	D	NR	RTA, RLG LTA	RTA, RLG LTA, LLG
10	D	NR	RTA, RLG LTA, LLG	RTA, RLG LTA, LLG
11	D	RTA, RLG	RTA, RLG LTA, LLG	RTA, RLG LTA, LLG

Note: NR = no response. Otherwise MEPs present in right tibialis anterior (RTA), left tibialis anterior (LTA), right lateral gastrocnemius (RLG), or left lateral gastrocnemius (LLG). [a] Detection of MEP questionable as response only occurred in the midst of ongoing EMG activity associated with non-specific muscle activation during attempted ankle dorsiflexion (but not plantarflexion).

3 ASIA D patients. In particular, 1 of these patients displayed late responses (latency = 82.5 ms) in the LG at rest despite the absence of a short latency response in this muscle or the ipsilateral TA (see Figure 15). One ASIA C patient (Subject 8) possessed a low amplitude late response (latency = 150.5 ms) in the TA that was difficult to discern during attempted target muscle contraction.

3.4.3.2 - Cortical conditioning of H-reflexes.

Overall results for each patient are tabulated in Table 12 outlining the presence or absence of enhanced H-reflexes at each C-T interval. In addition, the presence or absence of MEPs in the same muscle is indicated in this table. All subjects with incomplete SCI demonstrated enhanced H-reflexes for at least 1 C-T interval even though an MEP was not identified in the appropriate LG muscle in 2 of these patients (subjects 5 and 7) and the presence of a MEP was inconclusive in another (subject 6).

Mean H-reflex amplitudes for complete and incomplete subjects are presented in Figure 17. This figure shows that mean H-reflex amplitudes were particularly enhanced in the patients with incomplete SCI at C-T: 20, 50 and 80 ms and to a lesser extent at C-T: 150 ms. These means were 186%, 209%, 218%, and 148% greater than the control H-reflex amplitude for C-T: 20, 50, 80 and 150 ms respectively. There was no evidence of H-reflex enhancement in the patients with complete SCI at any C-T interval. A repeated measures ANOVA conducted on the mean, raw H-reflex data (i.e., actual mV values) revealed a significant main effect of C-T interval ($F(4,36) = 3.42$, $p = 0.018$) and a significant interaction effect of completeness and C-T interval ($F(4,36) = 3.20$, $p = 0.024$)

Table 12 - Enhanced H-reflexes Following Cortical Stimulation in Patients

Subject	ASIA Impairment Scale	MEPs	Conditioned H-reflex Amplitude (Normalized to mean control H-reflex - %)			
			C-T: 20 ms	C-T: 50 ms	C-T: 80 ms	C-T: 150 ms
1	A	NR	98.5	95.1	105.4	103.9
2	A	NR	102.3	105.4	95.2	98.6
3	A	NR	111.1	93.8	105.0	110.0
4	A	NR	108.9	93.6	113.3	106.2
5	B	NR	173.9 *	125.9	118.1	106.8
6	C	MEP[a, b]	152.7 *	160.6 *	137.9 *	122.1
7	C	NR	135.8 *	117.4	131.2 *	118.6
8	C	MEP[a]	231.9 *	496.6 *	571.0 *	264.7 *
9	D	MEP[a]	244.6 *	187.6 *	211.0 *	148.4 *
10	D	MEP[a]	135.5 *	121.8	132.1*	122.5
11	D	MEP	230.3 *	251.8 *	221.7 *	149.6 *

Note: MEP = MEP present in same LG as was tested with conditioning of H-reflex. NR = MEP absent. [a] MEP only able to elicited with attempted contraction, not at rest. [b]Detection of MEP questionable as response occurred in the midst of ongoing EMG activity associated with non-specific muscle activation during attempted ankle dorsiflexion (but not plantarflexion). * Conditioned H-reflex greater than 2 SE of control.

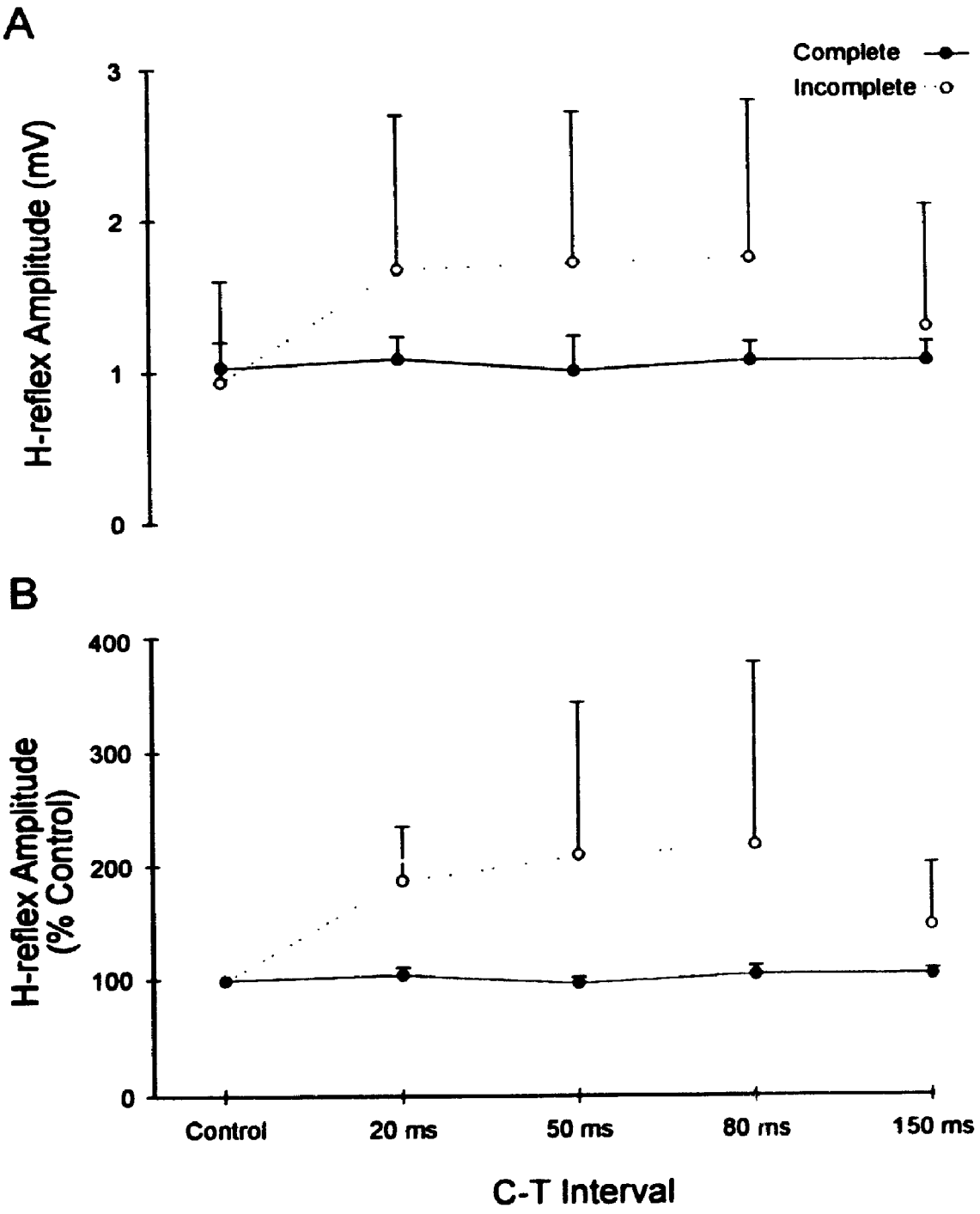

Figure 17: Mean H-reflex amplitudes in patients with complete (filled circles) vs. incomplete (empty circles) SCI. Note enhanced H-reflexes at C-T: 20, 50, and 80 ms in incomplete patients only. Vertical lines represent 1 SD about mean. Top graph (A) contains mean raw H-reflex amplitudes while bottom graph (B) contains H-reflex amplitudes normalized to the mean control.

Pairwise comparisons of the means of the conditioned H-reflex amplitude at each C-T interval with the mean of the control H-reflex amplitude revealed significant facilitation at C-T 20 ms ($t = 4.44$, $p < 0.01$), C-T: 50 ms ($t = 4.62$, $p < 0.01$), and C-T: 80 ms ($t = 4.89$, $p < 0.01$) for the patients with incomplete SCI. There were no significant differences noted in the mean H-reflex amplitudes for the patients with complete SCI.

The primary purpose of the present study was to enhance the probability of detecting preserved descending motor influences in patients with SCI. In particular, an attempt was made to determine if responses could be detected in patients who otherwise might not display evidence of preserved innervation. Therefore, the important results and clinical characteristics of those 3 patients (subjects 5, 6, and 7) demonstrating enhanced H-reflexes despite the absence (or uncertainty) of MEPs in the LG are elaborated below.

Case study of subject 5.

Subject 5, a 33 year old male, was tested 5 months following a motor vehicle accident in which he sustained a C4-5 dislocation. At the time of testing his diagnosis was C5 incomplete tetraplegia, ASIA B. His clinical features were indicative of anterior cord syndrome with preserved sensation (coarse touch and pressure but not pin prick) in his lower limbs including severe dysaesthetic pain particularly involving the left side of the body. Proprioception was absent in the wrist and fingers but some position sense was evident in the toes. No motor function was detected below the level of C7 including the right and left ankle plantarflexors. Manual muscle testing revealed motor index scores of 7 for the right and 6 for the left side of the body. H-reflexes were collected in the right LG for this patient. X-rays taken 111 days post-injury revealed minimal retrodisplacement of C-6 on C-7 in extension which reduced in flexion. An MRI (see Figure 18) taken 166 days post-injury (5 days post-testing) showed evidence of a focal syrinx at the level of injury (C4-5) which may be related to the finding of dysaesthetic pain.

Figure 18· **MRI of subject 5. Note increased density of signal at C4-5 in spinal canal indicative of syrinx formation (see arrow).**

As outlined in Figure 19, this patient demonstrated enhanced H-reflexes at C-T: 20 ms (M = 173.9% greater than the control H-reflex amplitude) despite the clinical designation of ASIA B (i.e., motor complete) and the absence of MEPs. Although this mean is slightly biased by one trial exhibiting an extremely large degree of facilitation (i.e., 267%), 4 of 5 trials exhibited facilitation at a level exceeding 2 SE of the control H-reflex amplitude. Facilitation at C-T: 20 ms is consistent with preserved corticospinal innervation to the LG motor neuron pool. However, in this patient these residual influences were incapable of mediating clinically detectable function and were not able to be detected by eliciting MEPs.

Another important finding with this patient was the absence of enhanced H-reflexes with cortical conditioning at later C-T intervals (i.e., C-T: 50, 80, and 150 ms). Of all the normal subjects and patients who demonstrated facilitation at C-T: 20 ms, this was the only subject who did not display a late period of enhanced motor neuron excitability. Earlier, it was suggested that the late period of enhanced motor neuron excitability might be mediated via bulbospinal pathways (see Experiment 3 Discussion). This is consistent with the clinical features of this patient which were indicative of damage to the anterior spinal cord. In such a patient, one would expect damage to bulbospinal pathways to be more severe than that found in the lateral corticospinal pathway. Therefore, the presence of an early period of enhanced excitability and the absence of a late period in this particular patient is consistent with the hypothesis that corticospinal and bulbospinal pathways may be mediating the early and late periods respectively.

Given the potential significance of this result, this patient was re-tested 4 months

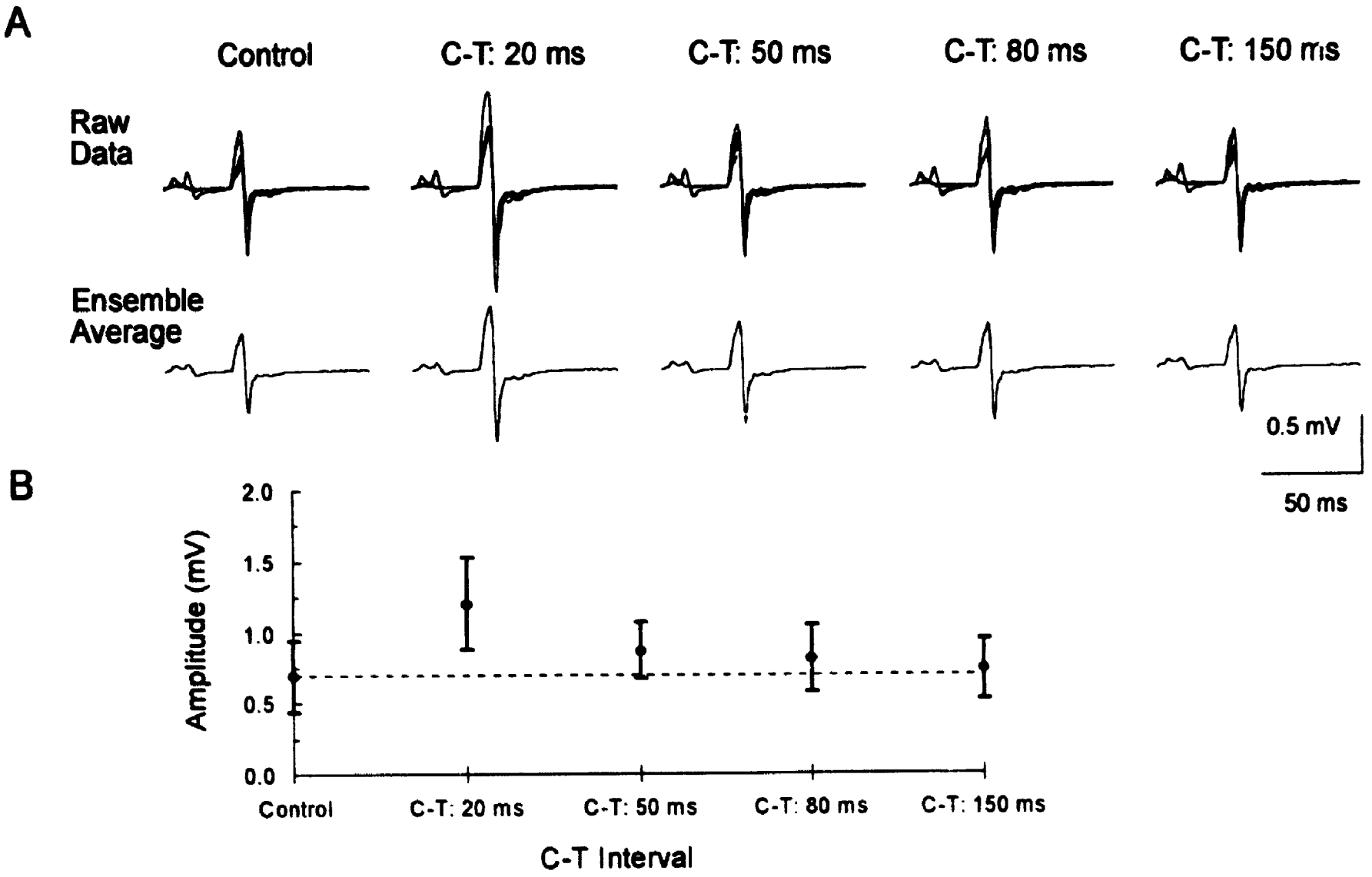

Figure 19: Cortical conditioning of H-reflexes in subject 5 (ASIA B). A) Raw data traces (5 trials superimposed) above and averaged traces below. B) Mean H-reflex amplitudes at each C-T interval. Vertical lines represent 2 SE. Horizontal dashed line denotes mean control H-reflex. Note enhanced H-reflexes at C-T: 20 ms.

following the original test. This patient, in the meantime, had undergone a syringo-subarachnoid shunt. This procedure did not result in any significant change of status. It was noted, however, during the clinical assessment on the second testing day, that this patient had marginal great toe movement in both feet, but only if these maneuvers were performed in extreme hip flexion. These subtle, yet detectable, movements were sufficient to reclassify this patient as ASIA C.

As before, MEPs were absent under all conditions with this patient. The pattern of H-reflex modulation with cortical conditioning was also quite similar to the previous testing day. Figure 20 shows the mean conditioned H-reflex amplitudes at each C-T interval. Again, enhanced H-reflexes were present at C-T: 20 ms (M = 145.9% greater than the control H-reflex amplitude) but not at C-T: 80 or 150 ms. However, the H-reflex amplitude at C-T: 50 ms was slightly greater than the control value by 2 SE. The reproducibility of this pattern demonstrates the robustness of these results even in a patient with quite severe motor impairment.

Case study of subject 6.

Subject 6, a 29 year old male, was involved in a motor vehicle accident resulting in C7 incomplete tetraplegia secondary to an anterior and lateral subluxation of C6 over C7. At the time of testing, 82 months post-injury, his diagnosis was incomplete tetraplegia, ASIA C with motor level of T1 on the right side and C8 on the left side. Evidence of a localized syrinx at the level of injury (C6-7) can be seen in an MRI taken 3 years post-injury (see Figure 21). Motor index scores of 30 and 20 were obtained for the right and left side respectively. H-reflexes were tested in the right LG for this patient with a muscle grade of 2 obtained for the right ankle plantarflexors. A period of increased motor unit activity was noted in this patient for the right LG following cortical stimulation with attempted ankle dorsiflexion (latency = 53.5 ms).

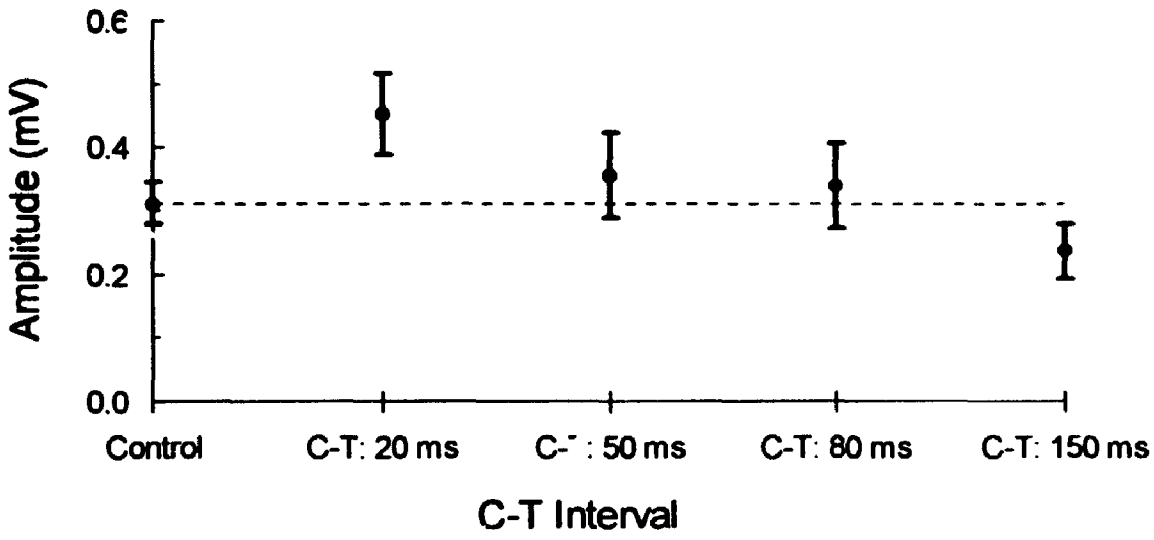

Figure 20: Mean H-reflex amplitudes in subject 5 obtained 4 months after original testing session. As before, H-reflex enhancement was noted primarily at C-T: 20 ms. Vertical lines represent 2 SE. Horizontal dashed line denotes mean control H-reflex.

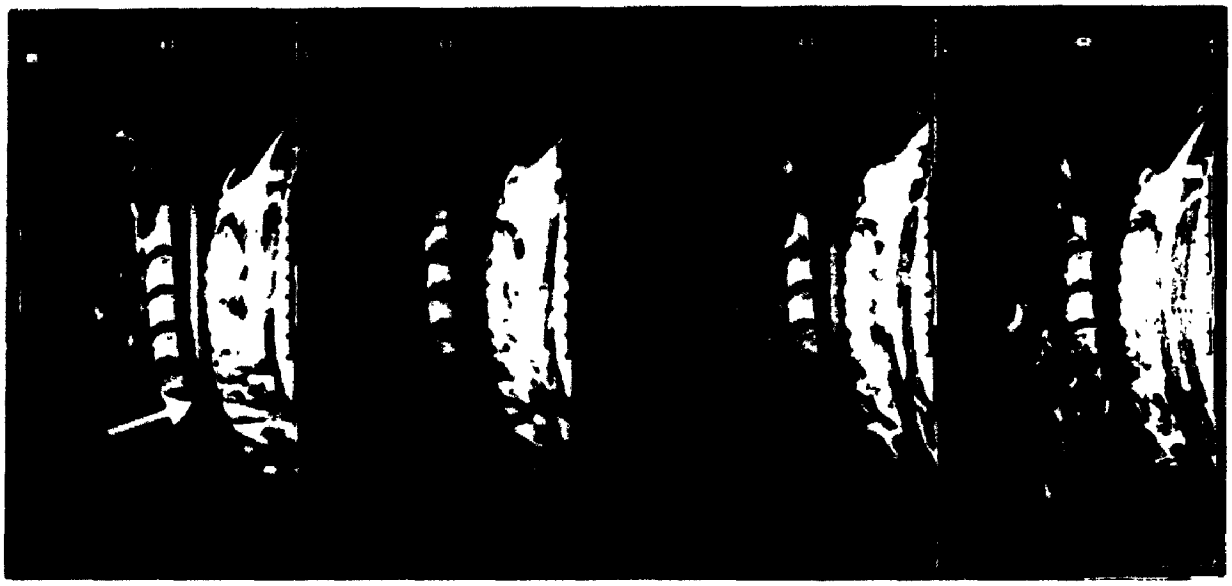

Figure 21 MRI of subject 6 Note evidence of syrinx in spinal canal at C6-7 (see arrow)

This patient demonstrated enhanced H-reflexes with cortical conditioning at C-T 20, 50 and 80 ms but not at C-T: 150 ms. Individual traces showing control and conditioned H-reflexes (A) and mean H-reflex amplitudes at each C-T interval (B) are displayed in Figure 22 for this patient. Mean normalized H-reflex amplitudes were 152.7%, 160.6%, and 137.9% of the control H-reflex amplitude at C-T: 20, 50 and 80 ms respectively. Peak H-reflex facilitation occurred at C-T: 50 ms with this patient. With normal subjects H-reflex facilitation was typically reduced at C-T: 50 ms relative to the early and late excitatory periods (i.e., C-T: 20 and 80 ms). It is not clear from the present data if this patient possessed 2 distinct periods of enhanced motor neuron excitability (i.e., early and late) separated by a definite reduction in motor neuron excitability or had one long period of enhanced motor neuron excitability following cortical stimulation.

Case study of subject 7.

Subject 7, a 43 year old male, suffered a C6-7 fracture with resultant C7 incomplete tetraplegia due to a motor vehicle accident. He was tested 69 months following the date of injury at which time his diagnosis was incomplete tetraplegia, ASIA C, with motor levels of T1 on the right and C8 on the left and sensory sparing bilaterally to C8. Manual muscle testing revealed residual motor function in the right ankle plantarflexors (grade 2) but not in the left. Motor index scores of 34 for the right and 21 for the left were obtained for this patient. H-reflexes were examined in the right LG for this patient. An MRI taken 154 days post-injury can be seen in Figure 23. This showed evidence of a localized syrinx at the level of injury (C6-7) and a fracture of the superior end plate of C7 with mild anterior wedging of the C5 vertebral body.

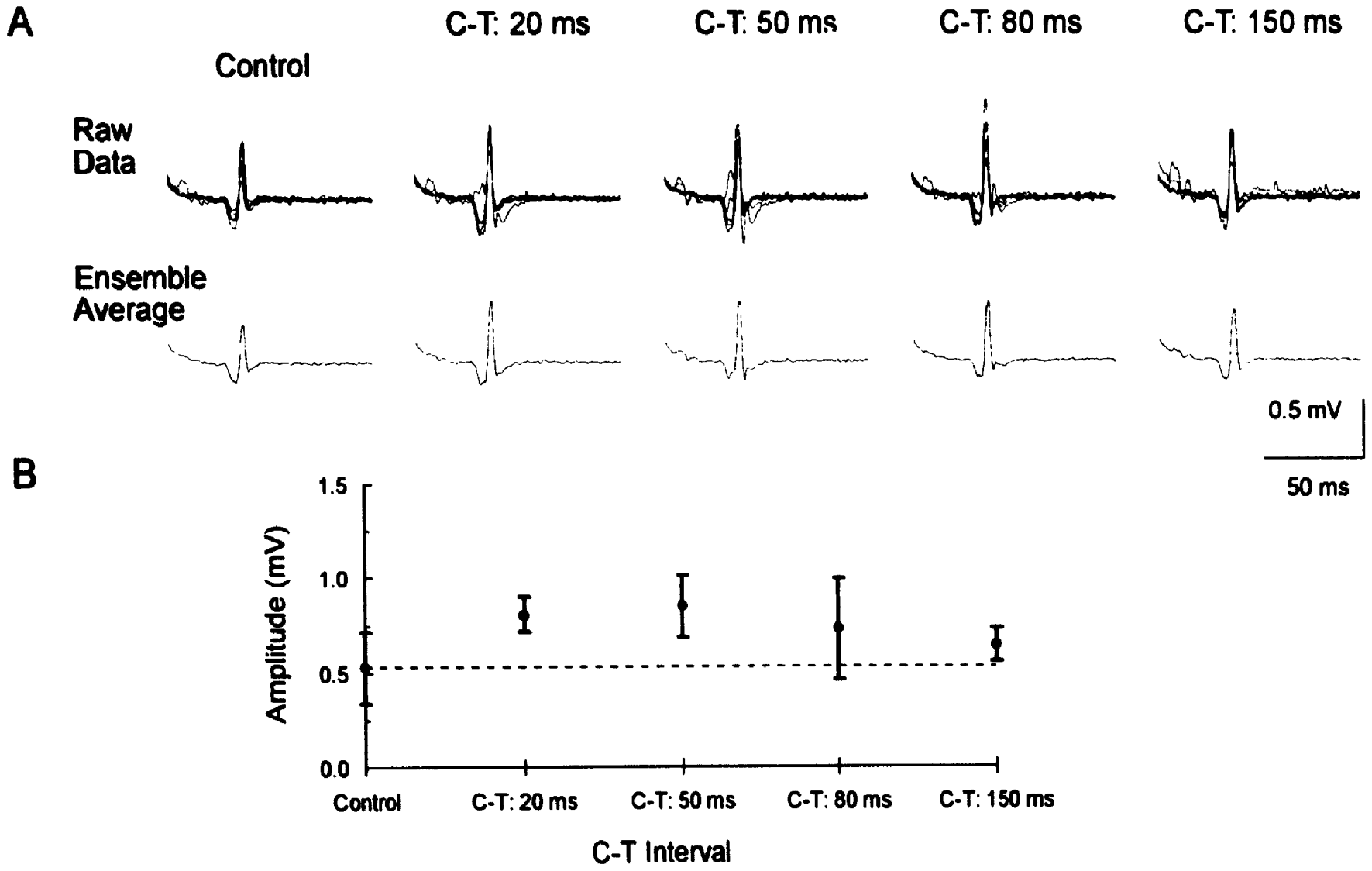

Figure 22: Cortical conditioning of H-reflexes in subject 6 (ASIA C). A) Raw data traces (5 trials superimposed) above and averaged traces below. B) Mean H-reflex amplitudes at each C-T interval. Vertical lines represent 2 SE. Horizontal dashed line denotes mean control H-reflex. Note enhanced H-reflexes at C-T: 20, 50, and 80 ms.

Figure 23 **MRI of subject 7. Note evidence of syrinx in spinal canal at C6-7 (see arrow)**

Raw traces of control and conditioned H-reflexes (A) and mean H-reflex amplitudes for each C-T interval (B) are displayed in Figure 24 for this patient. Conditioned H-reflexes were enhanced by at least 2 *SE* at C-T: 20 and 80 ms but not C-T: 50 and 150 ms. Mean normalized H-reflex amplitudes of 135.5% and 132.1% of the control H-reflex amplitude were attained at C-T: 20 and 80 ms respectively. This pattern was similar to that seen with normal subjects, although the level of facilitation was reduced with respect to that seen with the control subjects. The finding of increased excitability of the conditioned H-reflex at C-T: 20 ms despite the absence of MEPs and equivocal evidence of voluntary motor control of this muscle is suggestive of at least minimal preserved corticospinal connections.

The late period of enhanced excitability (i.e., C-T: 80 ms) following cortical stimulation evident with patients 6 and 7 is consistent with conduction in descending central as opposed to peripheral pathways. This follows from the observation that neither of these patients exhibited short latency MEPs at rest and therefore increased motor neuron excitability could not be due to the peripheral afferent consequences of any short latency responses. The clinical features of these patients were similar in that they were only able to produce poorly controlled voluntary muscle activity in the ankle plantarflexors. These muscle contractions were typically associated with non-specific muscle activation involving several muscle groups including ankle plantarflexors and dorsiflexors and the effort required to produce them often resulted in the elicitation of muscle spasms. These motor control characteristics have been associated with residual bulbospinal influences following SCI (Sherwood et al., 1992).

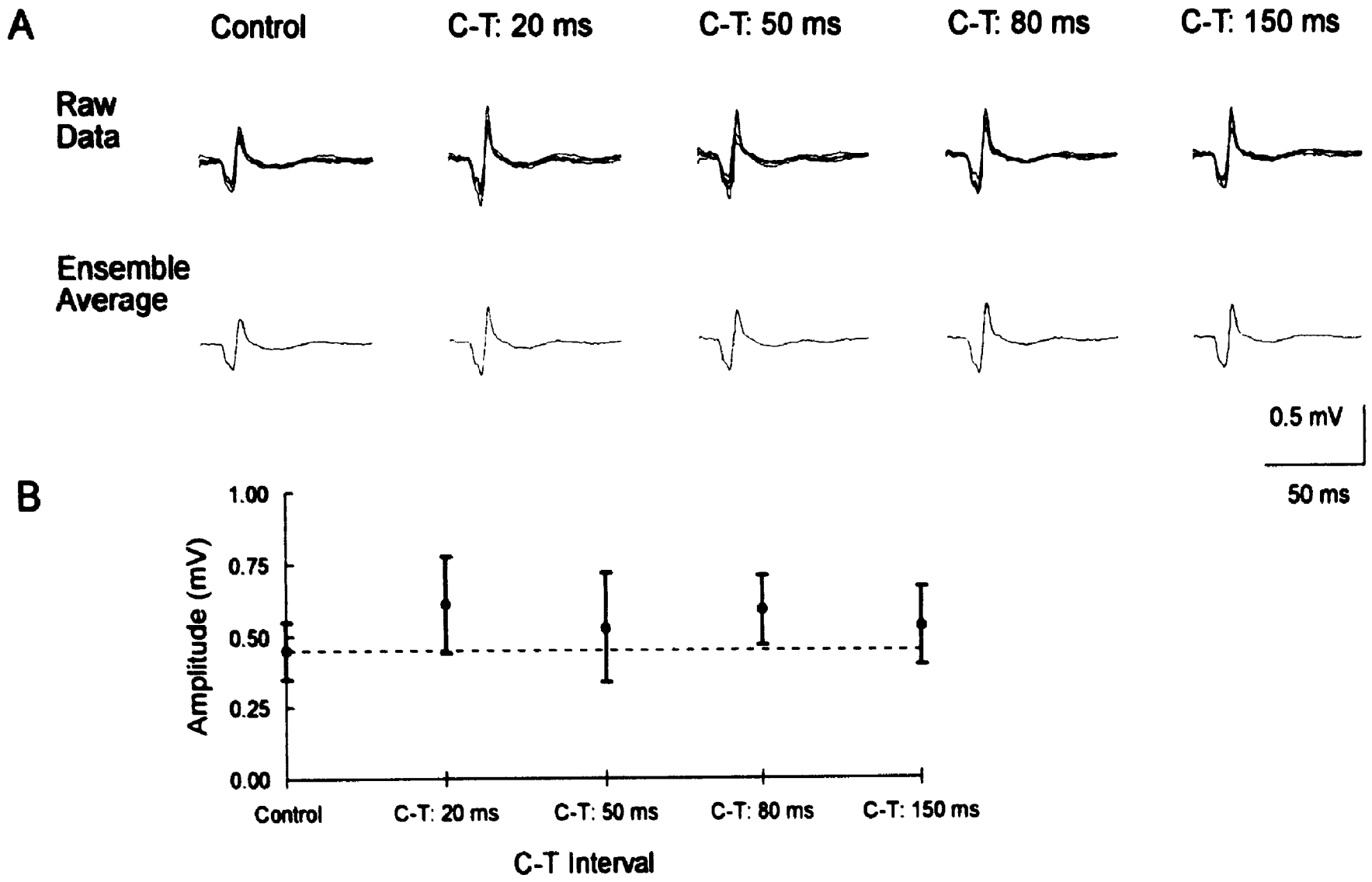

Figure 24: Cortical conditioning of H-reflexes in subject 7 (ASIA C). A) Raw data traces (5 trials superimposed) above and averaged traces below. B) Mean H-reflex amplitudes at each C-T interval. Vertical lines represent 2 SE. Horizontal dashed line denotes mean control H-reflex. Note enhanced H-reflexes at C-T: 20 and 80 ms. M-waves have been eliminated from traces for clarity of presentation.

3.4.4 - Discussion

The findings of Experiment 4 provided the most important observations related to the primary objective of the thesis of examining the presence and nature of preserved innervation in patients with SCI. In addition, there were several interesting results relating to late arriving inputs to the motor neuron pool following cortical stimulation.

Principally, the technique of cortical conditioning of H-reflexes resulted in increased sensitivity in detecting preserved descending innervation in patients with SCI. In the present study, all 7 patients with incomplete SCI demonstrated evidence of preserved motor innervation using this technique despite the fact that 3 of these patients displayed little or no evidence of MEPs even with maximal stimulation and attempts at neurologic reinforcement (i.e., attempted ankle dorsiflexion and plantarflexion). The finding of H-reflex facilitation at C-T: 20 ms in subject 5 is especially noteworthy as this patient was assessed as an ASIA B which denotes sensory sparing only and clinical examination revealed no motor function. Therefore, this finding provides support for the discomplete hypothesis as it has been suggested that ASIA B patients be termed *discomplete* with respect to the motor system if they demonstrate neurophysiological evidence of motor sparing (Sherwood et al., 1992). Further testing would be required to determine the proportion of patients with no clinically detectable motor function (i.e., ASIA A or B) who demonstrate enhanced motor neuron excitability following cortical stimulation.

Several important observations were also seen with respect to the late period of enhanced motor neuron excitability following cortical stimulation. Most notable was the finding that a late period of enhanced motor neuron excitability was seen in 5 patients

despite the absence of short latency MEPs at rest. Therefore, the late period could not have been due to the afferent consequences associated with the short latency response which provides support for the hypothesis that central descending pathways mediate late arriving inputs to the motor neuron pool following cortical stimulation. In addition, the only patient demonstrating an absence of the late period even though an early excitatory period was present was also the only patient with clinical features indicative of damage to the anterior spinal cord. This is consistent with the interpretation of a potential role for bulbospinal pathways in mediating the late period of enhanced motor neuron excitability following cortical stimulation.

A secondary objective of the present study was to relate the findings to the clinical features of the patients. The simplest distinction made with the present results is that of the complete vs. incomplete injury. All patients with complete SCI (ASIA A) demonstrated similar results in that there were no enhanced H-reflexes with cortical conditioning at any C-T interval. In addition, MEPs were not able to be elicited in any muscle under any condition for any of these subjects. This provides further evidence of the completeness of their injuries. On the other hand, all of the incomplete subjects (i.e., ASIA B, C, and D) demonstrated enhanced H-reflexes with cortical conditioning for at least 1 C-T interval. Although the limited number of subjects within each ASIA group did not allow comparisons between patients with incomplete SCI, as a group these patients demonstrated enhanced H-reflexes with cortical conditioning at C-T: 20, 50, and 80 ms ($p < 0.01$).

Typically, patients with SCI exhibit a wide degree of variability with respect to

their injuries and degree of preserved function (Guttmann, 1976). Therefore, it is not surprising that there was no consistent pattern of H-reflex modulation across all subjects as was seen with the normal subjects in Experiment 3. However, the pattern of H-reflex modulation in 3 (i.e., 2 ASIA D patients and 1 ASIA C patient) of the 7 subjects with incomplete SCI resembled that seen with normals in that the greatest facilitation was seen at C-T: 20 and 80 ms while the H-reflex was enhanced at C-T: 50 and 150 ms but to a lesser degree.

In general terms, this technique of cortical conditioning of H-reflexes may be a useful adjunct to a standard neurophysiological test battery. Presently, most neurophysiological approaches for detecting preserved motor innervation in patients with SCI focus on the identification of voluntary motor unit activation (Brandstater & Dinsdale, 1976; Shefner & Tun, 1991; Sherwood et al., 1992; Yang et al., 1990) or attempt to detect residual supraspinal influences through the initiation of spasms, non-specific muscle activation or the modulation of spinal reflexes (Dimitrijevic et al., 1984; Sherwood et al., 1992). Other approaches have employed cortical stimulation and methods to enhance the probability of eliciting MEPs in order to assess conduction in corticospinal pathways (Brouwer et al., 1992; Dimitrijevic, Hsu et al., 1992; Hayes et al., 1991, 1992). The primary advantage of the present technique in comparison to these approaches is the increased sensitivity due to the potential for detecting subthreshold descending inputs to the motor neuron pool. The cortical conditioning of H-reflexes produced results more closely paralleling the complete/incomplete distinction than the findings detected by the presence or absence of MEPs. As well, the detection of

preserved motor influences in patient 5 (ASIA B) suggests that the cortical conditioning of H-reflexes may be useful for detecting preserved influences in patients with complete SCI (i.e., demonstrating discompleteness). Secondly, the pattern of H-reflex modulation appears to reflect the severity of injury (i.e., ASIA A to D) to some extent and may provide corroborative evidence related to various clinical features of some patients. Finally, the results suggest the possibility that with this single examination, conduction in 2 distinct neural pathways may be assessed. It is clear that early periods of motor neuron excitability are mediated by conduction in corticospinal pathways, however, more study is required to determine if bulbospinal pathways are, in fact, responsible for late periods of motor neuron excitability.

Currently, there are very few techniques to assess conduction in these important bulbospinal pathways with respect to their role in the control of movement. It has been suggested that the elicitation of non-specific muscle activation (or spasms) by employing reinforcement maneuvers in patients with SCI provides evidence for the existence of residual bulbospinal influences (Dimitrijevic et al., 1984; Sherwood et al., 1992). However, the potential for increases in intra-abdominal or intra-thoracic pressure with these maneuvers and the resultant effect on segmental excitability was not addressed in these studies and therefore may have resulted in false positive results (Dobkin et al., 1994). Other approaches have included examination of tonic vibratory reflexes (Sherwood et al., 1993) and caloric augmentation of H-reflexes (Raffensperger & York, 1984) to assess function in the bulbospinal pathways. Recently, a pilot study used audiospinal responses, known to be mediated mostly via the reticulospinal tract (Davis et

al., 1982; Shimamura & Livingston, 1963; Wu et al., 1988), to condition H-reflexes as a means for assessing the completeness of SCI (Dobkin et al., 1994). In that study 1 ASIA B and 2 ASIA D patients displayed enhanced H-reflexes suggestive of preserved bulbospinal innervation while 1 ASIA B and 3 ASIA A patients demonstrated no evidence of enhanced H-reflexes. Future experiments examining the various reflex responses mediated by the brain stem, non-specific muscle activation, and late periods of motor neuron excitability with cortical stimulation might aid in the evaluation of these techniques for detecting preserved bulbospinal innervation.

The principal limitation of the H-reflex conditioning technique resides in the H-reflex itself. H-reflexes are only elicited reliably in a few muscles. Care must also be taken to control for factors that may affect motor neuron excitability such as posture, voluntary muscle contractions and eye closure. The presence of involuntary spasms may also alter motor neuron excitability and increase trial to trial variability. In the present experiment, it was necessary to monitor muscle activity to ensure trials were not conducted during or near a spasm.

In summary, the technique of cortically conditioning H-reflexes enhances the detection of preserved innervation in patients with SCI. The clinical implications of this are important in that the detection of preserved innervation not previously considered may alter treatment goals and strategies for a particular patient. More appropriately directed rehabilitation may result in improved functional outcome. In addition, this technique allows the effectiveness of therapeutic and experimental treatment strategies to be evaluated more sensitively and objectively. These methods would be most useful as part

of a neurophysiological test battery as an adjunct to routine clinical assessment. Although further evidence is required to validate the neuroanatomical pathways assessed by this technique, the possibility of assessing function in distinct pathways (i e., corticospinal and bulbospinal) would be of particular value.

Chapter 4 - General Discussion

4.1 - Introduction

The principal objective of the present thesis was to examine the presence and nature of preserved motor pathways in patients with SCI. Several techniques were employed to enhance the probability of eliciting MEPs following transcranial magnetic stimulation of the motor cortex thereby facilitating the detection of residual descending influences in these patients. In addition, cortical stimulation was used to condition H-reflexes as a means of identifying the presence and nature of descending modulation on the motor neuron pool following transcranial magnetic stimulation.

The experiments focused on obtaining new information pertaining to several theoretical issues. These included a) the presence and nature of late responses following cortical stimulation, b) the detection of subliminal corticospinal influences in patients with SCI, and c) the correspondence between the electrophysiological and clinical status of patients with SCI.

The nature of residual supraspinal influences on motor neuronal activity following cortical stimulation was examined using several different approaches. The initial experiment investigated the question of whether stimulation of cutaneous afferents in the lower limb (i.e., sural nerve stimulation) was capable of facilitating MEPs in a manner previously attributed solely to muscle afferents (Komori et al., 1992, Troni et al. 1988). Although cutaneous afferent stimulation provided a substantial enhancement of MEPs, the degree of facilitation was no greater than we have previously shown with stimulation of

the medial plantar nerve (i.e., mixed nerve) (Kasai et al., 1992). This technique was not examined further in the present thesis as we have previously established the usefulness of medial plantar nerve stimulation in documenting residual descending influences following cortical stimulation in patients with SCI (Hayes et al., 1992).

The second technique, using induced whole body hypothermia was generally ineffectual in enhancing MEPs in patients with severe SCI (i.e., ASIA A, B and C) There was, however, modest evidence for enhanced MEPs with cooling in patients with the least severe injuries (i.e., ASIA D) while normal control subjects demonstrated marked enhancements in MEPs following cooling.

The use of target muscle reinforcement (i.e., attempted ankle dorsiflexion or plantarflexion) was effective in enhancing the probability of eliciting MEPs in patients with SCI. In particular, MEPs were elicited in some patients (but not all) with more severe injuries (i.e., ASIA C) with this type of neurologic reinforcement. MEP detection was sometimes difficult with this method as MEPs occurred in the midst of ongoing muscle activity and therefore averaging techniques proved necessary.

The most sensitive technique for detecting preserved innervation proved to be the cortical conditioning of H-reflexes (Experiments 3 and 4). Evidence was obtained for residual supraspinal influences to the motor neuron pool in all patients with incomplete SCI including 1 patient previously thought to possess no motor function (i.e., ASIA B) These findings were obtained despite the fact that several of these patients demonstrated no evidence of MEPs even following neurologic reinforcement provided by target muscle contraction.

4.2 - Late Influences on the Motor Neuron Pool with Cortical Stimulation

Observations pertaining to the characteristics of late responses following transcranial magnetic stimulation represent some of the most important findings of the present thesis. In Experiment 1, late MEPs were noted in normal subjects with the convergence of high intensity cutaneous stimulation and cortical stimulation. These late responses, present in the TA and LG, occurred despite the absence of early MEPs in the LG. In Experiments 2 and 4, late MEPs were prevalent in patients with SCI and were elicited even when muscles were at rest. This was particularly evident in the least severely injured patients (i.e., ASIA D) and occurred even in the absence of early responses. The critical question surrounding these findings is whether or not these responses reflect conduction in descending motor pathways and therefore might aid in the detection of preserved central influences following SCI.

Evidence derived from experiments studying late responses by assessing the effect of altering muscle activation level or length (Sammut et al., 1995; Wilson et al., 1995) or by examining subjects with various peripheral or central nervous dysfunctions (Mills et al., 1991) suggests that late responses may be generated by afferent input associated with the twitch contraction of the target muscle, or its antagonist (i.e., the twitch associated with the short latency MEP). In particular, late motor neuron activity produced in this manner is especially evident when the muscles are preactivated (Mills et al., 1991; Sammut et al., 1995; Wilson et al., 1995). This was also the case in some of the present studies as late muscle activity following cortical stimulation was noted in several instances when subjects were asked to attempt ankle dorsiflexion or plantarflexion (e.g., see Figures 9, 10, and

16).

The results of the present thesis support the view that central motor pathways also exert late excitatory influences on the motor neuron pool following cortical stimulation There are several observations in both control subjects and patients with SCI that late periods of excitability persist despite the absence of early MEPs (e.g., following subthreshold cortical stimulation). Therefore, these late responses, or late periods of excitability, cannot be due to the afferent consequences associated with the muscle twitch of the short latency MEP. The determination of the descending tracts mediating these effects was beyond the scope of the thesis. Central structures that have been implicated in the generation of late periods of motor neuron excitability following cortical stimulation include bulbospinal pathways (Dimitrijevic, Kofler, et al., 1992; Holmgren et al , 1992) and slow conducting and/or oligosynaptic corticospinal pathways (Dimitrijevic, Kofler, et al., 1992). The finding of an absent late facilitatory period with the cortical conditioning of H-reflexes in the patient with suspected anterior spinal cord damage (i.e., ASIA B) is consistent with these responses being mediated by ventromedial pathways. These pathways may receive direct corticobulbar excitatory inputs following cortical stimulation Otherwise, proprioceptive inputs (Colebatch & Porter, 1987) from muscles of the scalp or auditory inputs (Delwaide et al., 1993; Dobkin et al., 1994; Holmgren et al., 1992, Rossignol & Melvill Jones, 1976; Rudell & Eberle, 1985) associated with the click of the stimulating coil may mediate late responses via the brain stem following cortical stimulation.

Another possibility for eliciting late responses with cortical stimulation involves the

activation of γ-motor neurons following stimulation of the motor cortex as has been documented with electrical cortical stimulation in a variety of animal models (Granit & Kaada, 1952; Grigg & Preston, 1971; Laursen & Wiesendanger, 1966). Increased Ia afferent discharge may result from γ-motor neuron activation and this increases α-motor neuron excitability. These effects are unlikely with the protocols employed in the present experiments as γ-motor neuron related spindle activity is only elicited at equivalent or greater stimulation intensities than required for activation of α-motor neurons with transcranial magnetic stimulation and even then requires pre-activation or lengthening of the homonomous muscle (Rothwell et al., 1990).

In summary, it appears that when an early MEP is elicited the late response is likely a combined effect of peripheral afferent inputs and descending excitatory inputs to the motoneuron pool. When late responses or late facilitatory influences are observed without a preceding short latency MEP, the late response is most likely attributable to inputs from descending motor tracts. It is this latter case that provides the opportunity for detection of preserved innervation in tracts other than the fast corticospinal pathway.

4.3 - Detection of Subliminal Descending Inputs in Patients with SCI

The demonstration of residual subthreshold descending influences in patients with SCI which were previously undetected by clinical assessment or cortical stimulation was also an important finding of the present thesis. In particular, evidence of preserved corticospinal fibers was obtained in an ASIA B patient by cortically conditioning H-reflexes. Clinical assessment and cortical stimulation had revealed no evidence of preserved motor function in this patient. In addition, cortical conditioning clearly

demonstrated preserved supraspinal (i.e., corticospinal and possibly bulbospinal) influences in 2 ASIA C patients despite previous evidence of only limited preserved motor function in these patients. These observations provide support for the emerging concept that many patients with SCI possess intact innervation despite the absence of useful sensory or motor function. The evidence obtained in the ASIA B patient was consistent with the hypothesis of discomplete lesions (i.e., patients with complete SCI yet electrophysiological evidence of preserved innervation - Dimitrijevic, 1987) The evidence obtained in the ASIA C patients suggests there may be some merit in extending the concept of the discomplete lesion to encompass the idea of the "***discomplete muscle***"; that is - electrophysiological evidence of preserved descending motor connections to a muscle otherwise thought to be devoid of descending inputs. This concept may prove useful as a hypothesis to test in future studies and would allow more comprehensive evaluations of patients with SCI.

These findings assume considerable importance with the realization that relatively few intact axons are necessary for useful function (Eidelberg, Story, et al., 1981; Eidelberg, Walden, et al., 1981; Kaelan et al., 1989; Noordenbos & Wall, 1976; Windle et al., 1958). Therefore, these and similar observations provide optimism for current therapeutic and experimental approaches seeking to enhance neuronal survival following trauma (e.g., methylprednisolone - Bracken et al., 1990, 1992) or enhance conduction in existing neural structures (e.g., 4-aminopyridine - Hansebout et al., 1993; Hayes, 1994 ; Hayes, Blight, et al., 1993; Hayes et al., 1994). In addition, techniques which allow the detection of subliminal influences will be critical for assessing potential benefits of these

and future therapies (e.g., nerve regeneration) and also may serve to detect which patients may benefit from such interventions.

4.4 - Electrophysiological and Clinical Status of Patients with SCI

In order to be clinically useful, neurophysiological findings must expand upon the results of the neurological assessment. The presence of MEPs alone may do this in that they verify the structural continuity of the corticospinal tract, however, MEPs are often difficult to elicit in patients with SCI. The present thesis demonstrated that the reinforcement of MEPs or the cortical conditioning of H-reflexes is required to demonstrate preserved innervation in all but the least severely injured patients. In these patients (i.e., ASIA D), MEPs were most easily elicited, although target muscle contraction was often necessary to ensure responses. These findings were paralleled by the results of the cortical conditioning of H-reflexes. These patients also exhibited voluntary motor control consistent with preserved corticospinal pathways. Therefore, MEPs only provided corroborating and not novel evidence of corticospinal function in these patients.

The cortical conditioning of H-reflexes clearly demonstrated preserved corticospinal influences in patients with more severe injuries (i.e., ASIA C). The presence or absence of MEPs , even with target muscle contraction to enhance the probability of eliciting responses, was less reliable as an indication of intact corticospinal pathways. These patients typically produced poorly controlled voluntary muscle activity which was equivocally under corticospinal control. The evidence from cortical conditioning of H-reflexes verified the presence of corticospinal influences in these patients. This technique

also demonstrated residual corticospinal connections in an ASIA B patient. These findings were not consistent with the clinical status for this patient as there was no evidence of preserved motor function with either attempted voluntary muscle activation or MEPs. Conversely, all electrophysiological measures and the clinical assessments in the most severely injured patients (i.e., ASIA A) were consistent. These patients, classified as patients with complete SCI, showed no evidence of MEPs, voluntary motor control, or enhanced motor neuron excitability following cortical stimulation.

4.5 - Conclusions and Future Directions

In summary, the experiments of the present thesis demonstrated several unique observations relating to the detection and nature of central motor pathways in patients with SCI. These experiments also addressed several methodological issues in control subjects and patients with SCI which served to identify the nature of preserved innervation as detected by the various techniques employed. The principal conclusions of these experiments were the following:

a) Preserved motor innervation may be detected in patients with SCI even in patients who show no other evidence of motor function. This is a relatively rare occurrence but has implications for the potential recovery of function with experimental or other therapeutic interventions. Even in the incomplete patient findings of intact innervation to individual muscles previously considered completely paralysed are encouraging and offer therapeutic potential. These observations were most easily achieved by identifying periods of increased

excitability of the motor neuron pool using cortical stimulation to condition H-reflexes which allows the detection of previously subliminal influences.

b) Cutaneous afferent conditioning stimulation facilitates short latency MEPs in both ipsilateral and contralateral muscles in normal subjects. Therefore, this technique may be useful in detecting preserved motor innervation. We have previously noted enhanced MEPs in SCI patients with mixed nerve stimulation.

c) Central motor pathways mediate long latency periods of enhanced motor neuron excitability following subthreshold cortical stimulation. These responses, identified by cortical conditioning of H-reflexes and observed following high intensity cutaneous conditioning stimulation in normal subjects, may prove beneficial in assessing conduction in descending motor pathways in addition to the corticospinal pathway (possibly bulbospinal).

d) Electrophysiologic results obtained with cortical stimulation augments the information obtained from clinical neurological assessments of patients with SCI. The techniques employed in the present thesis allow greater sensitivity in the detection of preserved motor innervation and assess the structural continuity of specific neuroanatomic pathways.

The results of the present experiments suggest several potential future directions.

Most importantly will be experiments which establish the precise identification of pathways mediating late responses following cortical stimulation. It is likely that several mechanisms are involved and that the precise combination of structures involved may vary under different circumstances. Future experiments may include animal experiments in which neural activity could be monitored in several ascending and descending systems or the use of microneurographic techniques in humans to monitor peripheral efferent and afferent activity following cortical stimulation. Ischaemic or pharmacological blockades to alter peripheral afferent inputs would prove useful in verifying central effects. The startle effects associated with the click of the stimulating coil or scalp muscle contraction should also be re-examined. Experiments involving auditory masking, or blocking, may prove helpful in identifying any contribution from auditory spinal responses.

Further investigation may also benefit from the employment of recently developed cortical stimulation coils (e.g., figure of eight design) which are thought to deliver more effective stimulation to the lower limb cortical areas. Similarly, to ensure that the maximum opportunity exists for detection of preserved innervation, patients should be weaned for any central depressant medications.

If it can be established that there is a tight correspondence between various electrophysiological measures (i.e., cortical conditioning of H-reflexes in particular) and clinical neurological status, at various stages of rehabilitation, then this information may be extremely beneficial for prognostic purposes.

Appendices

Appendix 1. Changes in PMCT and CMCT with Total Body Cooling (Experiment 2)

As expected, all response onset latencies were prolonged following cooling with these findings most evident in the distal musculature (i.e., EDB). This is consistent with the fact that temperature reductions were most pronounced distally. Conduction times in the peripheral pathway to the EDB (i.e., PMCT) were prolonged by ~ 2 ms with cooling This is less than the value of ~ 6 ms which may have been expected when noting the mean peripheral temperature reduction of 4.4 °C and assuming a temperature correction factor of 1.84 m/s/°C (see Appendix 2). These observations can be explained by the fact that the peripheral temperature reduction of 4.4 °C as measured on the dorsum of the foot was likely greater than that more proximally in the lower limb.

Unlike the prolongation seen with the PMCT, the CMCT was reduced with cooling. It is unlikely that this reduction reflects a true increase in central conduction velocity. CMCT is estimated by subtracting the PMCT from the MEP latency. It is possible that changes in the conduction times associated with the MEP latency are not due solely to cooling effects of slowing conduction velocity. Rather, changes in motor unit recruitment due to enhanced motor neuron excitability with cooling could have resulted in shorter conduction times in the MEP than otherwise expected. This possibility would have to be considered in future experiments assessing the potential of cooling in enhancing central conduction in patients with SCI.

Appendix 2. Predicted Changes in PMCT with Cooling (Experiment 2)

Measured conduction distance in an illustrative subject (1.75 m)	= d = 1.05 m.	
Conduction time	= $PMCT_{pre}$ = 24.6 ms.	(before cooling).
Mean conduction velocity (CV)	= CV_{pre} = d / $PMCT_{pre}$ = 1.05/0.0246 = 42.7 m/s	(before cooling).
Measured peripheral temperature change	= ΔT = - 4.4 °C.	
If use temperature correction factor	= T_{cf} = 1.84 m/s/°C	(de Jong et al., 1966).
Predicted change in CV	= ΔCV = $\Delta T \times T_{cf}$ = -4.4 x 1.84 = -8.1 m/s.	
Predicted CV	= CV_{post} = $CV_{pre} + \Delta CV$ = 42.7 + (-8.1) = 34.6 m/s	(after cooling).
Predicted PMCT	= $PMCT_{post}$ = d / CV_{post} = 1.05 / 34.6 = 0.03035 s or ~ 30.4 ms	(after cooling).
Therefore, predicted prolongation of PMCT	= PMCT = $PMCT_{post}$ - $PMCT_{pre}$ = 30.4 - 24.6 = 5.8 ms.	

Appendix 3. MEP Amplitudes and Latencies for the Right TA (Experiment 4)

Subject	ASIA Impairment Scale	Rest		Attempted Dorsiflexion		Attempted Plantarflexion	
		Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)
1	A	NR	NR	NR	NR	NR	NR
2	A	NR	NR	NR	NR	NR	NR
3	A	NR	NR	NR	NR	NR	NR
4	A	NR	NR	NR	NR	NR	NR
5	B	NR	NR	NR	NR	NR	NR
6	C	NR	NR	NR	NR	NR	NR
7	C	NR	NR	33.5	10	NR	NR
8	C	NR	NR	58.5	65	37.0	27
9	D	NR	NR	29.5	113	34.0	112
10	D	NR	NR	42.0	211	41.0	43
11	D	31.0	41	32.0	321	35.0	15

Note: MEP amplitudes were determined by calculating the AEMG value for 20 ms following the onset latency of the response. NR = no response

Appendix 4. MEP Amplitudes and Latencies for the Right LG (Experiment 4)

Subject	ASIA Impairment Scale	Rest		Attempted Dorsiflexion		Attempted Plantarflexion	
		Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)
1	A	NR	NR	NR	NR	NR	NR
2	A	NR	NR	NR	NR	NR	NR
3	A	NR	NR	NR	NR	NR	NR
4	A	NR	NR	NR	NR	NR	NR
5	B	NR	NR	NR	NR	NR	NR
6	C	NR	NR	53.5	132	NR	NR
7	C	NR	NR	NR	NR	NR	NR
8	C	NR	NR	39.5	35	32.5	41
9	D	NR	NR	31.0	14	26.0	202
10	D	NR	NR	34.0	38	35.5	73
11	D	37.0	5	31.0	21	34.0	25

Note: MEP amplitudes were determined by calculating the AEMG value for 20 ms following the onset latency of the response. NR = no response.

Appendix 5. MEP Amplitudes and Latencies for the Left TA (Experiment 4)

Subject	ASIA Impairment Scale	Rest		Attempted Dorsiflexion		Attempted Plantarflexion	
		Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)
1	A	NR	NR	NR	NR	NR	NR
2	A	NR	NR	NR	NR	NR	NR
3	A	NR	NR	NR	NR	NR	NR
4	A	NR	NR	NR	NR	NR	NR
5	B	NR	NR	NR	NR	NR	NR
6	C	NR	NR	NR	NR	NR	NR
7	C	NR	NR	NR	NR	NR	NR
8	C	NR	NR	30.5	120	41.5	39
9	D	[illegible]	NR	39.0	80	34.5	73
10	D	NR	NR	47.5	22	41.5	12
11	D	NR	NR	40.5	323	35.5	32

Note: MEP amplitudes were determined by calculating the AEMG value for 20 ms following the onset latency of the response. NR = no response.

Appendix 6. MEP Amplitudes and Latencies for the Left LG (Experiment 4)

Subject	ASIA Impairment Scale	Rest		Attempted Dorsiflexion		Attempted Plantarflexion	
		Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)	Latency (ms)	Amplitude (uV)
1	A	NR	NR	NR	NR	NR	NR
2	A	NR	NR	NR	NR	NR	NR
3	A	NR	NR	NR	NR	NR	NR
4	A	NR	NR	NR	NR	NR	NR
5	B	NR	NR	NR	NR	NR	NR
6	C	NR	NR	NR	NR	NR	NR
7	C	NR	NR	NR	NR	NR	NR
8	C	NR	NR	36.0	38	33.0	96
9	D	NR	NR	NR	NR	34.5	10
10	D	NR	NR	48.5	6	40	21
11	D	NR	NR	32.5	116	29.0	181

Note: MEP amplitudes were determined by calculating the AEMG value for 20 ms following the onset latency of the response. NR = no response.

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