MOTOR CORTICAL CONTROL OF HUMAN JAW MUSCLES

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page

ABSTRACT

The human jaw muscles exhibit precise control during mastication and speech. By analogy with the limbs, this control is probably mediated from the motor cortex via corticomotoneuronal (CM) cells, however CM cells have not been unambiguously demonstrated for the trigeminally innervated jaw muscles, and their characteristics have not been described in detail. In this study I have investigated the existence, nature and function of CM cells innervating the human masseter muscle during voluntary movements and reflexes.

Masseter CM projections were examined by a) comparing motor evoked potentials (MEPs) elicited concurrently in the surface electromyograms (EMG) of both masseter muscles by focal transcranial magnetic stimulation (TMS) of one hemisphere of the motor cortex; b) comparing responses of individual masseter motoneurons to TMS of the contra- and ipsi-lateral motor cortex, and; c) examining co-variation in left and right masseter MEP size on a trial-by-trial basis, to identify branched CM neurons (an analysis first tested in two situations where branched CM projections were known to exist). Masseter CM cell function was examined by assessing CM cell involvement during a) bilateral *vs.* unilateral biting and b) the masseter long latency stretch reflex (LLSR).

Two types of CM projections were identified in the control of human masseter. Larger MEPs were elicited in the surface EMG from the masseter contralateral to the TMS, and most low threshold motor units in masseter were excited at a monosynaptic latency. This suggested a population of CM neurons with exclusively contralateral projections to masseter motoneurons. However, bilateral masseter MEPs were elicited in the surface EMG following focal TMS and some masseter motoneurons were identified with

excitatory input from both hemispheres of the motor cortex. Co-variation in left and right masseter MEPs suggested that some CM neurons branch to innervate masseter motoneuron pools on each side.

CM cells from each hemisphere were shown to have distinct roles during the biting tasks; unilateral biting was associated with a reduced activity of CM cells in the contralateral, but not the ipsilateral cortex. By combining muscle stretch with TMS, I found no evidence for CM cell involvement in the masseter LLSR.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Sophie L Pearce

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LIST OF ILLUSTRATIONS

page

LIST OF TABLES

page

AIMS AND GENERAL INTRODUCTION

The role of the motor cortex in the control of precision movements in muscles of the hand has been widely studied, but comparatively few studies have investigated its role in the control of the jaw muscles. The hand muscles have a large representation in the motor cortex, and the fast, monosynaptic connections from the motor cortex to motoneurons (corticomotoneuronal (CM) cells) are known to be responsible for the precise control of independent finger movements in humans (Schieber, 1990; Lemon, 1993). The jaw and face muscles also have a large representation in the motor cortex, and studies point to the presence of CM cells controlling muscles of the jaw (Cruccu et al., 1989). The purpose of this thesis was to investigate the role of these CM cells in the control of voluntary and reflex movement in the human masseter muscle.

Precise control and coordination of the muscles acting on the mandible is essential for everyday activities such as speech and mastication. For example, during mastication chewing forces in humans average around 27 kg (Gibbs et al., 1981) and careful co-ordination of the jaw closing muscles is essential to protect the soft tissues in the mouth from damage. Also, the efficient breakdown of food requires a complicated asymmetrical coordination of jaw closing muscle activity, with the time course and level of muscle activation differing between sides (Hannam, 1976; Luschei and Goldberg, 1981). By analogy with their role in precision movements in the hand, it is hypothesised that CM cells are involved in the precise control of masticatory function.

Human CM cells can be studied painlessly using transcranial magnetic stimulation (TMS). When TMS is delivered using a figure-of-eight coil the CM cells originating from only one hemisphere of the motor cortex are exclusively activated (Rosler et al.,

1989; Cohen et al., 1990). An aim of the experiments reported in Chapter 2 was to determine the relative strength of the contralateral and ipsilateral CM projections to masseter motoneurons by comparing the size of the motor evoked potential (MEP) to focal TMS in the surface electromyogram of both muscles. This was of interest because the nature of the CM projections has implications for the ability of the motor cortex to mediate independent activation of the masticatory muscles on each side, especially since historically it has been accepted that the cortical projections to masseter motoneurons are bilateral and symmetrical (Kuypers, 1958a). This issue was explored in more detail in Chapter 3, where the response in single masseter motor units to TMS of either the contralateral and ipsilateral was examined. This allowed me to demonstrate CM projections to masseter motor units, and examine the organisation of inputs from each hemisphere of the motor cortex to individual masseter motoneurons.

The size of the response to TMS in a given muscle is a function of the excitability and number of CM cells which innervate that muscle (see Rothwell, 1997). In Chapter 2 the involvement of CM cells was compared during bilateral and attempted unilateral voluntary activation of one masseter muscle. Based on CM cell involvement in producing fractionated control of hand muscles, it was hypothesised that unilateral biting would be associated with a modulation of CM activity in the contralateral hemisphere, compared with the situation during bilateral activation of both masseter muscles.

CM cells that branch to monosynaptically excite motoneuron pools of several synergistic muscles are an important feature of the fine motor cortex control over the hand muscles in humans (see Porter and Lemon, 1993). The muscles on either side of the jaw act in synergy to produce jaw closing, but it is not known if CM cells exist which branch to

activate the muscles on each side. While synchronisation between motor units in each masseter muscle suggests these cells may exist (Carr et al., 1994), a further aim of this thesis was to provide more direct evidence for the presence of such cells in the neural control of left and right masseter. The basis of these experiments was that if single CM cells branch to innervate motor pools of several muscles, then the fluctuations in MEP size in these muscles are likely to co-vary as the excitability of the shared CM cells fluctuates.

To test the reliability of this technique in the detection of branched CM axons, it was first tested in two situations where branched axons were known to exist. First, covariations in MEP fluctuations were examined between synergistic muscles of the hand (Chapter 4). Secondly the technique was tested using MEPs elicited in the left and right muscles of the upper limb and the jaw in a patient with infantile hemiplegia and mirror movements (Chapter 5). The results of these studies confirmed that when the muscles were active, co-variation in MEP size fluctuations were present for muscle pairs sharing branched-axon CM inputs, and not present for muscle pairs without such projections. I was therefore confident of applying this technique to the trigeminal system where I aimed to provide evidence of branched CM projections to left and right masseter motoneurons (Chapter 6).

A final aim of this thesis was to establish the role of the CM projections in the long-latency stretch reflex (LLSR) of human masseter muscles. Stretch reflexes play an important role in motor control, providing compensation for sudden changes in muscle length, and the long latency component of the stretch reflex is thought to be the most important. There is considerable evidence, for muscles of the hand, that the delayed

nature of the LLSR is because it traverses a transcortical pathway (Hammond, 1960; Phillips, 1969; Matthews, 1991). An important line of evidence in support of this theory is that CM cells show an increased excitability during the LLSR (Day et al., 1991; Palmer and Ashby, 1992b). An advantage of a LLSR involving the motor cortex is the added flexibility that this would give the reflex response. Indeed there is evidence, although recently challenged (Capaday et al., 1994), that the LLSR is flexible and can be modulated by prior instruction to the subject (Calancie and Bawa, 1985). The experiments reported in Chapter 7 test the excitability of CM cells with TMS during the masseter LLSR, with the aim of testing if the masseter LLSR is transcortical. In addition, the flexibility of the masseter LLSR was tested by comparing its size under different conditions of instruction to the subject on how to react upon perception of the stretch.

The results of this thesis will provide a greater understanding of the function of the motor cortex in controlling human masseter muscles in normal subjects. In-depth knowledge of the neural control of masseter muscles in normal subjects is required as a foundation to understand the mechanisms of masticatory dysfunction in humans.

CHAPTER 1

LITERATURE REVIEW

Everyday oral activities, such as biting, chewing, and speech require the bilateral co-ordination of numerous muscles on either side of the mandible. The masseter muscles, together with the temporalis and medial pterygoid muscles, function to raise the mandible in a jaw-closing motion. Along with their antagonists, the jaw-opening muscles (digastric, lateral pterygoid, and the suprahyoid group), these muscles are known as the muscles of mastication (Luschei and Goldberg, 1981). The classification of the muscles of mastication into jaw-closers and jaw openers is in reality a great simplification (Luschei and Goldberg, 1981; Rowlerson, 1990), and the role of these muscles in mastication and other oral activities is complex. This review focuses on the cortical mechanisms responsible for the control of the human masseter muscle.

1.1 The masseter muscle

1.1.1 Structure

The masseter muscle has a complex pennate architecture consisting of separate portions with different fibre directions (Blanksma et al., 1992). Traditionally, masseter is subdivided into a superficial and a deep portion. The superficial portion originates by a strong tendon from the anterior two thirds of the zygomatic arch and inserts to the lower one-third of the lateral surface of the mandibular ramus. The deep masseter originates from the whole length of the zygomatic arch and inserts onto the upper two-thirds of the ramus (see Van Eijden et al., 1997). The deep portion is sometimes further subdivided

into an intermediate and deeper part (Hannam and McMillan, 1994). The separate portions of masseter are easily detectable in the posterior part of the muscle, but they fuse together in the anterior masseter (Lam et al., 1991; Goto et al., 1995; Zwijnenburg et al., 1999).

The superficial and deep parts of masseter are known to be activated differently according to task (Belser and Hannam, 1986; Tonndorf et al., 1989). The superficial fibres tend to be activated most during jaw elevation, elevation with protrusion, or movement on or towards the side contralateral to the muscle, while the deep fibres contribute strongly to jaw elevation and jaw retrusion on the side ipsilateral to the muscle (Hannam and McMillan, 1994).

The masseter muscle contains three to five internal aponeuroses that run roughly parasagitally and attach to the zygomatic arch and mandible (see Stalberg and Eriksson, 1987). The muscle fibres are arranged in a complex geometric pattern between the aponeuroses, with fibre orientation of the masseter differing in the deep and superficial regions (Hannam and McMillan, 1994). In the superficial part the general direction of fibres is downwards and slightly backwards, whereas fibre direction in the deep head is vertical (Scott and Dixon, 1972). The average muscle fibre length in superficial masseter is 24.6 ± 4.1 mm, and in deep masseter is 18.0 ± 2.8 mm (Van Eijden et al., 1997).

Like all jaw-closing muscles, masseter has architectural features that make it suitable for the production of high biting forces. These include short sarcomere lengths at the closed jaw, large masses of contractile and tendinous tissue, large physiological cross-sectional

areas, large pennation angles, short fibre lengths, short moment arms, and a low fibre length to muscle length ratios (Van Eijden et al., 1997).

1.1.2 Motor units and muscle fibre types

The motor unit is the smallest functional compartment of the motor system and consists of a single motoneuron, its motor axon and all the skeletal muscle fibres it innervates. Motor units can be classified according the ATPase activity of the muscle fibre myosin heavy chain isoforms, since all of the muscle fibres within a given motor unit are comprised of one histochemical type. Traditionally there are three groups of muscle fibres; type I fibres have low levels of ATPase activity and depend on oxidative metabolism, whereas type IIb have high levels of ATPase activity, and are specialised for glycolysis. Type IIa fibres, which are the other main fibre type in limb muscles, have properties between the Type I and Type IIb fibres.

Human jaw closing muscles are unusual in comparison to other skeletal muscles in that their fibres cannot always be classified simply by ATPase histochemistry, because characteristically they display heterogeneity of myosin heavy chain proteins. To summarise the main differences between masseter and limb muscle fibre types:

1. Masseter is primarily composed of Type I and Type IIb muscle fibres (Eriksson and Thornell, 1983) and contains very few type IIa fibres (Eriksson, 1982), although the proportion of IIa fibres has been shown to increase with age (Monemi et al., 1999).
2. The morphology of masseter muscle fibres is unusual in that the diameter of the type IIb fibres in masseter is less than that of type I fibres (Eriksson, 1982).

3. Masseter contains a number of muscle fibre types which have intermediate ATPase activity, and are considered transitionary in limb muscles (Rowlerson, 1990).
4. The correlation between the histochemical characteristics of motor units and their physiological properties does not seem to be as strong in masseter as in limb muscles. Masseter has a large proportion of Type I fibres, but contains very few motor units with a slow twitch time (Nordstrom and Miles, 1990).
5. In limb muscles the fibres from a single motor unit are scattered widely throughout the muscle. In contrast, the fibres of masseter motor units tend to be more localised within the muscle, and are contained to regions of the muscle with the same function (functional compartments) (see Hannam and McMillan, 1994).

1.1.3 Nerve supply

The masseter muscle is supplied by the mandibular division of the fifth cranial (trigeminal) nerve. The trigeminal nerve is the largest cranial nerve and is the sensory nerve of the head and face, and the motor nerve of the muscles of mastication. The mandibular division is the largest and most inferior of the three trigeminal projections, and the only branch to contain motor as well as sensory fibres. The large sensory root and smaller motor root leave the brainstem at the midlateral surface of pons, and descend through the foramen ovale into the infratemporal fossa from the medial part of the cranial fossa (Scott and Dixon, 1972).

Underlying the organisation of the trigeminal nerve is the general principle that the motor output and different modalities of sensation are processed by separate nuclei in the brain stem. Motor input to masseter is mediated via motoneurons located in the masticatory

motor nucleus of trigeminal nerve. The motor nucleus is located in the mid-pons of the brainstem, and also provides motor innervation to temporalis, the pterygoid muscles, anterior digastric, the mylohyoid, and the tensor tympani (Kelly, 1985). Bimodality of the size distribution of neurons and motor axons from the motor nucleus of the trigeminal nerve indicates that it contains both α and γ motoneurons (Limwongse and DeSantis, 1977). Proprioceptive information from masseter (and other jaw muscles) is mediated via the mesencephalic nucleus, the only example where cell bodies of peripheral afferent nerves lie within the adult vertebrate central nervous system (Kelly, 1985). A collateral branch from the mesencephalic nucleus goes directly to the motor nucleus of the trigeminal nerve, providing a monosynaptic reflex arc to the motoneurons (see section 1.1.5). Cutaneous sensation from the face and oral mucosa, along with tactile sensation from the teeth are meditated via the main sensory nucleus (Kelly, 1985). The nucleus of the spinal tract consists of the oral nucleus (mediates cutaneous sensation from the oral mucosa), the interpolar nucleus (mediates sensation of pain from the tooth pulp) and the caudal nucleus (mediates pain, temperature and light touch from the skin of the face, and sensation of pain from the tooth pulp) (Kelly, 1985).

1.1.4 Proprioceptors

Proprioceptors are receptive organs that signal to the CNS information regarding the positions of the body parts. They are located in the muscle (muscle spindles and Golgi tendon organs), joints and skin. In the case of the masticatory system, periodontal mechanoreceptors and receptors of the oral cavity should also be considered as proprioceptors. For a comprehensive review on the role of these proprioceptors in the control of jaw movement, see Taylor (1990a). This thesis is mainly concerned with the

role of the motor cortex in the control of movement in masseter. However, Chapter 7 does explore whether the masseter stretch reflex has a transcortical long-latency component, and so a brief description of the muscle spindle is given below.

Muscle spindles consist of a bundle of specialised muscle fibres (intrafusal fibres) which lie in parallel with the extrafusal fibres of the skeletal muscle. There are two types of intrafusal fibres in the muscle spindle: bag and chain fibres. On the basis of physiological properties, bag fibres have been subdivided into dynamic bag_1 and static bag_2 fibres (Saito et al., 1977; Rowlerson et al., 1988). The main motor innervation of the intrafusal fibres is from small diameter axons from the γ-motoneuron. In some cases the motor supply arises partially from branches of α-motoneurons innervating the extrafusal fibres, and these are known as β axons (see Rowlerson, 1990). It should be noted that the terms "α", "β" and "γ" are not strictly applicable in the case of the jaw muscle nerves, because the conduction velocities are not the same as in the hindlimb and the diameter spectra have not been shown to have peaks clearly related to function (Taylor, 1990b). The sensory innervation of the spindles is of two types: larger diameter Ia afferents and the smaller group II. Primary afferent endings terminate around the central area of all types of fibres, whereas the secondary endings are located adjacent to the central regions of the static bag and chain fibre afferents (see Pearson and Gordon, 2000). In masseter, the primary spindle endings are active during jaw opening (firing at higher rates when length is changing than when length is maintained) and silent during closing. In contrast, spindle group II endings fire tonically, at a lower frequency, throughout the chewing cycle (Taylor, 1976).

While the jaw-opening muscles contain few muscle spindles, the masseter muscles are richly supplied (Rowlerson, 1990). The majority of muscle spindles in masseter are located in the deep part of the muscle (Maier, 1979; Eriksson and Thornell, 1987; Rowlerson et al., 1988; Sciote, 1993) and are therefore in close association with the areas containing a high proportion of type I fibres. Masseter muscle spindles are larger and more complex than muscle spindles in limb muscles (Eriksson and Thornell, 1987), although the reason for this is not clear.

When a skeletal muscle is stretched, the intrafusal fibres of the muscle spindle lengthen and the sensory fibres innervating them are deformed and increase their discharge. The muscle spindle afferents make mono- and oligosynaptic contact with the motoneurons innervating the extrafusal muscle fibres, and their excitation causes contraction of the muscle.

The connectivity from a single spindle afferent onto homonymous motoneurons in masseter is 10-30% (Appenteng et al., 1978; Nozaki et al., 1985) which is lower than that found in motoneurons of limb muscles, where it approaches 100% (Mendell and Henneman, 1971; Watt et al., 1976). In humans, a non-uniform distribution of Ia afferents onto motoneurons is thought to account for the lack of stretch reflex responses in 35% of low threshold masseter motor units (Miles et al., 1995)

In limb muscles, muscle spindle input is most effective onto small motoneurons (Heckman and Binder, 1988), and is thought to be important in the maintenance of static posture. In contrast, masseter muscle spindle input has been shown to be more effective on the large motoneurons (Scutter and Türker, 2001). This suggests that muscle spindles

in masseter may be important in load compensation during chewing and for the development of powerful bite forces in aggressive or defensive situations.

1.1.5 Stretch Reflexes in the masseter

Downwards displacement of the jaw, either by tapping on the chin (Godaux and Desmedt, 1975; Murray and Klineberg, 1984) or by controlled depression of the mandible (Lamarre and Lund, 1975; Marsden et al., 1976; Cooker et al., 1980; Smith et al., 1985; Poliakov and Miles, 1994) results in a short-latency stretch reflex (the jaw-closing reflex), mediated by excitation of the muscle spindles in the stretched muscles. Although the short-latency component of the stretch reflex is often considered to be monosynaptic (Munro and Griffin, 1971), oligosynaptic transmission from group Ia afferents to homonymous spinal motoneurons has been demonstrated (Jankoswska, 1984), and is considered to be the likely mechanism for the later part of the short latency stretch reflex in limb muscles (Burke, 1989).

A careful analysis of the stretch reflex in human masseter was performed by Poliakov and Miles (1994), who found that slow stretch of the masseter muscle results not only in a short-latency stretch reflex (SLSR), but also in a long-latency stretch reflex (LLSR). This was similar to the pattern of the stretch reflexes reported in other muscles (see Deuschl and Lucking, 1990), but contrasted with earlier work which suggested that masseter did not respond to stretch at a long latency (Lamarre and Lund, 1975; Goodwin et al., 1978; Cooker et al., 1980). Poliakov and Miles (1994) suggested that the absence of a LLSR in previous studies was due to powerful disfacilitation of the motoneurons following the short-latency excitation caused by the brief stretches used in those studies.

Stretch reflexes are generally thought to function in the regulation of voluntary movement, adjusting the motor output to the biomechanical state of the body (Pearson and Gordon, 2000). The long-latency stretch reflex is considered to be the most important, contributing most of the force of the response (Hammond, 1960; Poliakov and Miles, 1994). In masseter, it has been suggested the jaw stretch reflex makes a significant contribution to the stability of the mandible, during such activities as mastication and locomotion (Goodwin et al., 1978; Cooker et al., 1980; Lund et al., 1984; Miles and Nordstrom, 2001). The origin of the long-latency component of the stretch reflex is complex, and may vary between muscles (Thilmann et al., 1991). In limb muscles it is usually believed to traverse the motor cortex (Hammond, 1960; Phillips, 1969; Cheney and Fetz, 1984; Day et al., 1991; Matthews, 1991; Palmer and Ashby, 1992b). However, the neural processes responsible for the long-latency stretch reflex in human masseter has not yet been determined. In Chapter 7 of this thesis, I examine evidence for a transcortical component of the masseter LLSR.

1.2 The motor cortex

In the mid-nineteenth century, it was generally accepted that the entire cerebral cortex functioned as a whole, with no localisation of function. The first suggestion that motor functions were localised to particular portions of the cortex came in the 1860s from Hughlings Jackson, following clinical observations on patients with focal seizures. This idea was later confirmed Fritsch and Hitzig (1870) and by David Ferrier (1873), who discovered that electrical stimulation of different parts of the cortex in dogs and monkeys produced contraction of different contralateral muscles. Later, Charles Sherrington

(1906) discovered in primates that motor effects were most readily elicited from the precentral gyrus (Brodmann's area 4). This region is now referred to as the motor cortex.

1.2.1 Organisation of the motor cortex

1.2.1.1 Topographical organisation

Penfield and Boldrey (1937) demonstrated that the human motor cortex is topographically organised. Areas subserving the legs are located near to the inter-hemispheric fissure and moving laterally across the precentral gyrus, shoulder, arm, hand and then face areas are sequentially represented. The different body parts are not represented equally (a difference depicted in the classical motor homunculus); the hand and face areas both occupy a large area of the motor cortex, whereas trunk and leg muscles have a smaller representation. This presumably reflects the complexity of movements performed by these muscles.

More recent studies indicate that the concept of a single systematic representation of the movement of a body part is too simple, and the motor cortex appears to be organised into numerous microzones representing particular movements (Sessle and Wiesendanger, 1982; Schieber, 2001).

1.2.1.2 Cellular organisation

The motor cortex, like the rest of the cerebral cortex, consists of six layers of neurons (layers I to VI). It is one of the thickest regions of the cerebral cortex, although it has a low cell density. Its widely-spaced neurones are separated by large masses of neuropil,

which may provide for the very rich and flexible synaptic connections between the neurons (Phillips, 1981). There are two basic types of neuron in the motor cortex; pyramidal cells and basket cells. The pyramidal cells are found in all cortical laminae from II to VI, but the majority are in layers III and V. Pyramidal cells have long apical dendrites and most project to other subcortical or cortical regions. The apical and basal dendrites of pyramidal cells are covered with spines that receive both excitatory and inhibitory synapses. It has been estimated that there are in the order of 60,000 synapses on a single pyramidal neuron (Cragg, 1975). The stellate or basket cells are found in laminae III, IV and V and have radial dendritic trees. Basket cell axons are myelinated and are predominantly horizontal in orientation, and make inhibitory GABAergic synaptic contacts with pyramidal neurons (Porter and Lemon, 1993).

As well as being organised into layers, the neurons in the cerebral cortex have a columnar organisation, with functional groups of cells arranged in a radial fashion normal to the pial surface (see Mills, 1999). Pyramidal and non-pyramidal cells are clustered into columnar aggregates which are approximately 300 μm wide and are separated by 100 μm cell-sparse zones (Mountcastle, 1997). Forty percent of neurons in such clusters project to a single motoneuron pool in the spinal cord, and the remainder project to the motoneuron pools of muscle groups active in similar movements. This provides a strong excitatory drive to adjacent neurons, and via inhibitory neurons, a columnar surround inhibition (Mountcastle, 1997).

1.2.2 The corticospinal and corticobulbar tracts

The motor cortex exerts its influence on motoneurons innervating limb and trunk muscles and cranial muscles via the corticospinal and corticobulbar tracts respectively. These tracts consist of neurons that originate mainly from pyramidal cells located within lamina V of the cortex. In humans, ~30% of corticospinal and corticobulbar fibres originate from the primary motor cortex (Brodmann's area 4), another 30% arise from the pre-motor cortex (Brodmann's area 6), and the remaining 40% arise from the parietal cortex (particularly the primary somatosensory cortex) (see Ghez, 1985).

The human corticospinal tract descends through the medullary pyramids, where ~90% of the fibres decussate at a level just below the dorsal column nuclei in the medulla. The crossed fibres then continue down to the spinal cord as the lateral corticospinal tract, synapsing principally on contralateral distal limb motoneurons. The 10% of fibres that do not decussate at the medullary level descend in the ventral columns to form the ventral corticospinal tract, and innervate axial and proximal muscles. The human corticobulbar fibres leave the tract at the pyramids and innervate the cranial motoneurons, including the trigeminal motoneurons (see Kuypers, 1958a; Porter and Lemon, 1993; Ghez and Krakauer, 2000).

All primates possess a large pyramidal tract, containing many corticospinal fibres, and in man the number of fibres has been estimated to be 1.1 million in total (Heffner and Masterton, 1975). Towe (1973) demonstrated that there is a precise relationship between body weight and the number of corticospinal fibres, although the number of fibres correlates poorly with dexterity (Heffner and Masterton, 1975). In all species most of the

corticospinal tract fibres have a small diameter; in man 92% of fibres are smaller than 4 μm, and only 2.6% are larger than 6 μm (see Porter and Lemon, 1993). The larger fibres, known as fast corticospinal fibres, are myelinated and have conduction velocities of approximately 50-60 ms^{-1} (up to 80 ms-^{1}, Levy et al., 1984). The smaller diameter fibres are called slow corticospinal fibres, are unmyelinated and have conduction velocities of approximately 14 ms^{-1} (Kuypers, 1981).

On the basis of differential corticospinal projections to various regions of the spinal cord, Kuypers (1981) divided the mammalian species into 4 main groups:

1. In group 1, which includes most marsupials, the corticospinal fibres extend only to cervical and mid-thoracic segments and terminate in the dorsal horn.
2. In group 2, which includes carnivores such as cats and dogs and some new world monkeys, the corticospinal fibres extend throughout the spinal cord and terminate in the dorsal horn and the intermediate zone.
3. In group 3, which includes most of the New and Old world monkeys, the corticospinal fibres extend throughout the spinal cord and terminate in the dorsal horn, intermediate zone and parts of the lateral motoneuronal cell groups.
4. In man and the great apes (group 4), the lateral corticospinal fibres project to sensory neurons in the dorsal horn (laminae IV and V), to interneurons in the intermediate zone and to alpha and gamma motoneuron pools.

Note that the influence of the cerebral cortex over the spinal cord via the corticospinal tract is largely restricted to the upper parts of the spinal cord in the lower mammals, where its principal action must be exerted on the sensory mechanisms of the dorsal horn.

In the higher mammals, the ventral shift of connections provides access, first to those regions of the intermediate zone which control the motoneurons innervating distal muscles (probably via propriospinal interneurons with short axons), and subsequently directly to these motoneurons, via the cortico-motoneuronal (CM) connections (see Porter and Lemon, 1993). CM cells are discussed more fully in section 1.2.4.

1.2.3 Techniques used to investigate the corticospinal and corticobulbar pathways

The techniques commonly used to investigate the motor pathways are described below. The studies that have employed these techniques to investigate the cortical control of masseter are described in section 1.3.

1.2.3.1 Neuroanatomical tracing

Neuronal tracing allows anatomical connections to be charted within the nervous system. Retrograde axonal transport allows identification of the cells of origin of afferent nerve fibres to a particular target zone. The tracer material is applied to a fibre tract or terminal field of innervation, becomes incorporated into the cell axons (usually by process of endocytosis), and is then carried back to the parent cell body. Anterograde axonal transport enables the projection target(s) of individual or groups of cells to be charted within the central nervous system. The uptake mechanisms involve the cell soma and/or its dendrites, and the tracer material is transported along the axonal microtubular system to the cell's synaptic terminals (Kobbert et al., 2000). A limitation to this type of study is that no indication of the functional characteristics of the projection is obtained.

1.2.3.2 Lesions/inactivation

Information regarding the role of descending fibres in the control of particular muscles can be achieved by lesion studies; the fibres are sectioned and the subsequent effects on motor control are observed. Similarly, the study of deficits in patients who have lesions as a result of clinical damage or disease (e.g. cerebrovascular accident or cerebral palsy) provides valuable information regarding normal motor control. The motor cortex can also be inactivated in a transient and reversible manner by cooling the brain tissue, thereby disrupting its normal function by interrupting the synaptic actions within the region (see Brooks, 1983).

The interpretation of deficits in movement performance that accompany lesions in the brain can be fraught with difficulties (see Porter and Lemon, 1993). This is due to the transient nature of some of the disturbances, and the fact that there is a great capacity for compensation, including plasticity in the brain itself. It is therefore sometimes difficult to deduce from changes in behaviour the exact functions that were subserved, or the information processing operations that were performed, by the tissue which has been damaged or removed.

1.2.3.3 Neuronal recording studies

Insights into the functional organisation of the motor cortex can be gained by recording the activity of single cortical neurons in awake, behaving animals or humans (Goldring and Ratcheson, 1972; Fetz and Cheney, 1978; Armstrong and Drew, 1984a; Lemon, 1984). These type of studies are limited by their inability to identify functional

connections between cortical neurons and the motoneurons of the target muscle. This becomes possible with a technique called spike-triggered averaging (STA).

STA involves recording from a single cortical neuron while also recording the EMG from a muscle. The effect the cortical neuron has on the EMG is determined by averaging the rectified EMG in relation to the discharge of the neuron. A functional connection between the cortical neuron and the motoneuron pool is indicated by a post-spike facilitation in the EMG. The latency, duration and amplitude of the post-spike facilitation in the EMG gives information regarding the characteristics of the innervation supplied by the cortical neuron to the muscle, such as whether the connection is monosynaptic or oligosynaptic (Fetz et al., 1976).

1.2.3.4 Electrical stimulation of the exposed motor cortex

Electrical stimulation of the surface or depths of the exposed motor cortex elicits distinct muscular contractions. While surface stimulation studies have provided valuable information (see Phillips and Porter, 1977) the disadvantage of the technique is that it does not allow discrete stimulation of small areas and is not effective in stimulating the deeper cortical layers. Intracortical microstimulation (ICMS) involves the insertion of microelectrodes into localised regions of the cortex and can be used to elicit well-defined discrete contractions of part or all of a muscle with very small currents (Asanuma and Sakata, 1967).

A single shock delivered to the exposed motor cortex results in a complex descending volley of waves separated by 1-2 ms (Patton and Amassian, 1954). The early component

is due to direct stimulation of the corticospinal neuron at or near the initial segment and is labelled the D- (direct) wave. Later waves are due to stimulation of excitatory inputs to the corticospinal neurons, which produces trans-synaptic re-excitation of some of the same corticospinal neurons that had previously responded in the D-wave, and termed I-waves. Katayama et al. (1988) demonstrated that direct stimulation of the exposed human motor cortex during neurosurgery results in a similar pattern of D-and I-waves as was recorded in the monkey.

1.2.3.5 Transcranial electrical stimulation

Transcranial electrical stimulation (TES) involves electrical activation of the motor cortex through the scalp. The first successful clinical application of TES was developed by Merton and Morton in 1980 (Merton and Morton, 1980), although researchers had been attempting to stimulate the human brain through the scalp for a number of decades previously (see Merton, 1981). Rather than using a train of smaller shocks, Merton and Morton (1980) used a single, high-voltage, capacitive discharge to activate the motor cortex underlying electrodes which were placed on the scalp, and they were able to elicit a twitch in distal hand muscles. Subsequently, various electrode montages and stimulation paradigms (bipolar *vs.* unipolar, anodal *vs.* cathodal) have been commonly used by researchers, each with differing advantages (see Rothwell, 1997).

As with direct cortex stimulation, TES results in multiple descending excitatory volleys (D- and I-waves) in both humans (Day et al., 1989; Burke et al., 1990) and monkeys (Edgley et al., 1990). This suggests that TES activates descending pathways in a manner similar to direct stimulation of the exposed brain surface.

The major disadvantage of TES is that only a small fraction of the current applied actually penetrates into the brain. The large currents that flow on the surface cause a contraction in nearby scalp muscles, making the stimulation uncomfortable.

1.2.3.6 Transcranial magnetic stimulation (TMS)

The transcranial magnetic brain stimulator was introduced in 1985 by Barker et al. (1985), and was originally developed as a non-contact peripheral nerve stimulator. It involves the passage of current through a flat, wire coil, creating a time-varying magnetic field that induces electrical currents in conductive structures nearby. When the coil is held over the scalp, the output pathways of the motor cortex are activated to evoke EMG responses in muscles (for reviews see Rothwell et al., 1991; Rothwell, 1997; Mills, 1999; Hallett, 2000). Currents induced on the scalp by magnetic stimulation are much smaller than those produced by TES, so that the sensation produced by TMS is very slight. This makes the procedure more comfortable for the subject.

Early work using TMS employed a flat, circular stimulating coil held over the subject's head. Although effective in activating the cortex painlessly, TMS did have the disadvantage that it was difficult to focus the site of activation. Many studies now use figure-of-8 stimulating coils, because they allow a more focussed site of activation, producing maximal current at the intersection of the two round components (Cohen et al., 1990).

At threshold, the responses in hand muscles to TMS have latencies which are 1-2 ms longer than the responses to TES (Day et al., 1987a; Hess et al., 1987; Amassian et al.,

1989; Day et al., 1989). This has led researchers to propose that TES activates corticospinal neurons directly, whereas TMS activates the corticospinal pathway trans-synaptically (Day et al., 1989). As TMS intensity is increased, a D-wave can appear which has the same latency as the threshold D-wave evoked with TES. However, unlike TES, further increases in TMS intensity do not lead to decreases in D-wave latency, indicating that excitation remains limited to cortical levels and does not spread down the corticospinal axons (see Mills, 1999). In addition to being affected by TMS intensity, there is evidence that the evocation of D or individual I waves by TMS depends on the direction of the induced current in the cortex (Day et al., 1989; Nakamura et al., 1996; Di Lazzaro et al., 2001). Not all muscles have the same pattern of response to TES and TMS as has been reported in hand muscles. For example in leg muscles TMS evokes a response which occurs at the same latency as TES (Priori et al., 1993; Nielsen et al., 1995).

Since TMS tends to activate the cortical cells transynaptically, the size of the response evoked by the TMS is highly dependent on the excitability of the cortical cells (compared with TES). TMS can therefore be used as a measure of cortical involvement in aspects of normal and abnormal motor control in human subjects (see Boylan and Sackeim, 2000; Fitzgerald et al., 2002). In addition, since TES and TMS activate the brain in different ways, and TES is less affected by cortical cell excitability, they are sometimes used concurrently to provide additional insights into brain activity (eg Datta et al., 1989; Day et al., 1991; Semmler and Nordstrom, 1998).

As well as producing excitatory effects, TMS is often used to study inhibitory processes within the cortex, such as the cortical silent period, transcallosal inhibition and intracortical inhibition (see Mills, 1999).

1.2.4 Corticomotoneuronal cells

A distinctive feature of hand function in primates is the highly developed capacity for relatively independent finger movements. The neural substrate for this ability is the fast-conducting corticospinal fibres that make direct monosynaptic connections with motoneurons innervating the distal limb muscles. These cells are known as corticomotoneuronal (CM) cells, and are involved in the fractionation of muscle activity, allowing precise control of the hand (Lemon, 1993). Evidence for CM cells which project to masseter motoneurons has been identified (see section 1.3), but to date this is largely indirect. One aim of my study was to obtain more direct evidence for CM projection to masseter motoneurons, and how these inputs are organised (see Chapters 2, 3 and 6). Virtually all of the research regarding CM cell function has been performed in relation to muscles of the hand.

1.2.4.1 Identification of CM cells

Electrophysiological evidence for the existence of cells providing a direct monosynaptic connection from the motor cortex to the motoneurons was obtained in monkeys by Bernhard et al. in 1953 (Bernhard et al., 1953) and in humans by Schoen in 1969 (Schoen, 1969).

All CM connections from the motor cortex are excitatory. This was shown by studies in which the monkey motor cortex was electrically stimulated and excitatory post-synaptic potentials (EPSPs) were recorded in motoneurons at latencies consistent with monosynaptic connections from the cortex (Preston and Whitlock, 1961; Landgren et al., 1962a, b). Short-latency inhibition is established via disynaptic pathways involving spinal interneurons (Jankowska et al., 1976) with a delay of 1.2 to 1.5 ms due to the extra synapse (Landgren et al., 1962a, b).

Neurons with a monosynaptic connection from the motor cortex to motoneurons can also be identified in the monkey by measuring the latency of the post-spike facilitation (PSF) following spike-triggered averaging of cortical neuron activity. For intrinsic hand muscles the latency of PSF is consistent with the estimated conduction time over the fast corticospinal pathway (Fetz and Cheney, 1980; Lemon et al., 1986; Lemon, 1993). In contrast, longer latencies are observed from cortical neurons which are known not to make monosynaptic connections with motoneurons, such as motor cortex non-corticospinal neurons (Lemon et al., 1986), corticospinal neurons which terminate in the dorsal horn of the spinal cord (Widener and Cheney, 1997) and corticospinal neurons from non-primates such as cats and rats (Armstrong and Drew, 1984b).

In awake human subjects, the latency of the response to TES and TMS and the pattern of responses evoked in single motor units can be used to assess whether there is a monosynaptic projection from the motor cortex to the muscle of interest (Mills, 1999). Narrow (1-2 ms) peaks in the response histograms of single motor units following TMS is a good indication that the unit is being excited via a monosynaptic connection. Excitation of a motoneuron via a pathway involving more than one synapse would result in a motor

unit response with greater temporal dispersion than one activated via a monosynaptic pathway (Mills, 1999).

1.2.4.2 Role of CM cells

Studies investigating the function of CM cells in allowing independent finger movements have been diverse in nature, and are discussed in more detail below.

a) Developmental studies

The CM system in monkeys is not present at birth, and adult patterns of monosynaptic connections are not observed until the 6th to 8th postnatal month (Kuypers, 1962). Parallel to the development of the CM system is the monkey's development of skill and dexterity (Flament et al., 1992).

b) Lesion studies

If the infant monkey's pyramidal tract is lesioned, normal skilled independent finger movement does not develop (Lawrence and Kuypers, 1968). In adult monkeys a bilateral pyramidotomy results in the loss of ability to produce independent finger movements, even though general motor behaviour is normal (Lawrence and Kuypers, 1968). Similarly, when the upper limb is affected by stroke or lesion of the motor cortex, human studies have revealed that hand movement is usually more seriously affected than movements involving more proximal muscles (Colebatch and Gandevia, 1989).

c) Anatomical studies

Animals with the highest levels of dexterity, such as primates, have numerous CM connections, in comparison to species that are less dexterous, such as cats and rats, which have no direct CM connections (Heffner and Masterton, 1975). Anatomically, corticospinal connections to the ventral horn are denser and more extensive in chimpanzees than monkeys (Kuypers, 1964) and are even more prominent in man (Schoen, 1969). Anatomical evidence suggests that it is the motoneurons innervating distal muscles (i.e., those involved in dexterity) that have the greatest number of CM cells (Porter, 1987).

d) Neuronal recording studies

Neuronal recording studies have shown that CM cell activity is not necessarily proportional to target muscle activity (Lemon, 1993). Cheney and Fetz (1980) recorded from CM cells which were recruited during a controlled wrist movement, but were silent during a ballistic movement. They also showed that the CM cells were more active at the start of the movement and during modulations in force, than they were during tonic holds. More recently it has been demonstrated that CM neurons facilitating hand muscles are particularly active during movements requiring a fractionated pattern of muscle activity (Muir and Lemon, 1983; Lemon et al., 1986). CM cells are active during a precision grip, but not a power grip, even though EMG is often greater during the power grip.

e) Cortical Stimulation

Clough et al. (1968) showed that the strongest monosynaptic facilitation is to distal muscles which are used for precision tasks. They used electrical stimulation to activate

the CM cells and made intracellular recordings from individual spinal motoneurons. It was shown that the largest monosynaptic excitatory post-synaptic potentials (EPSPs) generated by stimulation of the motor cortex were amongst the motoneurons supplying the finger muscles rather than the forearm muscles.

Studies using TMS suggest that CM cells are preferentially involved in the fine control of the digits. In humans, the motor evoked potential (MEP) evoked by TMS in hand muscles is larger during the performance of a precision grip than a power grip (Datta et al., 1989; Schieppati et al., 1996), or a simple finger abduction task (Flament et al., 1993). During a task requiring subjects to reach, grasp and lift an object using a precision grip, corticospinal excitability is greatest as the digit closes around the object, and just after the subject first touches the object (Lemon et al., 1995). Similar experiments using TES do not show these task-related variations in MEP amplitude (Datta et al., 1989; Schieppati et al., 1996), suggesting that it is a cortical mechanism responsible for the task related changes with TMS. This supports the observations in monkeys by Baker et al. (1995) who showed that during a precision grip the corticospinal volley evoked by TMS displayed a mean modulation of 13%, with the largest volley occurring during the hold phase of the task. This modulation was not seen following electrical stimulation of the corticospinal fibres via chronically implanted electrodes in the cerebral peduncle. Therefore, changes in cortical excitability are the most likely mechanism for variations in the response to TMS.

Together, these observations provide evidence that corticospinal projections from the primary motor cortex to the ventral horn of the spinal cord are, at least in part, necessary for the fine control of independent finger movements required during precision

movements. The issue of the presence of CM cells in the trigeminal motor system, and their functional role in control of masseter muscles is addressed in Section 1.3 of this literature review, and experimentally in Chapters 2, 3, 6 and 7.

1.2.5 Branching of CM cells

Techniques such as intra-spinal stimulation (Shinoda et al., 1976; Shinoda et al., 1979) and intra-axonal labelling (Shinoda et al., 1981) have demonstrated extensive branching of CM cell collaterals within the spinal cord. In fact, spike-triggered averaging combined with single motor unit recording has shown that single CMs branch to innervate most of the low threshold motoneurons innervating a given target muscle (Mantel and Lemon, 1987). This allows a single CM cell to exert a facilitatory influence over a range of EMG and force levels (Porter and Lemon, 1993).

As well as branching to innervate the motoneurons of one muscle, CM projections also branch to make contact with motoneurons of different muscles (Lemon, 1993). Evidence for this has come from studies using spike-triggered averaging, which have demonstrated that post-spike facilitation occurs in the EMG from several different hand and forearm muscles when averaged with respect to spikes from a single CM cell (Fetz and Cheney, 1980; Buys et al., 1986; Cheney et al., 1991). The group of muscles facilitated by a single CM cell is known as that cell's "muscle field" (Fetz and Cheney, 1980).

1.2.5.1 Functional significance of branched CM projections innervating multiple muscles

The organisation of CM cells which project to multiple muscles may represent a mechanism for reducing the large number of possible muscle contractions with which the motor cortex has to deal (Porter and Lemon, 1993). For example, during independent finger movements, the muscles of the hand show a fractionated pattern of activity in which the timing and amplitude of EMG activity varies considerably from one muscle to another (Long and Brown, 1964; Schieber, 1995). It is thought that the branched organisation of CM output may be important in the co-ordination of the fractionated muscle activity required for independent finger movements (Muir and Lemon, 1983; Lemon, 1993; Hoffman and Strick, 1995; Bennett and Lemon, 1996). Indeed, the muscle fields of some CM cells have been shown to resemble the synergies of muscular action required to produce independent finger movements (Buys et al., 1986; Bennett and Lemon, 1996).

1.2.5.2 Identification of branched CM projections in awake human subjects

In humans, synchronised discharge of motor units within the same muscle (Datta and Stephens, 1990) or different muscles (Bremner et al., 1991a, b; Carr et al., 1994) has been used to identify the presence of branched CM inputs common to both motor units. While there are a number of possible sources of common input to spinal motoneurons, the following evidence suggests that, at least for hand muscles, much of the synchrony in motor unit discharge has a corticospinal origin (see Porter and Lemon, 1993; Farmer et al., 1997):

1. Short-term synchrony is stronger in the intrinsic hand muscles than in the more proximal limb muscles. If synchrony were due to muscle spindle input, it would be stronger in proximal muscles (Datta et al., 1991).
2. Motor unit synchrony is still observed in the hand muscles of a deafferented patient (Farmer et al., 1991).
3. Short-term synchrony is absent in the affected hand of patients with stroke or spinal damage (Datta et al., 1991)
4. In mirror-movement patients with abnormal corticospinal axons which branch to left and right side homonymous motoneurons (see section 1.2.6), short-term synchrony was present between motor units from muscles on each side of the body (Farmer et al., 1990).

Another indirect yet effective method for detecting shared CM projections for two or more muscles in humans is to examine the co-variation in the response to TMS in the muscles. TMS produces responses which vary in size from one stimulus to the next (Amassian et al., 1989; Britton et al., 1991; Brasil-Neto et al., 1992; Kiers et al., 1993) due, at least partly, to fluctuations in cortical excitability (Ellaway et al., 1998; Funase et al., 1999). If two muscles share input from branched CM projections, then the fluctuations in MEP size in these muscles are likely to co-vary on a trial-by-trial basis as the excitability of the shared CM cells fluctuates. Previous studies have examined this issue for the upper limb and found that co-variation of excitatory responses to TMS occurs in intrinsic hand muscles which are acting synergistically (Ho et al., 1998) and in proximal arm muscles acting synergistically in a precision, but not a power, task (Schieppati et al., 1996). A detailed assessment of the co-variation of MEPs in multiple

hand and jaw muscles on each side of the body, to assess functional grouping of corticospinal and corticotrigeminal neuron populations in motor cortex under rest and active conditions is reported in Chapters 4, 5 and 6 of this thesis.

1.2.6 Mirror movements

Mirror movements are a type of associated, involuntary movement that occur on one side of the body when a voluntary movement is performed involving the homonymous muscles on the opposite side. They are also known as "contralateral imitative synkinesia" or "bimanual synkinesia". Mirror movements are most prominent in the muscles of the hand and while they are common in young children, they are considered abnormal if they persist past the first decade of life. There is evidence that the phenomenon of mirror movements is, at least in some cases, due to the presence of abnormally-branched corticospinal axons (Farmer et al., 1990; Farmer et al., 1991; Carr et al., 1993; Carr, 1996).

Mirror movements have been reported in various neurological conditions, including Kallmann's syndrome (Quinton et al., 1996) and Klippel-Feil syndrome (Bauman, 1932; Farmer et al., 1990). In this review I will focus on the type of mirror movement that results from the reorganisation of the corticospinal tract following infantile hemiplegia, since I was able to perform a series of experiments on such a patient (Chapter 5) to assess the organisation of the descending corticotrigeminal control of her masseter muscles. I have also included a discussion regarding the different types of reorganisation that can result from damage to the central nervous system.

1.2.6.1 Recovery of CNS following stroke

Following stroke, the human nervous system shows a remarkable capacity for functional recovery, especially if the damage is sustained in infancy (Kennard, 1940; Woods and Teuber, 1978; Farmer et al., 1991). The mechanisms of recovery can be different in each individual (Carr et al., 1993; Balbi et al., 2000), sometimes resulting in the development of mirror movements (Forget et al., 1986; Farmer et al., 1991; Carr et al., 1993; Carr, 1996; Kanouchi et al., 1997; Nirkko et al., 1997; Watson and Colebatch, 1997; Balbi et al., 2000) and sometimes not (Benecke et al., 1991; Carr et al., 1993; Lewine et al., 1994; Sabatini et al., 1994; Netz et al., 1997). Whether a patient develops mirror movements depends on the type of motor cortex reorganisation following the injury. Lesion studies in infant rats (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975; Barth and Stanfield, 1990), hamsters (Kalil and Reh, 1982; Kuang and Kalil, 1990; Merline and Kalil, 1990; Aisaka et al., 1999), cats (Gomez-Pinilla et al., 1986) and monkeys (Chapman and Wiesendanger, 1982; Kucera and Wiesendanger, 1985; Rouiller et al., 1998; Liu and Rouiller, 1999) have provided valuable information regarding the types of reorganisation that results from unilateral damage to the CNS. Characteristics of reorganisation depend on factors such as the age the injury was sustained, the site of lesion and the type and extent of injury (Kennard, 1940; Hicks and D'Amato, 1970; Gomez-Pinilla et al., 1986; Rouiller et al., 1998). There seem to be two major categories of reorganisation reported following unilateral damage to the CNS.

a) Recovery of damaged corticospinal tract

1) Regrowth of severed axons

Kalil and Reh (1982) lesioned the pyramidal tract on one side in neonatal hamsters and reported that the damaged fibres regrow around the injury to terminate, via a new pathway, at their normal sites in the spinal cord. They found a correlation between the presence of the new tract and the preservation of fine manipulatory skills (Reh and Kalil, 1982). There is now some doubt as to whether this is a likely mechanism of reorganisation, since more recent experiments indicate that corticospinal cells do not survive once their axons have been cut (Tolbert and Der, 1987).

2) Re-routing of axons which were not damaged

It is believed that axons within the developing corticospinal tract do not grow at the same rate, but develop in a staggered fashion (for a review, see (Joosten, 1997). Therefore, early injury to the corticospinal tract may not damage all the corticospinal cells, and recovery of function may be explained by the presence of uninjured, later-developing corticospinal axons which are redirected as a result of the injury (Tolbert and Der, 1987). Experiments in monkeys have confirmed that following unilateral neonatal lesions, recovery of function is due to cortical reorganisation in the areas surrounding the lesion, so that these adjacent areas take over the control of the muscles affected by the lesion (Rouiller et al., 1998).

b) Reorganisation so that the undamaged hemisphere takes over control

Many studies have indicated that when the CNS of neonates is injured unilaterally, the intact hemisphere compensates for the damage and takes over the control of functions lost

by the injury via an aberrant corticospinal pathway. This type of reorganisation is not seen following adult injury. There are differing opinions regarding the origin and trajectory of the aberrant pathway, and several of the mechanisms that have been reported in the literature are summarised below.

1) Novel corticospinal pathways develop from the undamaged hemisphere

Corticospinal axons from the undamaged hemisphere may develop *de novo* to innervate the denervated motoneurons. Anatomical studies in rats using silver-stain degeneration techniques have demonstrated that neonatal unilateral cortical lesions result in the formation of an aberrant ipsilateral tract, which deviates from the normal tract at the level of pyramidal decussation (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975; Leong, 1976). It is likely that this type of reorganisation only occurs if the injury is sustained at an age prior to the corticospinal projections reaching the pyramidal decussation (Leong and Lund, 1973; Rouiller et al., 1991; Aisaka et al., 1999). In rats and hamsters this occurs at 0 and 3 days respectively after birth, but in higher mammals it occurs prenatally (see Porter and Lemon, 1993).

During development in the opossum and the cat, transient populations of ipsilateral corticospinal projections have been demonstrated (Cabana and Martin, 1985; Alisky et al., 1992). One of the possibilities for reorganisation suggested by Carr et al (1993) to explain the cortical reorganisation in humans with hemiplegic cerebral palsy, was that this transient population becomes stabilised as a result of the cortical damage.

It is also conceivable that reorganisation could involve arborisation at the local spinal level of the normally-existing ipsilateral corticospinal axons. This was examined in

hamsters following unilateral neonatal lesions (Aisaka et al., 1999), but no evidence for this type of reorganisation was found.

2) Corticospinal cells from the undamaged hemisphere re-cross the spinal cord into the deinnervated areas

Anatomical studies of the corticospinal tract in hamsters and rats have shown that in response to unilateral neonatal lesions, corticospinal fibres from the intact hemisphere recross the spinal cord to innervate the denervated motoneurons (Barth and Stanfield, 1990; Kuang and Kalil, 1990; Aisaka et al., 1999). This type of reorganisation may be especially important in lesions that occur after the formation of the pyramidal decussation (Aisaka et al., 1999). The re-crossed corticospinal axons were seen to arborise in a normal pattern in the spinal cord deprived of cortical inputs (Kuang and Kalil, 1990; Rouiller et al., 1991).

3) Intact corticospinal axons may develop collateral branches in denervated spinal cord, thereby establishing bilateral connections

Individual corticospinal axons which give rise to arbours on both sides of the spinal cord have been identified in hamsters following neonatal unilateral CNS injury (Kuang and Kalil, 1990; Aisaka et al., 1999). The branching corticospinal fibres maintain their functional and topographic specificity (Kuang and Kalil, 1990). It has been suggested that these projections may be present at birth, but come under inhibitory control, unless unleashed by the injury. Transient fibres which recross the midline at the level of the spinal cord have been described in kittens (Theriault and Tatton, 1989), and following neonatal cortical damage, these may develop into the aberrant ipsilateral corticospinal projection (Barth and Stanfield, 1990).

1.2.6.2 *Evidence for branched corticospinal axons in hemiplegic patients with mirror movements.*

Neurophysiological testing in hemiplegic patients with strong mirror movements has suggested that branched corticospinal axons are responsible for the associated movements seen in these patients. In these patients, focal TMS of one hemisphere results in MEPs of identical latencies in the hand muscles of both sides (Farmer et al., 1991; Carr et al., 1993; Balbi et al., 2000). In addition, analysis of multi-unit EMG has revealed the presence of branched, last-order, presynaptic fibres to homonymous left and right motoneuron pools (Farmer et al., 1991; Carr et al., 1993). Taken together, these results suggest that in these patients, corticospinal axons from the unaffected hemisphere of the motor cortex have branched and innervated homonymous right and left motor pools.

In other hemiplegic patients, in whom mirror movements were either absent or weak, transcranial magnetic brain stimulation produced responses in ipsilateral and contralateral muscles, but the response on the ipsilateral side occurred at a longer latency than on the contralateral side (Benecke et al., 1991; Carr et al., 1993). In addition, in these patients cross-correlation analysis of multi-unit EMG recorded from homonymous left and right muscles did not reveal the presence of branched, last-order, presynaptic fibres to the two motoneuron pools (Carr et al., 1993). In these patients it seems that corticospinal axons from the motor cortex are distributed bilaterally as separate, non-branched projections (Carr et al., 1993).

There are no data on the cortical control of trigeminal muscles in patients with mirror movements. This is of interest because in the normal situation the jaw muscles are

innervated from each hemisphere. Does the lesion of one hemisphere early in development, which alters the corticospinal system to produce abnormal bilateral termination onto spinal motoneurons and mirror movements, affect the control of the masseter muscles from the unaffected hemisphere? This question was addressed in Chapter 5.

1.3 The cortical control of the masseter muscle

Information regarding the role of the motor cortex in the control of the masseter muscle comes from a number of different sources.

1.3.1 Anatomical investigations

Anatomical studies provide evidence for direct corticobulbar projections from the face motor cortex onto both trigeminal and facial nuclei. Kuypers (1958a; 1958b) examined nerve fibre terminal degeneration in the brainstem following ablation of restricted regions in primates or following stroke in humans and found evidence of direct projections from the lateral third of the precentral cortex to the trigeminal motor nuclei. These projections arise from one hemisphere of the motor cortex and terminate bilaterally at the trigeminal motor nuclei (Kuypers, 1958a; Iwatsubo et al., 1990). Jenny and Saper (1987) studied the facial (cranial nerve (CN) VII) nuclei rather than the trigeminal nuclei, and reported that unilateral injection of horseradish peroxidase into the face motor cortex of monkeys resulted in bilateral labelling of facial nuclei, with densest labelling in a region of the contralateral nucleus where motoneurons innervating the lower facial muscles are located.

1.3.2 Lesion studies

The study of lesions of the sensorimotor cortex in various animals has provided evidence that the cortex has some role in the control of mastication and voluntary jaw movements (Luschei and Goodwin, 1975; Larson et al., 1980; Luschei and Goldberg, 1981). Luschei and Goodwin (1975) reported that following bilateral lesion of the monkey face motor cortex, there was a permanent impairment in the monkey's ability to maintain a low, steady bite force, although the monkeys were still able to produce a phasic, forceful bite response. While Murray et al. (1991) did not find any deterioration in the monkey's ability to maintain a required force level when the motor cortex was inactivated with cooling, they did report subtle deficits on the rate of force development in the biting task. These studies suggest that while the motor cortex is not essential in the production of forceful jaw movements or mastication, it may be important for the fine control and modulation of jaw-closing muscle activity.

In humans, following unilateral lesions of the primary motor cortex, motor function in muscles of the jaw is relatively well-preserved (Willoughby and Anderson, 1984), presumably due to a bilateral innervation from the motor cortex. However, central projections onto masseter motoneurons are not symmetric, and voluntary EMG in masseter contralateral to the lesion has been shown to be reduced in stroke patients (Cruccu et al., 1988). These authors suggest that the defect may be inconspicuous clinically because it is of a minor degree and can be functionally compensated for by muscles on the unaffected side.

1.3.3 Single neuron recording

Numerous studies have recorded from single neurons in the face motor cortex of monkeys and have identified neurons involved in various orofacial movements (Luschei et al., 1971; Lund and Lamarre, 1974; Hoffman and Luschei, 1980; Murray and Sessle, 1992b; Martin et al., 1997; Yao et al., 2002). Neurons within the face motor cortex that alter their firing rates during jaw-closing movements have been identified (Luschei et al., 1971; Hoffman and Luschei, 1980; Murray and Sessle, 1992b), and have been implicated in the generation and control of voluntary jaw-closing forces.

To date, the spike triggered averaging and post-spike facilitation technique of Fetz et al. (1976) has not been used to study the CM projections to the trigeminal system in an experimental animal model.

1.3.4 Surface stimulation of the motor cortex

Penfield and Rasmussen (1950) by means of electrical stimulation of the human motor cortex were able to elicit bilateral movements of the jaw, tongue, eyebrows and eyelids. However, Clarke and Luschei (1974) described the difficulty in producing jaw movement using surface stimulation of the precentral cortex of an anaesthetised monkey, unless the stimulus current and train duration were increased to the point of seizure. As a consequence, most of the recent experiments that have electrically stimulated the exposed motor cortex have used the technique of ICMS (see below).

Electrical stimulation of the exposed cortex can induce rhythmic jaw movements (RJMs), which consist of a series of opening and closing movements of the jaw, usually accompanied by rhythmical tongue movements and secretion of saliva (Beevor and Horsley, 1894; Grunbaum and Sherrington, 1901; Walker and Green, 1938; Lund and Lamarre, 1974). RJMs are under the control of a central pattern generator (CPG) located in the brain stem (Lund, 1991), and the fact that they can be induced by stimulation of the cortex suggests that the CPG is under excitatory control from the cortex. The area of the cortex that most readily produces RJMs is called the cortical masticatory area, which in the primate is located in the precentral gyrus, immediately lateral to the primary motor cortex and adjacent to the Sylvian fissure (Lund and Lamarre, 1974). However, RJMs can also be induced by surface stimulation of the primate motor cortex (Woolsey et al., 1952).

1.3.5 Intra-cortical Micro-stimulation

Intra-cortical micro-stimulation (ICMS) of the face motor cortex reveals a complex electrically-excitable representation of the facial, jaw and tongue musculature. Discrete orofacial movements have been reported in primates (Clark and Luschei, 1974; McGuinness et al., 1980; Gould et al., 1986; Huang et al., 1988; Murray and Sessle, 1992a; Martin et al., 1997; Yao et al., 2002) and in cats (Iwata et al., 1985; Guandalini et al., 1990; Iwata et al., 1990). Consistent with observations for limb muscles (Asanuma, 1975; Sessle and Wiesendanger, 1982) there is evidence for multiple representation of particular face, jaw and tongue muscles in discrete, efferent microzones (Huang et al., 1988; Murray and Sessle, 1992a). This allows the integration of these muscles in the various activities in which they participate (Huang et al., 1988; Huang et al., 1989a).

Studies which have investigated jaw muscle activity using ICMS report that stimulation elicits jaw-opening movements much more frequently than jaw-closing (Clark and Luschei, 1974; Huang et al., 1988). Specifically, Huang et al. (1988) found that 85% of the stimulation sites which produced jaw movement resulted in jaw opening. Contralateral movements tended to dominate, but ipsilateral and bilateral movements were also been reported (Clark and Luschei, 1974; Huang et al., 1988). Jaw movements having a lateral component can be elicited, generally in association with unilateral activation of one or more jaw muscles (Clark and Luschei, 1974).

ICMS data has confirmed the involvement of the motor cortex in the production of rhythmical jaw movements (Huang et al., 1989b), swallowing (Martin et al., 1997; Martin et al., 1999; Yao et al., 2002), and tongue and facial movements (Murray and Sessle, 1992a, b, c), but suggests that the motor cortex has only a minor role in producing jaw closing (Huang et al., 1988; Murray and Sessle, 1992a).

1.3.6 Transcranial Electrical Stimulation in humans

With the development of TMS there has been little need for an extensive study of the cortical projections to masseter using the more painful method of TES. However, three studies have used TES to assess cortical control of human masseter muscles, mainly so that a comparison could be made with the results obtained from TMS (Cruccu et al., 1989; Macaluso et al., 1990; Guggisberg et al., 2001).

Cruccu et al. (1989) extensively studied the responses that occurred in both masseter muscles following TES, and they described excitatory responses that were very similar to

those which occurred following TMS (see section 1.3.7). TES of one hemisphere of the motor cortex evoked MEPs in both the ipsilateral and contralateral masseter muscles. The response in the contralateral masseter occurred at a latency of 5.6 ± 0.6 ms and was present only when the muscle was voluntarily activated. Two responses were recorded in the ipsilateral masseter; the first (the root MEP or rMEP) occurred at a latency 2 ± 0.3 ms, did not require muscle activation and was due to the direct activation of the trigeminal nerve. In the active masseter this response was followed by a later response which occurred at 5.7 ± 0.6 ms. Cruccu et al. (1989) labelled this the U-wave, due to its uncertain origin, but suggested it was most likely caused by the simultaneous activation of Ia afferents in the root and ipsilaterally projecting corticobulbar fibres.

Maculoso et al. (1990) were unable to elicit cortical MEPs following TES, and they describe only short-latency, ipsilateral responses which were consistent with direct activation of the trigeminal nerve. They suggest that their failure to obtain MEPs by TES was probably due to the type of stimulator they employed (a commercial stimulator with a maximal output of 99 mA, and not a high-voltage, low-output impedance device).

Guggisberg et al. (2001) recorded only from the contralateral masseter following TES, and reported a MEP with an average latency of 5.6 ± 0.5 ms. The response was identical in shape, amplitude and latency to that obtained with TMS, suggesting the same structures were activated with the two stimuli.

1.3.7 Transcranial Magnetic Stimulation in humans

The first report of MEPs elicited in human masseter using TMS was by Benecke and colleagues in 1988 (Benecke et al., 1988). A circular stimulating coil, held 4 cm lateral to the vertex, was used to activate the cortical projections to masseter, and MEPs were recorded in both ipsilateral and contralateral masseter. The contralateral response occurred at a latency of 10.5 ± 1.5 ms, and was, on average, larger and several milliseconds earlier than the ipsilateral MEP.

A more detailed examination of the corticobulbar projections to masseter motoneurons was carried out by Cruccu et al. (1989). They confirmed that bilateral MEPs could be elicited in masseter following TMS of the motor cortex using a circular stimulating coil. The contralateral MEP onset latency of 5.9 ± 0.4 ms reported in this study was somewhat shorter than those reported by Benecke et al. (1988) but was in the same order as that reported in a similar study at around the same time (Macaluso et al., 1990). The exact latency of masseter MEPs reported in subsequent studies has varied, and this is likely due to differences in TMS intensity. However all have recorded latencies shorter than those reported by Benecke et al. (1988). It is now generally accepted that technical differences in the triggering system probably accounts for the longer latency reported in that study.

The masseter muscle must be active to elicit a cortical MEP using TMS (Cruccu et al., 1989; Macaluso et al., 1990) and increases in muscle activation increase the size of the MEP, probably due to cortical and brainstem mechanisms (McMillan et al., 2001). Although the TMS threshold for evoking a MEP in masseter is similar to that in active hand muscles (see Cruccu et al., 1989), in hand muscles MEPs can also be elicited while

the muscles are at rest (Hess et al., 1987), while this is not seen for masseter. This difference may be because trigeminal masseter motoneurons are mainly of the high-threshold type, needing a high level of local excitability or temporal summation (see Cruccu et al., 1989), or because TMS evokes smaller compound excitatory post synaptic potentials in masseter motoneurons because the CM projection is weaker. A short-latency (~2-4 ms) response that does not require muscle activation is often observed in the masseter ipsilateral to the TMS (Benecke et al., 1988; Macaluso et al., 1990; Carr et al., 1994; McMillan et al., 1998a; McMillan et al., 2001). This response is due to direct activation of the trigeminal nerve root (Benecke et al., 1988; Cruccu et al., 1989) and is termed the rMEP. The rMEP often obscures the ipsilateral cortical response, and sometimes makes quantification of the cortical response difficult (Carr et al., 1994; McMillan et al., 1998a). The MEPs referred to in this thesis are cortical MEPs, unless specifically described as rMEPs.

Cruccu et al. (1989) calculated, based on the masseter cortical MEP latency measurements and conduction velocities, that the central delay at the trigeminal motoneuron synapse(s) was between 1.1 and 1.4 ms. Maculoso et al. (1990) calculated the central motor conduction time for masseter by comparing the MEP latency to the peripheral conduction time (approximated by calculating the latency of response following direct stimulation of the trigeminal nerve in its intracranial portion). They estimated a central conduction time of approximately 2 ms. This is sufficient time for no more than two synapses and Cruccu et al. (1989) argued that since the duration and latency variability of masseter MEPs were even shorter than for active hand muscles, which are served by direct corticomotoneuronal connections, that the corticomotoneuronal fibres project directly

onto trigeminal motoneurons. I have used single motor unit recordings (Chapter 3) to provide more compelling evidence for corticomotoneuronal projections to masseter.

All TMS studies that have examined the response in left and right masseter have found bilateral MEPs (Benecke et al., 1988; Cruccu et al., 1989; Carr et al., 1994; Cruccu et al., 1997b; Trompetto et al., 1998; McMillan et al., 2001), except for the study by Maculoso et al. (1990). The latter study reported only contralateral MEPs following TMS, but this may be due to differences in TMS intensity, since Cruccu et al. (1989) found that, at low intensities, the response to TMS in masseter was exclusively contralateral, whereas bilateral responses were evoked at higher TMS intensities.

Most of these studies have been performed using a circular stimulating coil, which can make their interpretation difficult. Although one hemisphere of the motor cortex is preferentially activated with TMS using a circular coil (Day et al., 1989), activation of both hemispheres cannot be excluded. Indeed, at the higher stimulus strengths used by Cruccu et al. (1989) to obtain bilateral responses in masseter, bilateral responses were also recorded in thenar muscles. Similarly, when activating the motor cortex with a circular TMS coil, Benecke et al. (1988) reported bilateral responses in both masseter and first dorsal interosseous (FDI) when the stimulating coil was placed over the vertex, although the bilateral responses in FDI disappeared when the coil was shifted laterally. These results indicate that TMS delivered via the circular stimulating coil does not activate the motor cortex exclusively on one side, and data obtained with this technique cannot be used to conclude that corticotrigeminal projection from one hemisphere supplies both masseter muscles.

A more focal stimulation of one hemisphere of the motor cortex can be achieved with TMS using a figure-of-eight stimulating coil (see section 1.2.3.6). Besides the studies performed in this thesis, there have been three other reports which have assessed the masseter response bilaterally following TMS with a figure-of-8 coil (Carr et al., 1994; Guggisberg et al., 2001; McMillan et al., 2001). All show that focal TMS of one hemisphere of the motor cortex produces MEPs in both left and right masseter, confirming that the corticotrigeminal projection to masseter from one hemisphere of the motor cortex is bilateral. Guggisberg et al. (2001) found no difference in the amplitude of MEPs in the ipsilateral and the contralateral masseter. However, both the other studies presented results suggesting that the contralateral masseter MEP is larger than the ipsilateral response, although this difference only reached statistical significance in the study by McMillan et al. (2001), and only when subjects were activating masseter in an isometric (compared with dynamic) contraction.

The MEPs evoked by TMS in the surface EMG of masseter are identical in shape, latency and amplitude to those evoked by TES, which suggests that the MEP results from direct, rather than trans-synaptic activation of the pyramidal cells (Guggisberg et al., 2001). This is in contrast to similar studies in hand muscles which have shown that the response to TES occurs approximately 2 ms before the response to TMS (Day et al., 1987a; Hess et al., 1987; Amassian et al., 1989; Day et al., 1989), suggesting that TMS activates the corticospinal cells transynaptically (see section 1.2.3.6). Prior to the experiments reported in this thesis (Chapter 3), there had been no study of the response to TMS in masseter single motor units, and therefore the characteristics of the descending corticotrigeminal volleys (D- and I-waves) have not been described. Similarly, analysis of the peaks of

excitation in the motor unit peri-stimulus time histogram (PSTH) following TMS (Chapter 3) is necessary to confirm the presence of CM projections to masseter motoneurons.

A number of studies have aimed to improve the reliability of recording TMS-evoked potentials in masseter. Turk et al. (1994) described a new recording electrode which was mounted onto a spatula and inserted into the pterygomandibular plica over the belly of masseter. McMillan et al. (1998b) described a novel method of locating neural stimulation sites, allowing accurate relocation for testing on different occasions. Guggisberg et al. (2001) tested various different figure-of-eight coil orientations and found that the best coil orientation for activating masseter with TMS was at an angle of 120° from the parasaggital plane, with induced current in the underlying coil flowing in a postero-medial direction. This current direction approximately paralleled the central sulcus, and was perpendicular to the current used in the present series of experiments (which were conducted prior to publication of the Guggisberg et al. (2001) paper.) Guggisberg et al. (2001) found no difference in the latencies of the responses with any coil orientation, and suggested that all orientations activated the CM cells directly (D-waves).

Recently TMS was used to map the cortical topography of masseter (McMillan et al., 1998a), and a discrete representation in the motor cortex and pre-motor cortex was demonstrated, as expected from anatomical studies. The area, volume and height of the map produced by TMS was shown to be highly reproducible over time (McMillan et al., 1998a) but varied with different biting tasks (Watson et al., 2000), possibly due to task-

related modulation of corticobulbar activity. I have addressed task-dependence of the masseter MEP in the present study (Chapter 2) to investigate the role of ipsilateral and contralateral hemispheres in unilateral biting (fractionated activation of masseter muscles on each side) and bilateral biting.

In addition to studying the neural pathways underlying the cortical control of masseter in healthy subjects, TMS has been used to functionally assess the central motor pathways to masseter in a number of pathological conditions: in hemiplegia (Cruccu et al., 1989), in patients with unilateral facial palsies (Turk et al., 1994), in patients following treatment for trigeminal neuralgia (Turk et al., 1994), in painful temporomandibular disorders (Cruccu et al., 1997b), in amyotrophic lateral sclerosis (Trompetto et al., 1998; Desiato et al., 2002), cervical spondylotic myelopathy (Trompetto et al., 1998), during pain (Romaniello et al., 2000) and in a infantile hemiplegic patient with abnormal mirror movements (Chapter 5).

Transcranial magnetic stimulation is a powerful technique for the study of the motor cortex and its projections to motoneurons. Considerable research has been performed using this technique in studying the corticospinal system, and much more limited number of investigations have been conducted for the trigeminal system. There are a number of unresolved issues regarding the role of the motor cortex in the control of human masseter muscles. These include:

1. Is the output from the primary motor cortex to masseter organised in a task-related manner, and does it differ in each hemisphere? An aim of the experiments reported in Chapter two was to investigate the task-dependency of corticobulbar

projections to masseter motoneurons during bilateral and attempted unilateral voluntary activation of one masseter muscle.

2. Are there CM projections from each hemisphere of the motor cortex to individual masseter motoneurons, and if so, how are they organised? This question was addressed at a whole muscle level and at a motor unit level in Chapters two and three, respectively. An aim of these experiments was to investigate the relative strength of the contralateral and ipsilateral projections to masseter motoneurons from the motor cortex.

3. Does TMS activate masseter CM cells directly (D-wave) or indirectly (I-waves)? An aim of the experiments reported in Chapter three was examine the nature of the excitatory response evoked in masseter motoneurons by focal TMS.

4. Is the bilateral response to TMS seen in masseter at least in part a consequence of CM cells that branch to innervate the masseter motoneuron pool on each side? The aim of the experiments reported in Chapter six was to examine the trial-by-trial fluctuations in the size of the MEPs elicited in masseter on each side by focal TMS of one hemisphere. The experiments reported in Chapters four and five suggest that correlations in MEP fluctuations in active muscles may result from the presence of branched corticospinal cells that innervate the motoneuron pools of both muscles.

5. Is the motor cortex involved in the masseter long-latency stretch reflex? The aim of the experiments reported in Chapter seven was to use TMS to establish the role of the motor cortex in the LLSR of masseter in man.

This thesis therefore advances knowledge regarding the organisation and function of the corticomotoneuronal input to masseter motoneurons.

CHAPTER 2

TASK-DEPENDENT CONTROL OF HUMAN MASSETER MUSCLES FROM IPSI- AND CONTRALATERAL MOTOR CORTEX

2.1 Introduction

The motor cortex is essential for the fine control of voluntary movement, as demonstrated by numerous studies investigating its role in movements of the limbs (for a review see Porter and Lemon, 1993). In contrast to the corticospinal system, much less is known about the cortical control of masticatory muscles via the corticotrigeminal projections. This is despite the fact that fine control and co-ordination of the mandibular muscles is essential during speech and mastication, in order to allow the efficient breakdown of food while still protecting the soft tissues of the mouth. The purpose of this study was therefore to investigate the role of the motor cortex in the control of the human masseter muscle.

It is likely that the general principles of motor control are similar for both the corticospinal and corticotrigeminal systems. Experiments in monkeys have suggested that the motor cortex does not play a major role in jaw-closing strength, but is involved in the fine control of jaw movements (Murray et al., 1991). This is consistent with the accepted role of the motor cortex in the control of hand muscles, where it is believed to be responsible for the production of fine, independent finger movements by permitting fractionated activation of different muscles moving the digits (Schieber, 1990; Lemon, 1993). By analogy, the cortical cells which project to trigeminal motoneurons may allow

fractionation of jaw muscle activity, such as that required for the precise control of mastication or speech.

How the corticotrigeminal projections might contribute to fractionated control of masticatory muscles is an interesting question, because it is generally accepted that the projections from the motor cortex to trigeminal motor nuclei in humans are bilateral (Kuypers, 1958a; Iwatsubo et al., 1990). Short-term synchronisation in the discharge of motor units from left and right masseter provide indirect evidence that at least some single corticotrigeminal neurons actually branch to innervate the masseter motoneuron pools of both sides (Carr et al., 1994). It has not been established in humans whether there are separate populations of ipsi- and contralaterally projecting corticotrigeminal neurons in each hemisphere. Transcranial magnetic stimulation (TMS) studies in humans suggest that contralateral projections to masseter are stronger, however the findings are not conclusive (Cruccu et al., 1989; Carr et al., 1994). The nature of the corticotrigeminal projections has obvious implications for the ability of the motor cortex to mediate independent activation of the masticatory muscles on each side. It is possible to activate the masseter muscle voluntarily on one side relatively independently of the other. It seems likely that the motor cortex contributes to this ability, although it is not known which type of projection is responsible, or which hemisphere controls this task.

The aim of the present study was to investigate the relative strength of the contralateral and ipsilateral projections to masseter motoneurons from the motor cortex, and the task-dependency of these projections during bilateral and attempted unilateral voluntary activation of one masseter muscle. By analogy with the role of the motor cortex in producing fractionated control of hand muscles, it was hypothesised that unilateral biting

would be associated with a modulation of corticotrigeminal neuron activity in the contralateral hemisphere, compared with the situation during bilateral activation of both masseter muscles.

2.2 Methods

Seventeen subjects (ten females and seven males, aged from 20 to 51 years) participated in the experiments. Subjects had no history of neurological disorders and all gave informed consent. Experiments were conducted with the approval of the Human Research Ethics Committee at the University of Adelaide.

2.2.1 Apparatus and recording

The surface electromyograms (EMG) of left and right masseter muscles were recorded using self-adhesive bipolar silver/silver chloride electrodes. One electrode of the pair was placed at the level of the lower border of the mandible, and the other about 2.5 cm above this, close to the motor point. Subjects were grounded by a lip-clip electrode (Türker et al., 1988).

Difficulties were encountered in early experiments due to contamination of the motor evoked potentials (MEPs) by the stimulus artefact. Later experiments were performed using a custom built artefact suppressing amplifier (ESK technologies) based on a design reported by Millard et al. (1992). The results from the earlier experiments were not included in the analysis, although it was noted that the results tended to support those reported here.

Surface EMG signals were amplified (1000 - 3000x) using the custom-built artefact suppressing amplifier. The stimulus artefact was suppressed by reducing the gain of the EMG amplifier to unity from 1 ms prior to the stimulus, until 2 ms afterwards. Surface EMG signals from the left and right masseter muscles were recorded onto separate channels of a 22 kHz PCM data recorder (Vetter 400, A.R. Vetter Co., Pennsylvania, USA). The records were filtered (bandwidth 20-500 Hz), digitised (2 kHz sampling rate per channel), rectified and averaged (n=50).

Focal TMS was used to activate the motor cortex of one hemisphere. This was achieved using a magnetic stimulator (Magstim model 200) and a figure-of-eight stimulating coil with outer coil diameters of 90 mm. The coil was placed over the face area of the motor cortex of one hemisphere at the optimal location for producing a MEP in the active masseter muscles. The coil was oriented at an angle of 45° relative to the parasagittal plane, with current induced in the underlying cortex flowing postero-anteriorly. The left motor cortex was stimulated in 10 subjects and the right motor cortex in 8 subjects (one subject had both left and right hemisphere stimulation, performed on separate occasions).

2.2.2 Focality of TMS

A number of tests were performed to ensure that the TMS stimulus was focal to the motor cortex of one hemisphere and did not activate the other hemisphere.

1. Electrodes were placed over left and right FDI as well as left and right masseter muscles. Bilateral responses in resting FDI would indicate that the stimulus was activating both hemispheres of the motor cortex, since TMS activation of one

hemisphere of the motor cortex evokes a MEP in contralateral FDI only. There is some evidence of ipsilateral projections to hand muscles in normal subjects, but these are only activated at very high TMS intensities, in active muscles, and the resulting MEP occurs at a much later latency than the contralateral MEP (Ziemann et al., 1999). Note also that the jaw somatotopic area in the motor cortex is more lateral than hand area. Therefore, the TMS would have to activate neurons further away from the stimulus to activate the jaw area of the other hemisphere than it would to activate the hand area on the opposite side.

2. The effect of moving the coil medially from the optimal scalp location was observed for MEPs in contralateral and ipsilateral masseter muscles. If TMS activated the other hemisphere, the size of ipsilateral masseter MEPs should increase as the coil was moved into a more medial position.

2.2.3 Protocol

Subjects were seated comfortably in front of two oscilloscopes that showed the rectified and smoothed EMG of the left and right masseter muscles as a horizontal line on separate screens. Subjects performed several maximum voluntary contractions (MVC) of the masseter muscles by biting with their teeth together in normal occlusion. The maximal rectified and smoothed EMG levels were used as a reference for subsequent contractions of the masseter muscles at a target level of 10% of maximal.

TMS was given in trials in which the subject performed one of three different voluntary isometric biting tasks using visual feedback of EMG from both masseter muscles.

1. *Task one* was a bilateral bite in which left and right masseter muscles were co-contracted to a level of 10% maximal EMG.
2. *Task two* was a unilateral bite in which the subject activated the masseter contralateral to the stimulated hemisphere at 10% maximal EMG, while keeping the ipsilateral masseter muscle as inactive as possible. This is referred to hereafter as a "contralateral bite".
3. *Task three* was also a unilateral bite but with the ipsi/contralateral muscle activations reversed. The masseter ipsilateral to the stimulated hemisphere was activated at 10% MVC while the contralateral masseter was kept as relaxed as possible. This is denoted "ipsilateral bite".

TMS were delivered in two blocks of 25 (<0.2 s^{-1}), during each biting task. The order of the biting tasks was randomised. In most subjects the protocol was performed at two suprathreshold TMS intensities (usually between 5-15% of stimulator output above threshold for a response in active muscles, ranging from 40–70% of maximum stimulator output). The data obtained using a single TMS intensity comprised a complete data set, consisting of 50 stimuli delivered during each of the three biting tasks.

In five subjects, a brisk tap was applied to the lower jaw using a tendon hammer, and the maximum size of the masseter tendon jerk reflexes was recorded from the surface EMG. This was then compared to the size of the masseter MEPs, to provide an indication of the proportion of the motoneuron pool recruited by the TMS.

In six subjects MEPs were recorded from contralateral and ipsilateral masseter during bilateral biting over a range of stimulus strengths (between 3 and 11 TMS intensities were

tested in each subject). This stimulus-response curve was performed to determine any differential effects of increasing stimulus intensity in the ipsilateral and contralateral masseter muscles.

2.2.4 Data Analysis

The mean level of the rectified averaged EMG was assessed for a 50-ms epoch preceding the stimulus to confirm the subject's ability to perform the biting tasks. A unilateral biting trial was considered successful if the mean EMG activity of one masseter muscle during this epoch was reduced to less than 55% of the bilateral bite condition, while activity in the other masseter remained at 10% of maximal EMG. Data were excluded from analysis if the pre-stimulus EMG during a unilateral bite did not fulfil these criteria. For some subjects, more than one data set (with different TMS intensities) satisfied the criteria. One data set from each subject was used to assess the task-dependence of the MEP. This was the set with the greatest difference in the pre-stimulus EMG of the masseter ipsilateral to the stimulus, for the bilateral and contralateral biting tasks (ie., the best performance of task two).

MEP onset latencies and duration were quantified off-line from the rectified averaged surface EMG records (n=50). MEP area was calculated from the rectified EMG average as the integral of the EMG activity for the identified duration of the MEP. The silent period following TMS was also measured as the time from the MEP onset to the consistent resumption of EMG activity at prestimulus levels. Differences in MEP area, onset latency or silent period during the different biting tasks and between the masseter muscles on each side were assessed with paired t-tests, with a significance level $p<0.05$.

2.3 Results

2.3.1 Performance of the biting tasks

All subjects were able to perform the bilateral biting task (task 1) and maintain an isometric contraction of both masseter muscles at 10% maximal EMG. After a period of practice, all but 2 of the subjects tested were capable of performing a relatively isolated contraction of the masseter muscle on each side (tasks 2 and 3). One subject could not perform the unilateral biting task with either left or right masseter and was excluded from the task-dependence comparison. The other subject could perform a satisfactory isolated contraction of right but not left masseter. This subject participated in the contralateral biting task (task 2) but not the ipsilateral biting task (task 3). In addition, one subject performed only tasks one and two during the experiment. Therefore 16 of the 17 subjects performed the contralateral biting task (task 2) and 14 of the 17 subjects also performed the ipsilateral biting task (task 3).

The average rectified EMG levels in the pre-stimulus period are summarised for the different biting tasks in Figure 2.1. The data included in Figure 2.1 are from the subjects and trials used for analysis of masseter MEP responses in the three biting tasks (see Figure 2.8). Sixteen subjects were tested with bilateral biting and unilateral activation of the masseter muscle contralateral to the stimulated hemisphere (contralateral bite). The pre-stimulus EMG levels were very similar in each masseter muscle during bilateral biting (Figure 2.1A). During the contralateral biting task, the pre-stimulus EMG in the contralateral masseter was similar to that seen in the bilateral biting task. The pre-stimulus EMG in the ipsilateral masseter during the contralateral biting task was only

31% of its mean amplitude during bilateral biting (10 ± 2 μV *vs.* 32 ± 4 μV; paired t-test, $p<0.001$, $n = 16$). The data from 9 subjects were included in the analysis of task 3 (in 5 of the 14 subjects who were tested during task 3, ipsilateral TMS directly stimulated the ipsilateral trigeminal root producing a response which precluded assessment of the later cortical MEP). Mean pre-stimulus EMG levels in the two masseter muscles were virtually identical during bilateral biting, and in the ipsilateral masseter during ipsilateral biting (Figure 2.1B). The pre-stimulus EMG in the contralateral masseter during the ipsilateral biting task was only 24% of its mean amplitude during bilateral biting (9 ± 2 μV *vs.* 37 ± 6 μV; paired t-test, $p<0.001$, $n = 9$). The data of Figure 2.1 demonstrate that the subjects were able to perform the required bilateral and unilateral biting tasks and that the mean pre-stimulus EMG in the masseter muscle of interest was very similar in the bilateral and unilateral biting tasks.

2.3.2 Focality of TMS

Stimulation of the motor cortex using the figure of eight coil during bilateral biting produced a MEP in contralateral and ipsilateral masseter. The same stimulus produced a MEP in contralateral, but not ipsilateral FDI. Data from one subject are shown in Figure 2.2. Although the data are not shown, it was noted during the experiments that movement of the coil into a more medial position abolished responses in all muscles.

2.3.3 Masseter MEPs during bilateral biting

Focal stimulation of the motor cortex elicited a MEP in both the contralateral and ipsilateral masseter muscles during bilateral biting, but not at rest. The average onset

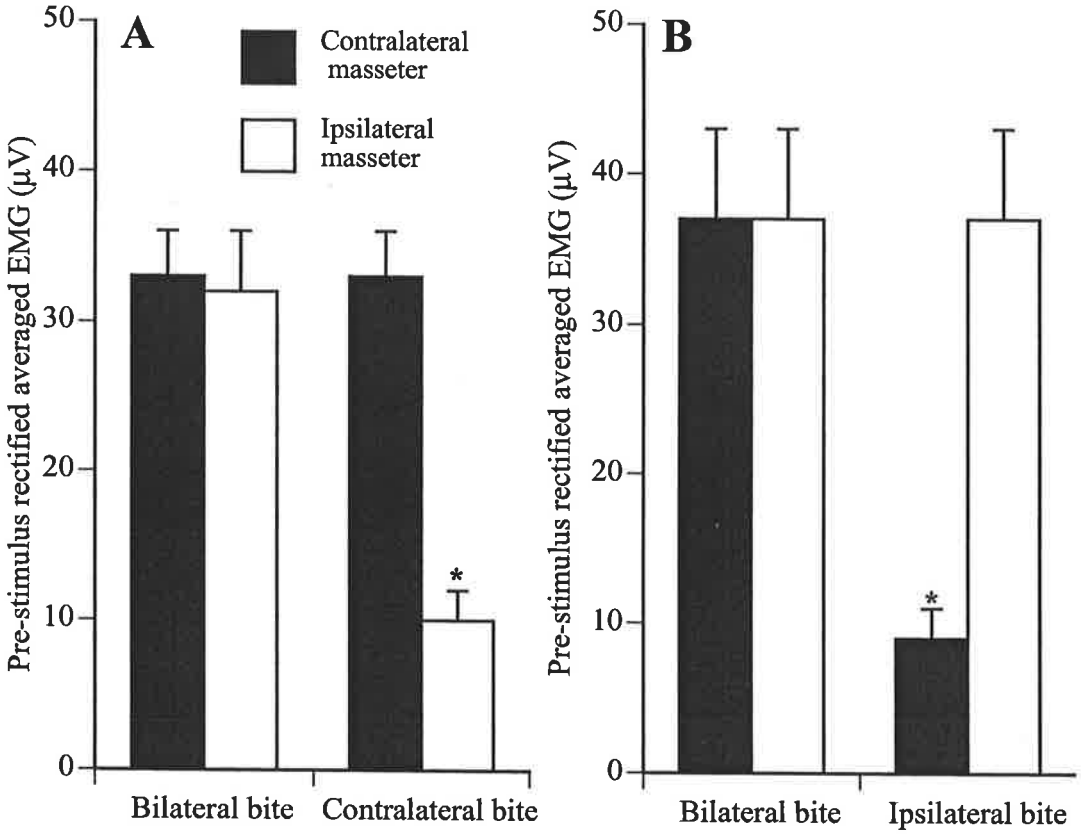

Figure 2.1

Pre-stimulus rectified EMG in contralateral and ipsilateral masseter muscles during various biting tasks. *denotes a significant reduction in EMG in a muscle between tasks (paired t-test, $P < 0.01$). **A,** mean pre-stimulus, rectified EMG (± s.e.) during bilateral biting and unilateral activation of contralateral masseter (n=16). There were no significant differences in baseline EMG levels in the two masseter muscles during bilateral biting. Activation of ipsilateral masseter was reduced by 69% during contralateral biting compared with bilateral biting (paired t-test, $P<0.001$). EMG levels in contralateral masseter were not significantly different in bilateral and contralateral biting. **B,** comparisons of mean, pre-stimulus rectified EMG (± s.e.) during bilateral biting and unilateral activation of ipsilateral masseter (n=9). Data arranged as in A. Activation of contralateral masseter was reduced by 76% during ipsilateral biting (paired t-test, $P < 0.001$). Subjects were successful in performing bilateral biting with equivalent activation of both masseter muscles, as well as relatively isolated activation of one masseter muscle at the target level during the unilateral biting tasks.

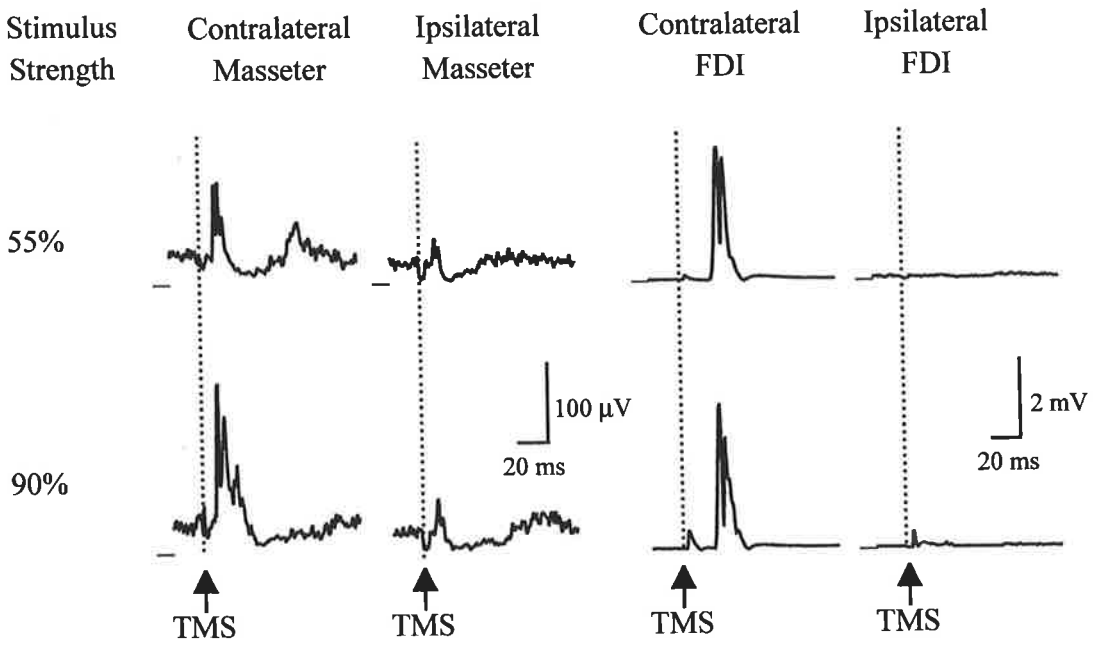

Figure 2.2

Rectified and averaged EMG responses from contralateral and ipsilateral masseter and FDI following focal TMS of one hemisphere of the motor cortex. Data are from one subject, and responses at two stimulus strengths are shown. Stimulus timing is indicated by the arrows. At both TMS intensities stimulation of one hemisphere produced MEPs in contralateral and ipsilateral masseter and in contralateral FDI. At neither strength was a response in ipsilateral FDI observed. This data provides evidence that the stimulus delivered via the figure of eight stimulating coil is focal to one hemisphere of the motor cortex.

latency for the response in the masseter contralateral to the stimulus was 7.0 ± 0.3 ms (n=16). This was followed by a silent period with a mean duration of 39.3 ± 4.0 ms (n=16). The responses to TMS in the ipsilateral masseter were more complicated. In some subjects a short latency response occurred at around 2 ms in the ipsilateral masseter. This response did not require activation of the muscle (Figure 2.3) and was considered to arise from direct stimulation of the ipsilateral trigeminal motor root (the rMEP of Cruccu et al., 1989). When present, the rMEP obscured any later responses in the ipsilateral masseter. This is shown in Figure 2.4, where 2 different stimulation sites were investigated. Stimulation at one site elicited MEPs of a similar latency (7 ms) in both contralateral and ipsilateral masseter muscles. Movement of the coil into a more lateral position still evoked a MEP in contralateral masseter at 7 ms, but the response in the ipsilateral masseter occurred at a much earlier latency (~2 ms). The presence of this rMEP obscured any later response in the muscle. It was possible to record the longer latency response in ipsilateral masseter without contamination from an ipsilateral rMEP in 12 subjects. The response had a mean onset latency of 6.7 ± 0.3 ms followed by a silent period of 32.8 ± 4.4 ms. This was not significantly different from the latency (6.6 ± 0.3 ms) or silent period (36.4 ± 4.8 ms) of the contralateral MEP obtained during the same bilateral biting task in those subjects (paired t-tests, $p>0.05$, $n = 12$). These ipsilateral responses were also considered to be cortical in origin.

Contralateral and ipsilateral MEPs had a similar threshold for TMS activation during bilateral biting. Increasing stimulus strength increased the size of the MEP in both contralateral and ipsilateral masseter (Figure 2.5). In all but two subjects, the size of the

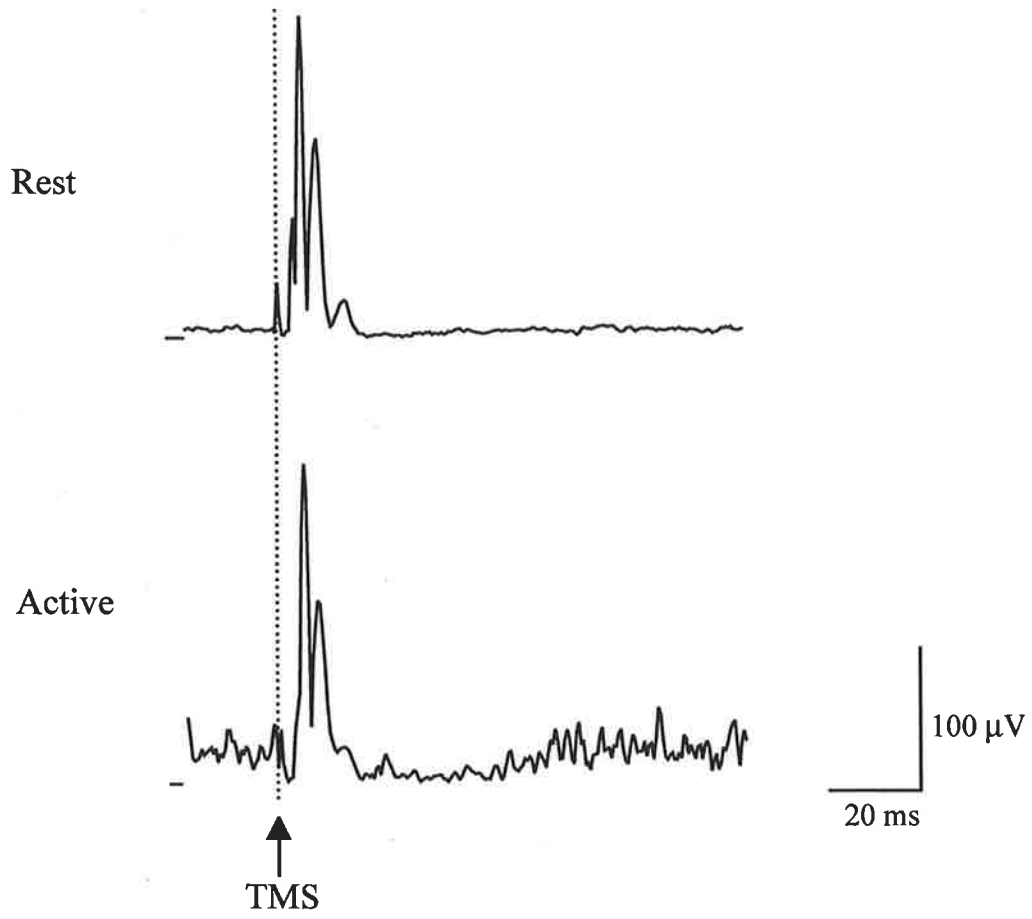

Figure 2.3

Short-latency responses in ipsilateral masseter at rest and when active. Traces are rectified and averaged EMG obtained from one subject. Stimulus timing is indicated by the arrow. Onset latency of the response was 3.0 ms at rest and 3.0 ms active. Activation of the masseter did not alter the size of the response or change its onset latency. This suggests it arises from direct stimulation of the ipsilateral trigeminal motor root.

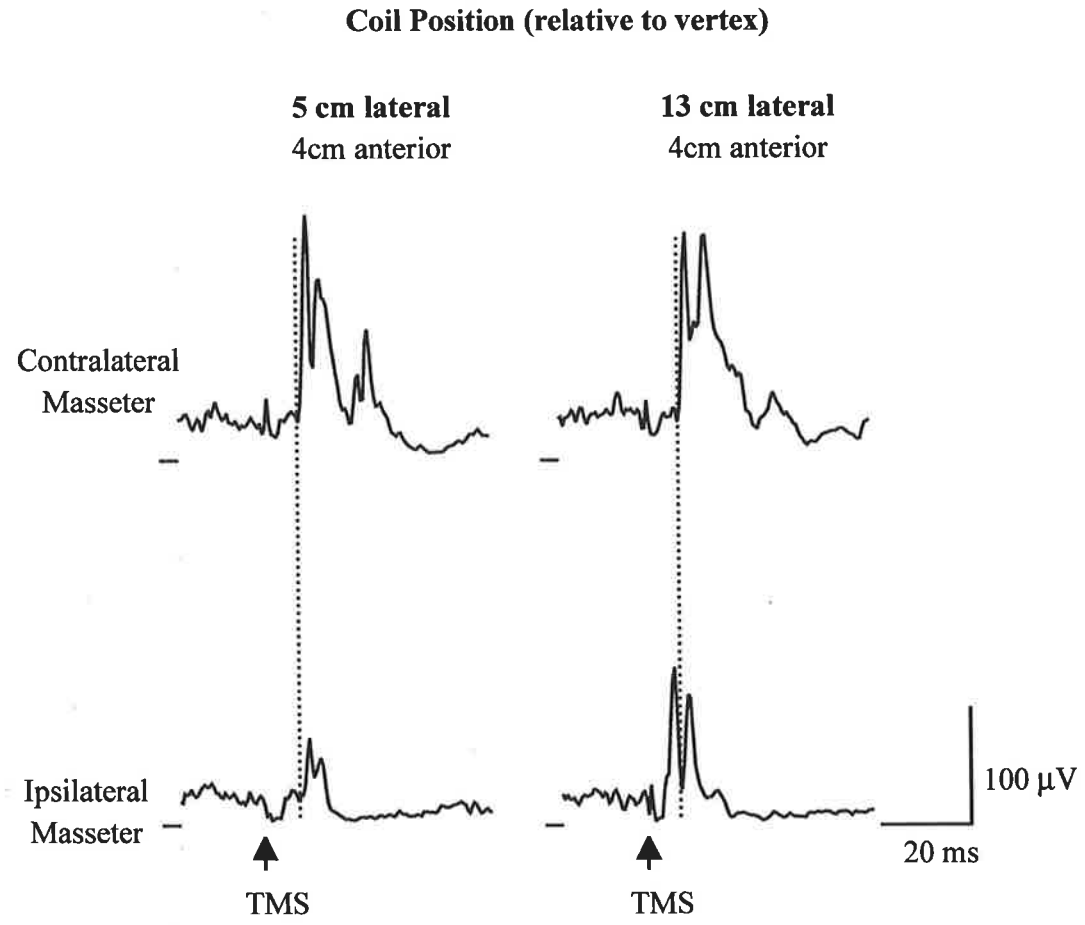

Figure 2.4

Effect of coil position on MEPs in masseter muscles. Traces are rectified and averaged EMG from the masseter contralateral (upper traces) and ipsilateral (lower traces) to the hemisphere stimulated with focal TMS. TMS was given at the time indicated by the arrow. Dashed line shows the onset of the MEP in the contralateral masseter arising from cortical activation. When the stimulating coil was positioned 5 cm lateral and 4 cm anterior to the vertex (left traces), cortical MEPs were obtained in both ipsilateral and contralateral masseter at a latency of 7 ms. When the stimulating coil was moved into a more lateral position (right traces), the cortically induced MEP in ipsilateral masseter was obscured by an earlier response with an onset latency of ~ 2 ms.

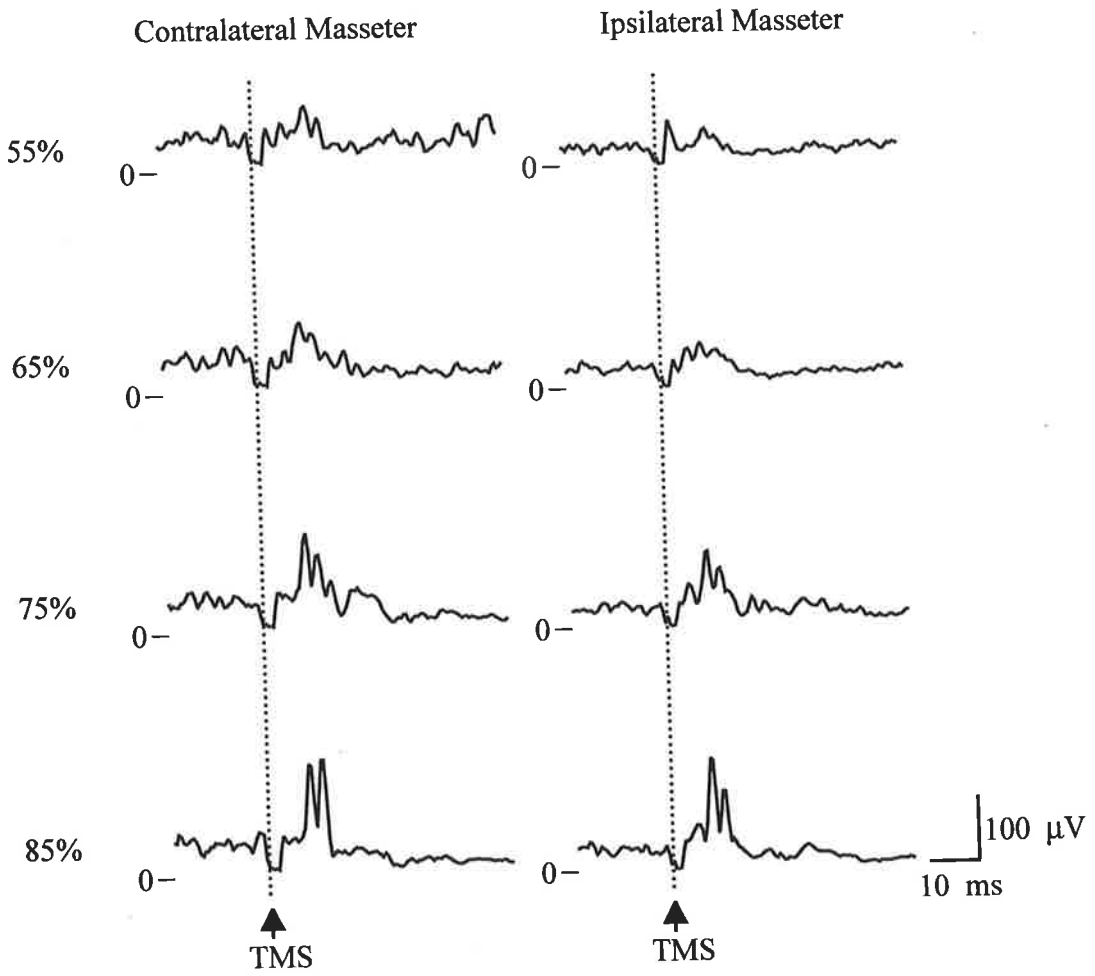

Figure 2.5

The effect of increasing TMS intensity on the responses obtained in contralateral and ipsilateral masseter. Averaged data (n = 25) from one subject. Responses from contralateral masseter are shown on the left, and ipsilateral masseter on the right. Stimulus timing is indicated by the arrows. In this subject the stimulus strength was increased from 55% to 85% in 10% increments. As TMS intensity increased, the size of the MEP increased in both masseter muscles.

MEP was larger in the contralateral masseter. When the MEP was normalised to pre-stimulus EMG activity, the contralateral MEP was larger than the ipsilateral MEP in every subject. In the data pooled from 12 subjects (Figure 2.6) during bilateral biting, the mean size of the contralateral MEP was 39% larger than the ipsilateral response during bilateral biting (0.71 ± 0.07 mV.ms *vs.* 0.51 ± 0.09 mV.ms; paired t-test, $p<0.005$, $n = 12$). By way of comparison, the size of maximal tendon jerk reflex in masseter of 5 subjects was 2.9 ± 0.8 mV.ms. The average size of the MEP was therefore about 25% of the maximal tendon jerk reflex in the contralateral masseter and 18% in the ipsilateral masseter.

2.3.4 Task-dependence of masseter MEPs during unilateral biting

Representative data from one subject showing MEPs elicited by TMS during the three biting tasks are shown in Figure 2.7. In this example, the MEP in the contralateral masseter was 8% smaller during contralateral biting (task 2) than during the bilateral biting task (task 1). This pattern of smaller MEPs in the contralateral masseter with contralateral biting was seen in every subject. The MEP in the ipsilateral masseter, however, was similar for bilateral and ipsilateral biting. Figure 2.8 summarises the task dependence of MEPs in contralateral (Figure 2.8A) and ipsilateral (Figure 2.8B) masseter muscles from the pooled data. On average, the MEP in the contralateral masseter (Figure 2.8A) was reduced by 15.5% during contralateral biting compared with bilateral biting (0.71 ± 0.07 mV.ms *vs.* 0.60 ± 0.06 mV.ms; paired t-test, $p<0.001$, $n = 16$). In contrast, the ipsilateral masseter MEP was not significantly different in bilateral and ipsilateral biting (0.59 ± 0.11 mV.ms *vs.* 0.57 ± 0.12 mV.ms; paired t-test, $p>0.05$, $n = 9$).

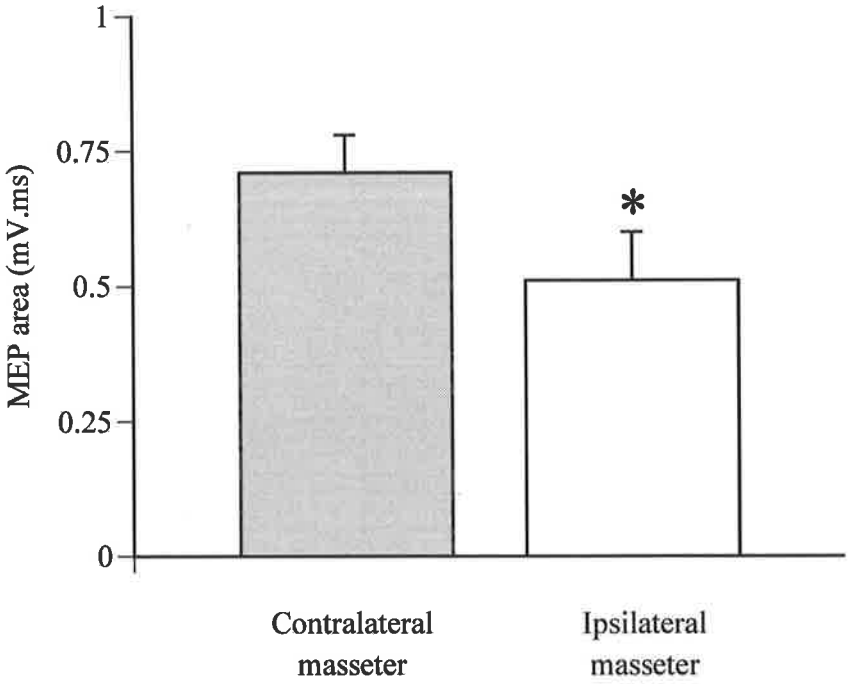

Figure 2.6

A comparison of the MEP area in contralateral and ipsilateral masseter during bilateral biting. Data are mean (± s.e.) MEP area from 12 subjects. * denotes that the MEP in ipsilateral masseter was significantly smaller than that obtained in contralateral masseter (paired t-test, $p<0.005$).

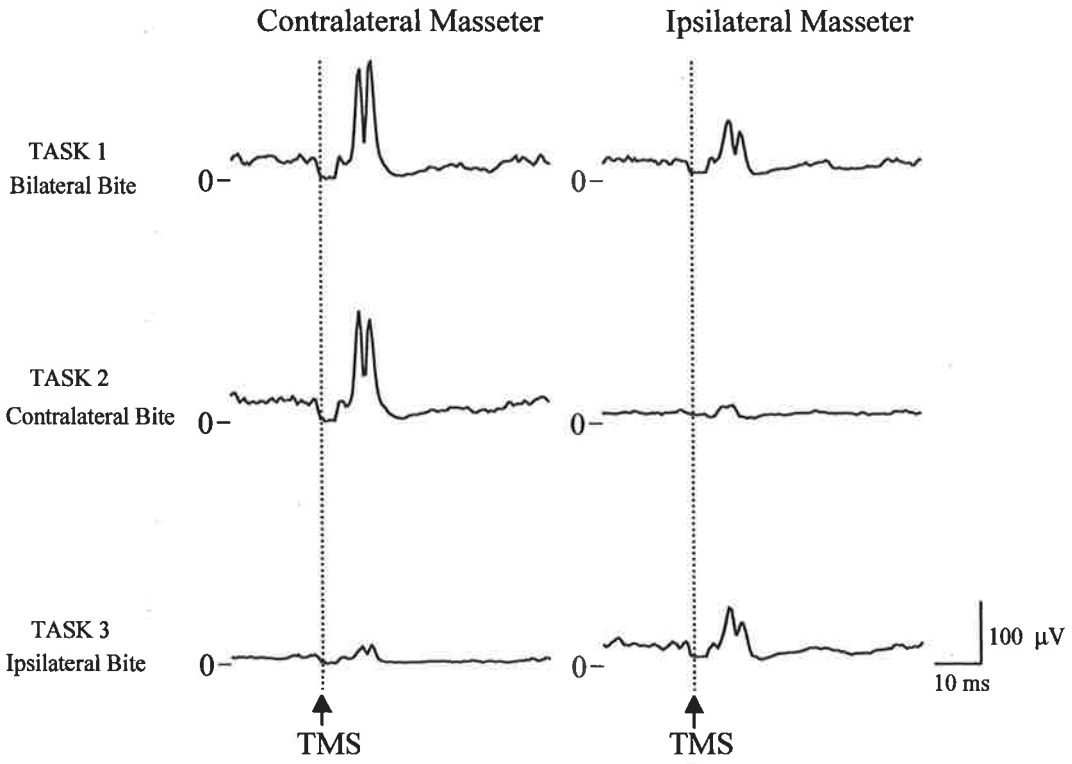

Figure 2.7

MEPs from one subject following focal TMS of one hemisphere during different biting tasks. Traces are rectified and averaged (n=50) surface EMG records with stimulus timing indicated by the arrows. An artefact-suppressing amplifier gated the signal from 1 ms before the stimulus, until 2 ms after it. TMS intensity was 50% of maximal stimulator output. Responses in masseter contralateral to the stimulated hemisphere are shown on the left and the ipsilateral masseter on the right. Masseter responses to TMS are shown during the bilateral bite (Task 1; uppermost traces), during attempted unilateral activation of contralateral masseter (Task 2; middle traces) and during attempted unilateral activation of ipsilateral masseter (Task 3; lowermost traces).

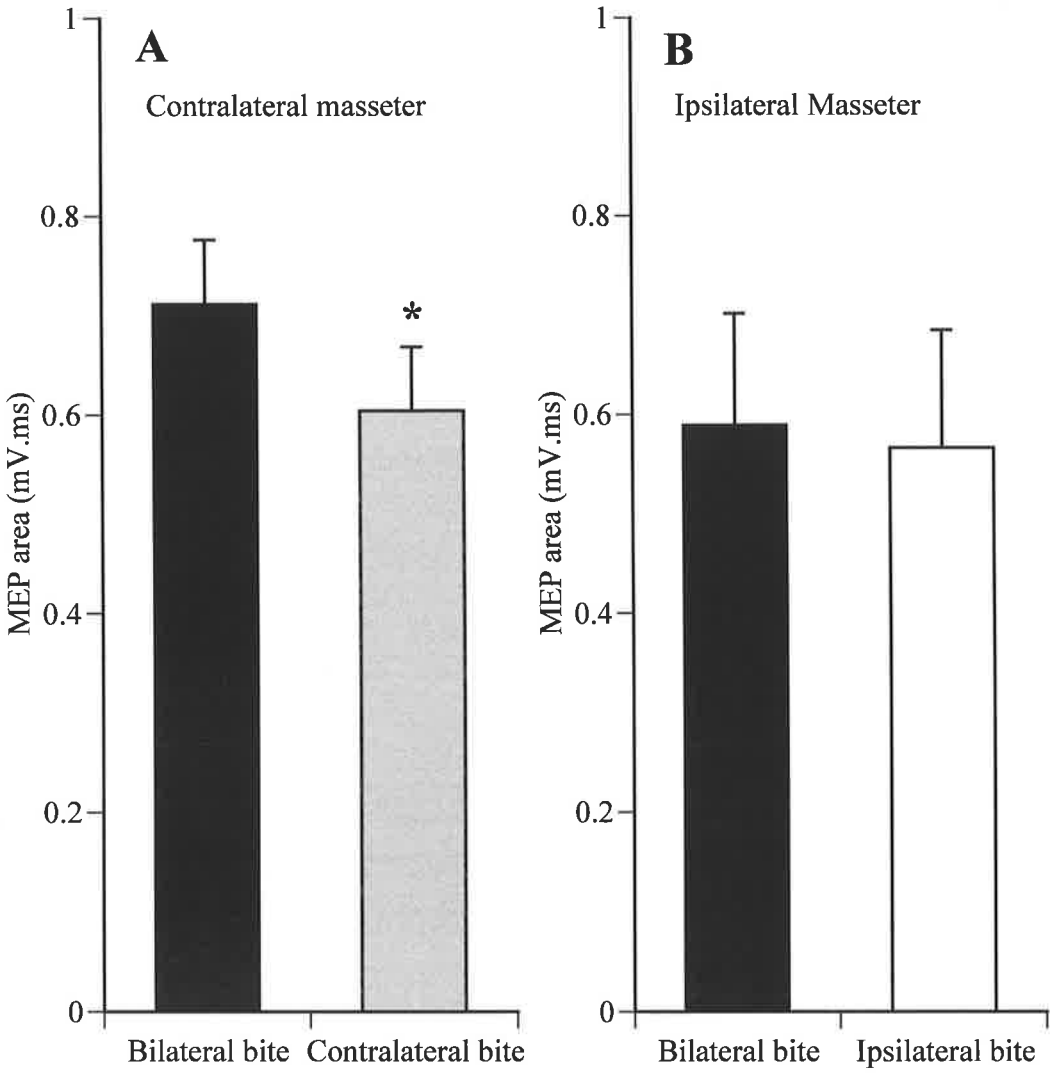

Figure 2.8

Effect of biting task on masseter MEP size. A, pooled data showing mean (± s.e.) MEP area in the contralateral masseter during bilateral and contralateral biting (Task 1 *vs.* Task 2; n = 16). **B**, pooled data from ipsilateral masseter showing mean (± s.e.) MEP area during bilateral and ipsilateral biting (Task 1 *vs.* Task 3; n=9). * denotes significant difference: unilateral *vs.* bilateral biting (paired t-test, $p<0.001$). The MEP in contralateral masseter was significantly smaller during contralateral biting compared with bilateral biting. The MEP in ipsilateral masseter was not affected by the biting task.

Neither the onset latency of the MEP nor the silent period following was influenced by biting task in either contralateral or ipsilateral masseter (Figure 2.9). For contralateral masseter, MEP onset latency was 7.0 ± 0.3 ms with bilateral biting and 7.1 ± 0.3 ms with the contralateral bite (paired t-test; $p>0.05$). Silent period in the contralateral masseter was 39.3 ± 4.0 ms with bilateral biting and 39.9 ± 3.5 ms with ipsilateral biting (paired t-test; $p>0.05$). For ipsilateral masseter, onset latency was 6.6 ± 0.3 ms during bilateral biting and 6.7 ± 0.3 ms during the ipsilateral bite (paired t-test; $p>0.05$). Silent periods were 35.5 ± 5.4 ms with bilateral biting and 32.1 ± 6.8 ms with ipsilateral biting (paired t-test; $p>0.05$).

2.4 Discussion

The principal finding of the present study is the asymmetric nature of the motor cortical control over the masseter muscles. First, although focal TMS evokes MEPs in both masseter muscles during bilateral biting, the MEP is significantly larger in the contralateral muscle. Second, the motor cortex excitability varies with biting task in an asymmetric manner. The MEP in the contralateral masseter is reduced when the muscle is activated during unilateral biting, but no modulation was seen in the MEP in ipsilateral masseter when it was activated for unilateral biting. These results suggest that the corticotrigeminal component of the command for unilateral biting originates from the contralateral hemisphere.

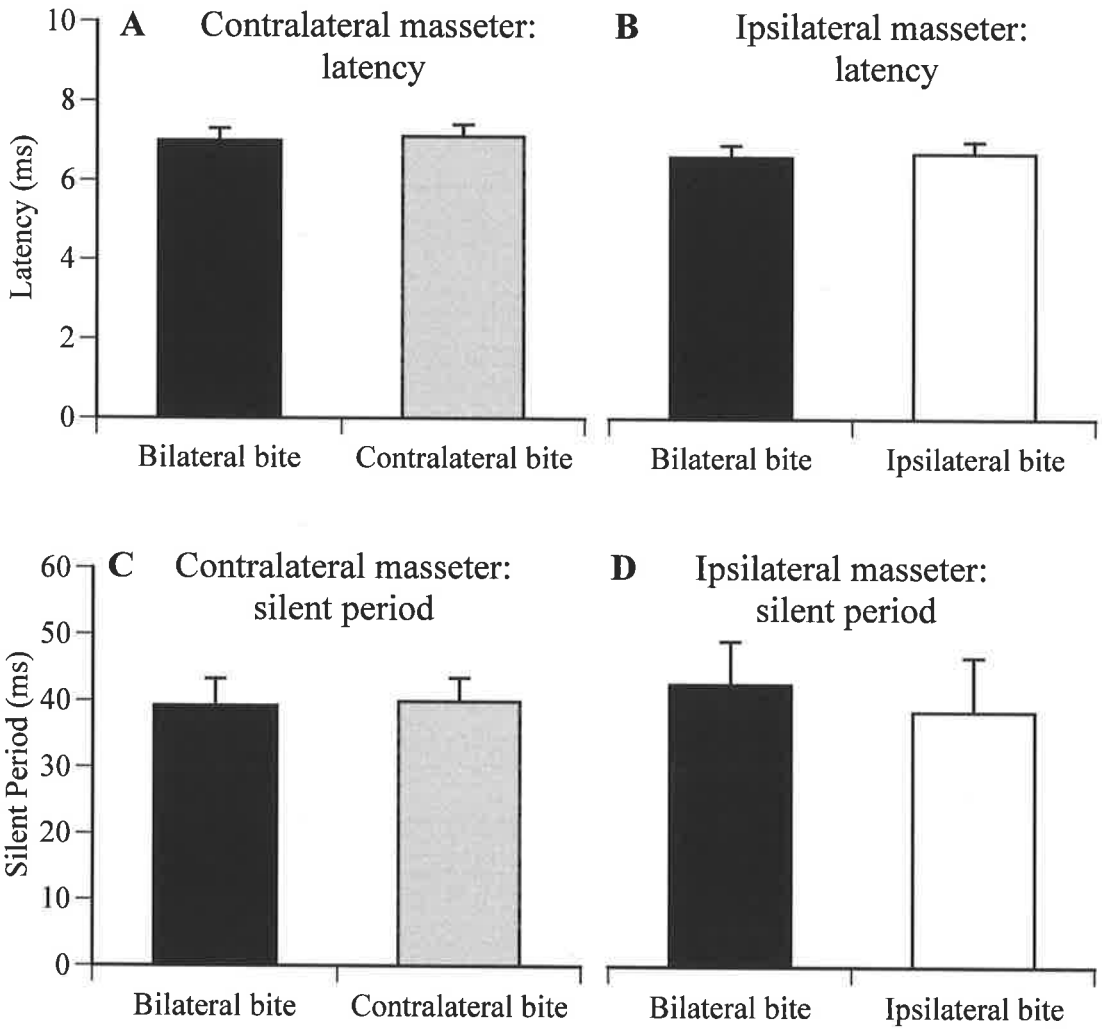

Figure 2.9

Effect of biting task on masseter MEP latency and silent period duration. A, pooled data showing mean (± s.e.) MEP latency in the contralateral masseter during bilateral and contralateral biting (Task 1 *vs.* Task 2; n = 16). **B**, pooled data showing mean (± s.e.) MEP latency in the ipsilateral masseter during bilateral and ipsilateral biting (Task 1 *vs.* Task 3; n =9). **C**, pooled data from contralateral masseter showing mean (± s.e.) silent period duration in the during bilateral and contralateral biting (Task 1 *vs.* Task 2; n = 16). **D**, pooled data from ipsilateral masseter showing mean (± s.e.) silent period duration in the during bilateral and ipsilateral biting (Task 1 *vs.* Task 3; n = 9). Biting task did not significantly affect latency or silent period in either ipsilateral or contralateral masseter (paired t-tests, $p>0.05$).

2.4.1 The nature of corticotrigeminal projections to masseter motoneurons

Focal TMS of one hemisphere of the motor cortex produced bilateral masseter MEPs, with a larger MEP in the contralateral muscle. The response in the masseter contralateral to the stimulus had an onset latency of 7.0 ± 0.3 ms (n=17). This is comparable with the onset latencies reported by Macaluso et al. (1990), Cruccu et al. (1989), Carr et al. (1994) and Turk et al. (1994) and is consistent with a monosynaptic connection from the motor cortex to the masseter motoneurons. Cruccu et al. (1989) calculated, based on masseter MEP latency measurements and conduction velocities, that the central delay at the trigeminal motoneuron synapse(s) was between 1.1 and 1.4 ms. This was sufficient time for no more than two synapses and it was argued that the projection was likely to be monosynaptic since the duration and latency variability of masseter MEPs were even shorter than for active hand muscles, which are served by direct corticomotoneuronal (CM) connections. Further evidence that the MEP is due to activation of monosynaptic CM cells is the brief nature of the peaks in the masseter PSTH following TMS (see Chapter 3).

Two types of responses were produced in the masseter ipsilateral to the TMS. The first response had a very short latency and was not affected by activation of the muscle (Figure 2.3). This response is due to direct activation of the trigeminal root (Benecke et al., 1988; Cruccu et al., 1989; Macaluso et al., 1990; Gooden et al., 1999).

The second response occurred at a longer latency (6.7 ± 0.3 ms, n=9) and is comparable with the response seen in contralateral masseter. Previous authors have referred to this response as the ipsilateral long latency response (Benecke et al., 1988) or the U wave

(Cruccu et al., 1989). There are three main theories that would account for this response. First, the response may be due to spread of the stimulus to the other hemisphere thereby activating cortical cells that cause activation of the muscle via contralateral projections. This is not likely in the present study as a figure of 8 coil was used to apply focal stimulation to one hemisphere. This is discussed further below. Second, the response may result from the activation of Ia fibres in the trigeminal nerve causing an H-reflex in masseter. This is unlikely to account for the entire response since in related experiments (Chapter 5 and Butler et al., 1997) a patient who had suffered a stroke in infancy was tested and stimulation of the damaged hemisphere with TMS failed to produce responses in either left or right masseter. If the response in ipsilateral masseter was a reflex following activation of the trigeminal nerve in origin it would be expected to occur following TMS of the damaged hemisphere in the patient. A third explanation for the response is that it may be due to the activation of ipsilaterally projecting cortical neurons. This is the most likely explanation and is supported by the finding that the ipsilateral response had the same threshold, latency, and silent period duration as the response in contralateral masseter, suggesting that the origin of the two responses is similar.

There is anatomical evidence for bilateral corticotrigeminal projections in humans (Kuypers, 1958a; Iwatsubo et al., 1990). A circular coil was first used with TMS to investigate the nature of the cortical projections to human masseter. Benecke et al. (1988) reported that TMS evokes bilateral responses in masseter, whereas Macaluso et al. (1990) obtained cortically evoked responses only in the masseter contralateral to the TMS. This could possibly be due to a difference in stimulus strength since Cruccu et al. (1989) found

that, at low stimulus strengths, the response to TMS in masseter was exclusively contralateral, whereas bilateral responses were evoked at higher TMS intensities.

Previous studies with a circular coil are difficult to interpret because although one hemisphere may be preferentially stimulated, activation of both hemispheres cannot be excluded. Indeed, at the higher stimulus strengths used by Cruccu et al. (1989) to obtain bilateral responses in masseter, bilateral responses were also recorded in thenar muscles (see their Figure 6). Similarly, when activating the motor cortex with a circular TMS coil, Benecke et al. (1988) reported bilateral responses in both masseter and first dorsal interosseous (FDI) when the stimulating coil was placed over the vertex, although the bilateral responses in FDI disappeared when the coil was shifted laterally. These results indicate that TMS was not activating the motor cortex exclusively on one side. In order to overcome this problem I used a figure-of-eight coil. The magnetic field produced by this coil is more focussed than that produced by a circular coil (Cohen et al., 1990). Rosler et al. (1989) compared the responses in abductor digiti minimi (ADM) to TMS using circular and figure-of-eight coils. At high stimulus strengths they reported bilateral responses in ADM when the circular stimulating coil was used, but only contralateral responses with the figure-of-eight coil, suggesting that there is no current spread to the other hemisphere when using the figure-of-eight coil. The present study has found that bilateral MEPs were observed in active masseter muscles with a figure-of-eight coil. When the coil was moved medially from the optimal scalp location, both ipsi- and contralateral masseter MEPs disappeared. This suggests that the ipsilateral response evoked in masseter by TMS is not due to spread of the stimulus to the motor cortex of the

other hemisphere, and that it does in fact result from corticotrigeminal projections from the ipsilateral motor cortex to masseter motoneurons.

There have been three other studies which have used a figure-of-eight coil to investigate the bilateral nature of cortical projections to masseter (Carr et al., 1994; Guggisberg et al., 2001; McMillan et al., 2001). All showed that focal TMS of one hemisphere of the motor cortex produced MEPs in both left and right masseter. While Guggisberg et al. (2001) found no difference in the amplitude of MEPs in the ipsilateral and the contralateral masseter, both the other studies presented results suggesting that the contralateral masseter MEP is larger than the ipsilateral response. This difference did not reach statistical significance in the sample of 7 subjects studied by Carr et al. (1994), but was significant in the 10 subjects studied by McMillan et al. (2001), when subjects were activating masseter in an isometric (compared with dynamic) contraction. The present study employed an even larger sample (12 subjects) and demonstrated that during an isometric contraction the contralateral MEP in masseter is significantly larger (39% on average) than the ipsilateral MEP. The size of the masseter MEPs in the present study is similar to the values reported by Carr et al. (1994) and McMillan et al. (2001), and also comparable to those reported under similar recording conditions by Guggisberg et al. (2001). The average MEP size in contralateral masseter in the present study was about 25% of the maximal tendon jerk reflex in masseter (~3 mV.ms) measured under similar recording conditions.

2.4.2 Differences in the control of masseter motoneurons from each hemisphere

TMS is thought to activate pyramidal tract neurons either transynaptically or at the initial segment, and is therefore highly dependent on cortical excitability (reviewed by Rothwell, 1997). In hand muscles, the size of the MEP varies, depending on the nature of the task performed, despite equivalent EMG levels (Flament et al., 1993; Schieppati et al., 1996). This is believed to reflect a flexibility of CM cell activation with task which has been observed in motor cortex of the monkey (see Lemon, 1993). While CM cells appear to be more active in precision tasks, it is not yet clear which aspect of the task engages greater CM cell activity. Datta et al. (1989) found the FDI MEP to be larger when FDI was abducted in isolation, compared with a grip involving index finger and thumb. In contrast, Flament et al. (1993) found that FDI MEPs were larger in "complex" tasks requiring control of multiple muscles, than in simple index finger abduction using FDI alone. They suggested that isolated activation of FDI may require less CM activity because of the need to inhibit some CM cells which excite both FDI and its synergists. Schieppati et al. (1996) found larger MEPs in FDI when the task required precise control of pincer grip force using visual feedback, rather than supporting a static load with a pincer grip.

The present study has shown that the contralateral masseter MEP is smaller when the muscle is activated during a contralateral bite than during a bilateral bite. This was only the case for the masseter contralateral to the hemisphere stimulated, but not with ipsilateral stimulation. Other aspects of the response to TMS, such as MEP latency and silent period duration were unaffected by the biting task. It is concluded from this that the

ipsilateral motor cortex is not directly involved in the descending command mediating unilateral activation of masseter. The contralateral motor cortex is involved, as the excitability of motor cortex neurons projecting to contralateral masseter is reduced during the performance of the unilateral bite.

The fact that the more demanding unilateral biting task was accomplished with *reduced* activity in the corticotrigeminal projection to masseter from the contralateral hemisphere seems rather at odds with the presumed role of CM cells in promoting fractionated muscle activation for precision tasks. I propose that the reduction in MEP size with the more independent activation of masseter is related to the nature of the corticotrigeminal projections to masseter motoneurons. This is explained further below.

The observations that 1) the MEP in contralateral masseter is larger than the response in ipsilateral masseter, and 2) only the contralateral MEP is modulated during unilateral biting, suggest that there is a separate population of corticotrigeminal neurons with exclusively contralateral projections to masseter. The ipsilateral MEP could arise from activation of single corticotrigeminal neurons which branch to innervate both masseter motoneuron pools, or from a separate population of cells with exclusively ipsilateral projections. Short-term synchrony in the discharge of motor units in right and left masseter muscles (Carr et al., 1994) is evidence for the former class of corticotrigeminal cells. My findings provide no evidence for the existence of the latter class of neurons, however the experiments cannot exclude the possibility that they exist. A representation of these three classes of neurons is shown in Figure 2.10A.

Consider the MEP obtained in masseter contralateral to the TMS (right side of diagram in Figure 2.10A). The MEP resulting from TMS may be due to activation of the corticotrigeminal cells which project to contralateral motoneurons exclusively, or due to the activation of the corticotrigeminal cells which branch to innervate the masseter motoneuron pool on both sides. The size of the MEP will depend on the number of these projections which are activated during the biting task. During bilateral biting both types of projections are likely to be active in the motor cortex, and therefore both will contribute to the MEP elicited by TMS. Figure 2.10B depicts the situation during the independent activation of masseter contralateral to the stimulus. To perform this task it is desirable to reduce excitability of corticotrigeminal cells with bilateral projections, since their activity promotes contraction of both masseter muscles. Reduced activity of the bilaterally projecting corticotrigeminal cells would result in a smaller MEP in the independently active contralateral masseter muscle. The input from the ipsilateral hemisphere must also be considered and Figure 2.10C represents the situation during independent activation of masseter ipsilateral to the TMS. The size of the MEP resulting from TMS was no different when ipsilateral masseter was activated alone or in unison with the other masseter muscle. This suggests that in the ipsilateral motor cortex the same corticotrigeminal cells which are active during a bilateral bite are also active during the unilateral bite and the ipsilateral hemisphere therefore plays no part in the modulation of unilateral biting. Presumably, in order to accomplish isolated activation of the ipsilateral masseter, the contralaterally projecting CM projections are turned off. However, the results of the present study do not allow direct evidence of that since the response in contralateral muscle during the ipsilateral bite is affected by the decreased level of EMG activity.

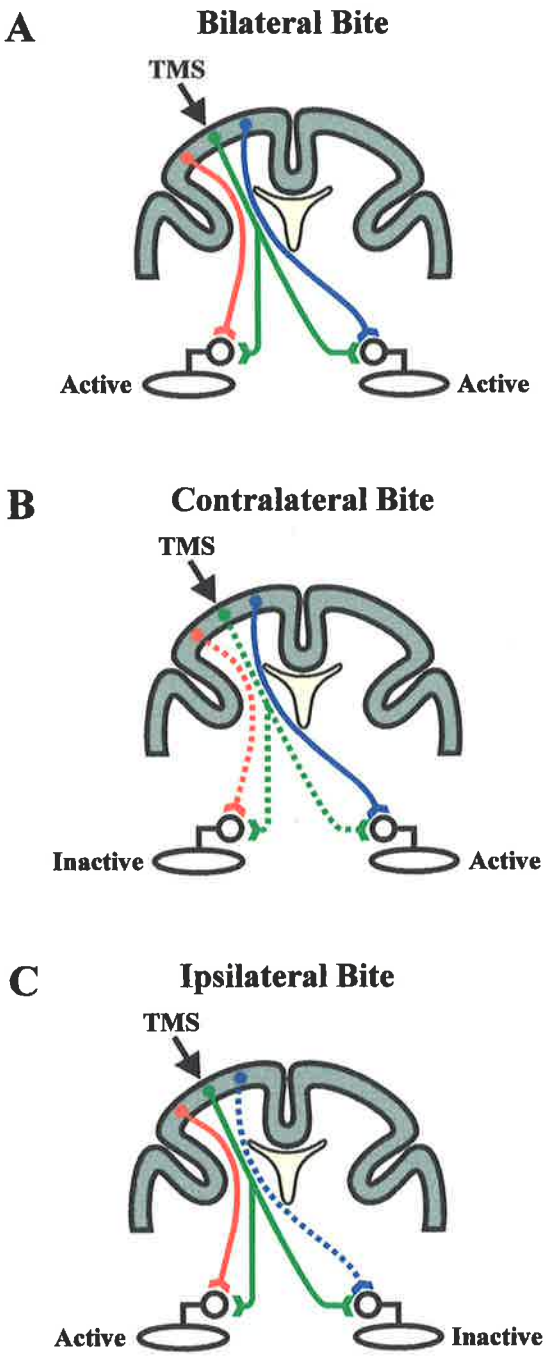

Figure 2.10

A model of corticobulbar projections to account for the asymmetry in the task dependence of MEP size. Corticobulbar projections innervating ipsilateral motoneurons are shown in red, those innervating contralateral motoneurons are shown in blue and those that branch to innervate the motoneurons on both sides are shown in green. Projections shown with a solid line are likely to be active during the biting task. A dotted line indicates that the activity in the projection is likely to be reduced. **A**, Bilateral biting (task one). **B,** Contralateral biting (task two). **C,** Ipsilateral biting (task three).

It is not known whether there are corticotrigeminal neurons with exclusively ipsilateral projections to masseter motoneurons (the red projections in Figure 2.10). This study has not been able to confirm or deny the existence of these cells. The absence of modulation of the ipsilateral MEP between the bilateral and ipsilateral biting tasks argues against a role for exclusively ipsilaterally projecting neurons. However, a transition from a bilateral to a contralateral bite could be accomplished with the aid of reduced activity of CM cells with exclusively ipsilateral projections (see Figure 2.10B); quantitative analysis of the ipsilateral MEP to assess this is confounded by the reduced EMG of the ipsilateral muscle during the contralateral biting task.

The absence of modulation of corticotrigeminal neuron activity in ipsilateral cortex with unilateral biting may contribute to the inability to activate one masseter muscle completely independently of the other (Figure 2.1). If the task is to activate the right masseter, for example, corticotrigeminal neurons in the right (ipsilateral) hemisphere remain active to the same extent as that seen during the bilateral bite. Corticotrigeminal neurons in the right hemisphere with bilateral projections will continue to excite the left masseter motoneuron pool, and contribute to activation of the left masseter muscle.

2.4.3 The role of the motor cortex in controlling movements of the hand *vs.* the jaw

The cortical control over hand muscles has been the subject of much research and TMS has often been used as a tool to study the cortical projection to the motoneurons innervating hand muscles. These experiments employ techniques similar to those described in the present study. A comparison of the results obtained in hand studies with

the results obtained in the present study of the masseter muscle highlight some important differences in the cortical control of human hand and jaw muscles.

Activation of masseter by TMS is technically more difficult than the activation of hand muscles. We found that MEPs could readily be produced in relaxed FDI but we were unable to elicit MEPs in relaxed masseter, despite the use of high TMS intensities. This was true even in subjects who displayed a very low active threshold to TMS in masseter. The threshold for eliciting MEPs in resting FDI was always less than that needed to elicit MEPs in the active masseter muscle and the responses were much larger in resting FDI. This suggests that the projections from the cortex to motoneurons innervating the hand are more numerous and/or stronger than those which innervate masseter, or that the elements activated by TMS which trans-synaptically excite the hand CM cells are more powerful and/or accessible than for masseter CM cells.

The cortical projections to hand muscles are almost exclusively contralateral so that the corresponding muscles on each side of the body are controlled completely independently from each other. In comparison, our results have demonstrated that masseter motoneurons receive input from both hemispheres allowing the muscles on each side to be activated in unison. This may be important during basic jaw movements where co-activation of masseter is necessary. However for efficient mastication some asymmetry of jaw movement is essential, and therefore, the masseter muscles on each side also receive cortical input which is independent from the other. The differences in the cortical innervation of masseter compared with hand muscles explains why the results of stroke are so much more damaging in the contralateral hand muscles than they are in contralateral jaw muscles (Willoughby and Anderson, 1984).

The motor cortex seems to be responsible for the production of fractionated muscle activation in both the hand (Lemon, 1993) and in masseter (the present study). However, the mechanism by which the motor cortex accomplishes this is different for the two muscle groups. During fine movements of the hand it has been suggested that intracortical GABAergic inhibitory circuits within the motor cortex may focus the motor command to the appropriate CM cells (Ridding et al., 1995b; Ridding et al., 1995a; Nordstrom and Butler, 2002). The CM cells are more active during fine tasks compared with during power tasks (Muir and Lemon, 1983; Lemon et al., 1986). In contrast, rather than altering the excitability of the contralaterally projecting corticotrigeminal cells, independent contraction of one masseter muscle (e.g. a unilateral bite), may involve a reduction in the excitability of the bilaterally projecting corticotrigeminal cells when compared to bilateral biting. Further study is required to determine if this is also accomplished through intracortical inhibitory circuits.

2.4.4 The silent period

While the main purpose of this study was to examine the excitatory response in masseter following TMS, it was noted that the excitatory response was followed by a period of reduced muscle activity. The silent period was bilateral, and unlike the MEP, was symmetrical and not altered by task. The present study has not investigated the neural process underlying the silent period. Studies of the silent period induced in hand muscles by TMS (Inghilleri et al., 1993; Roick et al., 1993; Triggs et al., 1993; Ziemann et al., 1993; Schnitzler and Benecke, 1994; Brasil-Neto et al., 1995) and cranial muscles (Werhahn et al., 1995; Cruccu et al., 1997a) suggest that much of the silent period following TMS is due to the activation of cortical inhibitory circuits. Changes in

segmental excitability may also play a role, but only in the early part of the cortical silent period (less than 60 ms) (Cantello et al., 1992; Inghilleri et al., 1993; Ziemann et al., 1993). The masseter silent period following TMS is examined and discussed in more detail in Chapter 3.

2.4.5 Conclusion

In summary, this study has shown that there is a corticotrigeminal projection from the motor cortex of one hemisphere to both masseter motoneuron pools. It is concluded that this input is asymmetric due to the existence of a population of corticotrigeminal cells with exclusively contralateral projections. The results show that the ipsilateral and contralateral motor cortex differ in their control over masseter motoneurons. The corticotrigeminal component of the command for unilateral biting originates from the contralateral hemisphere only, and I suggest that this may be accomplished in part by reduced activity of the population of corticotrigeminal neurons in the contralateral hemisphere with branched-axon projections to both masseter motor pools.

CHAPTER 3

CONTROL OF MASSETER SINGLE MOTOR UNITS FROM MOTOR CORTEX OF EACH HEMISPHERE

3.1 Introduction

Transcranial magnetic stimulation (TMS) can be used to study the projections from the motor cortex to motoneurons in awake human subjects. To date, the responses to TMS have been studied in human masseter muscles using surface electromyography (EMG) (see Chapter 1.3.7), but not at a single motor unit level. Earlier studies using surface EMG have suggested that there are corticomotoneuronal (CM) projections from one hemisphere of the motor cortex to motoneurons innervating both masseter muscles, but that the projection is stronger to the contralateral muscle (Chapter two and Cruccu et al., 1989; Butler et al., 2001; McMillan et al., 2001). There is evidence of individual corticotrigeminal cells that exclusively project to contralateral masseter motoneuron pool (Cruccu et al., 1989; Butler et al., 2001), and also evidence of cells which are branched and project bilaterally (see Chapter 4 and Carr et al., 1994). It is not known if there are corticomotoneuronal cells which exclusively project to ipsilateral masseter motoneurons. Analysis of single motor unit responses to TMS are required to confirm whether CM projections are present, and how these are organised to the motoneuron pool from each hemisphere.

In Chapter 2 it was shown that the masseter muscles are capable of relatively independent activation, and there were asymmetries in the level of motor cortex activation during

bilateral and unilateral biting (see Chapter 2 and Butler et al., 2001). At the level of the motoneuron pool, it is not known whether a masseter single motor unit receives an excitatory projection from both hemispheres, or whether motor units within a masseter muscle may receive differential input from the two hemispheres. A better understanding of the nature of the projections from the motor cortex of each hemisphere to single masseter motoneurons will assist in understanding how the motor cortex might mediate differential control of the masseter muscles on each side.

The aim of the present study was to examine the corticobulbar inputs to single masseter motoneurons from the contra- and ipsilateral motor cortex. Unilateral focal TMS was used and the responses evoked in masseter were studied at a single motor unit level to provide further information regarding (a) the presence and relative strength of excitatory projections from the ipsilateral and contralateral hemisphere to single masseter motoneurons; (b) whether there are corticomotoneuronal projections to masseter single motor units from each hemisphere; and (c) the nature of inhibitory responses evoked in masseter single motor units by TMS of either hemisphere.

3.2 Methods

The experiments were conducted with the approval of the Human Research Ethics Committee of the University of Adelaide. Thirty motor units were tested in six experiments with four male and two female subjects. The subjects, aged between 24 and 32, had no history of neurological disorders, and all gave their informed consent before participating in the experiments.

3.2.1 Apparatus and recording

Surface electromyograms of the left and right masseter muscles were recorded using self-adhesive gel-filled bipolar Ag/AgCl electrodes placed along the long axis of the muscle fibres. One electrode was positioned at the level of the lower border of the mandible, and the other about 2.5 cm above this, close to the motor point. Single motor unit (SMU) activity was recorded with intramuscular fine-wire electrodes inserted into the right masseter. These consisted of three fine, Teflon^{TM}–insulated wires (45-μm diameter) threaded into the needle of a disposable 24 or 25 gauge needle. The needle was inserted into the masseter and then removed, leaving the fine wires in place. Three wires were used to allow the choice of three electrode pairs per needle insertion; the pair of wires that gave the clearest discrimination of one or more single unit action potentials was used. Subjects were grounded by a lip-clip electrode (Türker et al., 1988). The surface EMG and the SMU activity was amplified (1000 X) using a custom made stimulus artefact suppressing amplifier and recorded on video tape (Data recorder model 400 PCM, A.R. Vetter Co., Pennsylvania, USA) at a sampling rate of 22 kHz/channel.

The action potentials of a voluntarily activated masseter single motor unit were discriminated on-line with a hardware device and converted to TTL pulses. Inter-pulse interval determined the timing of the TMS stimulation (details below). More stringent discrimination of single motor units which allowed resolution of waveform superimpositions in multi-unit recordings was performed offline (see below) using a computer and proprietary waveform template-matching algorithms (SPS-8701: Signal Processing Systems, Malvern, Australia).

The jaw area of the motor cortex was stimulated with a standard Magstim 200 stimulator using a figure-of-eight coil with outer coil diameters of 9.5 cm. The coil was positioned to obtain the best motor evoked potential (MEP) in both the contralateral and ipsilateral masseter surface EMG at the lowest stimulus intensity. Coil orientation was at an angle of 45° relative to the parasagittal plane, with current flowing in the underlying cortex in a postero-anterior direction. This orientation preferentially elicits direct (D) or early indirect (I_1) waves in hand muscles (Sakai et al., 1997). In some trials the hemisphere contralateral to the monitored motor unit was stimulated and in others the ipsilateral hemisphere was stimulated. Usually this was alternated for a single motor unit, while it was tested with a range of different TMS intensities.

3.2.2 Protocol

Subjects maintained a tonic contraction of a masseter motor unit at a comfortable firing rate that was usually in the range 10-12 Hz. This was achieved by the subject with the help of visual feedback of the mean motor unit discharge frequency, displayed as a horizontal line on an oscilloscope screen. In most experiments subjects wore headphones and listened to white noise to ensure that the discharge noise of the stimulator did not evoke an inhibitory reflex in the motor unit (Meier-Ewert et al., 1974; Sato et al., 1994). Earlier studies have shown that the response probability of a motor unit to TMS is greater when the unit is firing at a slow compared to fast rate (Brouwer et al., 1989; Bawa and Lemon, 1993). Therefore TMS were delivered ($< 0.2\ s^{-1}$) under computer control to restrict stimulation to periods in which the subject controlled the motor unit within acceptable limits (usually $\pm$ 2 Hz) of the target rate for two consecutive inter-pulse intervals (cf. Nordstrom et al., 1995).

Stimulus timing was incremented on successive trials in 1- or 2-ms steps with respect to the last discharge time of the motor unit. The number of stimuli delivered per block of trials (commonly 40-50) varied depending on the target motor unit mean interspike interval (ISI) (see Nordstrom et al., 1995). This ensured that stimuli were evenly dispersed within the motor unit mean ISI.

Separate blocks of trials were performed at various TMS intensities, and with stimulation of the contralateral and ipsilateral hemispheres. The number of blocks of trials performed for each motor unit depended on the continued ability to discriminate the motor unit activity from other motor units.

For many motor units a late increase in the probability of motor unit discharge was observed, approximately 50 ms after the stimulus. For two motor units this was examined in more detail, with the aim of determining whether this was a true "excitation" of the motor unit, and whether the motor unit fired both at a short latency and then again at long latency in response to the same stimulus. To accomplish this, the stimulus paradigm was changed so that TMS was delivered at a fixed time during the motor unit ISI. Subjects were instructed to activate masseter so that the motor unit discharged at approximately 12 Hz, giving an ISI of around 80 ms. TMS was delivered 65 ms after the preceding motor unit discharge, when the two preceding ISIs were within ±2 Hz of the target firing level. This ensured that in most trials the compound excitatory post synaptic potentials (EPSPs) induced in the motoneuron by the TMS would occur when the motoneuron membrane potential was approaching threshold, and thus be more likely to cause the motoneuron to discharge an action potential at short-latency on each trial.

3.2.3 Data Analysis

Motor unit action potential discrimination for all analyses was achieved off-line from the taped records with a computer-based discrimination system that used a template-matching algorithm (SPS-8701). Discharge times of the motor unit were measured with a resolution of either 25 or 100 μs. Motor unit discharge times were referenced to the onset of the motor unit waveform, to minimise the effect of waveform shape on the latency measurements. On some occasions, motor units other than the motor unit controlled by subject feedback were identified in records from the same electrode, and these data were also analysed, provided discrimination accuracy was acceptable. Great care was taken to resolve superimpositions and discrimination accuracy using iterative analysis of unmatched spikes, so that discrimination accuracy approached 100%.

The peri-stimulus time histogram (PSTH) and cumulative sum (CUSUM, Ellaway, 1978) of motor unit discharges were constructed for each run. The criterion for accepting that TMS produced a short-latency excitation was that the average of 2 consecutive 1-ms bins within the 5-15 ms epoch had a mean bin-count greater than three times the mean value in the pre-stimulus epoch. Bin-widths of 1 ms were used in the first instance to assess response probability, which was calculated as the number of counts in the 5-15 ms bins expressed as a percentage of the total number of stimuli. Measurements of response latency and duration were performed on data sorted into 0.2 ms bins. The spike trains were also displayed as trial-by-trial rasters. In this presentation each row in the raster represents a single trial, and the dots represent the discharge time of the motor unit with respect to the stimulus. Plots of instantaneous ISI *vs.* the peri-stimulus discharge time

were also constructed to assess whether peaks in the PSTH or CUSUMs were due to excitatory (shortened ISIs) or inhibitory events (lengthened ISIs) (Poliakov et al., 1994).

Paired t-tests (α=0.05) were used to compare the onset time of the short-latency excitation evoked in a motor unit by contralateral and ipsilateral TMS. To determine the effect of TMS intensity on the excitatory response, motor units tested with at least two contralateral TMS intensities, differing by at least 5% maximum stimulator output, were used to compare the response probability and onset latency for the lowest and the highest TMS intensities using paired t-tests (α=0.05).

3.3 Results

3.3.1 Patterns of masseter single motor unit responses to TMS

Figure 3.1 shows the most common pattern of responses in a masseter single motor unit to focal TMS applied to each hemisphere. Contralateral TMS produced an increased firing probability at short latency, ~7 ms in this case. This was followed by a period of decreased firing probability (silent period). The size of the short-latency excitatory response and the duration of the silent period (SP) tended to increase with increasing stimulus strength. In this example, contralateral TMS at the lower stimulus intensities also resulted in an increased discharge probability beginning approximately 50 ms after the stimulus, which disappeared at the highest TMS intensity. This "late" increase in firing probability was evident in a number of motor units following both contralateral and ipsilateral TMS, and was analysed further in two motor units (referred to later in section

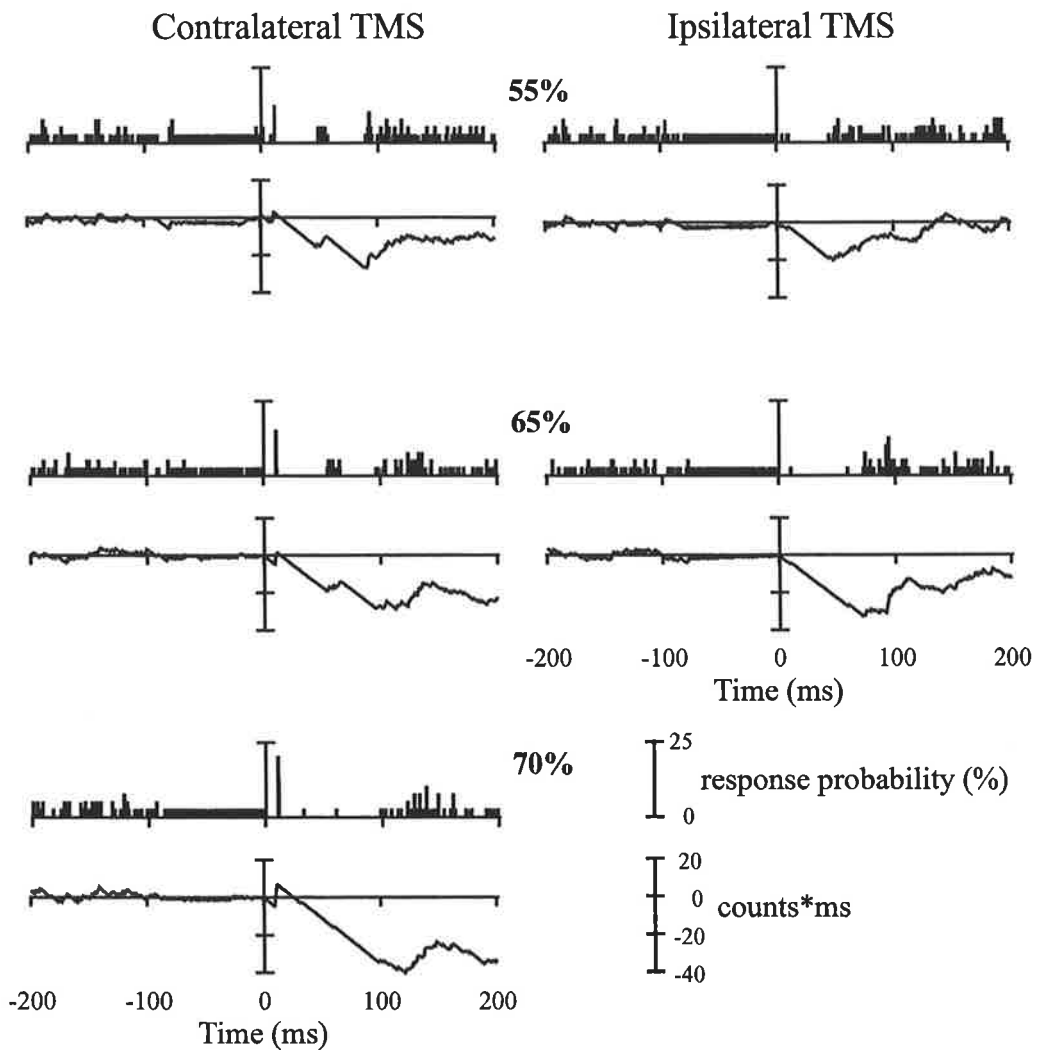

Figure 3.1

Responses of a masseter single motor unit to focal TMS applied to either hemisphere. TMS was given at time 0. The PSTHs and CUSUMs show that this motor unit was excited at ~ 7 ms latency by stimulation of the contralateral motor cortex at three different TMS intensities (55%, 65% and 70% of maximal stimulator output). Response probability increased, with increasing TMS intensity, from 13.5% to 30%. A silent period followed the excitation, which was interrupted by a period of increased firing probability at around 50 ms, at the two lower stimulus strengths. Stimulation of the ipsilateral hemisphere at TMS intensities of 55% and 65% produced a silent period lasting 50-70 ms without short latency excitation.

3.3.2). No short-latency excitatory response was evoked in this motor unit by ipsilateral TMS, rather a clear reduction in motor unit discharge probability until 50-70 ms after the stimulus. The ipsilateral SP also tended to lengthen with increasing TMS intensity.

While 26 of 30 (87%) motor units showed a short-latency excitation with contralateral TMS, only 4 of 16 (25%) masseter motor units displayed short-latency excitation with ipsilateral TMS (Table 3.1). Figure 3.2 shows an example of a masseter single motor unit that was excited at short latency by both contralateral and ipsilateral TMS. Three stimulus intensities were tested for each side. TMS of either hemisphere produced a short-latency increase in the discharge probability of the motor unit. For this motor unit the threshold for an excitatory response was lower for ipsilateral TMS than for contralateral TMS.

For 2 of the 4 masseter motor units excited by ipsilateral TMS, further increases in TMS intensity abolished the excitation and produced inhibition of the unit. An example is shown in Figure 3.3. The motor unit was tested with four intensities of contralateral TMS, and the short-latency excitatory response probability increased with increasing TMS intensity from 14% at 60% TMS to 20% at 70% TMS. The same motor unit was tested with ipsilateral TMS at 2 intensities. At the lower stimulus strength, the motor unit had an increased firing probability (response probability 10%) at a latency of ~6 ms following TMS, which was followed by a SP of around 75 ms. At the higher TMS intensity the motor unit was inhibited for a period of 75 ms, with no excitation.

	Number of SMUs tested	MU response No. (%)			Latency of excitatory response
		+	nil	-	
Contralateral TMS	30	26 (86.7%)	2 (6.7%)	2 (6.7%)	7.0 ± 0.3 ms (n=26)
		+	nil	-	
Ipsilateral TMS	16	4 (25%)	3 (19%)	9 (56%)	6.7 ± 0.6 ms (n=4)

+, excitation; nil, no effect; - , inhibition

Table 3.1

Summary of responses of masseter motor units to focal TMS applied to either hemisphere. Latency data are mean ± se.

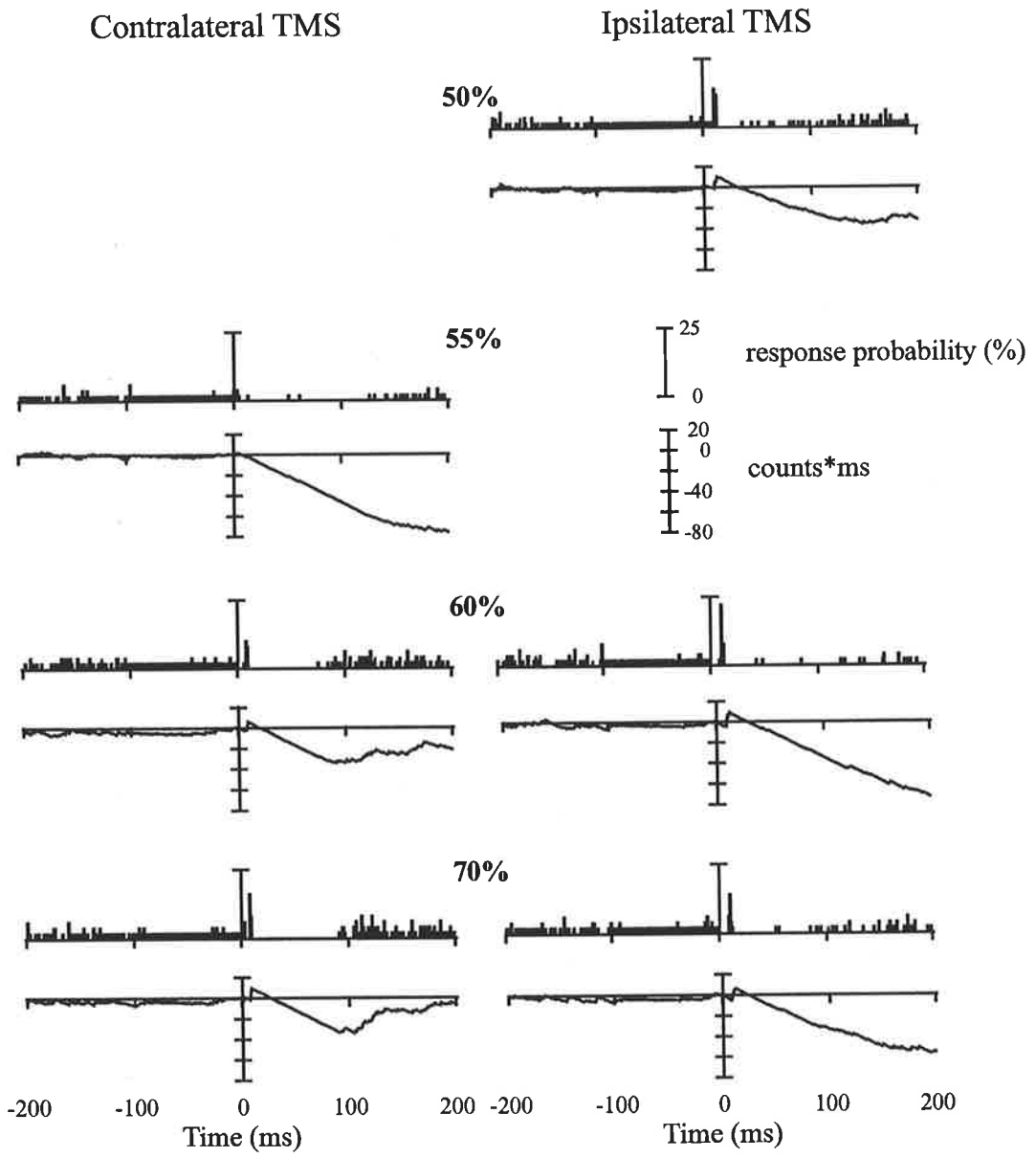

Figure 3.2

Excitation of a masseter single motor unit by TMS applied to either hemisphere. TMS was given at time 0. The PSTHs and CUSUMs show that this unit was excited at a corticobulbar latency by TMS of the contralateral motor cortex at 55%, 60% and 70% of maximal stimulator output and the ipsilateral motor cortex at 50%, 60% and 70% of maximal stimulator output. Threshold for excitation was lower with ipsilateral stimulation than contralateral stimulation for this motor unit. Response probability was higher with ipsilateral stimulation (26% compared with 18% at 65% TMS; 23% compared with 21% at 70% TMS).

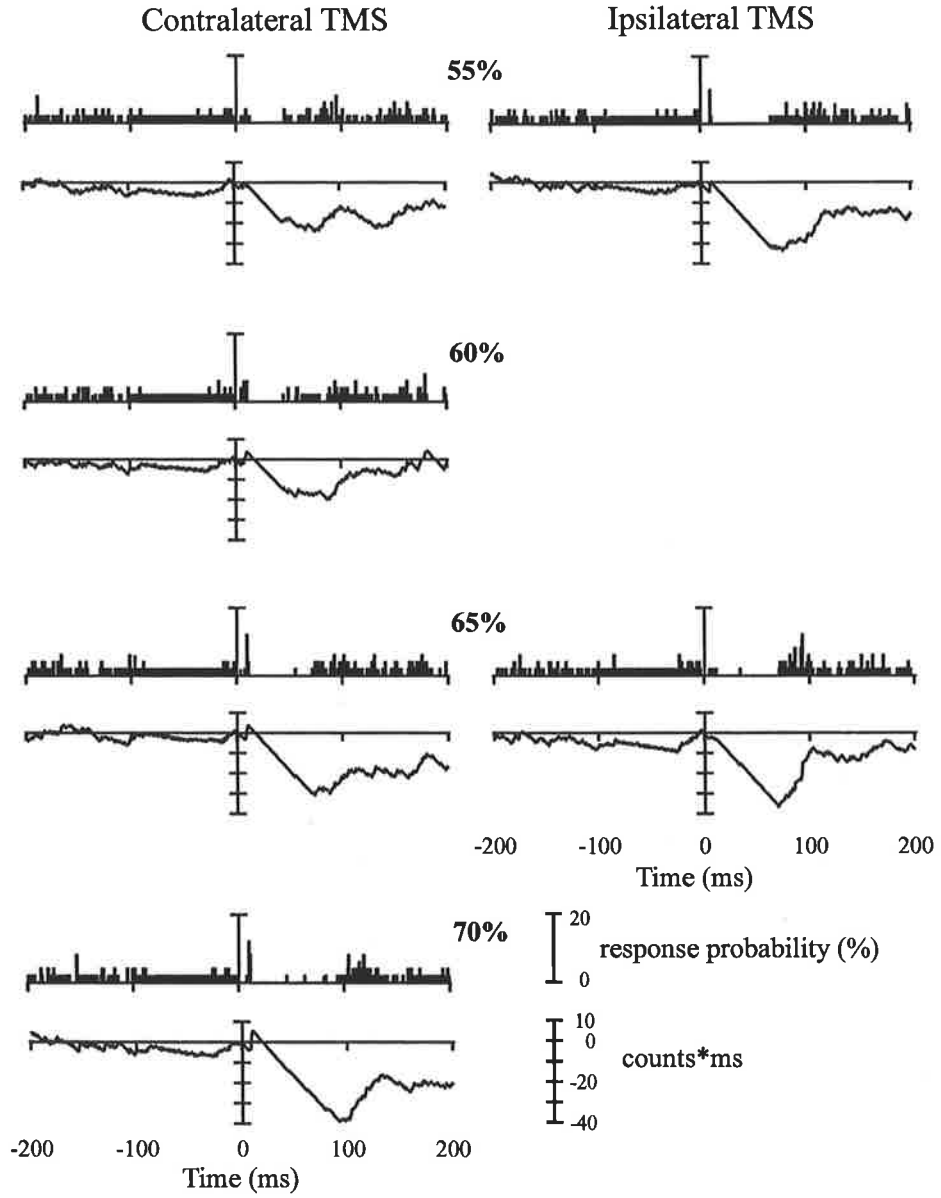

Figure 3.3

Varying stimulus-response characteristics of a single motor unit in masseter with contralateral and ipsilateral TMS. TMS was given at time 0. The responses of this motor unit to different TMS intensities of the contralateral or ipsilateral motor cortex are presented as PSTHs and CUSUMs. The motor unit was excited at short latency in a stimulus-dependent manner by contralateral TMS. The excitation was followed by a silent period, which became longer at higher intensities. Ipsilateral TMS produced a short latency excitatory response followed by a silent period at a low TMS intensity (55%), but suppression of firing without excitation at the higher TMS intensity (65%). An increased firing probability was observed at the end of the silent period after both contralateral and ipsilateral TMS.

The pattern of responses are summarised in Table 3.1 for the masseter motor units studied. With contralateral TMS, 26 of 30 (87%) were excited, 2 of 30 (7%) did not respond at the intensities tested (which were suprathreshold for whole muscle responses) and 2 of 30 (7%) had reduced firing probabilities without a preceding excitation. With ipsilateral TMS, 4 of 16 motor units (25%) showed short-latency excitation. Firing probability was reduced in 9 of 16 (56%) without a preceding excitation, and there was no response in 3 of 16 motor units (19%) despite a MEP evident in the surface EMG.

The nature of the short-latency excitation following TMS was analysed from the PSTH using 0.2 ms bins. Figure 3.4 shows typical examples of the excitatory response to contralateral TMS from 8 motor units, and an excitatory response to ipsilateral TMS in 3 of these same units. The excitation consisted of a single peak, with a duration of 1.5 ± 0.2 ms. The mean onset latency of the excitation in motor units following contralateral TMS was 7.0 ± 0.3 ms (Table 3.1). For the four motor units that produced an excitatory response to both contralateral and ipsilateral TMS, the latency of the response was slightly longer with ipsilateral stimulation, but the differences were not significant (contralateral 6.0 ± 0.2 ms *vs.* ipsilateral 6.7 ± 0.6 ms; paired t-test $p>0.05$). The duration of the peaks in these four units was the same with contralateral and ipsilateral TMS (contralateral 1.4 ± 0.3 ms *vs* ipsilateral 1.5 ± 0.3 ms, paired t-test $p>0.05$).

Thirteen motor units were tested with a range of contralateral TMS intensities, differing by at least 5% of maximal stimulator output. MEP latency was not influenced by increasing stimulus intensity; with weak TMS (48 ± 2% maximal stimulator output)

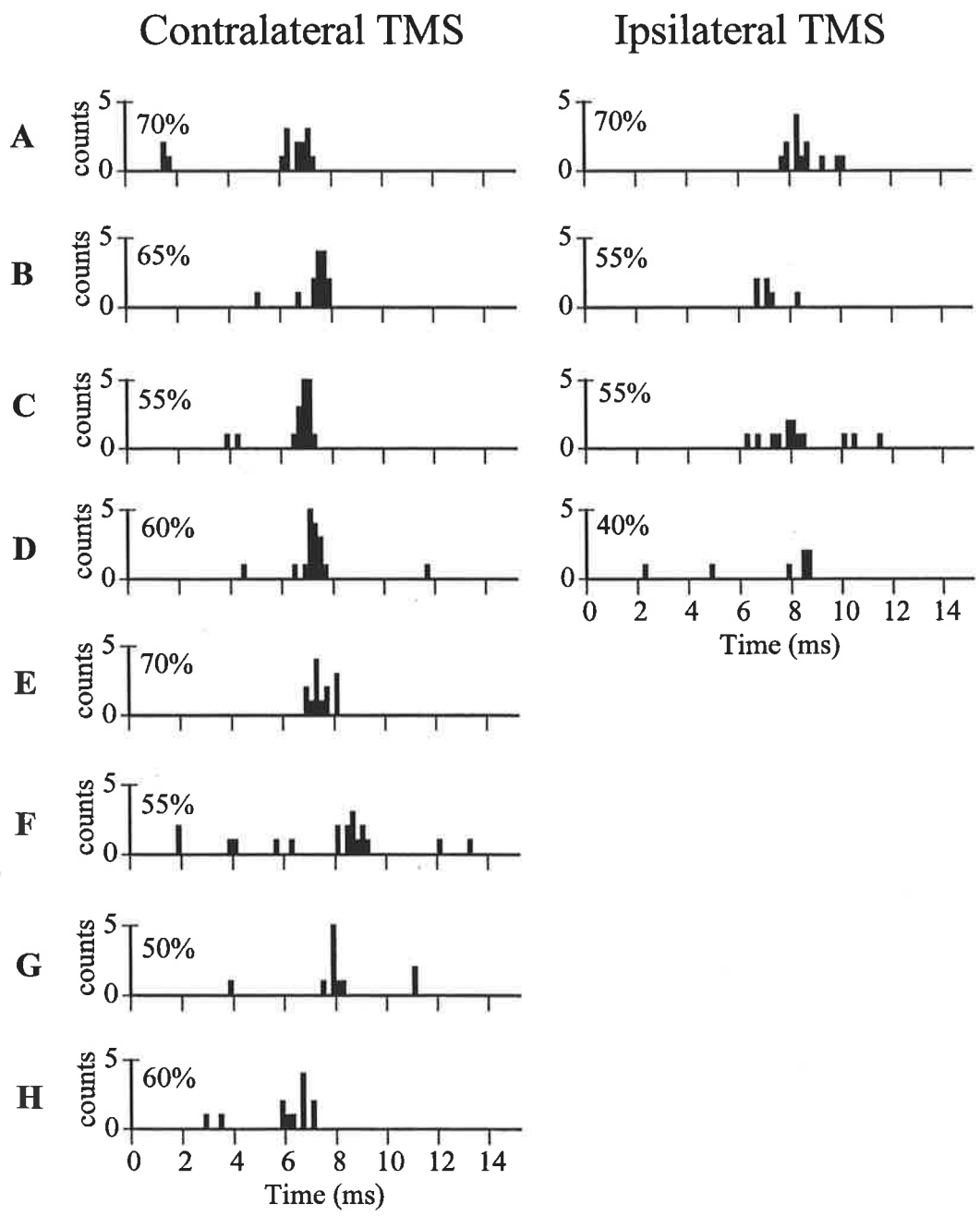

Figure 3.4

Analysis of the peaks of short-latency increased discharge probability induced by contralateral and ipsilateral TMS. TMS was delivered at time 0. PSTHs are presented with 0.2 ms bins for 8 different motor units (A-H). Responses are shown to contralateral TMS (all units) and ipsilateral TMS (A-D). TMS intensity is indicated as percentage of maximal stimulator output. The short latency excitation following TMS consisted of a single peak of activity, usually less than 2 ms duration.

response latency was 6.6 ± 0.4 ms, and with strong TMS (61 ± 2% maximal stimulator output) it was 6.7 ± 0.4 ms (paired t-tests, $p>0.05$). Response probability was significantly increased at the higher TMS intensities (15.4 ± 2.1% with weak TMS and 23.1 ± 2.2 % with strong TMS; paired t-test, $p<0.05$).

3.3.2 "Late" increase in motor unit firing probability induced by TMS

Figure 3.5 shows the PSTH and raster plots from a motor unit tested with TMS delivered at a fixed interval of 65 ms following the preceding discharge, while the subject controlled the mean ISI of the motor unit at 80 ms. The PSTHs demonstrate a brief, short-latency excitation, which increased in size with increasing TMS intensity. At the two lower TMS intensities an increase in firing probability is evident ~50 ms following the stimulus in the PSTH (also evident in Figure 3.1 and 3.2.) The raster plots show that the ISI following TMS was either shortened by the TMS (resulting in the peak at 6 ms latency) or it was lengthened (resulting in the peak at 50 ms latency). On no occasion did the motor unit fire at both 6 ms and at 50 ms following TMS in the same trial. The peak in the PSTH at 6 ms results from an excitation of the motor unit, as evidenced by the shortened ISI. The peak in the PSTH at 50 ms following TMS is not caused by excitation of the motor unit, as the ISI was lengthened in these trials. The peak at 50 ms is therefore a rebound from a period of inhibition (or disfacilitation) of the motor unit.

3.3.3 Slowing of masseter motor unit discharge by TMS

Additional analyses were performed to examine the reduced motor unit discharge probability which followed TMS. Figure 3.6 shows a motor unit for which contralateral

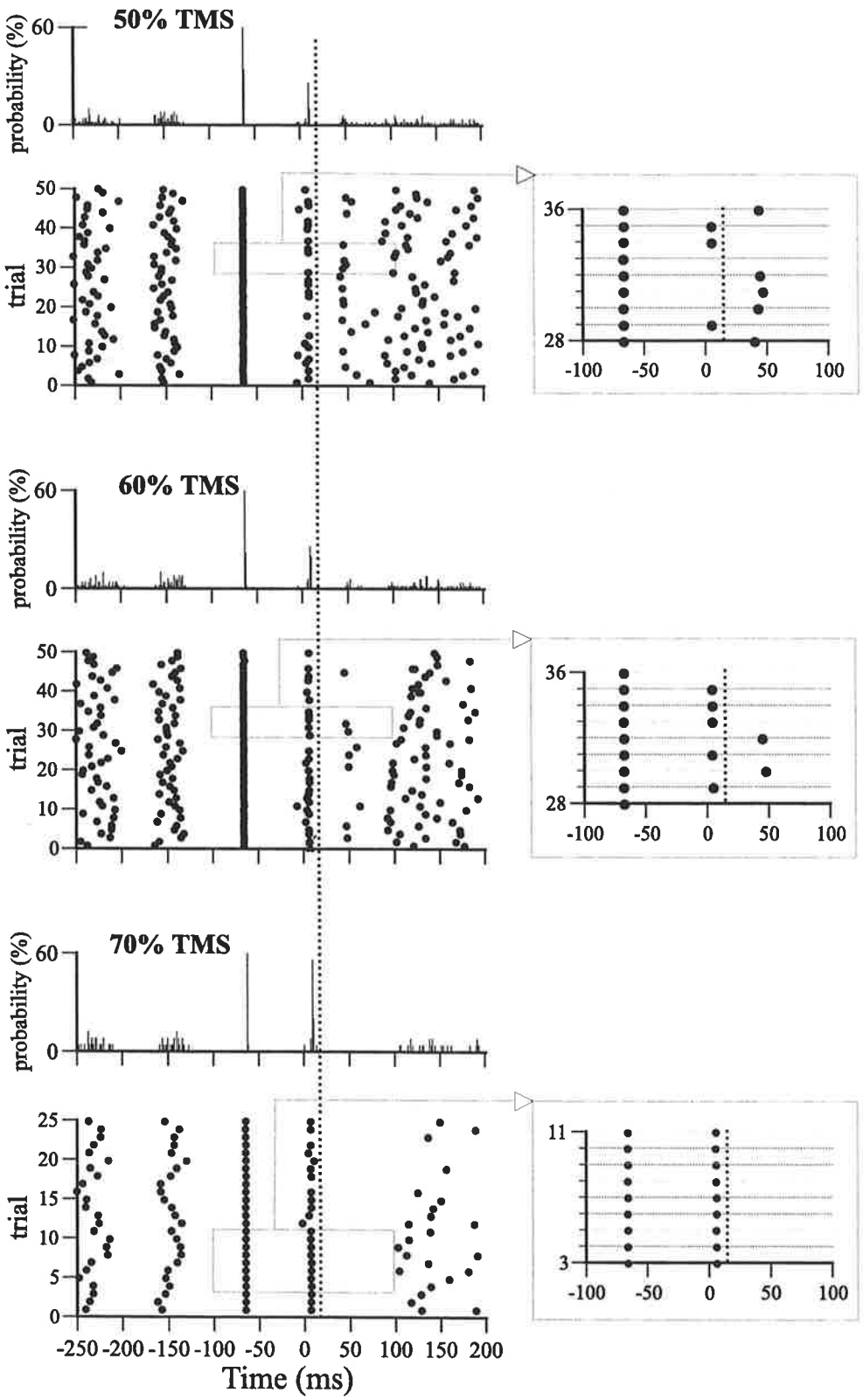

Figure 3.5

Responses of a masseter single motor unit to focal TMS applied to the contralateral hemisphere at a fixed interval during the ISI. PSTHs (1 ms bins) are shown (left

Figure 3.5 cont.

panel) for the three TMS intensities (50%, 60% and 70%). Stimulus at time 0. The large peak in the PSTH at –65 ms has been truncated and is a consequence of the stimulus paradigm. The vertical dotted line at +15 ms indicates the expected time of discharge of the motor unit in the absence of a stimulus. A brief, short latency excitatory peak was observed at ~6 ms, which increased in size with increasing stimulus strength. A small second peak at ~50 ms was also observed in the PSTH with 50% and 60% TMS intensity. The raster plots for all stimuli are shown (left), and on an expanded time scale for 10 successive stimuli (right). Note only 25 stimuli were given with 70% TMS intensity. Following TMS the motor unit discharge was either advanced from the expected time of arrival (dotted line) to discharge at ~6 ms, or it was delayed until ~50 ms. At no time did the motor unit fire at both 6 ms and 50 ms in a single trial. As the stimulus intensity increased, the motor unit tended to discharge at 6 ms in most trials, and therefore the peak at 50 ms disappeared.

TMS produced an excitation followed by a SP of ~50-70 ms. At the lowest stimulus strength, contralateral TMS did not produce excitation of the motor unit, but a SP was still observed. The plots of ISI vs time indicate that the ISI preceding the motor unit discharge at the end of the SP (beyond 100 ms) is much longer than the ISIs observed in the pre-stimulus period. This indicates that the SP was due to a delay in motor unit firing induced by the TMS. Ipsilateral TMS produced only slowing of the motor unit discharge, with no excitation observed at any TMS intensity.

3.4 Discussion

The present study is the first to examine the responses of single motor units in human masseter to transcranial magnetic stimulation. The main finding of this study is that the low threshold motor units in masseter receive predominantly excitatory projections from the contralateral motor cortex, and most do not receive excitatory projections from the ipsilateral hemisphere. A small percentage of masseter motor units, however, are excited at short latency by both the ipsilateral and contralateral motor cortex. TMS produces a single excitatory volley in the descending corticotrigeminal pathway resulting in a single brief excitatory peak in the PSTH of a masseter motor unit. The features of the PSTH peak suggest it is produced by D-wave activation of the cortical neurons and subsequent monosynaptic compound EPSP in the masseter motoneurons.

3.4.1 Corticomotoneuronal projections to human masseter

The mean onset latency of the short latency excitation elicited in the active masseter motor units by focal TMS was 6.8 ± 0.3 ms in the contralateral muscles and 6.5 ± 0.4 ms

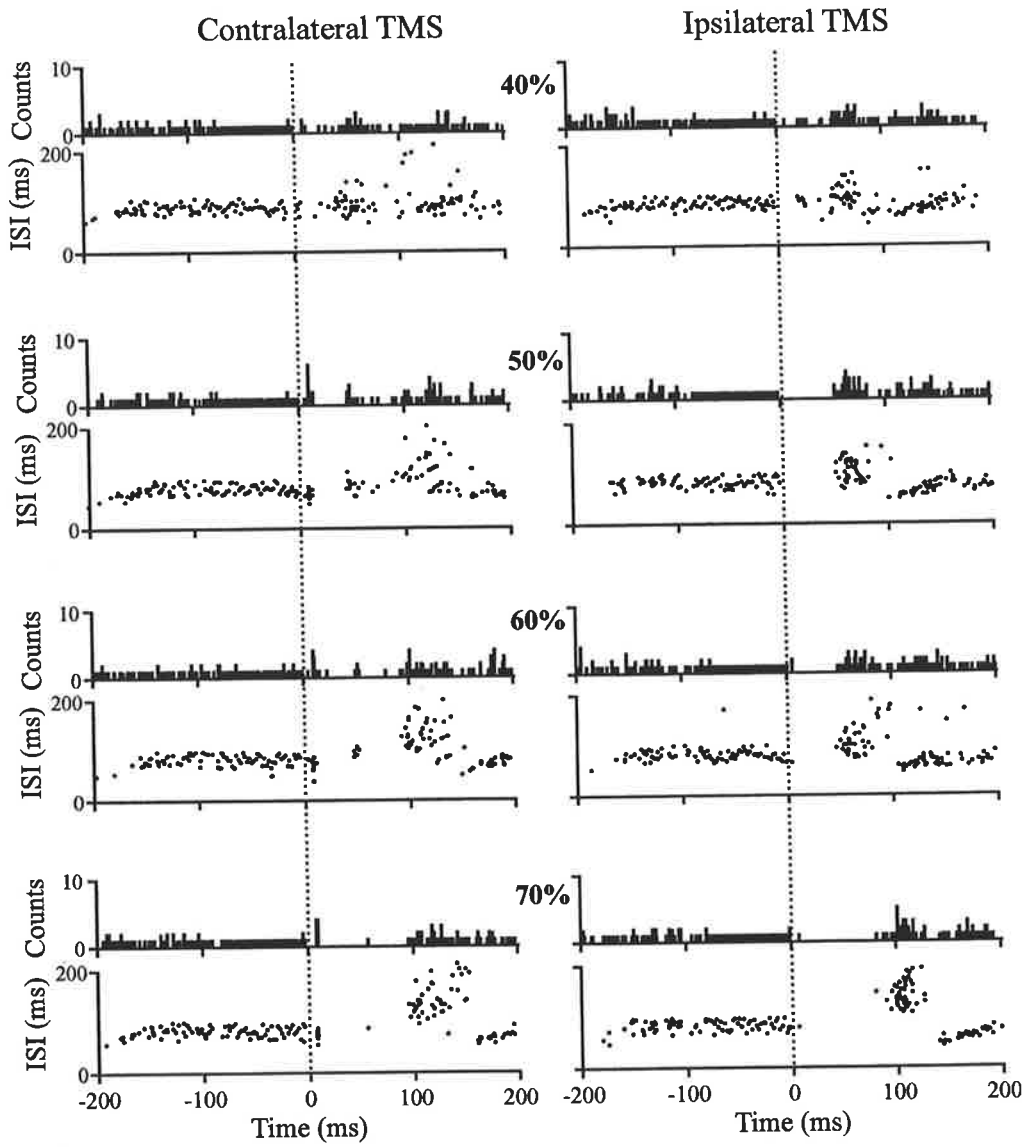

Figure 3.6

Delayed discharge of a masseter single motor unit following focal TMS applied to either hemisphere. The PSTHs show that this motor unit was excited at short latency by stimulation of the contralateral motor cortex at four different TMS intensities (40%, 50%, 60% and 70% of maximal stimulator output). A silent period followed the excitation, which was interrupted by a late peak of increased discharge probability at around 50 ms. With ipsilateral TMS there was no short-latency excitation, but a silent period and late peak at 50 ms. Silent period duration increased with stronger TMS applied to either hemisphere. The plots of ISI *vs* peristimulus time indicate that the peak at 50 ms is due to delayed discharge of the motor unit, as the ISI is longer here than in the pre-stimulus period. The late peak at 50 ms is therefore due to a realignment of spikes following inhibition (or disfacilitation), rather than an excitation of the motor unit.

in the ipsilateral muscles. These values are within the range of latencies (5.5 - 8.9 ms) reported in previous studies for masseter MEPs using surface EMG (Cruccu et al., 1989; Macaluso et al., 1990; Carr et al., 1994; McMillan et al., 1998a, Turk et al. 1994; Butler et al., 2001) and are consistent with conduction via a fast corticobulbar projection. Cruccu et al. (1989) calculated that the central delay at the trigeminal motoneuron synapse(s) was between 1.1 and 1.4 ms, leaving time for no more than two synapses. These authors argued that since the duration and latency variability of masseter MEPs were even shorter than for active hand muscles, which are served by direct corticomotoneuronal connections, that at least some of the corticobulbar fibres project directly onto trigeminal motoneurons.

The present study provides further evidence for the monosynaptic nature of the corticotrigeminal cells activated by TMS. TMS produced excitatory peaks in the PSTH of masseter motor units that were brief (1.5 ± 0.2 ms following contralateral TMS, 1.5 ± 0.3 ms following ipsilateral TMS; see Figure 3.4), with temporal dispersion comparable with peaks in published data from motor units of intrinsic hand muscles, which are known to have strong corticomotoneuronal projections (cf. Day et al., 1989; Boniface et al., 1991; Schubert et al., 1993). For example, the duration of D-wave peaks following anodal stimulation is 1.5 ± 0.4 ms in FDI (Day et al., 1989). Further, the duration of peaks in the masseter PSTH following TMS is comparable to that seen in masseter motor units with H-reflex testing (see Figure 3 in Scutter et al., 1997)

3.4.2 The nature of the excitatory response to TMS induced in masseter motor units

Figure 3.4 shows that excitatory peaks in high resolution PSTHs (0.2 ms bins) are different in masseter from those reported for muscles of the hand using TMS. It is well established that TMS produces multiple peaks of increased short latency firing probability in the PSTH from single intrinsic hand muscle motor units (Mills, 1988; Day et al., 1989; Boniface et al., 1991; Palmer and Ashby, 1992a; Schubert et al., 1993; Sakai et al., 1997, Hanajima et al., 1998). This is due to the arrival at the motoneurons of a sequence of descending impulses, usually referred to as D-waves ("direct activation of CM cells") and I-waves ("indirect activation of CM cells"). Different I-waves are preferentially activated according to the direction of current flow induced in the brain by TMS (Sakai et al., 1997), and are differentially modulated by cortical inhibitory systems (Hanajima et al., 1998). This suggests that TMS activates a number of different cortical structures, which synapse onto CM cells innervating hand muscles, thus producing excitation at slightly different latencies, in the hand muscle. In contrast, it can be seen in Figure 3.4 that the response to TMS in masseter consists of a single peak of excitation. To confirm unambiguously that this response is a D-wave, it would be necessary to compare its latency to that obtained with transcranial electrical stimulation (TES), thought to produce D-waves in corticomotoneuronal cells (Day et al., 1987b). Due to the unpleasant nature of TES, it was not performed in the present study. However, studies that have compared the masseter response latencies in the surface EMG to TMS and TES report little difference in response latencies (Cruccu et al., 1989; Guggisberg et al., 2001). This is in contrast to what has been reported in the hand, where differences of up to 2 ms were found for a motor unit activated with TES and TMS (Hess et al., 1987; Day et al., 1989).

Guggisberg at al (2001) suggested that the presynaptic projections to pyramidal cells of the masticatory muscles are less abundant than in hand muscles, and therefore less accessible to trans-synaptic stimulation. It seems likely, therefore, that the single peak of excitation in the masseter PSTH is due to direct activation of the corticomotoneuronal cells, producing a D-wave.

In hand muscles, an increase in TMS intensity decreases the latency of the response. It is thought that at near-threshold TMS intensities with the "preferred" coil orientation, TMS evokes only I-waves in the corticospinal cells innervating these muscles, and as the TMS intensity increases, D-waves begin to be evoked (see Mills, 1999). In the present study moderate increases in TMS intensity did not affect the response latency in masseter, even though response probability increased. This is in accordance with the suggestion that TMS evokes only D-waves in the CM cells innervating masseter, and that the site of stimulation did not move deeper (for example distant nodes of Ranvier on the CM cell axon) with TMS at these intensities.

3.4.3 The late increase in discharge probability

In addition to the short-latency response evoked by TMS in hand muscles, a longer latency increase in discharge probability occurs at about 55 ms in the EMG after (or during) the SP with relatively weak stimuli (Garland and Miles, 1997, Mills et al., 1991). A similar peak, also around 50 ms latency, was often seen in the PSTH of masseter motor units during the SP (Figures 3.1, 3.5, 3.6) and after the SP (Figure 3.3, 3.5). The "late" peak in both FDP (Garland and Miles, 1997) and FDI (Mills et al., 1991) is due to shortening of the motor unit ISI, and is not the result of a "rebound" in activity of

motoneurons that are inhibited by the TMS, nor is it the result of the next discharge in a motoneuron in which the spike was evoked at MEP latency in that trial. It is thought that the "second" peak is secondary to an event induced in the muscle, via a long loop reflex (Mills et al., 1991) or through cortical activation of γ (Mills et al., 1991) or β (Garland and Miles, 1997) motoneurons. However, this is not the case for the late increase in firing probability in masseter motor units, as the peak was associated with prolongation of the ISI. The rasters in Figure 3.5 (right) show that the first post-stimulus discharge time of the motoneuron was either advanced by TMS, resulting in a discharge at around 6 ms (shortened ISI), or it was delayed by the TMS, resulting in a discharge at around 50 ms (lengthened ISI). The shortened ISI is unambiguous evidence of an excitation of the motoneuron. The lengthened ISI indicates either inhibition of the motoneuron (e.g. from segmental inhibitory interneurons activated by di- or oligosynaptic effects of the descending volley), or disfacilitation (due to cortical inhibitory processes activated by TMS interrupting ongoing descending drive during the SP, cf. Hallett, 1995).

The peak of increased firing probability seen in the PSTH at 50 ms was therefore a result of an alignment of post-stimulus motor unit discharge following an inhibition (or disfacilitation), not a second wave of excitation. Similarly, the ISI plots in Figure 3.6 indicate that the peak of activity during the SP (with contralateral TMS in this unit) or following the SP (with ipsilateral TMS in this unit) resulted from the motoneuron discharging after an elongation of the ISI. Had the peak been due to excitation, the ISIs at that time would be shortened from the pre-stimulus level. The peak of activity in the PSTH in the middle of, or at the end of the SP, was therefore not due to excitation of the

motoneurons, but rather caused by their segmental inhibition or disfacilitation by interruption of cortical drive. This is discussed further below.

3.4.4 The nature of the inhibitory response to TMS induced in masseter motor units

The main purpose of this study was to examine the pattern of excitation in masseter motor units following focal TMS of the motor cortex. However a SP followed the excitatory response, which was also present even in motor units using a TMS intensity that was below threshold for an excitatory response (Figure 3.5, contralateral TMS, upper trace). Ipsilateral TMS often produced a pronounced silent period, without any excitation at all. Examination of the rasters and ISI plots (Figures 3.5 and 3.6) in the present study indicates that the SP in all these situations resulted from a lengthening of the peri-stimulus ISI. The data of the present study does not distinguish whether the SP in masseter was due to segmental or cortical inhibition, however several possibilities can be excluded, as described below.

At a segmental level motoneuron excitability following TMS may be influenced by 1) Renshaw cell inhibition from motoneuron recurrent collaterals; 2) motoneuron after-hyperpolarisation (AHP) and refractoriness following an action potential; 3) changes in the proprioceptive input produced by the muscle twitch; 4) inhibition induced by a cutaneous, periodontal or auditory reflex; or 5) activation of segmental inhibitory interneurons by the corticofugal volley (see Fuhr et al., 1991; Cantello et al., 1992; Hallett, 1995). Renshaw inhibition can be excluded for the masseter, since motor axon recurrent collaterals and Renshaw inhibitory interneurons are not present in the trigeminal

system (see Taylor, 1990b). Motoneuron AHP and refractoriness can be excluded as a major contributor because the slowing of motor unit discharge could be seen without a discharge in the short-latency excitatory peak on single trials (Figure 3.5), and with TMS that did not produce a short-latency excitation in the motor unit with contralateral (Figure 3.2, 55% and Figure 3.6, 40%) or ipsilateral stimulation (Figures 3.1 and 3.6). Inhibition induced by unloading of muscle spindles in the jaw-closing muscles would occur around 10 ms following the twitch (see Poliakov and Miles, 1994), which in turn occurs 7 ms following the TMS. This latency is too long to account for the earliest components of the inhibition seen in the present study. Similarly, inhibitory periodontal, cutaneous or acoustic reflexes all have a latency too long (at least 14-15 ms) to account for the onset of inhibition here (Meier-Ewert et al., 1974; Cruccu et al., 1986; Türker et al., 1994). An acoustic reflex caused by the sound of the stimulator discharge does not appear to be an important consideration, since subjects wore headphones playing white noise to mask the noise of the stimulator discharge. In addition, preliminary experiments suggested that auditory masking did not alter the masseter SP following TMS.

It is possible that the descending volley in corticobulbar axons activated segmental inhibitory interneurons via di- or oligosynaptic connections that resulted in IPSPs in masseter motoneurons at short latency. Indeed, there is evidence in primates that the corticospinal tract projects to spinal Ia inhibitory interneurons (Jankowska et al., 1976). If such an arrangement does occur in the trigeminal system, it would not use the Ia inhibitory interneuron as this class of interneuron is not found in the trigeminal system (see Luschei and Goldberg, 1981). Inhibitory interneurons that are part of the chewing

pattern generator and under cortical control could be engaged in such a circuit (see Nakamura and Katakura, 1995).

The main evidence in limb muscles that decreased motoneuron excitability is not responsible for the SP following TMS is that for much of the SP the size of induced H-reflexes are not affected (Fuhr et al., 1991). Similarly in facial muscles, the size of blink-like reflexes induced by cutaneous trigeminal stimulation is not affected during the SP induced by TMS, indicating that facial motoneurons (CN VII) are not inhibited (Cruccu et al., 1997a). Similar studies have not been performed for the trigeminally innervated masseter muscles, but would provide direct evidence for or against a segmental inhibition during the masseter SP.

Since changes in motoneuron excitability cannot account for the entire silent period, many researchers have concluded that the SP following TMS in the hand (Inghilleri et al., 1993; Roick et al., 1993; Triggs et al., 1993; Ziemann et al., 1993; Schnitzler and Benecke, 1994; Brasil-Neto et al., 1995) and cranial muscles (Werhahn et al., 1995; Cruccu et al., 1997a) is, at least partly, of cortical origin. While there have been no in-depth studies of the TMS-evoked SP in masseter, there are several other lines of evidence to support a cortical origin for the SP in other muscles. First, patients with lesions of the primary motor cortex who have normal excitatory responses have absent silent periods (Schnitzler and Benecke, 1994). Second, facial muscles (CN VII) have a silent period following TMS which is comparable to the silent period muscles of the limb, despite the fact that facial muscles have few stretch receptors and their motoneurons receive neither reciprocal nor recurrent inhibition (Cruccu et al., 1997a). Finally, the silent period is altered in situations of altered cortical excitability (see Mills, 1999). For example, the TMS evoked

SP is shorter in patients with Parkinson's Disease, and anti-parkinsonian medication has been shown to lengthen the SP, even in normal subjects (Priori et al., 1994). Silent period evoked by TMS is reduced in the masseter muscle of patients with amoytrophic lateral sclerosis (ALS) (Desiato et al., 2002). This is in accordance with results from hand muscles in ALS patients (Siciliano et al., 1999) and presumably reflects a deficit in cortical inhibitory mechanisms in these patients.

A cortically induced silent period could potentially be caused by 1) refractoriness of the CM cells; 2) activation of segmental inhibitory interneurons by CM cells; or 3) activation of intracortical inhibition circuits by the TMS. Refractoriness of the CM neurons is unlikely to be a major factor since these cells are capable of producing trains with inter-spike intervals of only 2-3 ms (see Mills, 1999). As discussed earlier, activation of segmental inhibitory interneurons by the CM cells could not account for the fact that motoneurons are not inhibited during the whole of the SP (Fuhr et al., 1991; Cruccu et al., 1997a). Intracortical inhibition seems the most likely mechanism for a cortically induced silent period. Intracortical inhibitory neurones use γ-Aminobutyric acid (GABA) as their neurotransmitter, and it has been shown that when GABA re-uptake is blocked (enhancing transmission at GABA synapses), the silent period induced by TMS is increased (Werhahn et al., 1999). This has not been tested for masseter, but if the same mechanisms are operating, then a similar result would be expected.

The masseter silent periods observed in the present study are considerably shorter than those reported for hand muscles following TMS (see also Desiato et al., 2002). This may be because the intracortical inhibitory circuits that project onto hand CM cells are stronger

than those that project onto masseter CM cells. To date, no studies have investigated the intracortical inhibitory circuits for masseter. Alternatively, neural structures contributing to the intracortical inhibition may be more accessible for hand muscles than for masseter. Different coil orientations may produce better activation of these circuits, although coil orientation does not appear to affect activation of intracortical inhibitory circuits influencing hand area CM cells (Ziemann et al., 1996; Hanajima et al., 1998). These notions could be tested in further studies.

3.4.5 Functional implications

Studying the corticomotoneuronal input to a single masseter motoneurons allows a much more direct examination of the nature of individual corticomotoneuronal cells than can be obtained using surface EMG. The limitation of this technique, however, is that only low threshold motor units can be studied, since the greater the number of motor units recruited, the more difficult discrimination of a single unit becomes. The information regarding the nature of corticomotoneuronal cells obtained in this study is therefore restricted to low-threshold masseter motor units.

The results obtained in the present study confirm that the majority of the low-threshold motor units tested were excited by TMS of the contralateral hemisphere only, and do not receive excitatory projections from the ipsilateral hemisphere (Table 3.1). This is consistent with the observation in surface EMG studies that the MEP evoked in masseter using focal TMS is larger in the contralateral than the ipsilateral muscle, and that the motor cortex exerts *differential* control over the contralateral and ipsilateral masseter (Chapter 2 and Butler et al., 2001). This organisation of cortical inputs to masseter motor

nuclei would contribute to the capacity for each hemisphere to independently activate the masseter muscles on each side, particularly at low bite forces. This may assist controlled biting on one side when food is held between the teeth.

A small percentage of motor units were excited at short latency by stimulation of the ipsilateral hemisphere. This is consistent with the observation in previous studies, using surface EMG, that bilateral responses are evoked in human masticatory muscles using TMS (Benecke et al., 1988; Cruccu et al., 1989; Macaluso et al., 1990; Carr et al., 1994; Turk et al., 1994; Butler et al., 2001). Given the very small percentage of motor units that were excited by ipsilateral TMS, it seems likely that a greater number of higher threshold motor units are activated by the ipsilateral hemisphere and contribute to the ipsilateral MEP in the surface EMG. It is not possible to determine from the present results whether the ipsilateral CM cells identified here were exclusively ipsilateral, or whether their axons branched to innervate both contralateral and ipsilateral masseter motoneuron pools (evidence for this is presented later in Chapter 6).

3.4.6 Comparison to digastric muscle

A study similar to the present one has been performed by our group for the anterior digastric, a trigeminally innervated jaw-opening muscle (Gooden et al., 1999). Like masseter, the projection from the motor cortex to anterior digastric motoneurons is bilateral, but not symmetric. For digastric, the contralateral projection was stronger than the ipsilateral projection, although the difference was not so pronounced as it is in masseter. All 17 anterior digastric motor units tested were excited at fast corticobulbar latency by contralateral TMS, and 80% were also excited by ipsilateral TMS. Unlike

masseter, the anterior digastric did not have to be active to obtain a response to TMS, suggesting a stronger excitatory cortical projection to digastric than to masseter. Temporal distribution of excitatory peaks in the PSTH following TMS in contralateral digastric suggested that the contralateral projection may involve more than one synapse. This was not found in masseter, where the data suggests a monosynaptic connection from the motor cortex to both ipsilateral and contralateral masseter motoneurons. In only one anterior digastric single motor unit, ipsilateral TMS produced inhibition, while contralateral TMS produced excitation. This suggests that while most low threshold motor units in masseter receive excitation from the contralateral hemisphere only, most low threshold anterior digastric motor units receive excitatory input from both hemispheres. These results may indicate that while the cortex has the capacity to contribute to independent activation of the anterior digastric on each side, this ability is not so pronounced as it is for masseter, at least at low biting strengths.

3.4.7 Conclusion

The present study has investigated the effect of focal contralateral and ipsilateral TMS on single motoneurons innervating masseter. Evidence is presented that the motor cortex has bilateral control over the masseter muscles, but that the control is not symmetrical. For most low threshold motoneurons excitatory input comes only from the contralateral hemisphere, while stimulation of both hemispheres produces inhibition, the nature of which requires further investigation.

CHAPTER 4

SIMULTANEOUS FLUCTUATIONS IN SIZE OF RESPONSES TO FOCAL TMS IN MULTIPLE MUSCLES. I. MUSCLES OF THE UPPER LIMB

4.1 Introduction

A fundamental principle underlying the organisation of the corticomotoneuronal (CM) output appears to be one of multiple muscle control (see Porter and Lemon, 1993). Spike triggered averaging techniques in a variety of hand and forearm muscles have revealed that the majority of CM cells facilitate EMG activity in more than one muscle (Fetz and Cheney, 1980; Kasser and Cheney, 1985; Lemon et al., 1991). This is achieved through divergence of individual CM cells to monosynaptically excite motoneurons of different synergistic muscles (Porter and Lemon, 1993).

Activation of the motor cortex with transcranial magnetic stimulation (TMS) produces motor evoked potentials (MEPs) in target muscles that vary in amplitude from stimulus to stimulus (Amassian et al., 1989; Britton et al., 1991; Brasil-Neto et al., 1992; Kiers et al., 1993). A source of this variability, at least in part, comes from fluctuations in CM cell excitability (Ellaway et al., 1998; Funase et al., 1999). It seems reasonable to assume that if motoneuron pools of two muscles share input from the same CM cells, then the variability in MEP sizes in the two muscles will be correlated. This is the basis of the experiments reported in Chapter 6, where evidence is sought for branched-axon projections from single CM cells to motoneurons innervating left and right masseter

muscles. However, the presence of branched CM projections is not the only factor that may cause correlations in the MEP size of two muscles. Ellaway et al. (1998) revealed a positive correlation in the trial-by-trial fluctuation in size of MEPs obtained from left and right hand muscles at rest with bilateral motor cortex stimulation using TMS. This result is clearly not due to branched CM projections innervating both motoneuron pools, since CM projections to hand muscles are contralateral (Porter and Lemon, 1993). This result is most likely due to changes in cortical excitability that are linked between the two hemispheres. Indeed, MEP size is known to be influenced by rhythmic oscillations in cortical excitability (Rossini et al., 1991), some of which are synchronous in each hemisphere (Nikouline et al., 2001). A number of cortical oscillations which are present at rest are desynchronised with muscle activation (Crone et al., 1998), and so it is not known whether the processes producing the MEP fluctuations at rest also operate when the muscle is activated by voluntary commands.

The aim of the present study was therefore to examine the correlation in trial-by-trial fluctuations in MEP size reported by Ellaway et al. (1998), for muscle pairs within- and between-limbs, under rest and active conditions. It was expected that between-limb correlations in MEP fluctuations would be eliminated by voluntary activation, reflecting the desynchronisation of cortical excitability fluctuations in the two hemispheres. Within-limb correlations in MEP fluctuations were expected to be weaker with voluntary activation, but still evident due to the presence of shared, branched-axon CM inputs to the muscle pairs.

4.2 Methods

A total of 8 healthy subjects (3 females and 5 males, aged from 21 to 43 years) participated in the experiments. None had a history of neurological disorders. Informed consent was obtained prior to the study and experiments were conducted with the approval of the University of Adelaide Human Research Ethics Committee.

4.2.1 Apparatus and recording

4.2.1.1 Electromyography

Surface electromyograms (EMG) of the first dorsal interroseous (FDI), abductor digiti minimi (ADM) and extensor digitorum communis (EDC) muscles of both hands were recorded using self-adhesive gel-filled bipolar silver/silver chloride electrodes. One electrode was placed over the belly of the muscle, and the other on the tendon. Subjects were grounded by a lip-clip electrode (Türker et al., 1988). Surface EMG signals were amplified (1000-2000x) and filtered (bandwidth 5-500 Hz). The EMG for 50 ms preceding the stimulus and 200 ms following was digitised at 1 kHz sampling rate, and stored on computer for later analysis. In addition, EMG from left and right FDI was recorded on a four-channel PCM data recorder (Vetter 400, A.R. Vetter co., Pennsylvania, USA) sampling at 22kHz/channel. The remaining two tape channels were used to record force from either left or right FDI or ADM muscles, depending on the task performed (see section 4.2.2).

4.2.1.2 Transcranial magnetic stimulation

Transcranial magnetic stimulation was achieved using one or two separate magnetic stimulators (Magstim 200), each connected to a figure-of-eight stimulating coil with outer coil diameters of 90 mm. For the single TMS protocol, one coil was placed over the motor cortex contralateral to the test limb. For the dual TMS protocol, the coils were hand-held separately by two individuals over either side of the subject's head. The coils were positioned along the parasagittal plane, with the centre of each coil positioned approximately 5 cm lateral to the vertex. The direction of current induced in the brain under the cross over region of each coil flowed in a lateral to medial direction. These were the same stimulus conditions used by Ellaway et al. (1998), and allowed room for both coils to be positioned on the head without overlap. Stimulus intensity and coil position were adjusted until clear responses were observed in all 6 muscles. TMS resting threshold (T) for left and right FDI was determined as the stimulus intensity producing a 50 μV MEP in 3 out of 5 consecutive stimuli. TMS test intensity was adjusted to produce MEPs in all muscles, and averaged 1.3 T for the 8 subjects.

A preliminary study was performed to examine the effect on MEP size when the two TMS stimulators discharged simultaneously, or at different intervals between 1-5 ms. This was prompted by the findings of Ellaway et al. (1998) that discharging the stimulators simultaneously resulted in smaller MEPs than when either stimulator was discharged independently. They reasoned that the magnetic fields produced by the coils may become distorted due to the close proximity of the coils. Details of the protocol used to examine this issue in the present study are given in section 4.2.2.1. Although no effect on MEP size was found when the simulators were discharged simultaneously, compared

to when they were separated by up to 5 ms (see Figure 4.1), it was decided to use 1 ms separation for the main series of experiments, to facilitate direct comparison with the results obtained by Ellaway et al. (1998).

4.2.1.3 F-waves

Supramaximal stimulation of the ulnar nerve was used to induce F-waves (Magladery and McDougal, 1950) in left and right FDI in 3 subjects. The F-wave predominantly results from antidromic activation of α-motoneurons following maximal stimulation of the nerve, and its amplitude provides a measure of motoneuron excitability (Kimura, 1989). Two self-adhesive silver/silver chloride electrodes were placed on the left and right wrist, over the ulnar nerve. Electric pulses (between 100 and 200 μs duration) were delivered to the ulnar nerve of each arm via the electrodes using separate stimulators (Digitimer models DS7 and D180, Digitimer Limited, Hertfordshire, England).

4.2.2 Protocol

Subjects were seated comfortably with both hands supported in a manipulandum. EMG activity from left and right FDI and ADM muscles was displayed on oscilloscopes in front of the subject. Force transducers were positioned on either side of the hand so that subjects could contract FDI and/or ADM in abduction of the 2nd or 5th digit to a target of 0.5 N. When appropriate, subjects were given visual feedback of force from FDI and ADM on an oscilloscope.

4.2.2.1 Effects of timing of dual TMS on MEP size

Four of the 8 subjects participated in experiments to examine the effect of the timing of dual stimulation on MEP size. Subjects were 2 females and 2 males, aged between 21 and 35 years. For 3 subjects the left motor cortex was designated as the test hemisphere, in the remaining subject the test hemisphere was the right motor cortex. MEPs were recorded from FDI, ADM and EDC in both hands. TMS intensity was determined for each stimulator separately so as to produce a 0.5 – 1 mV signal in the contralateral FDI. Responses to TMS in the test muscles were examined with unilateral TMS applied to the test hemisphere, and when a conditioning stimulus was applied to the opposite hemisphere, 0-5 ms prior to the test TMS. Single or dual TMS were delivered randomly by the computer for a block of 30 trials (<0.02 s^{-1}). Inter-stimulus intervals (ISIs) of 0, 1, 2, 3, 4 and 5 ms were tested in separate blocks. For statistical analysis, an additional control block was performed with the conditioning stimulator switched off. For all conditions, the area of the conditioned MEP in the test muscles was expressed as a percentage of the unconditioned responses.

4.2.2.2 Trial-by-trial fluctuations in MEP size

All 8 subjects participated in these experiments. TMS (n=50, <0.02 s^{-1}) was given in trials of single (hemisphere contralateral to test muscles only) or dual stimulation (both hemispheres, 1ms ISI) during various different contraction tasks. These were:

1. All muscles at rest
2. Activate FDI muscle on test side (0.5 N index finger abduction).
3. Activate FDI muscles on both sides (0.5 N index finger abduction with each hand).

4. Activate both FDI and ADM muscles in test hand (0.5 N abduction performed with 2^{nd} and 5^{th} digit).

The order of the tasks was randomised for each experiment. During activation tasks 3 and 4 the TMS intensity was adjusted for the coil contralateral to the test muscles, so that MEP size in the active muscles were approximately matched to the MEP in the resting muscles (0.5-1mV).

The EMG from all muscles was monitored carefully throughout the experiment to ensure that the subjects were successfully performing the activation tasks required of them. Two subjects were unable to successfully activate FDI and ADM together, and the data from this task, in these subjects were excluded. Care was taken during the experiment to ensure that the muscles not involved in the activation task remained inactive during the task.

4.2.2.3 F-waves

In a separate experimental session, three of the 8 subjects participated in F-wave experiments. Subjects were 2 males and 1 female aged between 25 and 37 years. F-waves were simultaneously produced in left and right FDI. One hundred stimuli were given (in two separate blocks of 50 stimuli) for each condition. Two conditions were tested: a) both FDI muscles at rest

b) Both FDI muscles slightly active (< 5% MVC).

4.2.3 Data Analysis

4.2.3.1 TMS Thresholds

TMS thresholds for left and right FDI were compared using paired t-tests (α=0.05)

4.2.3.2 Effects of timing of dual TMS on MEP size

MEP areas in muscles contralateral to the test hemisphere were measured from the averaged unrectified EMG. For each ISI and the control trial (with the conditioning stimulator turned off) the MEP area from the conditioned trials (dual TMS) was expressed as a percentage of the MEP area obtained from the unconditioned (single TMS) trials. For each muscle, one way analysis of variance (ANOVA; α=0.05) was used to identify differences in the size of the MEPs resulting from the different stimulus conditions.

4.2.3.3 Regression analysis of trial-by-trial variation in size of MEPs elicited in pairs of muscles

Stimulus intensity was adjusted to produce MEPs in all muscles at rest on all trials. TMS intensity was reduced during the active tasks to match the size of the MEP in the active muscle to its value at rest. As a consequence, the MEPs could be smaller in the resting muscles. In these instances, only data from muscles with MEPs on > 75% of trials were used.

The averaged MEP was calculated for the 50 trials, and cursors were used to identify the onset and duration of the MEP. The epoch identified by the cursors was used to measure the area of MEPs for each of the 50 trials contributing to the average. For each muscle,

the mean and standard deviation (SD) of MEP area was calculated for the 50 trials, and expressed as the coefficient of variation (CV; SD/mean) as a measure of the variability in MEP size. One way ANOVA (α=0.05) was used to compare the CV in the three muscles. Student's t-tests (α=0.05) allowed a comparison between CV in resting and active muscles, and the CV when single or dual TMS was used.

Linear regression analysis was used to assess the independence of fluctuations of MEP size on a trial-by-trial basis for all available pairs of muscles. Muscle pairs within the same limb (within-limb comparisons) were examined with both single and dual TMS. Muscle pairs from different limbs (between-limb comparisons) were compared, by necessity, only with dual TMS. Chi-squared analysis was used to compare the incidence of significant correlations when all muscles were at rest compared to when one or both muscles were active. The strength of the correlation in MEP size for each muscle pair was quantified by the coefficient of determination (r^2). Student's t-tests (α=0.05) were used to compare:

1. The mean r^2 values obtained when muscle pairs were at rest and when one or both of the muscles were active.
2. The mean r^2 values for muscle pairs within a limb and muscle pairs between limbs.

Two strict criteria were applied to the data used for regression analyses to ensure that any correlated changes in MEPs for the pair of muscles was not due to factors such as coil movement or parallel changes in the level of voluntary muscle activation over the duration of the block of trials.

a) MEP "time effect" criteria

For each muscle, the mean MEP area from the first 25 stimuli was compared with the mean MEP area resulting from the second 25 stimuli using unpaired t-tests. Data were excluded from further analysis if a significant difference in MEP area was found between the first and second series of 25 stimuli, in both muscles of a pair. Whether the MEPs may have changed over time due to some physiological process, or perhaps due to altered coil position, the non-stationarity would have produced a spurious correlation in the pairwise regression of MEP size. Of the 562 pairs available for regression analysis, 83 were excluded on this basis.

b) Pre-stimulus EMG time effect

Unpaired t-tests were used to compare the level of pre-stimulus EMG in the 50 ms preceding the first 25 stimuli with that of the second 25 stimuli of each block. If the pre-stimulus EMG was significantly different for the first and second series of 25 trials in both muscles, regression analysis was not performed on the data. Six of the 562 pairs available for regression analysis were excluded on this basis.

MEP data from 473 pairs survived the exclusion criteria and were subject to linear regression analysis.

4.2.3.4 Regression analysis of trial by trial variation in size of F-waves simultaneously elicited in left and right FDI

F-wave amplitudes were measured from the unrectified EMG of left and right FDI. The amplitude of the F-wave in the left FDI was compared to that obtained on the right FDI on a trial-by-trial basis using regression analysis.

4.3 Results

4.3.1 Effect of discharging two TMS coils in close proximity

Figure 4.1 shows that there was no significant effect on the MEP amplitude in FDI, ADM or EDC when conditioning TMS was applied to the ipsilateral hemisphere between 0-5 ms prior to the test TMS, and in the control trial with the conditioning stimulator turned off (one way ANOVAs, $p>0.05$).

4.3.2 MEP thresholds

There was no significant difference in the TMS threshold for producing a MEP in relaxed FDI on either side. The threshold for the left hemisphere was 46 ± 3% of maximal stimulator output, and threshold for the right hemisphere was 50 ± 4% (paired t-test, $p>0.05$).

4.3.3 MEP variability

Figure 4.2 shows an example of the variability in MEP size for ten consecutive stimuli, recorded concurrently from left and right FDI in one subject with the dual TMS protocol. Data are shown when the muscles were at rest (left), and when active (right). There was considerable variability of MEP size from trial to trial for each muscle. At rest, there was a clear correlation in size of MEPs in left and right FDI in different trials. This was not evident with both FDI muscles active.

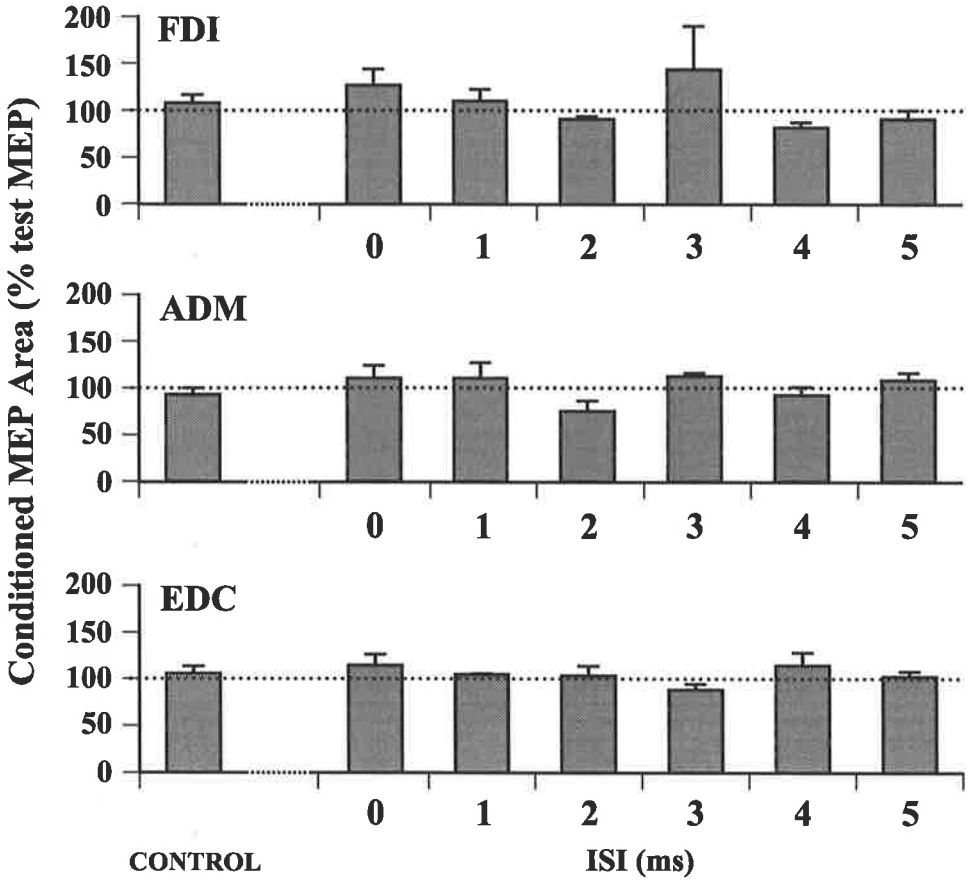

Figure 4.1

Size of MEPs conditioned by discharge of the magnetic stimulator over the ipsilateral motor cortex at different ISIs. Bars are mean (± SE) size of the conditioned MEP normalised to the test MEP. ISI is the delay between discharge of the conditioning TMS (applied to the hemisphere ipsilateral to the test muscles) and the test TMS (applied to the hemisphere contralateral to the test muscles). For Control trials the conditioning magstim was switched off. Conditioning TMS had no significant effect on the MEPs elicited in any muscle (ANOVA, $p>0.05$).

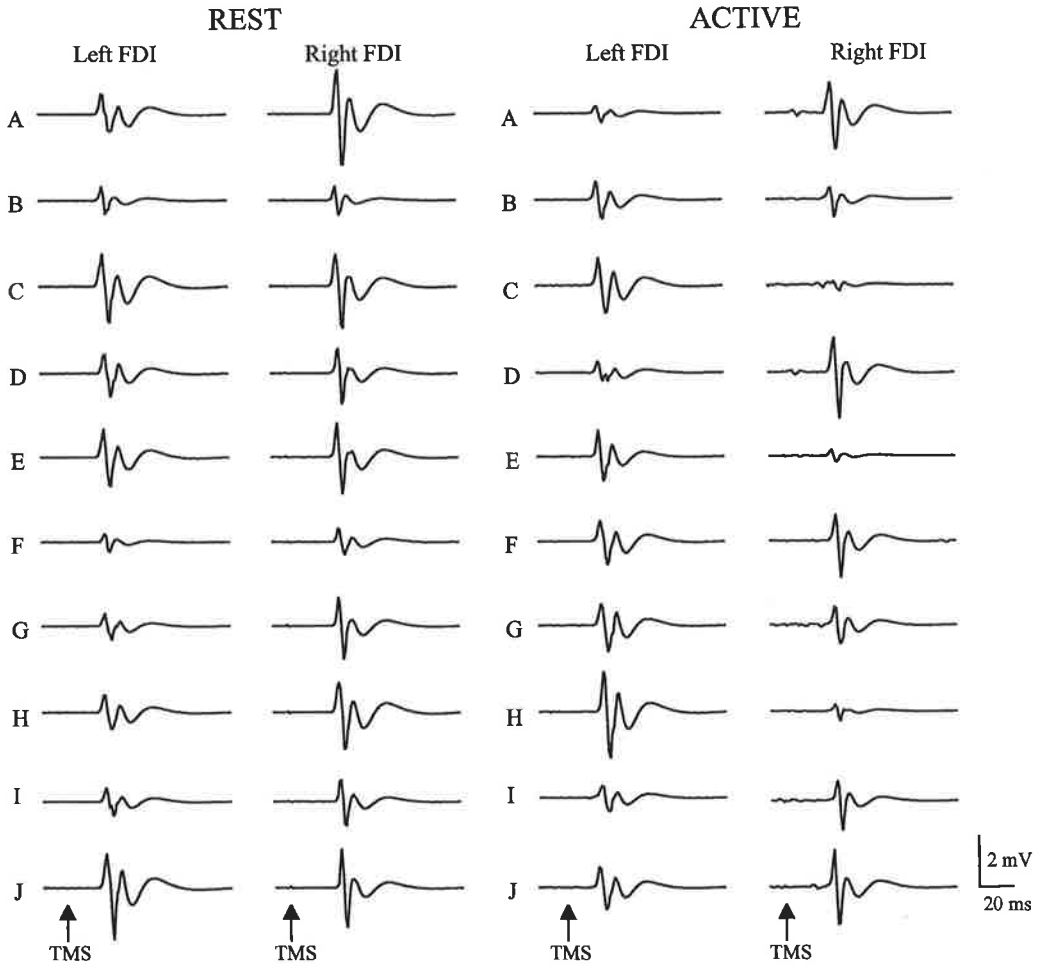

Figure 4.2

Representative examples of MEPs from left and right FDI following dual TMS in one subject, at rest and with both muscles active. Responses to 10 consecutive stimuli (A - J) are shown. Timing of TMS is indicated by the arrow. At rest (LHS), TMS intensity was 41% for right FDI and 45% for the left FDI. With both muscles active (RHS) TMS intensity was 34% for right FDI and 38% for left FDI. Note the trial-by-trial variability in MEP size under both rest and active conditions. At rest, MEP size fluctuated in parallel for left and right FDI, but this was not evident when both muscles were active.

There was considerable trial-to-trial variation in MEP size for all muscles in all subjects. Figure 4.3 shows distribution histograms of the MEP area in six muscles of one subject for 50 consecutive trials with the dual TMS protocol. The degree of variation in MEP area for this subject, as expressed by the coefficient of variation (CV), was 0.40 and 0.91 for left and right FDI, respectively; 0.25 and 0.56 for left and right ADM and 0.32 and 0.37 for left and right EDC.

The exclusion of data showing a MEP time effect ensured that the analysis was not affected by long-term changes in MEP size, such as might be observed if there were changes in the coil position or the level of muscle activation over the course of the trial. Figure 4.4 shows the data from one subject (same data as Figure 4.3) and shows that the MEP variations were similar in the first and second block of 25 trials, and did not show a progressive change with time.

The MEP variance for all muscles at rest and when active is summarised in Table 4.1. In the upper half of the Table the data are separated according to muscle and side of body; there was no difference in variability between sides (t-tests, $p>0.05$). In the lower half of the Table the data are separated according to whether single or dual TMS was used; MEP variability was the same with the two forms of stimulation (t-tests, $p>0.05$). MEP variability differed between the three muscles (one way ANOVA, $p<0.05$), and post-hoc t-tests showed that the variability in EDC was significantly less than the variability in ADM ($p<0.01$). Variability was lower during trials in which the muscles were active, compared to when they were at rest (t-test, $p<0.05$).

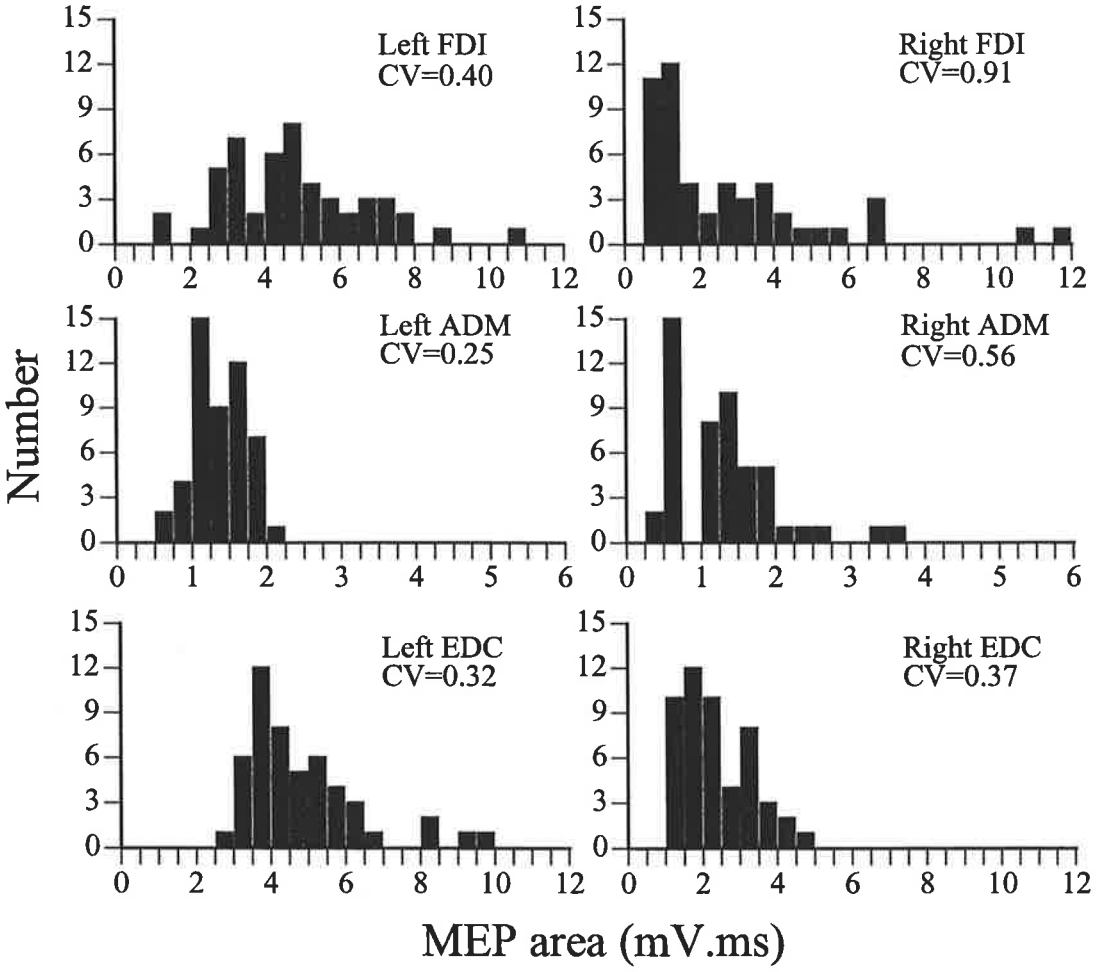

Figure 4.3

Distributions of MEP areas in the left and right FDI, ADM and EDC muscles elicited by 50 consecutive stimuli with the dual TMS protocol in one subject. TMS intensity was 80% for R hemisphere and 74% for L hemisphere. All muscles were at rest. There was considerable variation in the size of the MEPs obtained with the 50 stimuli in all six muscles.

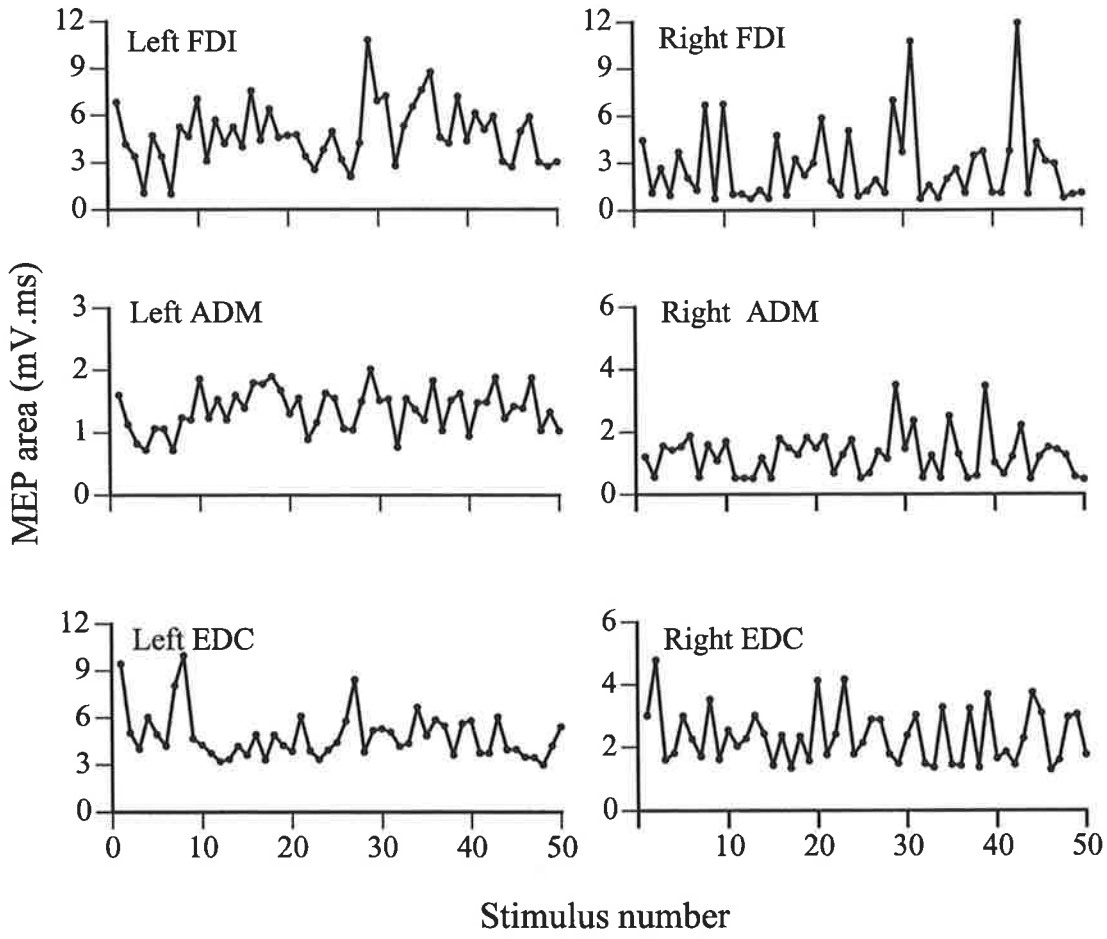

Figure 4.4

Data from one subject showing no progressive change in MEP size over time. For each muscle, MEP area is shown as a function of stimulus number. TMS intensity in this subject was 80% for R hemisphere and 74% for L hemisphere. All muscles were at rest. There was no significant difference in the size of mean MEP areas for stimuli 1-25 *vs.* those obtained with stimuli 26-50 in any muscle (paired t-tests, $p>0.05$). In this example trial-by-trial fluctuation in size of MEPs in FDI and ADM were significantly correlated within the same hand (left, $r^2 = 0.54$, $p<0.001$; right, $r^2 = 0.39$, $p<0.001$), and for homonymous pairs between limbs (FDI, $r^2 = 0.23$, $p<0.001$; ADM, $r^2 = 0.18$, $p<0.01$).

	MEP Variance (CV)			
	Rest		Active	
	LHS	RHS	LHS	RHS
FDI	0.51 ± 0.04	0.52 ± 0.06	0.49 ± 0.10	0.42 ± 0.04
ADM	0.58 ± 0.09	0.64 ± 0.05	n.a.	0.45 ± 0.04
EDC	0.42 ± 0.03	0.37 ± 0.03	n.a.	n.a.
	Unilateral TMS	Bilateral TMS	Unilateral TMS	Bilateral TMS
FDI	0.50 ± 0.08	0.52 ± 0.05	0.32 ± 0.05	0.48 ± 0.05
ADM	0.65 ± 0.05	0.61 ± 0.06	0.47 ± 0.06	0.44 ± 0.06
EDC	0.37 ± 0.03	0.39 ± 0.03	n.a.	n.a.

Table 4.1

Variance in MEP area recorded from left and right FDI, ADM and EDC in all subjects. Data are mean (± s.e.) coefficient of variation of MEP areas for 50 consecutive stimuli at rest (left) and with the muscles active (right). Data pooled from 8 subjects. In the upper half of the Table the data are separated for left (LHS) and right (RHS) limb. In the lower half of the Table the data are arranged according to whether the data were obtained with single or dual TMS. n.a. = data not available.

4.3.4 Trial-by-trial correlations in MEP size

4.3.4.1 Between-limb comparisons

Figure 4.5 shows data from one subject illustrating the co-variation of MEP size for pairs of muscles in left and right upper limb using dual TMS with all muscles at rest. In this subject there were significant positive correlations in two out of the three homonymous muscle pairs (left and right FDI, $p<0.001$; and left and right EDC, $p<0.001$) and in three of the six heteronymous muscles pairs (right FDI and left EDC, $p=0.01$; right EDC and left FDI, $p<0.01$; Right EDC and left ADM, $p<0.05$). Figure 4.6 contains data from the same subject as Figure 4.5, obtained with right FDI active during index finger abduction. In this subject none of the pairwise comparisons of MEP size between muscles of different limbs were significant. This pattern of reduced MEP correlations occurred whether one or both muscles of the regression were active. Figure 4.7 shows data from left and right FDI in one subject, showing a significant correlation in MEP size at rest (A, $r^2=0.19$, $p<0.002$), and the disappearance of this relationship when either one (B) or both (C) of the muscles were activated.

The data in Figures 4.5, 4.6 and 4.7 are representative of those obtained in all subjects. Table 4.2 summarises the between limb comparisons during the various activation tasks. When all muscles were at rest, there were significant positive correlations in 20 of 36 comparisons (56%) of homonymous muscle pairs between limbs (mean $r^2 = 0.16 \pm 0.02$), and in 33 of 59 between-limb comparisons (56%) of heteronymous muscle pairs (mean $r^2 = 0.13 \pm 0.02$). When both muscles of the pair were at rest, but other muscles in the hand were being activated, the incidence of significance was reduced for homonymous muscle

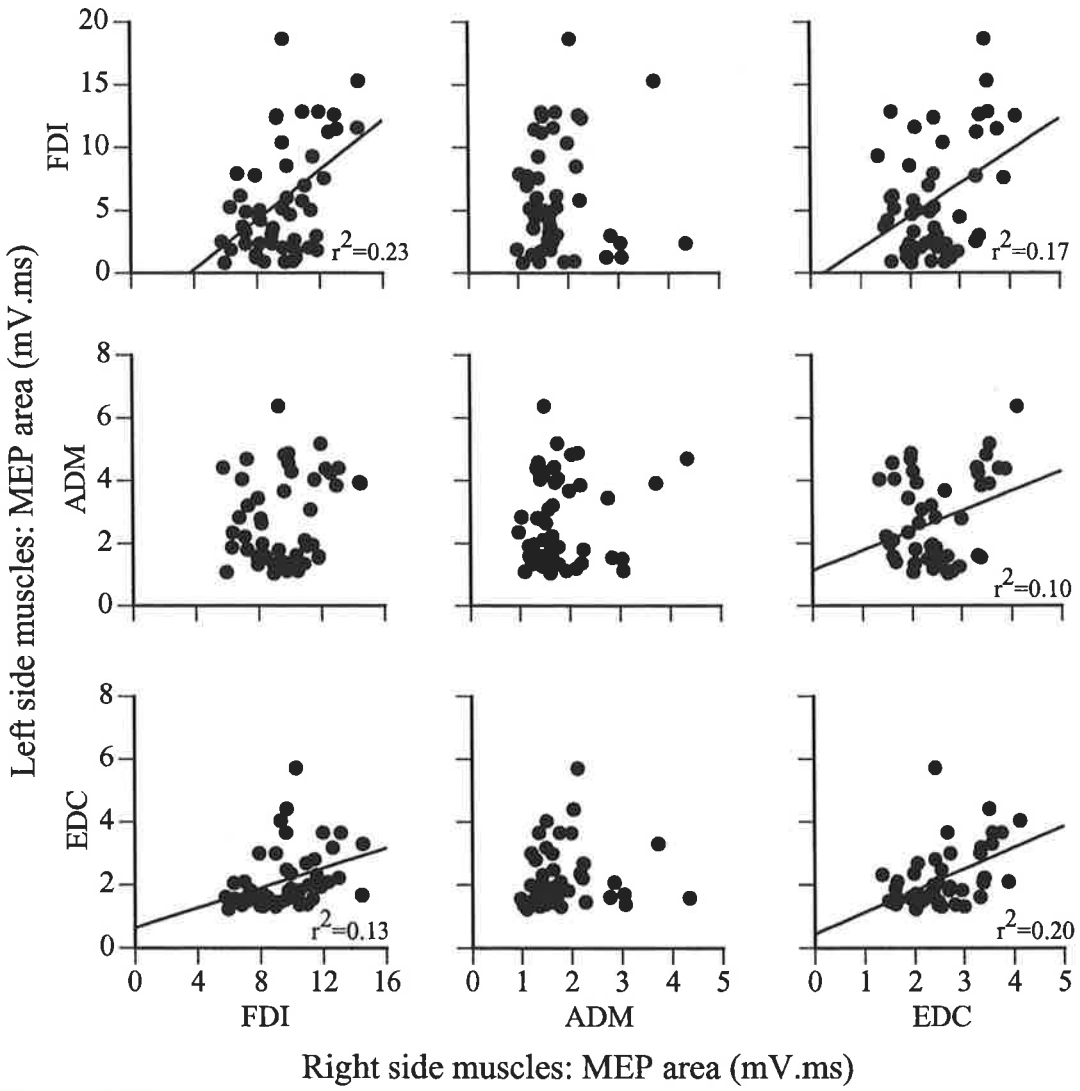

Figure 4.5

Co-variation in MEP size for muscles of the left and right upper limb at rest, using the dual TMS protocol. All data are derived from a single block of 50 consecutive TMS stimuli in one subject. All between-limb comparisons of muscle pairs are shown. Significant linear regression lines and r^2 values are shown ($p < 0.05$). In this example, two of the three between-limb comparisons involving homonymous muscles were significant, and three of the six between-limb comparisons involving heteronymous muscles were significant. These data are representative of those obtained in all subjects at rest.

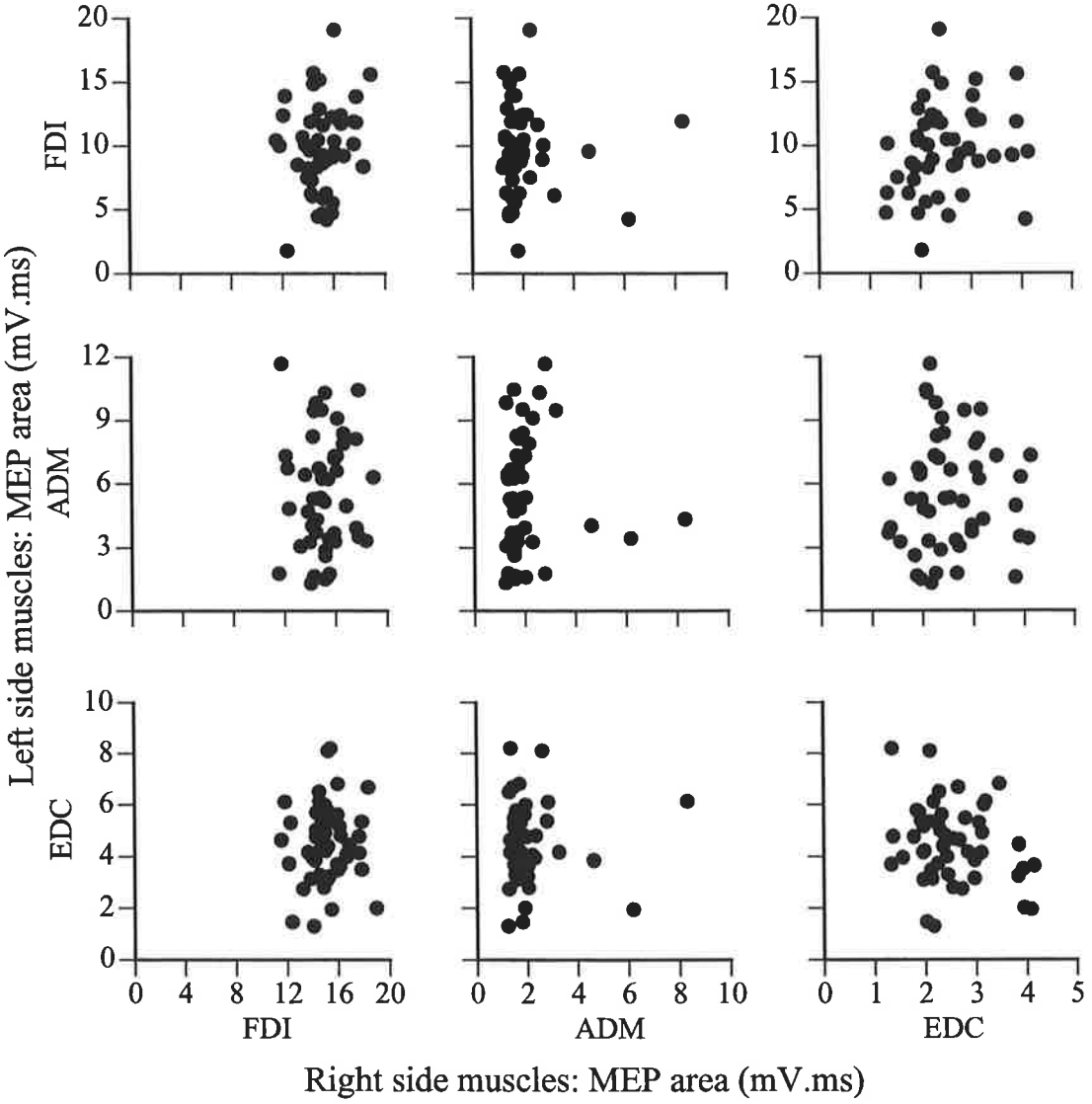

Figure 4.6

Absence of co-variation in MEP size for muscles of the left and right upper limb with right FDI active. Data are derived from a single trial of 50 consecutive stimuli in the dual TMS protocol with weak voluntary activation of right FDI. All between-limb comparisons of muscle pairs are shown (same subject as Figure 5). With right FDI active, none of the between-limb comparisons of MEP size exhibited a significant correlation.

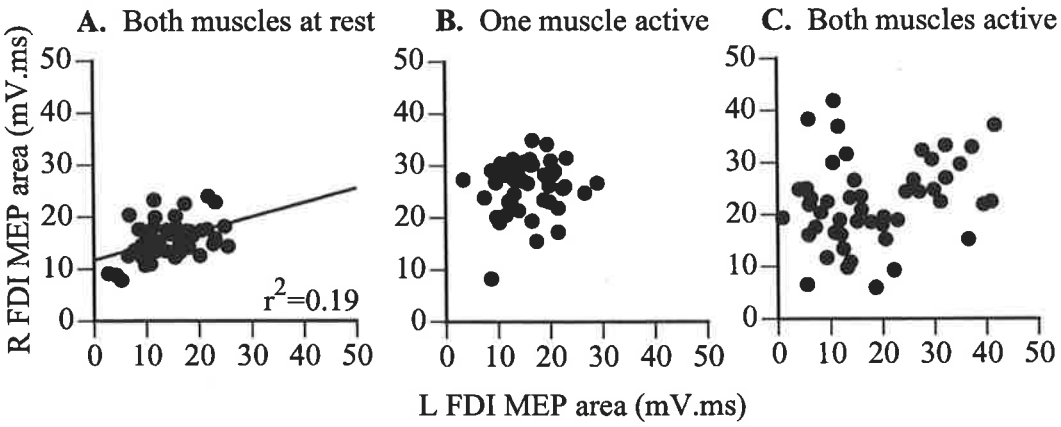

Figure 4.7

Relation between size of MEPs in left and right FDI in one subject at rest, and with one or both muscles active. Data in each panel are derived from a separate block of 50 consecutive TMS in the dual stimulation protocol. **A**, Both FDI muscles at rest (TMS for right FDI 75%, left FDI, 58%). There is a significant positive linear relationship in MEP area for the two muscles (r^2=0.19, $p < 0.002$). **B**, right FDI active (TMS for right FDI 62%, left FDI 58%), showing no significant correlation of MEP size in the two muscles ($p>0.05$). **C**, both FDI muscles active (TMS for right FDI, 62%, left FDI, 43%), showing no significant correlation of MEP size for the two muscles ($p>0.05$).

pairs (20% compared with 56%, Chi-squared analysis, $p<0.01$) and heteronymous muscle pairs (28% compared with 56%, Chi-squared analysis, $p<0.01$). The mean r^2 values were significantly smaller in the resting muscles when other muscles were active compared to when all muscles in the upper limb were at rest. This was true for both homonymous muscle pairs (0.16 ± 0.02 *vs.* 0.06 ± 0.02, t-test, $p<0.001$) and heteronymous muscle pairs (0.13 ± 0.02 *vs.* 0.07 ± 0.01, t-test, $p<0.01$). These data indicate that for resting muscles pairs between sides, activation of other muscles within the upper limb reduces the correlation of MEP size in the resting muscles.

When one muscle of the regression was active, there was a significant positive correlation with the homonymous muscle of the other limb in only 1 of 19 comparisons (5%). For heteronymous muscle pairs in opposite limbs, only 4 of 59 (8%) comparisons were significant when one muscle of the pair was active. When both muscles involved in the comparison were active, there was no significant correlation between the MEPs obtained in those muscles (0 of 4 regressions were significant).

To summarise the effect of voluntary muscle activation on the between-limb comparisons, when one or both muscles of the pair were active the incidence of significant correlation in MEP size was much reduced compared to the situation with all muscles at rest. This was the case for pairings of homonymous muscles (4% *vs.* 56%) and heteronymous muscles (7% *vs.* 56%). Chi-squared analysis confirmed that the incidence of significant regressions was significantly greater when the muscles were at rest than when one or both of the muscles were active ($p<0.001$). The r^2 values were significantly greater with both

Between-limb Comparisons	Mean r^2 (all comparisons)	Incidence of significant correlation in MEP size	
		Proportion of total comparisons	%
All muscles @ rest			
L-R homonymous muscles	0.16 ± 0.02	20 / 36	56%
L-R heteronymous muscles	0.13 ± 0.02	33 / 59	56%
FDI active (unilateral)			
L-R homonymous muscles (one muscle active)	0.02 ± 0.01	0 / 8	0%
L-R heteronymous muscles (one muscle active)	0.01 ± 0.004	0 / 14	0%
L-R homonymous muscles (both @ rest)	0.04 ± 0.02	3 / 13	23%
L-R heteronymous muscles (both @ rest)	0.07 ± 0.02	9 / 25	36%
FDI active (bilateral)			
L-R homonymous muscles (both active)	0.04 ± 0.01	0 / 4	0%
L-R heteronymous muscles (one muscle active)	0.06 ± 0.02	2 / 17	12%
L-R homonymous muscles (both @ rest)	0.08 ± 0.04	1 / 7	14%
L-R heteronymous muscles (both @ rest)	0.10 ± 0.06	2 / 7	29%
FDI and ADM active (unilateral)			
L-R homonymous muscles (one muscle active)	0.04 ± 0.01	1 / 11	9%
L-R heteronymous muscles (one muscle active)	0.03 ± 0.01	2 / 28	7%
L-R homonymous muscles (both @ rest)	0.05 ± 0.01	1 / 5	20%
L-R heteronymous muscles (both @ rest)	0.05 ± 0.01	1 / 11	9%

Table 4.2

Summary of correlations in MEP sizes for muscles in opposite limbs. Strength of correlation given by mean r^2 (± s.e.) for available comparisons from the different activation tasks in eight subjects. Incidence of significant correlation expressed as a fraction and percentage of the total number of comparisons made in each category.

muscles at rest, than when one or both of the muscles were active. This was true for homonymous muscle pairs (0.16 ± 0.02 *vs.* 0.04 ± 0.01, t-test, $p<0.001$) and heteronymous muscle pairs (0.13 ± 0.02 *vs.* 0.04 ± 0.01, t-test, $p<0.001$).

4.3.4.2 *Within-limb comparisons*

Trial-by-trial fluctuations in MEP area were significantly correlated between FDI, ADM and EDC muscles within the same limb at rest and when one or both of the muscles was voluntarily activated. An example from one subject with all muscles at rest is shown in Figure 4.8. In this example the MEPs were significantly correlated for all resting muscles within the same limb. Correlations between muscle pairs within a limb were observed even when one or both of the muscle pairs were active. Figure 4.9 shows examples of the MEP regression analysis between FDI and ADM in the same hand at rest and during two activation tasks. At rest there was a significant correlation in MEP fluctuations in the two muscles ($r^2=0.36$, $p<0.001$). During FDI abduction, when ADM was at rest, MEP areas were still significantly correlated in the two muscles ($r^2=0.55$, $p<0.001$). Similarly, during activation of both FDI and ADM, MEP areas were still significantly correlated ($r^2=0.13$, $p<0.05$).

Table 4.3 summarises the within-limb comparisons during the various activation tasks. With both muscles of the pair at rest, and no other muscles active, 75% of 100 comparisons showed a significant positive correlation of MEP size, with mean r^2 of 0.19 ± 0.05. When both muscles of the pair were at rest but another muscle within the same hand was active, mean r^2 was 0.26 ± 0.05 and 12 out of 18 regressions (67%) were

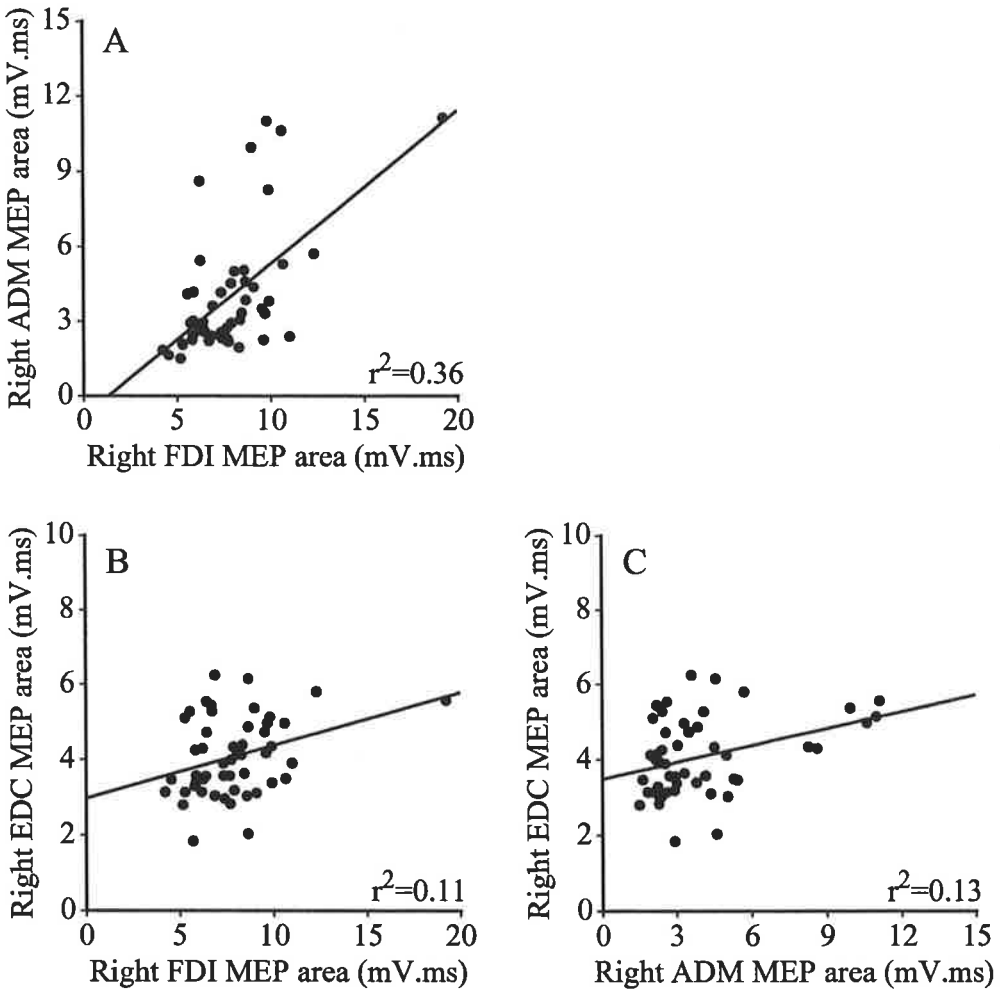

Figure 4.8

Correlation in size of MEPs in three muscles of the same limb at rest. All data are derived from a single block of 50 consecutive TMS applied to the contralateral hemisphere in the single TMS protocol. TMS intensity was 68% of maximum stimulator output. Significant linear regression lines and correlation coefficients are shown on each plot ($p < 0.05$).

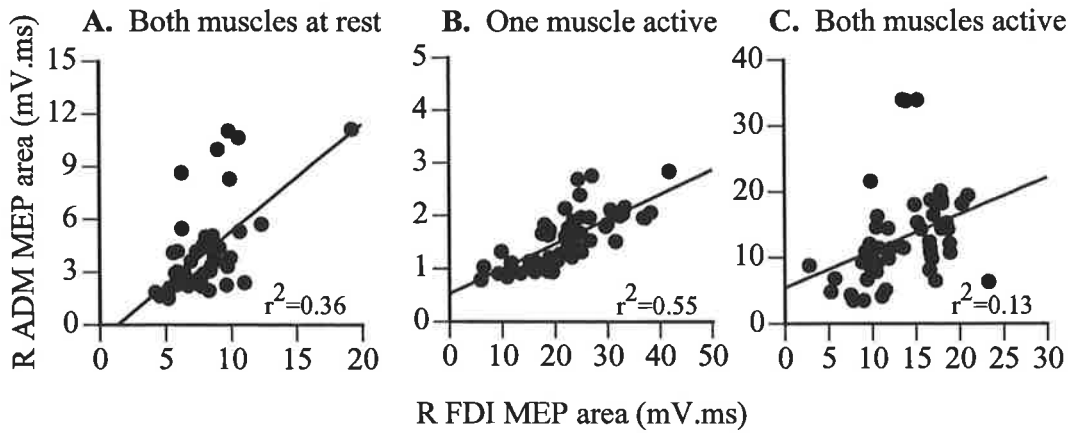

Figure 4.9

Correlation of MEP areas for FDI and ADM muscles of the same limb at rest and with activation of one or both muscles. Data for each panel are derived from a separate block of 50 consecutive TMS stimuli delivered focally to the left hemisphere (same subject as Figure 4.8). Significant linear regression lines and r^2 values are shown on each plot. **A**, trial with both muscles at rest. TMS intensity 68% of maximum stimulator output. **B**, trial in which the subject was activating FDI in a simple abduction task. ADM was at rest. TMS intensity 68% % of maximum stimulator output **C**, trial with both FDI and ADM active. TMS intensity 55% of maximum stimulator output. In all cases, linear regression revealed a significant positive correlation of MEP size in the two muscles.

significant. When both muscles of the pair were at rest, but muscle(s) in the opposite hand were active, mean r^2 was 0.19 ± 0.02, with 27 out of 35 regressions (77%) significant. The mean r^2 for these 3 conditions were not significantly different (t-tests, $p>0.05$). These data indicate that for muscles at rest within the same limb, activation of another muscle within that limb or the opposite limb had little effect on the strength of the correlation of MEP size in the resting muscles.

When one muscle of the pair was active, and the other at rest, mean r^2 was 0.12 ± 0.02, with 34 out of 63 regressions (54%) significant. When both muscles of the pair were active, mean r^2 was 0.12 ± 0.04, and the regressions were significant in 5 out of 12 (52%) cases. Chi-squared analysis showed that the incidence of significant correlations was reduced when one or both muscles of the pair were active compared to the situation where both muscles of the pair muscles were at rest. The r^2 values were significantly higher when both muscles of the pair were at rest than when one or both muscles were active (0.20 ± 0.01 *vs.* 0.13 ± 0.02, t-test, $p<0.05$). These data indicate that activating one or both muscles of the pair within the same limb reduced the strength of the correlation in MEP size for the two muscles, but did not eliminate it completely.

The strength of MEP correlation for muscle pairs within the limb were compared to those obtained between limbs (homonymous and heteronymous muscles combined) for the resting and active conditions. Student t-tests revealed that there was no difference in the mean r^2 values when all muscles were at rest for within-limb (0.19 ± 0.01) *vs.* between-limb (0.15 ± 0.01) comparisons (t-test $p>0.05$). There was a difference, however, in the r^2

Within-limb Comparisons	Mean r2 (all comparisons)	Incidence of significant correlation in MEP size	
		Proportion of total comparisons	%
All muscles @ rest			
Both muscles @ rest	0.19 ± 0.05	75 / 100	75%
FDI active (unilateral)			
One muscle active	0.11 ± 0.03	16 / 25	64%
Both muscles @ rest , in working hand	0.16 ± 0.04	7 / 10	70%
Both muscles @ rest , in non-working hand	0.18 ± 0.03	15 / 19	79%
FDI active (bilateral)			
One muscle active	0.19 ± 0.06	10 / 15	67%
Both muscles @ rest	0.37 ± 0.09	5 /8	63%
FDI and ADM active (unilateral)			
Both muscles active	0.12 ± 0.04	5 / 12	42%
One muscle active	0.09 ± 0.03	8 / 23	35%
Both muscles @ rest	0.20 ± 0.03	12 / 16	75%

Table 4.3

Summary of correlations in MEP sizes in muscle pairs within the same limb. Strength of correlation given by mean (± s.e.) r^2 for available comparisons from the different activation tasks in eight subjects. Incidence of significant correlation expressed as a fraction and percentage of the total number of comparisons made in each category.

values obtained when one or both of the muscles were active for within-limb comparisons (0.13 ± 0.02) *vs.* between-limb comparisons (0.04 ± 0.01; t-test $p<0.001$).

4.3.5 F-waves

Stimulation of the ulnar nerve on one side produced only ipsilateral F-waves. Bilateral stimulation of the ulnar nerve produced F-waves in FDI which were unrelated in amplitude between sides, with FDI at rest and when it was active. An example from one subject of linear regression of F-wave amplitudes in left and right FDI at rest and during a weak voluntary contraction of both muscles is shown in Figure 4.10. These data were representative of those obtained in all three subjects, and suggests that the motoneuron pools on each side did not show simultaneous, parallel fluctuations in excitability.

4.4 Discussion

The present study involved the analysis of MEPs from various hand muscles on a trial by trial basis to identify correlations in MEP size fluctuations between muscle pairs on opposite sides of the body, and between muscles on the same side. The results show that when all muscles are at rest there are significant correlations in MEP size for muscles within the same upper limb (75% of comparisons), and between upper limb muscles on opposite sides of the body (56% of comparisons). MEP correlations were reduced to near-chance levels for muscle pairs in opposite limbs when one or both muscles of the pair were active (6% of comparisons were significant). In contrast, for within-limb pairs activation of one or both muscles of the pair weakened the strength of MEP correlations, but did not eliminate them, as correlation coefficients were significant in 52% of these

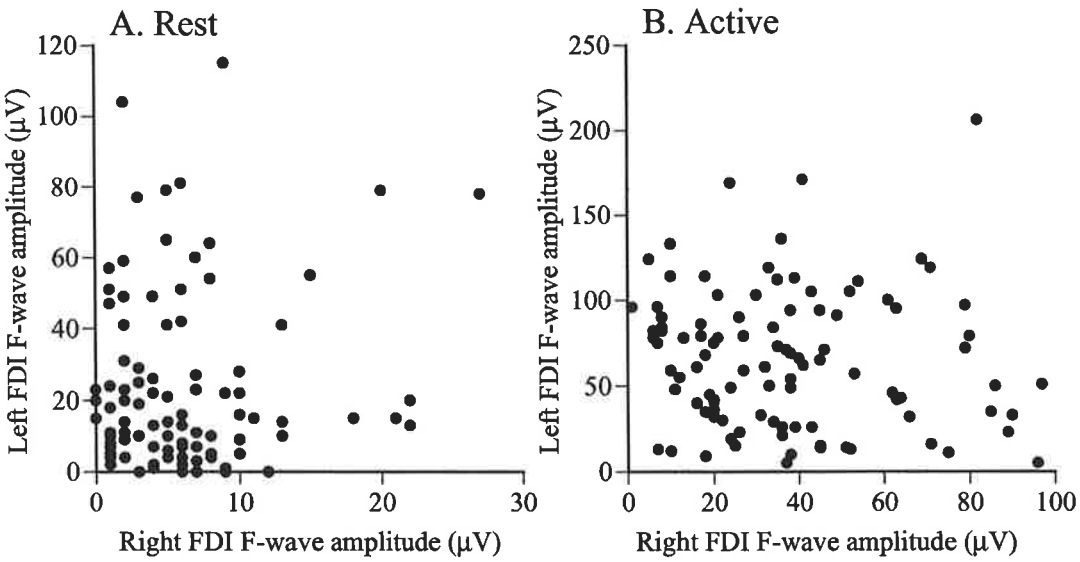

Figure 4.10

Amplitude of F waves evoked in left and right FDI of one subject following simultaneous supramaximal stimulation of left and right ulnar nerves. Data are derived from 100 stimuli in one subject at rest (**A**) and with weak voluntary activation of both FDI (**B**). There were no significant correlations in F wave amplitudes in the two FDI muscles in either condition.

pairs. These data suggest that the process responsible for common fluctuations of MEPs in muscles of opposite limbs is suppressed during voluntary activation. The relative insensitivity of within-limb MEP correlations to voluntary activation suggests that different processes are largely responsible for the common fluctuations in MEP size for muscles of the same limb.

4.4.1 MEP variability

It is well known that the MEP resulting from TMS exhibits considerable variability in size with each stimulus (Amassian et al., 1989; Britton et al., 1991; Brasil-Neto et al., 1992; Kiers et al., 1993). The coefficient of variation (CV), which is computed by dividing the standard deviation of a sample by the mean, can be used to quantify the variability in MEP responses in a given block of stimuli. The CV reported in the present study are consistent with those reported in previous studies of MEP variability: variability of MEP amplitude in resting EDC muscles has been reported to range between 0.2 and 0.8, and in resting thenar muscles, between 0.25 and 1.3 (Ellaway et al., 1998, Figure 4); in resting FDI muscles, at various levels of stimulus intensity, the CV in MEP area ranged from 0.16 to 0.77, while in active FDI it was between 0.03 and 0.40 (Kiers et al., 1993, Table 1). This level of variability is also comparable to that seen by Burke et al. (1995), who elicited D- and I-waves by electrically stimulating the motor cortex in anaesthetised patients and found that the CV of I-wave amplitudes fell in the range of 0.32 and 0.35. MEP variance was reduced in the active state, in accordance with previous observations (Kiers et al., 1993).

The source of the moment-to-moment changes in cortical excitability remains unknown. The variation is not due to changes in the effectiveness of the stimulation (Reutens et al., 1993) or noise in the recording system (Burke et al., 1995), nor is it related to the phase of the cardiac or respiratory cycles (Amassian et al., 1989). Motoneuron excitability cannot solely explain the fluctuations (Kiers et al., 1993; Funase and Miles, 1999), and variations in motor cortex excitability are believed to be responsible, at least in part, for the fluctuations in MEP size (Ellaway et al., 1998; Funase et al., 1999).

Kiers et al. (1993) concluded that the variation was relatively specific to functional groups of cortico-motoneurons, since they found no correlation in MEPs from left and right FDI, induced using a single circular stimulating coil, nor correlations in MEP size in FDI and soleus. This, and the fact that they found no change in MEP variability during constant mental alertness induced by mental calculation, suggested that global changes in the degree of cortical arousal were not responsible for the fluctuations in MEP size. More recently, however, Ellaway et al. (1998) found that MEPs in muscles on the same and on opposite sides of the body, elicited using two figure-of-eight magnetic stimulating coils, showed correlations in MEP fluctuation. They suggested, in contrast to the conclusions of Kiers et al. (1993) that fluctuations in the subject's state of arousal might alter the overall excitability of the motor cortex, affecting both hemispheres. Other possibilities suggested by Ellaway et al. (1998) were indirect changes in the level of excitability to the muscles studied through changes in cortical drive to other muscles such as postural muscles, excitability changes within a hemisphere affecting both contralateral and ipsilateral corticospinal pathways and transcollosal inhibitory and facilitory pathways.

4.4.2 MEP fluctuations in muscles on opposite sides of the body

The present study has replicated the techniques employed by Ellaway et al. (1998) in order to investigate further the bilateral nature of the MEP variability in upper limb muscles. In the experiments performed by Ellaway et al. (1998) all muscles were at rest. This was repeated in the present study, but additional trials were added in which subjects performed various activation tasks involving the muscles in the hand.

The present study has confirmed that, when all muscles are at rest there is a bilateral component to the fluctuations in the excitability of the corticospinal pathway, which gives rise to the variability in the response to TMS. This result is in accordance with that published by Ellaway et al. (1998), and of comparable strength, accounting for 16% of MEP variance in the present study and 19% in the Ellaway et al. (1998) study. The finding that F-waves were not correlated suggests that the source of variability is at a cortical rather than a spinal level.

These results differ from those of Kiers et al (1993), who concluded that there was no correlation in left and right MEP size fluctuations. They used a circular stimulating coil, and were able to elicit bilateral MEPs in 20 experiments in 6 subjects. Significant correlations between left and right MEPs were found in only 4 trials. It was not reported whether these trials were at rest, or whether they were with the muscles activated. This may account for the differences in results, since in this study, activation of the muscles decreased the correlations in MEP variability across sides. There were also differences in the way bilateral MEPs were elicited in the different studies; the present study and the study of Ellaway et al (1998) used two figure of eight stimulating coils, whereas Kiers et

al (1993) used a circular stimulating coil. When using a circular stimulating coil, one side of the cortex is preferentially activated. MEPs resulting from activation of the other side of the cortex are smaller, they occur at a longer latency and are thought to be due to the activation of different cortical neurons than those activated with the preferential coil orientation (Day et al., 1989). This may account for the lack of correlation between sides, since the MEPs on the left side and on the right side result from activation of different cortical elements. In contrast, using two figure of eight coils to stimulate the motor cortex bilaterally in the manner of the present study causes activation of the equivalent neural elements in each hemisphere of the motor cortex, producing MEPs of similar amplitude and latency.

The new finding of this study is that the processes producing the bilateral correlated MEP fluctuations do not appear to contribute significantly to MEP fluctuations when one or both of the muscles from which MEPs are being recorded are active. Bilateral MEPs that showed synchronous fluctuations in size were desynchronised with unilateral or bilateral muscle activity. Some desynchronisation also occurred in resting muscle pairs if other muscles in the upper limb were being activated. While it is beyond the scope of the present study to determine the origin of the bilateral synchronous MEP fluctuation and subsequent desynchronisation that occurs with muscle activation, it is possible to speculate as to a cause. Neurons in the human brain exhibit intrinsic oscillations that form the basis for macroscopic rhythms, detectable with electroencephalography (EEG) and magnetoencephalography (MEG) (see Hari and Salmelin, 1997). The size of MEPs induced by TMS has been shown to be affected by these cortical oscillations (Rossini et al., 1991). The results of the present study could potentially be explained by a cortical

rhythm that showed coherent behaviour in both hemispheres when the subject was at rest, but was desynchronised during movement. When at rest, the bilateral fluctuations in cortical excitability caused by the cortical rhythm would affect the size of the MEPs on both sides of the body. The event-related desynchronisation in the rhythmic activity that accompanied muscular activation would disrupt the bilateral coherence in cortical excitability so that the MEPs from the muscles on each side of the body would no longer be correlated. The sensorimotor cortex exhibits rhythmic activity over a broad range of frequencies, including the rolandic mu and central beta rhythms. Both of these oscillations need to be considered as potential candidates in explanation of the results presented in this paper.

The mu rhythm is the most prominent rhythm of the sensorimotor cortex, and is characterised by desynchronisation during movement (Chatrian et al., 1959; Pfurtscheller and Aranibar, 1979; Nashmi et al., 1994). Several studies have reported that the "classical" mu rhythm does not show bilateral coherence between the left and the right hemispheres (Storm van Leeuwen et al., 1978; Schoppenhorst et al., 1980; Andrew and Pfurtscheller, 1996). However, a recent examination of the mu rhythm has demonstrated that there are at least two types of mu rhythms, differentiated by their frequency in the alpha band, their patterns of desynchronisation and their somatotopic specificity (Pfurtscheller et al., 2000). At this time it is not clear if one of these rhythms, or perhaps even a yet undescribed mu rhythm, may display coherent behaviour in both hemispheres. The existence of such a rhythm could explain the results seen in the present study.

There is evidence that central beta rhythms are attenuated during movement (Pfurtscheller, 1981; Stancak and Pfurtscheller, 1996). A recent study examined

interhemispheric phase synchrony and amplitude correlation of beta oscillations using MEG at rest, and found evidence that beta activity was synchronous in the left and right hemisphere (Nikouline et al., 2001). Further study investigating the effect of beta rhythms on MEPs and the effect of movement on the phase synchrony would help to confirm if this rhythm is responsible for the correlations in left and right side resting muscle MEP fluctuations.

4.4.3 MEP fluctuations in upper limb muscles on the same side.

The present study has confirmed that, when all muscles are at rest, the fluctuations in excitability of the corticospinal pathway, which gives rise to the variability in the response to TMS, is correlated for muscles within the upper limb. About 19% of the MEP variance is explained by this process influencing both motor pools, a figure slightly less than the 26% observed by Ellaway et al. (1998). Further, the results show that with voluntary activation the within-limb fluctuations are only reduced marginally. This indicates that the process causing the common MEP fluctuations between limbs must not make a major contribution to the within-limb common MEP fluctuations. The weakening of the within-limb correlations in active muscles may be influenced by a worsened signal to noise ratio as the MEP is superimposed on the EMG interference pattern in the active state. It is difficult to assess the influence of this factor, however it may not be large because the MEP variance actually declined in the active muscles. These results do confirm that the disappearance of MEP correlation in muscle pairs on opposite sides of the body is not simply due to a deterioration in the signal to noise ratio. If this were the mechanism behind the results obtained in muscles on opposite sides of the body, then the MEP

correlations would have similarly disappeared for pairs of active muscles within the same limb.

One obvious difference between muscle pairs of the same limb, and between limbs, is the presence of shared, branched-axon CM inputs to motoneuron pools supplying muscles of the same limb (see Porter and Lemon, 1993). This neural substrate would be very effective in transmitting common fluctuations in size of TMS-evoked descending volleys to motoneuron pools of muscles of the same limb. This would be true regardless of whether the muscles are at rest or whether they are active. In Chapter 6, I show that active muscles on the same side of the body lacking shared, branched-axon CM inputs (masseter, FDI), also lack significant correlation in MEPs. The co-fluctuations in MEP size in muscles within the hand may therefore be explained by the presence of branched corticospinal axons innervating the muscles.

While branched-axons are likely to contribute to the MEP correlations in muscle pairs within the limb, other factors may be involved. The fluctuations in excitability driving the within-limb correlations could be aperiodic, or could have some oscillatory nature, such as the 20-32 Hz oscillations in the primary motor cortex that increase in size during a tonic hold (Baker et al., 1997). There is also recent evidence for weak, but widespread synchrony among primary motor cortex output neurons supplying hand muscles (Baker et al., 2001). This has oscillatory and non-oscillatory components, and suggests that the primary motor cortex neurons are not discharging completely independently. It is not known whether this would be sufficient to produce correlated MEPs in the absence of shared CM projections to the muscle pair.

4.4.4 Conclusion

The mechanisms operating to produce correlated MEP size fluctuations in pairs of muscles when they are active differ from those operating when they are at rest. In pairs of muscles within the same limb, significant simultaneous co-fluctuations in MEP size are observed, even when one or both muscles are active. This may be, at least in part, due to fluctuations in the excitability of CM projections that branch to innervate both muscles. In contrast, MEPs in muscles of opposite limbs are often correlated when the muscles are at rest, but not when one or more muscles are active. This is presumably due to cortical oscillations that synchronise the excitability of CM cells at rest, but are desynchronised with the voluntary command for muscle activation. These results have important implications for the interpretation of results in Chapter 6, where correlations in MEP size in left and right masseter were examined.

CHAPTER 5

SIMULTANEOUS FLUCTUATIONS IN SIZE OF RESPONSES TO FOCAL TMS IN MULTIPLE MUSCLES. II. A CASE STUDY IN A PATIENT WITH INFANTILE HEMIPLEGIA AND MIRROR MOVEMENTS

5.1 Introduction

Mirror movements are caused by involuntary contraction of the muscles on one side of the body during a voluntary movement of the homonymous muscles on the opposite side. While common in young children, they are considered abnormal if they persist past the first decade of life. Mirror movements are most prominent with movements of the fingers. They have been described in conjunction with a number of different congenital conditions (Schott and Wyke, 1981; Forget et al., 1986; Farmer et al., 1990; Cohen et al., 1991) and also in patients displaying no other neurological abnormality (Haerer and Currier, 1966; Regli et al., 1967). However, mirror movements may also arise as a consequence of corticospinal reorganisation following early damage to the central nervous system (Forget et al., 1986; Farmer et al., 1991; Carr et al., 1993; Carr, 1996; Kanouchi et al., 1997; Nirkko et al., 1997; Watson and Colebatch, 1997; Balbi et al., 2000).

Reorganisation of the nervous system following damage has been well documented in both animals (Kuang and Kalil, 1990; Merline and Kalil, 1990; Joosten, 1997; Rouiller et al., 1998; Aisaka et al., 1999; Z'Graggen et al., 2000) and humans (Benecke et al., 1991; Farmer et al., 1991; Lewine et al., 1994; Sabatini et al., 1994; Carr, 1996; Netz et al., 1997; Nirkko et al., 1997; Balbi et al., 2000). Characteristics of reorganisation depends

on factors such the age at which the injury was sustained and the type and extent of injury (Woods and Teuber, 1978; Carr et al., 1993; Joosten, 1997; Balbi et al., 2000). One mode of reorganisation following unilateral damage to the motor cortex involves branching of the corticospinal axons that originate from the intact hemisphere, to innervate the motoneurons normally controlled by the damaged contralateral hemisphere. There is evidence in hamsters for this type of reorganisation following unilateral pyramidotomy or unilateral cortical ablation, where single corticospinal neurons originating from the intact hemisphere have been shown to branch to innervate motoneurons bilaterally (Kuang and Kalil, 1990; Aisaka et al., 1999). The branching corticospinal fibres maintain their functional and topographic specificity (Kuang and Kalil, 1990). Cross-correlation analyses and reflex testing in infantile hemiplegic patients have provided evidence that these abnormally branched corticospinal cells contribute to the mirror movements seen in many of these patients (Farmer et al., 1991; Carr et al., 1993; Carr, 1996).

While the presence of branched corticospinal axons to homonymous distal hand muscles on left and right sides is obviously abnormal, corticospinal axons that branch to the motoneuron pools of more than one target muscle within a limb do play a role in normal motor control (see Porter and Lemon, 1993). Spike triggered averaging techniques have identified corticospinal axons which diverge to innervate several synergistic muscles within the hand (Fetz and Cheney, 1980; Kasser and Cheney, 1985; Buys et al., 1986; Cheney et al., 1991). Further, motor unit synchronisation studies suggest that branched corticomotoneuronal (CM) axons are a feature of the bilateral cortical control of muscles which are normally co-activated, such as left and right masseter, diaphragm and rectus abdominus (Carr et al., 1994).

Alterations in the cortical control of a muscle that is normally bilaterally innervated, such as masseter, have not been assessed in a patient with mirror movements. In the present study I have investigated the presence and strength of branched-axon CM projections to masseter in a patient with mirror movements, using the trial-by-trial analysis of motor evoked potentials (MEPs) already described in Chapter 4. These data serve as a comparison with data obtained from masseter muscles in normal subjects and reported in Chapter 6. I have also studied responses in hand muscles of each limb in the mirror movement patient, and compared these to responses obtained in normal subjects (reported in Chapter 4).

5.2 Methods

Experiments were performed on a 55-year-old female patient with left-sided infantile hemiplegia and mirror movements of the hands. MRI revealed long-standing right frontoparietal atrophy associated with atrophy of the ipsilateral cerebral peduncle and medullary pyramid. Mirror movements were evident during voluntary movement of the patient's hands, and occurred on the affected side when the subject moved the fingers of her "good" side. The patient was capable of some movement in her left hand (affected side), but she was unable to perform fractionated finger movements unless she also moved the fingers on her right hand. Experiments were conducted with the approval of the Human Research Ethics Committee at the University of Adelaide and written consent was obtained from the patient.

5.2.1 Apparatus and Recording

5.2.1.1 Surface electromyography

Surface electromyograms (EMG) were recorded in the same manner as reported in previous chapters. Self-adhesive bipolar silver/silver chloride electrodes were placed in a belly-tendon montage over the muscles of interest (these were a combination of first dorsal interroseous (FDI), abductor pollicis brevis (APB), abductor digiti minimi (ADM), extensor digitorum communis (EDC) and masseter in different experiments; details below). The EMG from homonymous muscles on either side of the body were recorded. The patient was grounded by a lip-clip electrode on the lower lip. EMG signals were amplified (1000 - 3000x) and recorded onto separate channels of a 22 kHz PCM data recorder (Vetter 400, A.R. Vetter Co., Pennsylvania, USA). The records were filtered (bandwidth 20-500 Hz) and digitised at 2 kHz sampling rate per channel (between 50 and 100 ms preceding the stimulus and 150 - 400 ms following the stimulus was digitised). Digitised EMG signals were rectified prior to analysis of MEPs while F-waves were analysed from unrectified EMG records.

5.2.1.2 Single unit electromyography

In one experiment Teflon^{TM}-insulated fine wire electrodes (45 μm core diameter) were inserted into the FDI muscles on either side to detect single motor unit activity. EMG signals were amplified (1000 - 2000X) and recorded on tape for off-line analysis.

5.2.1.3 Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) with a figure of eight coil was used to activate the motor cortex of the patient's intact (left) hemisphere. The coil was oriented at an angle of 45° relative to the parasagittal plane, with induced current in the underlying cortex flowing postero-anteriorly. The coil was always placed over the area of the motor cortex that produced the best response in all muscles being studied. Preactivation was needed in order to elicit MEPs in masseter muscles, and clear responses could only be recorded when the stimulus artefact was suppressed using a custom built artefact suppressing amplifier (ESK technologies). This was also true in normal subjects (Chapter 2).

5.2.1.4 F-waves

Two self-adhesive silver/silver chloride surface electrodes were placed over the ulnar nerve at the wrist on the left and right sides. Supramaximal stimulation of the ulnar nerve was used to elicit F-waves in the left and right FDI muscles. Electric pulses (between 100 and 200 μs duration) were delivered to the ulnar nerve via the electrodes using two digitimer stimulators (Digitimer models DS7 and D180, Digitimer Limited, Hertfordshire, England), each positioned to activate the ulnar nerve on one side.

5.2.2 Protocol

The patient participated in five experiments performed on separate occasions. The experiments were aimed at investigating four main issues. Details of the protocols used are given below.

5.2.2.1 Trial-by-trial fluctuations in masseter MEP size

The presence and strength of MEP correlations in the masseter muscles was assessed for the patient, for comparison with normal subjects (Chapter 6). This was to provide evidence about abnormal branching of CM inputs to the trigeminal motor system in the patient. The subject was given feedback of EMG activity from left and right masseter muscles. She was asked to maintain a contraction of 10% MVC in both masseter muscles. TMS was given in two blocks of 25 stimuli at 3 supra-threshold intensities. The subject had difficulty biting evenly; the left side masseter EMG tended to be larger than the right side (relative to MVC). The patient was asked to maintain this more natural biting task (which equated to approximately 10% MVC on the right side, and 20% MVC on the left side) while a further 50 TMS stimuli (2 blocks of 25) were given.

To allow a comparison of MEPs elicited from single TMS in muscles which are functionally unrelated, the EMG from resting left and right FDI muscles was recorded throughout this experiment. There are no branched corticospinal axons between masseter and FDI motor pools, so this provided a control to eliminate the possibility that MEP correlations between homonymous muscles was due to trial-by-trial variations in coil position or non-specific fluctuations in motor cortex excitability.

5.2.2.2 Trial-by-trial fluctuations in MEPs from upper limb muscles on the same, and opposite sides

The presence of abnormal branched-axon CM projections to both left and right upper limbs in the patient was expected to produce strong MEP correlations for muscle pairs in opposite limbs, comparable to the within-limb comparisons. Unlike normal subjects

(Chapter 4), these between-limb correlations were expected to remain during voluntary activation in this patient.

Two experiments were performed. Surface EMG from left and right FDI and APB muscles was recorded in both experiments. In one experiment the surface EMG from left and right ADM and EDC was also recorded.

The subject was seated comfortably and given feedback of EMG activity from the right side FDI and APB muscles. The rectified and smoothed EMG levels during maximum voluntary contraction (MVC) of both the right FDI and right APB was assessed and used as a reference for subsequent contractions. TMS was applied to the left motor cortex at various supra-threshold intensities, and during three different tasks:

1) Task one: All muscles at rest
2) Task two: Right FDI active at 10% of MVC (activity mirrored in left FDI)
3) Task three: Right FDI and APB active in a pincer grip, both at 10% MVC (activity mirrored in left FDI and APB)

Stimuli were given in two blocks of 25 so that a total of 50 stimuli were given at each stimulus intensity.

5.2.2.3 F-waves

F-waves were elicited first by stimulation applied to each arm in isolation (single stimulation) and then simultaneously to both (dual stimulation). One hundred stimuli

were given (in two separate blocks of 50 stimuli) for each condition. Two conditions were tested, both with single and dual stimulation:

a) both FDI muscles at rest.

b) both FDI muscles slightly active (<5% MVC).

5.2.2.4 *Synchronisation of motor unit discharge*

This experiment was performed with fine-wire intramuscular electrodes in both FDI muscles to detect single motor unit activity during right index finger abduction. Separate trials were performed in which visual feedback of tonic motor unit activity was given from the left and right FDI muscle. During the task motor units in the left and right hand were monitored to ensure suitable data was available for cross-correlation analysis. A total of 8 motor units (4 from left FDI, 4 from right FDI) were examined, and ten cross-correlograms were performed. Short-term synchrony was assessed from the cross-correlogram (Nordstrom et al., 1992) for motor units recorded in separate muscles.

5.2.3 Data Analysis

5.2.3.1 *Regression analysis of trial-by-trial variation in size of MEPs elicited in pairs of muscles*

MEP area (mV.ms) resulting from the 50 TMS stimuli were measured in both contralateral and ipsilateral muscles for each block of trials. For each muscle, the mean and standard deviation (SD) of MEP area was calculated for the 50 trials, and expressed as the coefficient of variation (CV; SD/mean) as a measure of the variability in MEP size. One way ANOVA was used to compare the CV in the different muscles. Student's t-tests

allowed a comparison between CV in left and right side muscles, and between resting and active muscles.

Linear regression was used to examine the correlation in size of MEPs elicited by unilateral TMS for all available muscle pairs, both within- and between-limbs. The following comparisons were performed using Student's t-tests (α=0.05):

a) coefficient of determination (r^2) for MEP size fluctuations in pairs of muscles under rest and active conditions.

b) r^2 values for homonymous muscle pairs *vs.* heteronymous muscle pairs in opposite limbs.

c) r^2 values for MEP fluctuations from right side muscle pairs *vs.* left side muscle pairs.

Chi-squared analysis was used to compare the incidence of significant correlations when all muscles were at rest compared to when one or both muscles were active.

In the trials involving active muscles, the mean EMG level for 50 ms prior to the stimulus was measured and compared for each muscle, using linear regression. In addition, the relationship between MEP size and pre-stimulus EMG activity was assessed using linear regression analysis.

Several criteria were applied to the data to eliminate possible factors such as coil movement or changes in the level of muscle activation in producing false MEP correlations. These are described in detail in Chapter 4. Briefly:

a) MEP "time effect" criteria

For each muscle unpaired t-tests were performed comparing the MEP areas obtained from the first 25 stimuli to those obtained with the second 25 stimuli. Data were excluded from regression analysis if a significant difference in MEP area was found between the two blocks of 25 trials, in both muscles of the pair. 52 of 210 regressions were excluded on this basis.

b) Pre-stimulus EMG time effect

Unpaired t-tests compared the level of muscle activation during the first 25 stimuli to that during the second 25. Data were excluded if the prestimulus EMG was different between blocks of 25 trials, for both muscles of the regression. 16 of 210 regressions were excluded on this basis.

MEP data from 142 pairs survived the exclusion criteria and were subject to linear regression analysis.

5.2.3.2 Regression analysis of trial by trial variation in size of F-waves simultaneously elicited in left and right FDI

F-wave amplitudes were measured from the unrectified EMG for each trial. The amplitude of the F-wave in the left FDI was compared to that obtained on the right FDI on a trial-by-trial basis using regression analysis, as described above.

5.2.3.3 Correlations in motor unit firing times in left and right FDI

The discharge times of concurrently active motor units in the left and right FDI were cross correlated to assess motor unit short-term synchronisation (Nordstrom et al., 1992). The

firing time of the right FDI motor unit was used as reference trigger, and cross-correlation histograms were constructed with a bin width of 1 ms and a pre- and post-trigger duration of 100 ms. The cumulative sum (CUSUM Ellaway, 1978) allowed identification of peaks in the histogram.

5.3 Results

5.3.1 Responses to focal TMS

Focal TMS of the damaged right hemisphere failed to elicit responses in any of the muscles monitored. When the intact left motor cortex was activated with TMS, MEPs were obtained in muscles contralateral *and* ipsilateral to the stimulus. The bilateral muscle responses showed similar properties to the contralateral muscle responses obtained in normal subjects. Typical averaged MEPs recorded from four different muscles in the patient are shown in Figure 5.1. As in normal subjects (refer to Chapter 2), masseter needed to be active during TMS to produce a MEP. All other muscles are shown at rest. The ipsilateral MEPs tended to be larger than the contralateral responses, and always occurred at the same short latency. MEP latencies were 7.0 ms for contralateral and ipsilateral masseter, 26.0 ms for contralateral and ipsilateral FDI, 26.0 ms for contralateral and ipsilateral APB, 20.0 ms for contralateral and ipsilateral EDC, and 25.0 ms for contralateral and ipsilateral ADM (data not shown). Muscle evoked potentials in the active hand muscles (data not shown) occurred at a shorter latency than in the resting muscles (24 ms in left and right FDI; 23 ms in left and right APB).

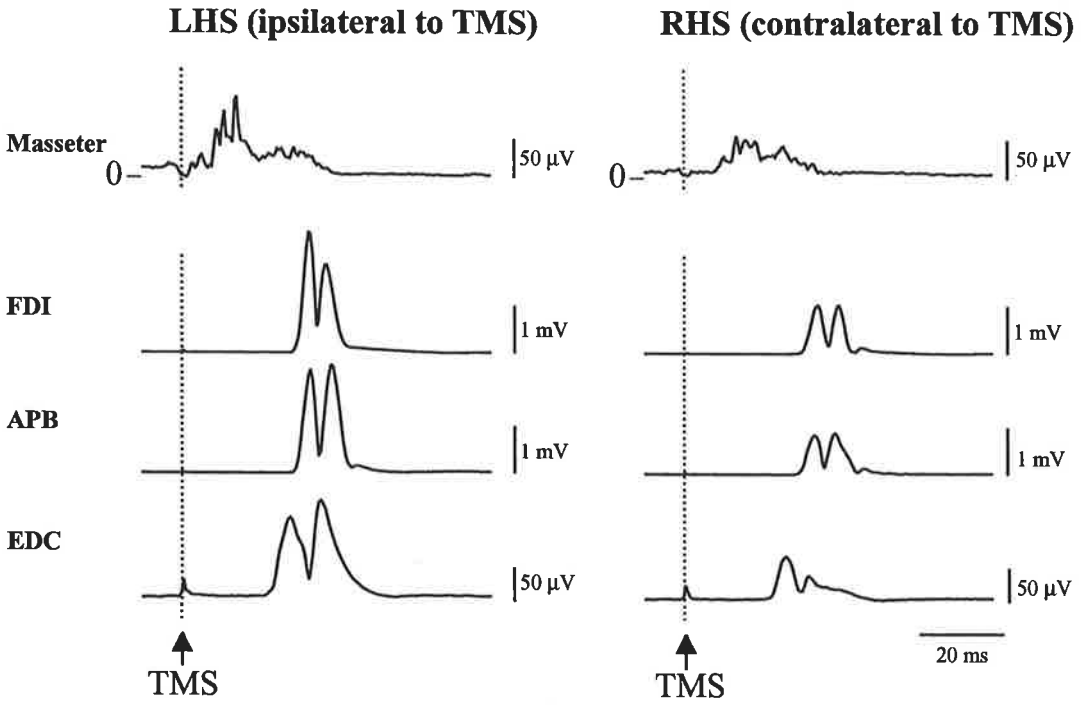

Figure 5.1.

MEPs from contralateral and ipsilateral masseter, FDI, APB and EDC following focal TMS of the left motor cortex in the mirror movement patient. Data are rectified and averaged (n=50) EMG traces. Stimulus timing is indicated by the arrows. Masseter responses (upper traces) were recorded in a separate experiment to the hand responses (lower three traces). Stimulus intensity in the masseter experiment was 60% with masseter active at 10% MVC. Stimulus intensity for the hand muscles was 75%, with all muscles at rest. Magnetic stimulation of one hemisphere produced MEPs in contralateral *and* ipsilateral muscles. Contralateral and ipsilateral latencies were identical, and responses tended to be larger in the ipsilateral muscle.

5.3.2 MEP variability

The MEPs varied in size from one stimulus to the next. This is a well-documented feature of the contralateral response to TMS seen in normal subjects (Amassian et al., 1989; Britton et al., 1991; Brasil-Neto et al., 1992; Kiers et al., 1993). Figure. 5.2 shows an example of the responses to ten consecutive stimuli, recorded concurrently from the patient's masseter and FDI muscles on both sides. The variance in MEP size, expressed as the coefficient of variation, is summarised for all muscles in Table 5.1. All muscles showed some degree of variability in MEP size from stimulus to stimulus, which was not different in the five muscles (ANOVA, $p>0.05$). The variance was similar on the left and right sides (t-tests, $p>0.05$) and was larger in resting muscles than in active muscles (t-test, $p<0.05$).

5.3.3 Trial-by-trial correlations in MEP size

5.3.3.1 MEP correlations in left and right Masseter

It is clear from Figure 5.2 that fluctuations in MEP size were correlated in the right and left FDI muscles, although this is not evident upon simple visual examination of the masseter data. Figure 5.3 shows the data from one block of 50 TMS and illustrates the co-variation in MEP size in homonymous muscles (left and right masseter, $r^2=0.40$, $p<0.001$; left and right FDI, $r^2=0.79$, $p<0.001$). There was no significant relationship between the masseter and FDI on each side. Four blocks of 50 stimuli were performed at different stimulus intensities in the patient, and regression analysis showed that in 3 of the blocks the MEP size in left and right masseter was positively correlated, with a mean

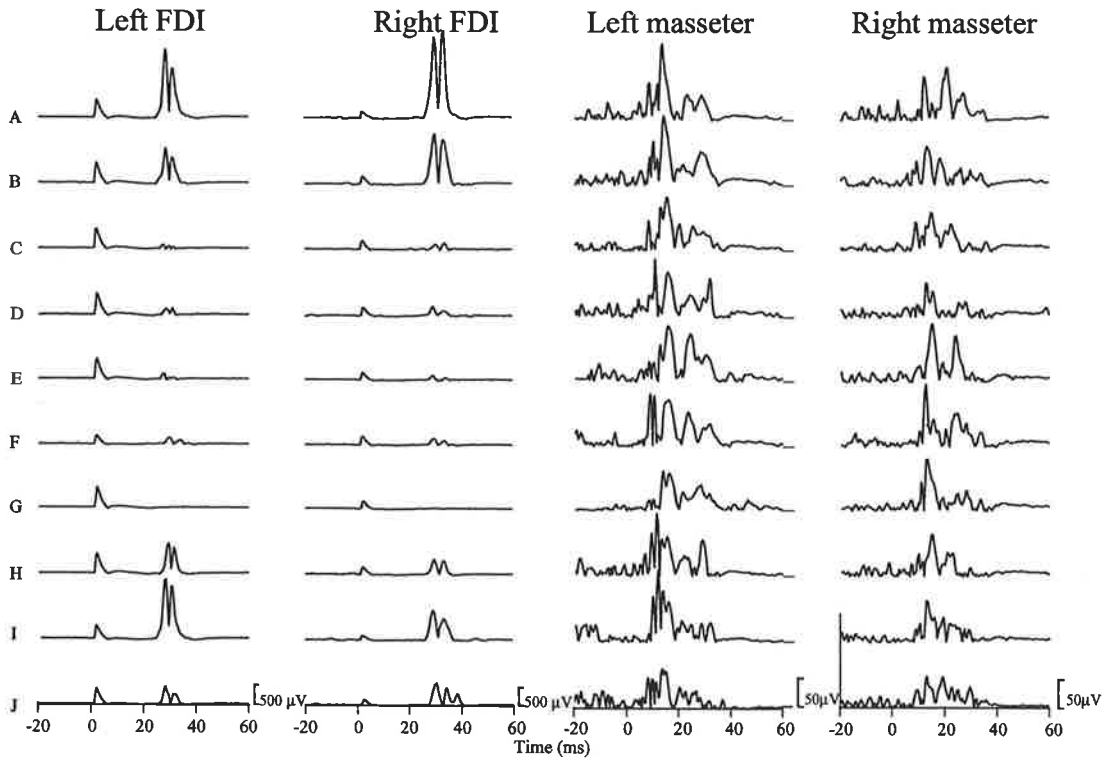

Figure 5.2

Representative examples of MEPs in both FDI and masseter muscles following TMS of the patient's left hemisphere. TMS intensity was 65% maximal stimulator output. Rectified EMG showing responses to 10 consecutive stimuli (A - J). TMS was delivered at 0 ms, producing bilateral MEPs in resting FDI and active masseter at latencies consistent with the activation of fast corticospinal (26 ms) and corticobulbar (7 ms) pathways. Note the clear correlation in MEP amplitudes in both FDI muscles in different trials.

	MEP Variance (CV)			
	Rest		Active	
	LHS	RHS	LHS	RHS
Masseter	n.a.	n.a.	0.35 ± 0.05	0.32 ± 0.05
FDI	0.86 ± 0.15	0.94 ± 0.21	0.59 ± 0.10	0.40 ± 0.04
APB	0.80 ± 0.16	0.72 ± 0.12	0.77 ± 0.09	0.60 ± 0.08
ADM	0.35 ± 0.02	0.54 ± 0.05	n.a.	n.a.
EDC	0.76 ± 0.20	0.53 ± 0.08	n.a.	n.a.

Table 5.1

The average coefficients of variation in MEP area in the mirror movement patient's left and right masseter, FDI, APB, ADM and EDC. Data are mean (± s.e.) CV shown for the resting and the active muscles. Between 4 and 10 trials of 50 TMS stimuli for each muscle were recorded in each condition (rest and active), and the coefficients of variation shown are the mean and SEM observed in these trials. n.a. = data not available.

coefficient of determination (r^2) of 0.32 ± 0.07 (all $p<0.01$). The fourth block also showed a significant correlation in left and right masseter MEPs, but was excluded from analysis due to a MEP time effect. The MEPs elicited in left and right FDI for the same stimulus were also recorded and while the responses in left and right FDI were significantly correlated (mean r^2 =0.76 ± 0.01, $p<0.001$), the responses in FDI and masseter were not (left side, mean r^2 = 0.03 ± 0.01; right side mean r^2 = 0.03 ± 0.1, all $p>0.05$).

The pre-stimulus EMG levels in left and right masseter were positively correlated in all trials (r^2 = 0.29 ± 0.08). There was a positive correlation between prestimulus EMG and MEP size in 6 of 8 comparisons (r^2 = 0.29 ± 0.08). To minimise any influence of background EMG, data were re-analysed after normalising the MEPs to the baseline EMG level, even though it is known that EMG has a weak effect on MEP size in this protocol (see Chapter 6). Normalised MEPs in left and right masseter were still strongly correlated (r^2 = 0.16 ± 0.05).

5.3.3.2 *MEP correlations in the upper limb – between-limb comparisons*

Figure 5.4 shows the relationship of MEP size in pairs of upper limb muscles from the left and right side when 50 TMS stimuli were delivered while all muscles were at rest. The EMG from four muscles on each side was recorded, and homonymous and heteronymous muscle pair correlations are shown. In this example there were strong positive correlations of MEP size in both left and right homonymous and heteronymous muscles (r^2 ranged from 0.29 to 0.61, $p<0.001$). This is representative of the data obtained at all TMS intensities in this subject.

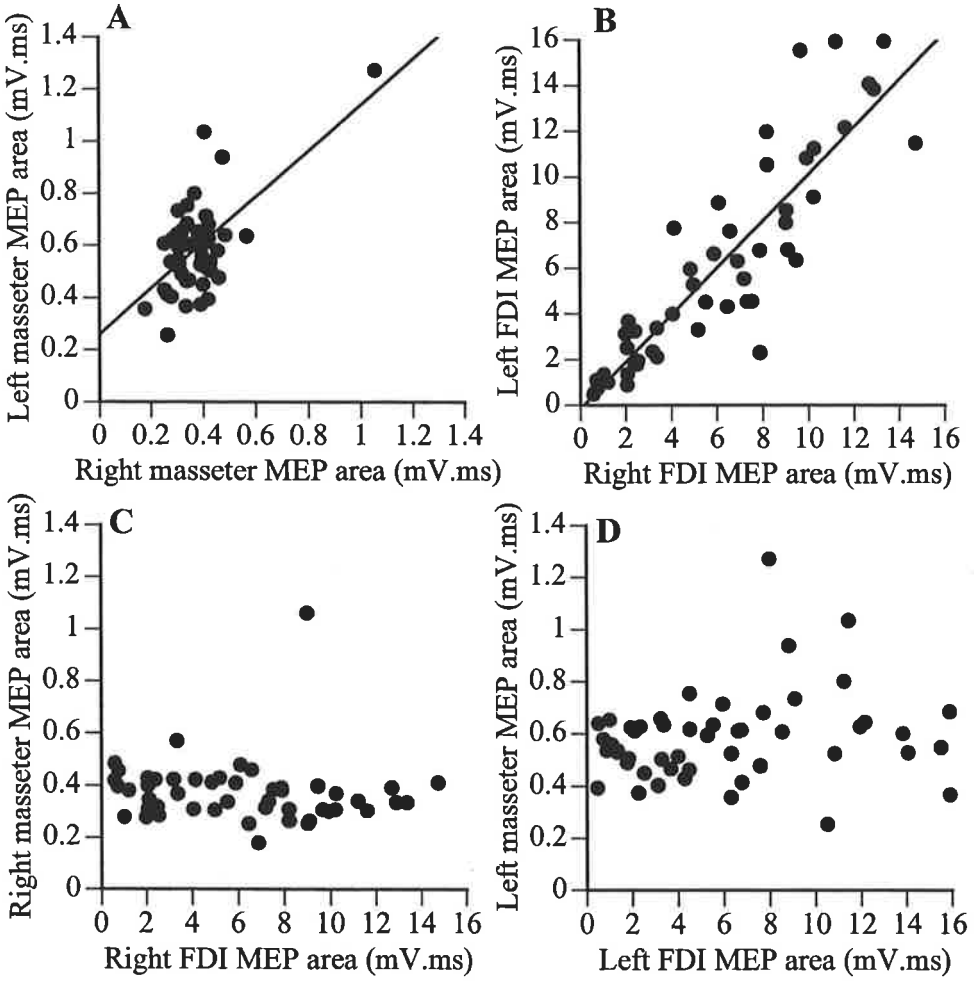

Figure 5.3

Correlation in size of MEPs in different muscles in the mirror movement patient for 50 consecutive focal TMS stimuli over the left motor cortex. TMS intensity was 65% of maximal stimulator output. **A**, significant positive correlation in size of MEPs in left and right masseter ($r^2 = 0.40$, $p < 0.001$). **B**, significant positive correlation in size of MEPs in left and right FDI ($r^2 = 0.79$, $p < 0.001$). **C**, **D**, lack of significant correlation in size of MEPs in FDI and masseter muscles on the right (**C**) or left (**D**) side of the body for the same 50 trials. The absence of correlations in MEP size for muscles on the same side of the body suggest that trial-to-trial variations in coil position (effectiveness of the stimulus) or global cortical excitability were not responsible for the significant correlations of bilateral MEPs in homonymous muscles found in A and B. These data are representative of results obtained in the patient with several different stimulus intensities.

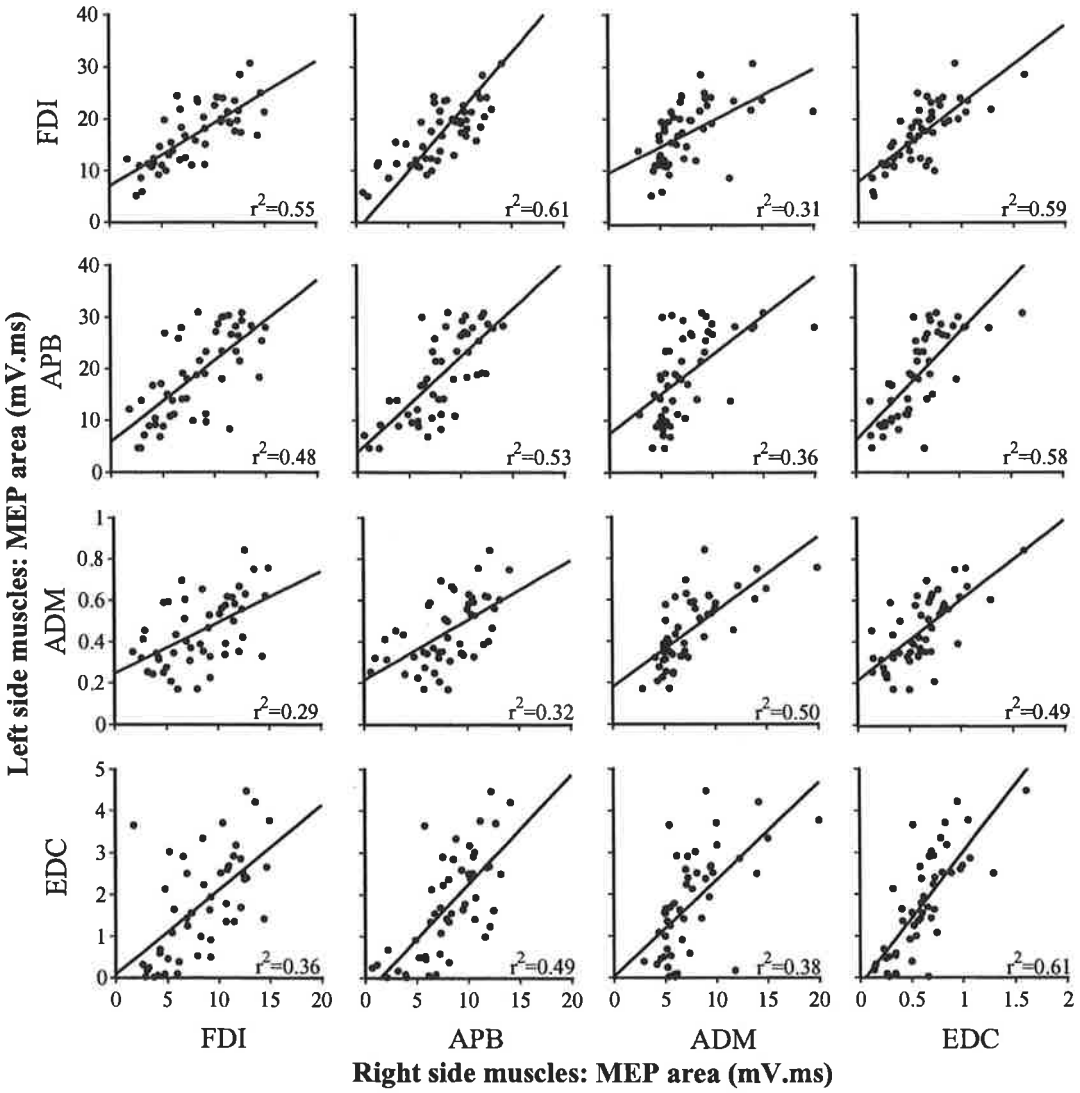

Figure 5.4

Relation between size of MEPs in different upper limb muscles to focal TMS stimuli of the left motor cortex in the mirror movement patient. TMS intensity was 75% of maximal stimulator output. All data are derived from a single trial of 50 consecutive TMS stimuli with all muscles at rest. All correlations are between muscle pairs on opposite sides of the body. Linear regression lines and coefficients of determination (r^2) are shown on each plot. All were statistically significant ($p < 0.001$). These data are representative of those obtained in the patient at all stimulus strengths.

In total, 7 blocks of 50 TMS stimuli were performed at various stimulus intensities while all hand muscles were at rest. These data are summarised in Table 5.2. Eleven between-limb comparisons were made for homonymous muscle pairs, and all showed a positive significant correlation in MEP area (mean $r^2 = 0.56 \pm 0.04$). In addition, between-limb comparisons of heteronymous muscle pairs (31 comparisons) all showed a significant correlation in MEP size (mean $r^2 = 0.36 \pm 0.03$). The mean r^2 was higher for homonymous muscle pairs than for heteronymous muscle pairs (t-test, $p<0.001$).

When the muscle pairs were at rest, but other muscles in the upper limb were active, MEP size fluctuations in the resting muscles during the activation tasks showed a significant positive relationship between sides. For homonymous muscles, 5 out of 5 regressions were significant (mean $r^2 = 0.47 \pm 0.10$), and for heteronymous muscles, 4 out of 6 regressions were significant (mean $r^2 = 0.24 \pm 0.03$). The r^2 values were no different to those obtained when all muscles were at rest (t-tests, $p>0.05$).

Figure 5.5 shows examples of the relationship between MEPs simultaneously elicited in FDI and APB muscles of both hands with focal TMS of left motor cortex when the subject voluntarily activated one or both muscles of the right hand. During right index finger abduction (Figure 5.5A), there was a significant correlation of MEPs in active FDI muscles on each side ($r^2=0.36$, $p<0.001$) and between the resting APB muscles on each side ($r^2=0.49$, $p<0.001$). The between-limb comparisons for active FDI and resting APB were also significant ($r^2=0.41$ and 0.20, $p<0.01$). When both FDI and APB were active during the pincer grip (Figure 5.5B), all between limb correlations of MEP size remained significant (r^2 ranged from 0.35 to 0.45, $p<0.001$).

Between-limb Comparisons	Mean r^2 (all comparisons)	Incidence of significant correlation in MEP size	
		Proportion of total comparisons	%
All ***muscles*** @ rest			
L-R homonymous muscles	0.56 ± 0.04	11 / 11	100%
L-R heteronymous muscles	0.36 ± 0.03	31 / 31	100%
FDI active (bilateral)			
L-R homonymous muscles (both active)	0.37 ± 0.01	4 / 4	100%
L-R heteronymous muscles (one muscle active)	0.18 ± 0.06	5 / 7	71%
L-R homonymous muscles (both @ rest)	0.43 ± 0.16	3 / 3	100%
L-R heteronymous muscles (both @ rest)	0.13 ± 0.04	3 / 3	60%
FDI and APB active (bilateral)			
L-R homonymous muscles (both active)	0.40 ± 0.08	5 / 5	100%
L-R heteronymous muscles (both active)	0.26 ± 0.03	7 / 7	100%
L-R heteronymous muscles (one muscle active)	0.28 ± 0.04	12 / 13	92%
L-R homonymous muscles (both @ rest)	0.53 ± 0.13	2 / 2	100%
L-R heteronymous muscles (both @ rest)	0.50	1 / 1	100%

Table 5.2.

Summary of correlations in MEP sizes in muscles in opposite limbs in the mirror movement patient. Strength of correlation given by mean r^2 for available comparisons from the different activation tasks. Incidence of significant correlation expressed as a fraction and percentage of the total number of comparisons made in each category. All available comparisons from FDI, APB, ADM and EDC muscles are summarised. TMS was given to the left motor cortex.

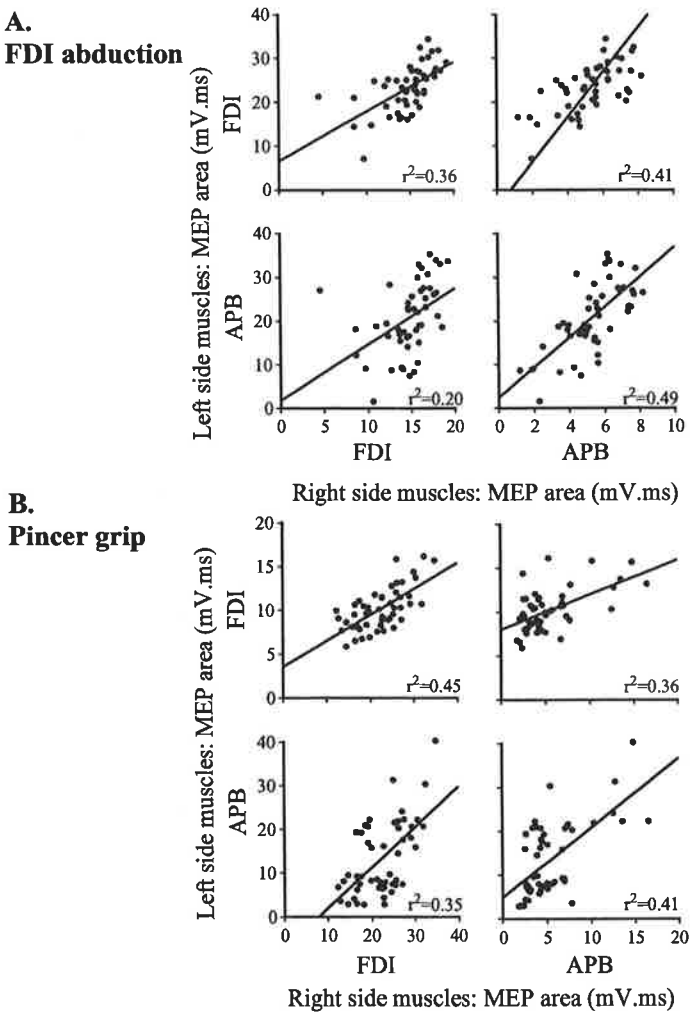

Figure 5.5

Correlation between MEP areas in left and right FDI and APB during different activation tasks in the mirror movement patient. All correlations are the result of 50 consecutive TMS stimuli delivered focally to the patient's left hemisphere. Linear regression lines and coefficients of determination (r^2) are shown on each plot. **A**, data from a trial in which the patient activated right FDI to 10% MVC during index finger abduction. TMS intensity was 65% of maximum stimulator output. All correlations were significant ($p<0.001$). **B**, data from a trial in which both FDI and APB of the right hand were voluntarily activated in a pincer grip task to 10% of their maximum. TMS intensity was 40% maximum stimulator output. All correlations were significant ($p<0.001$). These data are representative of those obtained at all stimulus strengths in this subject.

The data in Figure 5.5 are representative of that obtained across all trials. A summary of the between-limb regression analyses in active muscles is presented in Table 5.2. In 9 out of 9 comparisons in active homonymous muscles between limbs there was a significant positive relationship in MEP size (mean $r^2 = 0.39 \pm 0.04$). For active heteronymous muscle pairs in opposite limbs, 7 out of 7 of the regressions were significant (mean $r^2 = 0.26 \pm 0.03$). When only one muscle of the heteronymous muscle pair was active, and the other was at rest, 17 out of the 20 regressions were significant (mean $r^2 = 0.24 \pm 0.03$). The mean r^2 values were greater for the active homonymous muscle pairs than for the active heteronymous muscle pairs (t-test, $p<0.05$).

To summarise the effect of voluntary contraction on the between-limb comparisons, when one or both of the pair were active the incidence of significant correlation in MEP size was similar to what was seen when all muscles were at rest. This was true for homonymous muscle pairs (100% *vs.* 100%) and heteronymous muscle pairs (89% *vs.* 100%). These distributions were not significantly different (Chi-squared, $p>0.05$). The r^2 values, however, were greater for muscle pairs when all muscles were at rest than when one or both of the muscles in the pair were active, for both homonymous (0.56 ± 0.02 *vs.* 0.39 ± 0.02, t-test, $p<0.05$) and heteronymous muscle pairs (0.36 ± 0.02 *vs.* 0.26 ± 0.02, t-test, $p<0.05$).

Pre-stimulus EMG levels were usually correlated in homonymous muscles during the activation tasks (7 of 9 regressions were significant; mean $r^2 = 0.21 \pm 0.05$). Pre-stimulus EMG levels in active heteronymous muscles between sides were less likely to show a

significant positive relationship; 3 of 7 comparisons were significant (mean $r^2 = 0.12 \pm 0.06$). The size of MEPs was significantly correlated to pre-stimulus EMG activity in 8 out of 14 (57%) correlations in muscles on the left side (mean $r^2 = 0.13 \pm 0.03$) and 9 of 14 (64%) correlations in muscles on the right side (mean $r^2 = 0.15 \pm 0.03$).

5.3.3.3 *MEP correlations in the upper limb – within-limb comparisons*

Significant MEP correlations were obtained between resting muscles of the same limb. An example of the data obtained in FDI, APB, ADM and EDC on the right side is shown in Figure 5.6, and on the left side in Figure 5.7. In these examples, muscles on the right side showed significant correlations with r^2 values ranging from 0.19 to 0.50 ($p<0.002$) and on the left side with r^2 values ranging from 0.59 to 0.71 ($p<0.001$). The summary of all within-limb correlations performed at rest is shown in Table 5.3. In total, when all muscles were at rest, 28 out of 31 (90%) within-limb comparisons of MEP size were significant (mean $r^2 = 0.37 \pm 0.04$). The within-limb MEP correlations were stronger in the left hand than in the right hand, as evidenced by a significantly larger r^2 value obtained in left side muscle pairs than in right side muscle pairs (0.59 ± 0.04 *vs.* 0.23 ± 0.04, t-test $p<0.001$).

When both muscles of the regression were at rest, but other muscles within the upper limb were active, 4 of 4 regressions were significant in the left limb (mean $r^2 = 0.46 \pm 0.03$), but neither of the two regressions were significant in the right limb (mean $r^2 = 0.02 \pm 0.02$).

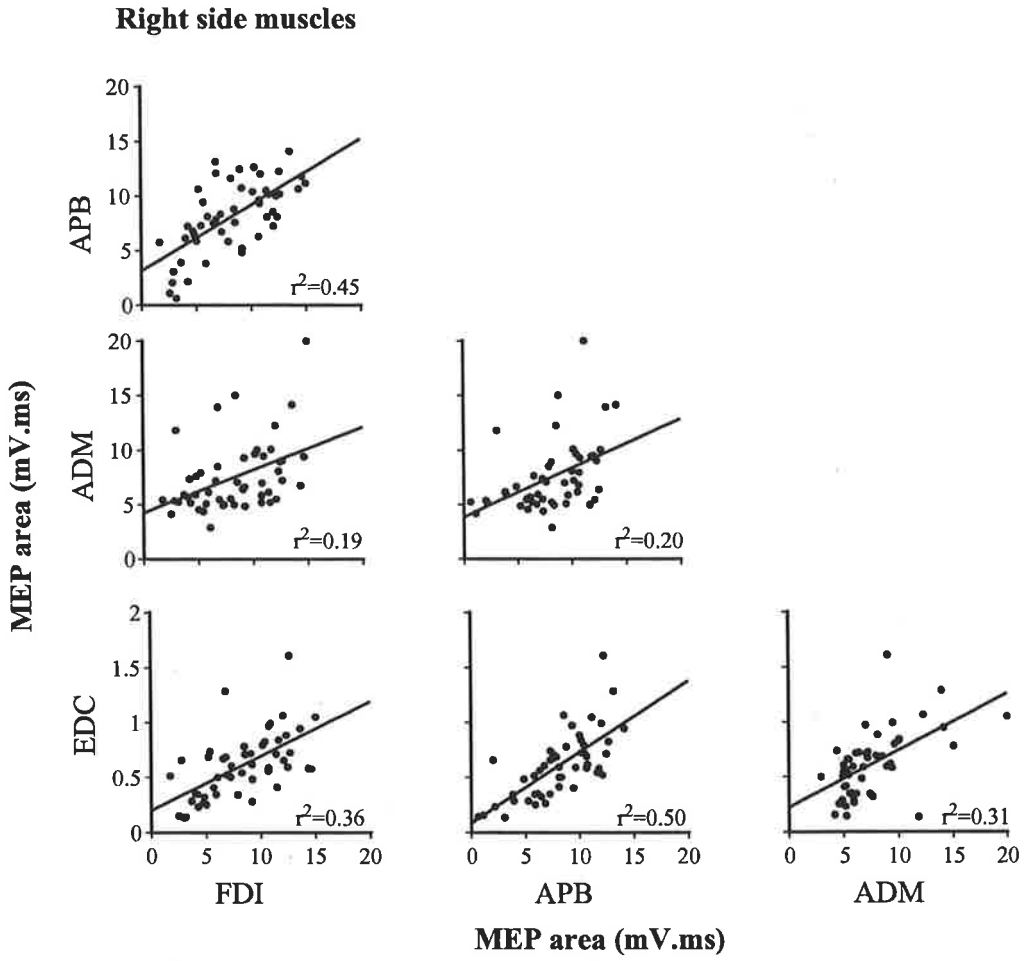

Figure 5.6

Relation between size of MEPs in different upper limb muscles on the right side to focal TMS stimuli of the left motor cortex in the mirror movement patient. TMS intensity was 75% of maximal stimulator output. All data are derived from a single block of 50 consecutive TMS stimuli with muscles at rest (same trials as Figure 5.4). Linear regression lines and correlation coefficients are shown on each plot. All were statistically significant ($p < 0.002$). These data are representative of those obtained at all stimulus strengths in this subject.

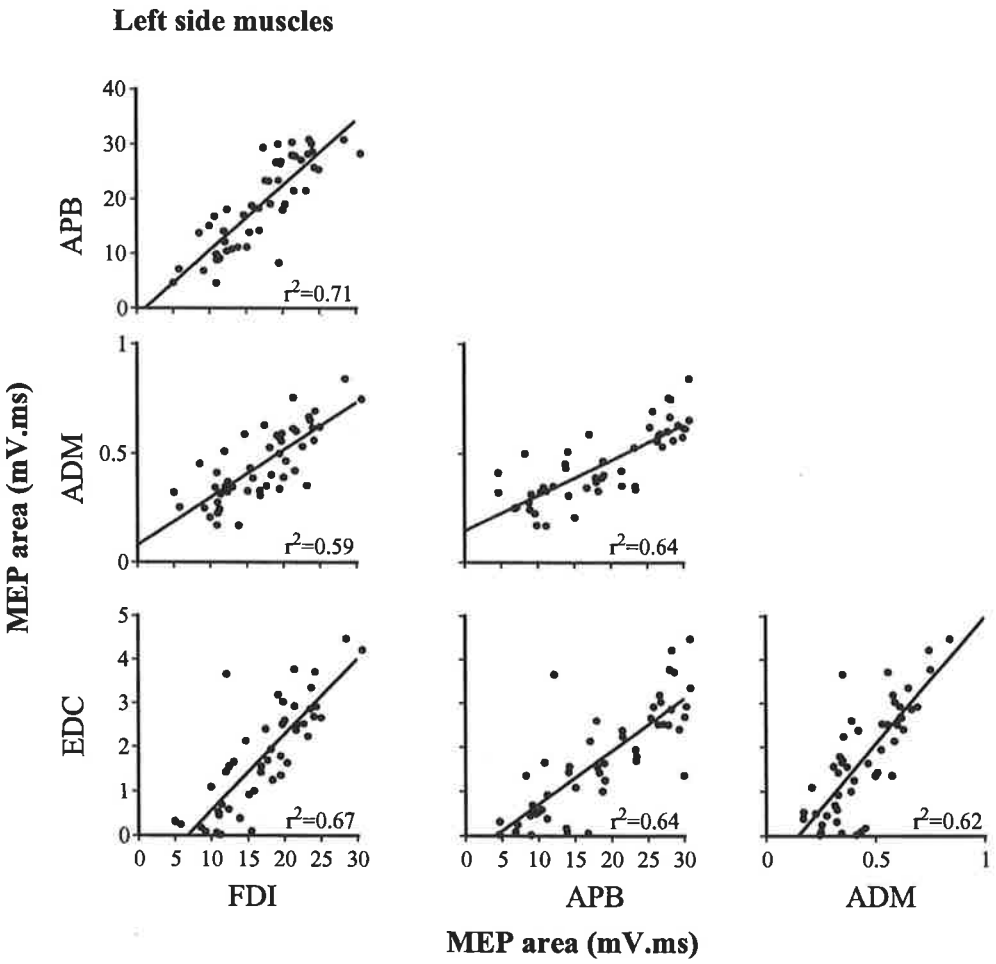

Figure 5.7

Relation between size of MEPs in different upper limb muscles on the left side to focal TMS stimuli of the left motor cortex in the mirror movement patient. TMS intensity was 75% of maximal stimulator output. All data are derived from a single block of 50 consecutive TMS stimuli with muscles at rest (same trials as figures 5.4 and 5.6). Data are arranged as in figure 5.6. All correlation coefficients were statistically significant ($p < 0.001$). These data are representative of those obtained at all stimulus strengths in this subject.

Within-limb Comparisons	Mean r^2 (all comparisons)	Incidence of significant correlation in MEP size	
		Proportion of total comparisons	%
All muscles @ rest			
R limb muscle pairs	0.23 ± 0.04	16 / 19	84%
L limb muscle pairs	0.59 ± 0.04	12 / 12	100%
Right FDI active			
R limb muscle pairs, one muscle active	0.07 ± 0.03	2 / 5	40%
L limb muscle pairs, one muscle active	0.39 ± 0.18	2 / 3	67%
R limb muscle pairs, both muscles @ rest	0.02 ± 0.02	0 / 2	0%
L limb muscle pairs, both muscles @ rest	0.43 ± 0.02	3 / 3	100%
Right FDI and APB active			
R limb muscle pairs, both muscles active	0.19 ± 0.04	2 / 3	67%
L limb muscle pairs, both muscles active	0.48 ± 0.05	3 / 3	100%
R limb muscle pairs, one muscle active	0.21 ± 0.06	5 / 7	71%
L limb muscle pairs, one muscle active	0.49 ± 0.05	6 / 6	100%
R limb muscle pairs, both muscles @ rest	-	-	-
L limb muscle pairs, both muscles @ rest	0.54	1 / 1	100%

Table 5.3

Summary of correlations in MEP sizes in muscle pairs within the same limb in the mirror movement patient. Strength of correlation given by mean r^2 for available comparisons from the different activation tasks. Incidence of significant correlation expressed as a fraction and percentage of the total number of comparisons made in each category. All available comparisons from FDI, APB, ADM and EDC muscles are summarised. TMS was given to the left motor cortex. Comparisons for left and right limb are shown separately.

Figure 5.8 shows an example of the within-limb regression analysis for FDI and APB MEPs during two activation tasks. During FDI abduction, when APB was at rest there were significant correlations between the two muscles of the same limb, both on the right and left sides (r^2=0.36 and 0.62 respectively, $p<0.005$). During the pincer grip, when both muscles were active, the MEPs in FDI and APB within the same limb were still significantly correlated (r^2=0.36 on the right side and 0.42 on the left side, $p<0.005$).

The combined data from all within-limb comparisons are summarised in Table 5.3. When both muscles of the regression were active, 3 out of 3 regressions were significant in the left hand (mean $r^2 = 0.48 \pm 0.05$) and 2 out of 3 regressions were significant in the right hand (mean $r^2 = 0.19 \pm 0.04$). The r^2 values were higher on the left side than on the right side (t-test, $p<0.05$). When one muscle of the pair was active, and the other at rest, 8 out of 9 regressions were significant in the left hand (mean $r^2 = 0.45 \pm 0.04$), and 5 out of 6 were significant in the right hand (mean $r^2 = 0.15 \pm 0.02$). Again, the r^2 values were higher on the left side than on the right side (t-test, $p<0.01$).

To summarise the effect of muscle activation on the within-limb comparisons, the incidence of significance in resting muscles was no different to the situation when one or both muscles of the pair were active, in either the left or right side muscle pairs (Chi-squared analysis; $p<0.05$). The mean r^2 values tended to be lower under active conditions for both hands, although differences were significant for the left hand (rest, 0.59± 0.04 *vs.* active, 0.46± 0.05; t-test $p<0.05$) but not the right (rest, 0.23± 0.04 *vs.* active, 0.16± 0.03; t-test $p>0.05$).

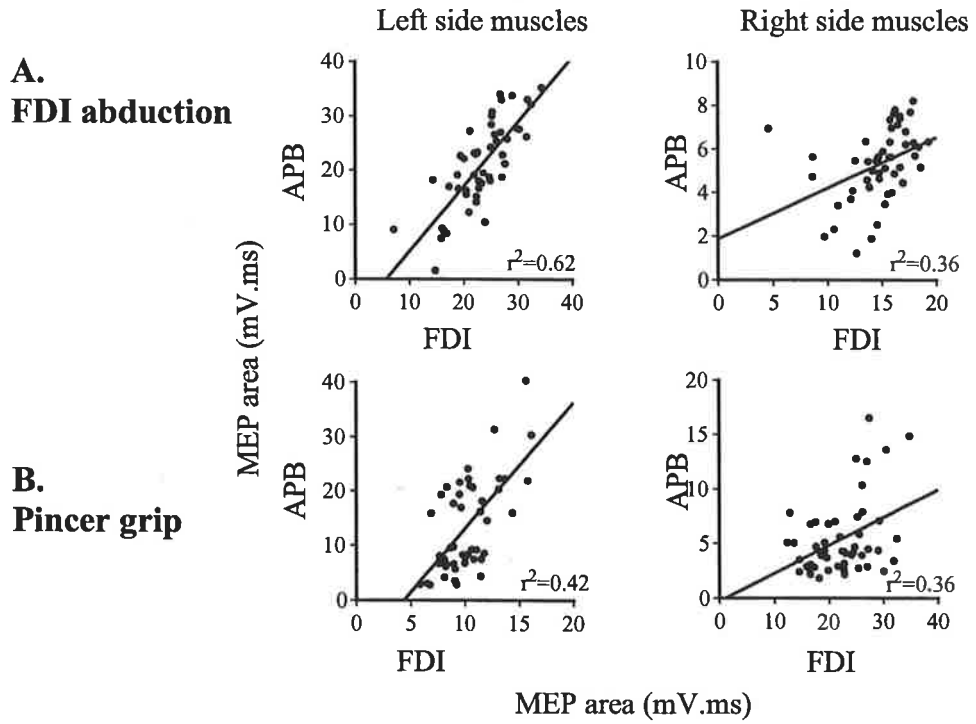

Figure 5.8

Within-limb comparisons of MEP areas in FDI and APB during different activation tasks in the mirror movement patient. All data are derived from a single block of 50 consecutive TMS stimuli delivered focally to the left hemisphere (same trial as figure 5.5). Linear regression lines and correlation coefficients are shown on each plot. Data from the left hand are in the left column; those from the right hand are in the right column. **A**, data from a trial in which the patient activated right FDI to 10% MVC during index finger abduction. TMS intensity was 65% of maximal output. MEP size in FDI was significantly correlated with MEP size in the resting APB of the same limb, for both left and right hands ($p<0.005$). **B**, data from a trial in which both right FDI and APB were voluntarily activated in a pincer grip task to 10% of their maximum. TMS intensity was 40% of maximal output. MEPs in FDI and APB of the same hand were significantly correlated, for both right and left sides ($p < 0.005$). These data are representative of those obtained at all stimulus strengths in this subject.

5.3.4 F-waves

Stimulation of the ulnar nerve on one side in isolation produced only ipsilateral F-waves, indicating that motor axons did not branch to innervate muscles bilaterally. Bilateral stimulation of the ulnar nerve produced F-waves that were unrelated in amplitude between sides. F-waves recorded during weak voluntary activation of FDI were also unrelated between sides. An example of the relationship in F-wave amplitude on the left and right sides at rest and during a weak contraction is shown in Figure 5.9.

5.3.5 Synchronisation of FDI motor unit discharge

Cross-correlograms (1 ms bin width) were constructed from discharge times of single motor units active in left and right FDI. These between-limb cross-correlograms of motor unit discharge revealed a central synchronous peak of short duration. A typical example of a cross-correlogram (histogram and CUSUM) from two motor units is shown in Figure 5.10. There was a central synchronous peak in CUSUMs of 8 of the 10 cross-correlograms.

5.4 Discussion

Focal TMS of the intact hemisphere in a patient with infantile hemiplegia and mirror movements resulted in MEPs in muscles on both sides of the body, which occurred at identical latencies in homonymous muscles. Analysis of MEPs on a trial-by-trial basis revealed correlations in MEP size fluctuations between muscle pairs on opposite sides of the body and between functionally related muscles on the same side. Activation of one or

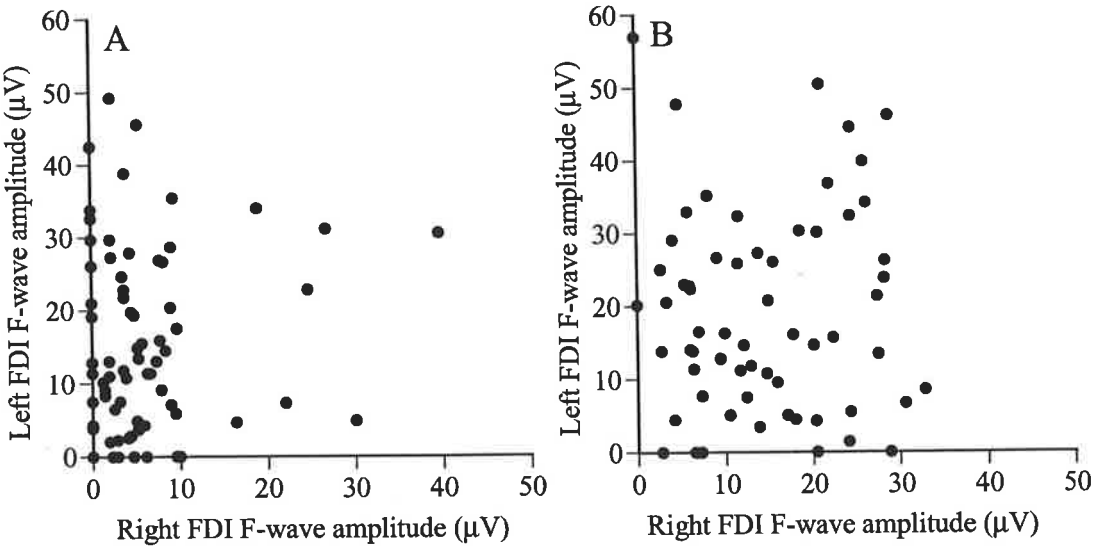

Figure 5.9

Amplitude of F-waves evoked in the patient's left and right FDI following simultaneous supramaximal stimulation of left and right ulnar nerves. Data are derived from 100 stimuli and are shown at rest (**A**) and with weak voluntary activation of right FDI (**B**). There were no significant correlations in F wave amplitudes in FDI muscles with simultaneous stimulation in either condition. These data suggest that motoneuron pools on each side did not show simultaneous, parallel fluctuations in excitability.

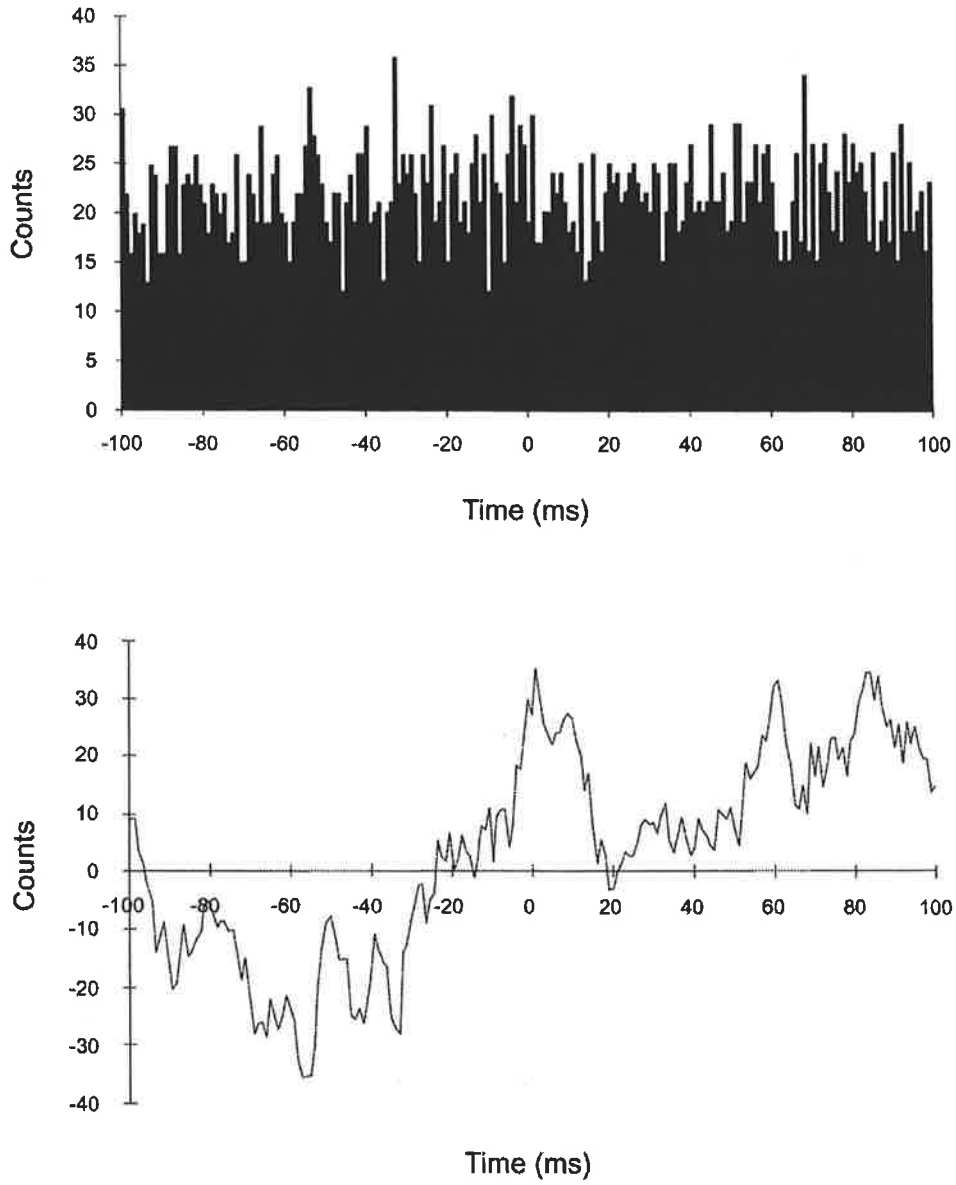

Figure 5.10

Cross-correlogram constructed from single motor units from simultaneously contracting left and right FDI muscles in the mirror movement patient. Upper trace shows the cross correlation histogram of the individual discharges of the two motor units. Lower trace shows the cumulative sum of the same data. The y-axis indicates the number of times the left FDI motor discharged at various times before and after the right FDI motor unit. Bin width is 1 ms. The central peak in the CUSUM indicates the presence of short-term synchronisation in the discharge of these motor units.

both muscles tended to weaken the strength of the MEP correlations, but did not eliminate them. This was true for upper limb muscle pairs on the same side and opposite sides of the body. MEP size correlations were stronger for homonymous muscle pairs than for heteronomous muscle pairs on either side, and were stronger between muscle pairs in the limb contralateral to the lesion (left muscles) than in ipsilateral muscle pairs. The correlation in MEP fluctuation was not present for functionally unrelated muscle pairs (masseter and FDI). Together with the observation that the discharge times of FDI motor units on each side were synchronised, these results may reflect the presence of intact corticospinal axons that branch to innervate the motoneuron pools on each side of the body.

5.4.1 The nature of MEPs elicited by focal TMS

In normal subjects focal TMS of one hemisphere results in bilateral responses in masseter (see Chapter 2 and Carr et al., 1994) but only contralateral responses in upper limb muscles (Carr et al., 1994). Previous studies have demonstrated ipsilateral responses in upper limb muscles following TMS (Wassermann et al., 1994; Ziemann et al., 1999), however the response characteristics are quite different from those obtained in contralateral muscles: 1) threshold is almost double, with target muscles having to be activated to at least 20% of maximum; 2) the responses are much smaller, with response latency delayed by nearly 6 ms; and 3) the optimal current direction for eliciting ipsilateral responses is 45-135° from the preferred current direction for contralateral MEPs (Ziemann et al., 1999). Such ipsilateral MEPs are thought to be due to activation of an oligosynaptic ipsilateral pathway, such as a corticoreticulospinal or corticopropriospinal projection (Ziemann et al., 1999).

With the stimulus parameters used in the present study, only the contralateral muscles of the upper limb were activated in normal subjects (see Chapter 4) while masseter was activated bilaterally by focal, unilateral TMS (see Chapter 2). In contrast, the present study has shown that unilateral, focal TMS in the mirror movement patient resulted in bilateral activation of both arm and masseter muscles. The abnormality appears to be at a cortical level, since unilateral stimulation of the ulnar nerve evoked F-waves only on the stimulated side, confirming that the pathway from the motoneurons to the muscles in normal in this patient. The latencies of the responses to TMS were the same in contralateral and ipsilateral muscles, and were comparable to the latencies of the contralateral response obtained in normal subjects (see Chapters 2 and 3, and Rothwell et al., 1991). These latencies are consistent with the activation of fast conducting corticospinal and corticobulbar axons to motor pools on both sides.

The MEPs recorded in the mirror movement subject showed many properties similar to those seen in normal subjects. It was not possible to activate masseter with TMS unless the muscle was active, as reported for normal subjects (Chapters 2 and 3). Activation shortened the latency of the response for hand muscles, as has been well documented in normal subjects (Rothwell et al., 1987). The size of responses varied considerably from one stimulus to the next, a feature well documented in normal subjects (Amassian et al., 1989; Britton et al., 1991; Brasil-Neto et al., 1992; Kiers et al., 1993). MEP variability, as expressed by the coefficient of variation, was comparable to that obtained in normal subjects for masseter and upper limb muscles (see Chapters 4 and 6). Taken together, these data suggest that the processes responsible for activating the intact motor cortex and

generating the MEP, and the conduction velocity of the fastest descending axons appear normal in the mirror movement patient.

5.4.2 Correlations in MEP fluctuations

In the present study, strong correlations in fluctuations of MEP size were seen in muscle pairs within the upper limb. These correlations had similar characteristics to those observed in normal subjects (Chapter 4). In particular, correlated MEP fluctuations were present whether the muscles were at rest or whether one or both of the muscles were active. The strength of MEP correlations (r^2) obtained within the patient's right (good) limb were comparable to those seen in normal subjects, both at rest (patient 0.23 ± 0.04 *vs.* normal subjects, 0.19 ± 0.03) and when one or both of the muscles were active (patient 0.16 ± 0.03 *vs.* normal subjects, 0.13 ± 0.05). MEP correlations between muscle pairs on the affected side of the patients body (left side) were much stronger (rest, 0.59 ± 0.04 and active, 0.46 ± 0.04). One reason for MEP correlations in synergistic muscles within the upper limb may be the presence of CM cells which branch to innervate the motoneuron pools of both muscles (see Chapter 4). Branched CM cells are known to play an important role in the fractionation of movement in normal motor control (see Porter and Lemon, 1993). The reason why the muscle pairs in the patients affected limb have stronger correlations than normal is not clear, but may indicate a more extensive branching of individual CM cells to muscles within this limb.

In the present study, strong correlations of MEP size fluctuations were also seen in homonymous and functionally related heteronymous upper limb muscles between sides. While similar results have been observed in normal subjects at rest, there are a number of

striking differences. In the present study, the MEP correlations in upper limb muscle pairs between sides were present under resting and active conditions. This is in contrast to the situation in normal subjects (Chapter 4) where the correlations disappear when one or both of the muscles are activated. This suggests that the mechanism responsible for producing the MEP correlation in upper limb muscles on opposite sides is different in the mirror movement patient than in normal subjects. Even at rest the strength of MEP correlation between-limb was much stronger in the mirror movement patient, both for homonymous muscle pairs (r^2 was 0.56 ± 0.04 compared with 0.16 ± 0.02 in normal subjects), and heteronymous muscle pairs (r^2, 0.36 ± 0.03 *vs.* 0.13 ± 0.02). The short-term synchrony in motor unit discharge observed in the present study, and in other studies on mirror movement patients (Carr et al., 1993) suggests that CM cells which originate from the intact hemisphere branch to innervate motoneuron pools on either side of the body. If present, fluctuations in the excitability of these cells may account for the fluctuations in MEP size in muscles on either side of the body. Correlations were stronger for homonymous muscle pairs than for heteronymous muscle pairs. This suggests that the abnormal axons branch to innervate the correct motoneuron pool on the opposite side of the body.

In the study of patients with infantile hemiplegia by Carr et al. (1993), two types of reorganisation was described. First were patients who had strong mirror movements, presumably due to abnormally branched CM axons. Second were patients with weak or absent mirror movements, who were reported to have abnormal ipsilateral projections, which were distinct from the contralateral projections. In these patients, focal TMS produced bilateral MEPs, but there was no correlation in motor unit firing rate in muscles

on opposite sides of the body. A comparison of the MEP correlations in muscle pairs between sides in this group of patients compared to the results presented here would provide further evidence that the MEP correlations are caused by branched CM cells.

Strong correlations in MEP size between left and right masseter were observed in the mirror movement subject. In related experiments in normal subjects (Chapter 6) I found that the MEP size in left and right masseter was correlated in 6 of 19 comparisons (32%). The mean r^2 from these 19 correlations from masseter muscles in normal subjects was 0.05 ± 0.01, much weaker than the correlations observed in the patient for masseter MEPs (0.32 ± 0.07). The correlations in masseter MEP size observed in normal subjects and in the mirror movement patient may reflect the presence of branched CM cells innervating the masseter motoneuron pool on each side. Normal subjects also have a population of CM cells that project to the contralateral masseter motoneurons only (see Chapter 3). On the affected side in the mirror movement patient these cells are not functional, and a stronger branched CM input to masseter motoneurons from the intact hemisphere seems likely. This would account for the stronger correlation in left and right masseter MEP size observed in the patient compared to normal subjects.

While branched CM axons are likely to contribute to the MEP correlations seen in muscle pairs on the same side and between sides in the present study, a number of other factors must be considered. These are discussed briefly below.

5.4.2.1 Fluctuations in MEP size due to movement of the stimulating coil.

MEP size varies with the position and orientation of the stimulating coil. Care was taken to ensure the coil was held firmly in position over the patient's head, so that minimal movement occurred. There were no significant correlations in the size of MEPs elicited from the same stimuli in masseter and FDI. Movement of the coil would cause changes in MEP size that would be reflected in both FDI and masseter as their corticomotor representations are close, and so this suggests that coil movement was not responsible for the MEP variations. As an extra precaution, trials in which both muscles showed a difference in the size of the MEP from the first 25 stimuli compared with those from the second 25 were excluded (MEP time effect, 52 of 210 trials excluded).

5.4.2.2 Fluctuations in motoneuron excitability

In the resting muscles there was no correlation in the amplitude of F-waves induced by bilateral maximal stimulation of the ulnar nerves (Figure 5.9). However, there were strong correlations in the MEP sizes in resting muscles on each side. This suggests that the source of the correlations, at least in the resting condition rest, is not fluctuations in motoneuron excitability.

The situation in the active muscles is a little more complex. Although the F-waves were not correlated on the left and right sides in active muscles (Figure 5.9), significant correlation in the fluctuations of muscle activity was observed in 7 of 9 comparisons involving homonymous muscles, and 3 of 7 comparisons involving heteronymous muscles. In normal subjects, small fluctuations in MEP size has little effect on the size of the MEP (Funase et al., 1999). However, in this patient significant correlations between

MEP size and muscle activity were observed in 61% of correlations. Analysis of the MEP size correlations after normalisation to baseline EMG may allow a more accurate assessment of the cortical contributions to MEP correlations. This was done in the present study for the masseter regressions, and strong correlations in left and right masseter MEPs were still observed.

Longer-term parallel change in EMG activity over the course of a block of trials was not a factor in correlations between active muscle pairs. The level of muscle activation was monitored carefully during the experiment and the patient was given a target EMG level for each muscle that was the same throughout the trial. In addition, the exclusion of data showing an EMG time effect (16 of 210 trials excluded) ensured that long-term changes in EMG were not contributing to MEP correlations.

5.4.2.3 *There may be separate populations of contralaterally and ipsilaterally projecting CM neurons.*

Although the MEP correlations behave in the mirror movement patient as expected if they were driven by branched CM axons, it is possible that they may be due to synchronous changes in the excitability of separate cortical neurons. These changes, if present, must be specific to a particular somatotopic area, since no correlations in MEP size from masseter and FDI were observed. Also, whatever is driving the synchronous changes is not suppressed with movement, as compared to the process responsible for the MEP correlations between sides in normal subjects (Chapter 4).

It is likely that any effects on MEP correlations mediated by separate populations of contralaterally and ipsilaterally projecting CM neurons be weaker than effects mediated

by branched CM projections. Also, the fact that the MEP correlations were observed in situations where motor unit synchronisation were present (present study), and in situations where branched axons are known to exist (within the hand, Chapter 4) provides further support that the MEP correlations are caused by branched axons rather than a separate population of ipsilateral and contralateral CM projections.

5.4.3 Conclusions

In conclusion, the present study has shown that:

1. The patient demonstrates a similar pattern of cortical reorganisation to that reported in other infantile hemiplegic patients, in which single corticospinal axons from the intact hemisphere have branched abnormally to innervate motoneurons on both sides.
2. Fluctuations in MEP size co-vary in muscles that are believed to be innervated by branched axons of single CM cells (as evidenced by motor unit short-term synchrony or known anatomical connections). MEP fluctuations do not co-vary for muscles that lack these shared inputs (masseter and FDI).
3. In the patient, within-limb MEP correlations were stronger on the left side, which receives the abnormal CM projection from the ipsilateral motor cortex. The within-limb correlations on the right side were comparable to the within limb correlations in normal subjects. This suggests a more extensive branching of CM projections to motoneurons innervating muscles on the patients affected side.
4. Between-limb correlations were stronger for homonymous muscle pairs than for heteronymous muscle pairs, suggesting that the abnormal branched axons are specific in the motoneurons they innervate.

5. In the patient, the between-limb correlations in MEP size are present in resting and active muscles and seems likely to arise from an abnormal branching of CM projections to motor pools of both upper limbs. In normal subjects, between-limb correlations in MEPs are not seen with voluntary activation, presumably because the motor pools do not share branched-axon projections from single CM cells, and a process synchronising excitability of separate neuronal populations in the two hemispheres is suppressed with voluntary activation.
6. In the patient, the MEP correlations between masseter muscles were stronger than in normal subjects. This presumably reflects a greater number of branched CM projections to these muscles, in the absence of the normal population of contralaterally projecting CM cells from the damaged hemisphere in the patient.

CHAPTER 6

SIMULTANEOUS FLUCTUATIONS IN SIZE OF RESPONSES TO FOCAL TMS IN MULTIPLE MUSCLES. III. LEFT AND RIGHT MASSETER MUSCLES

6.1 Introduction

Corticomotoneuronal (CM) cells that branch to monosynaptically excite motoneuron pools of several synergistic muscles are an important feature of the fine motor cortex control over the hand muscles in humans (see Porter and Lemon, 1993). It is not known if CM cells within the corticotrigeminal system also branch to activate synergistic muscles. Unlike limb muscles, the muscles on either side of the jaw act synergistically in most jaw movements, and it is known that the motor cortex of each hemisphere innervates masseter motoneurons bilaterally (Kuypers, 1958a; Iwatsubo et al., 1990; Carr et al., 1994; Butler et al., 2001). This may be due in part to the presence of corticobulbar axons that branch to innervate the masseter motoneuron pool on either side. Synchronisation between motor units in each masseter muscle has provided indirect evidence for the existence of these cells in humans (Carr et al., 1994). Since the presence of these cells has important implications for the understanding of how the motor cortex controls the jaw (see Chapter 2), the purpose of the present study was to provide more direct evidence in support of their existence using transcranial magnetic stimulation (TMS).

The experiments reported in this chapter are based on a well-documented feature of TMS. When the motor cortex is stimulated using TMS, the motor evoked potentials (MEPs)

which result vary considerably in size from one stimulus to the next, even when a constant stimulus intensity is used (Amassian et al., 1989; Britton et al., 1991; Brasil-Neto et al., 1992; Kiers et al., 1993). This variation cannot be wholly attributed to changes in the effectiveness of the stimulation (Reutens et al., 1993) or noise in the recording system (Burke et al., 1995). Nor is the fluctuation occurring only at the level of the motoneurons (Funase et al., 1999). Fluctuations in motor cortex excitability are believed to be responsible, at least in part, for the fluctuations in MEP size (Ellaway et al., 1998; Funase et al., 1999).

If single CM cells branch to innervate motor pools of several muscles, then the fluctuations in MEP size in these muscles are likely to co-vary as the excitability of the shared CM cell fluctuates. Previous studies have examined this issue in the upper limb and found that co-variation of excitatory effects occurs in intrinsic hand muscles (Ho et al., 1998; Rossini et al., 1999) and in proximal arm muscles acting synergistically (Schieppati et al., 1996). In related experiments I have demonstrated co-variation in MEP size from pairs of muscles known to be innervated by CM cells with branched axons (Chapters 4 and 5).

The present study aimed to apply this technique to the trigeminal system and examine trial-by-trial correlations in the size of MEPs elicited in the masseter muscles on each side by focal TMS of one hemisphere. A significant positive correlation in the size of MEPs in the two muscles may indicate single corticotrigeminal neurons with branched-axon projections to both masseter motor pools.

6.2 Methods

A prerequisite for subjects to be included in this study was the ability to elicit cortical MEP responses in both masseter muscles following focal magnetic stimulation of one hemisphere of the motor cortex. This was achieved in 12 subjects of the 16 tested. In the other subjects the cortical MEP in the ipsilateral masseter was obscured by direct stimulation of the ipsilateral trigeminal root. The 12 subjects were 10 females and 2 males, aged between 20 and 51. All subjects gave informed consent to the procedures, and the experiments were conducted with the approval of the Human Research Ethics Committee at the Adelaide University.

6.2.1 Apparatus and recording

Details of the apparatus and recording techniques used in these experiments have been reported in Chapter 2. Briefly, the electromyogram from the left and right masseter muscles was recorded using bipolar surface Ag-AgCl electrodes. In 4 subjects electrodes were also placed over the first dorsal interosseous muscle on the side contralateral to the TMS. Surface EMG signals were amplified using the stimulus artefact suppressing amplifier (1000-3000 X), filtered (bandwidth 20-500 Hz) and recorded onto separate channels of a 22 kHz PCM data recorder (Vetter 400, A.R. Vetter Co., Pennsylvania, USA). The EMG signals 50 ms preceding and 150 ms following each stimulus were digitised (2 kHz sampling rate per channel), rectified and stored on computer for later analysis.

Focal stimulation of one hemisphere of the motor cortex was achieved using transcranial magnetic stimulation (Magstim 200) delivered through a figure-of-eight stimulating coil. The coil was oriented at an angle of 45° relative to the parasagittal plane, with current induced in the underlying cortex flowing postero-anteriorly, and was positioned so as to produce optimum responses in the active masseter muscles on both sides.

6.2.2 Protocol

Subjects were seated in front of two oscilloscopes showing rectified and smoothed EMG feedback from the left and right masseter muscles. They were instructed to bite maximally on their molar teeth and the maximum voluntary contractions (MVCs) of each masseter muscle were recorded. Subjects were then required to maintain a bilateral steady contraction of their masseter muscles at 10% of their maximal EMG activity. TMS intensity was adjusted so that a response was evoked in both masseter muscles with every stimulus. Two subjects were tested at three TMS intensities, seven subjects at two intensities and the remainder were tested with only one TMS intensity. At each TMS intensity 50 stimuli were given, delivered in two blocks of 25.

6.2.3 Regression analysis of trial-by-trial variation in size of MEPs elicited in pairs of muscles

The area of MEPs resulting from the 50 TMS stimuli were measured in contralateral masseter, ipsilateral masseter and contralateral FDI from the digitised rectified EMG signals. The coefficient of variation (CV) was recorded as a measure of the variability in MEP sizes throughout the 50 stimuli and compared between FDI and masseter using a

one way ANOVA (α=0.05). Linear regression was used to examine the correlation in size of MEPs elicited by unilateral TMS in both masseter muscles, and in FDI, where appropriate, on a trial-by-trial basis.

To exclude factors such as coil movement or changes in the level of masseter activation from producing spurious correlations in MEPs between the masseter muscles on each side, two strict criteria were applied to the data.

a) MEP "time effect" criteria

For each muscle unpaired t-tests were performed to compare the mean MEP area obtained with the first 25 stimuli and those obtained with the second 25 stimuli. This was done to ensure that the effectiveness of the stimulus did not change over the course of the 50 trials (perhaps due to slight shifts in coil positioning between blocks of trials). Data were excluded from further analysis if a significant difference in mean MEP area was found between blocks of trials, in both muscles of the regression, as this could potentially introduce a false correlation in MEP size in the two muscles over the 50 trials. This was called the "MEP time effect." Three of 23 bilateral biting task trials were excluded on this basis.

b) Pre-stimulus EMG time effect

The level of activation of a muscle may affect the size of the MEP elicited by TMS (Rothwell et al., 1991). Unpaired t-tests were therefore used to compare the level of pre-stimulus EMG in the 50 ms preceding the first 25 stimuli with that of the second 25 stimuli. If the mean pre-stimulus EMG was found to differ between the two blocks of

trials, the data were not included in the analysis. This effect was called the "pre-stimulus EMG time effect" and one of the 23 regressions performed was excluded on this basis.

Since the masseter muscles must be active to obtain a MEP, it might be argued that correlations in masseter MEPs were due to parallel fluctuations in EMG activity. The mean EMG level for 50 ms prior to the stimulus was therefore measured from the rectified EMG records and compared for both masseter muscles, using linear regression. In addition, linear regression analysis was performed to determine if the small fluctuations in prestimulus EMG from stimulus to stimulus were related to the fluctuation in MEP size.

In four subjects, MEPs were also recorded from the resting FDI muscles during the bilateral biting task. The face and hand areas of motor cortex are situated close to each other, and TMS at the intensities used to produce a MEP in active masseter muscles was always suprathreshold for a response in the contralateral FDI at rest. Trial-by-trial correlations between FDI and masseter MEPs contralateral to the stimulated hemisphere were examined in these subjects to be confident that significant MEP correlations between masseter muscles were not due to trial-by-trial variations in coil position or non-specific fluctuations in motor cortex excitability. The same exclusion criteria were applied to these regressions as for the left-right masseter regressions. This resulted in one of the ten pairs of FDI/masseter regressions being excluded due to a MEP time effect in both muscles.

6.3 Results

6.3.1 MEP variation

Focal TMS of one hemisphere of the motor cortex produced responses in left and right masseter, and in the FDI contralateral to the stimulated hemisphere (see Figure 6.1). TMS of constant intensity produced MEPs with considerable variation in size from one stimulus to the next. A representative example of the masseter and FDI responses to four consecutive stimuli, recorded in one subject is shown in Figure 6.1. Distribution histograms showing the variations in MEP area in each muscle over the 50 stimuli are shown in Figure 6.2 for this subject. The degree of variation in MEP area for this subject, as expressed by the coefficient of variation, was 0.31 in contralateral masseter, 0.50 in ipsilateral masseter and 0.33 in contralateral FDI. The coefficient of variation of MEP area in each of the three muscles in all subjects is shown in Figure 6.3. On average the coefficient of variation for contralateral masseter was 0.40 ± 0.01, for ipsilateral masseter was 0.38 ± 0.01 and for contralateral FDI was 0.34 ± 0.09. There was no difference between the coefficients of variation for any of the muscles (ANOVA, $p>0.05$).

The exclusion of data showing a MEP time effect ensured that only muscles in which the MEP variations were independent of time were included in the analysis. Figure 6.4 shows the data from one subject and shows that the MEP variations were similar in the first and second block of 25 trials (same subject as Figures 6.1 and 6.2).

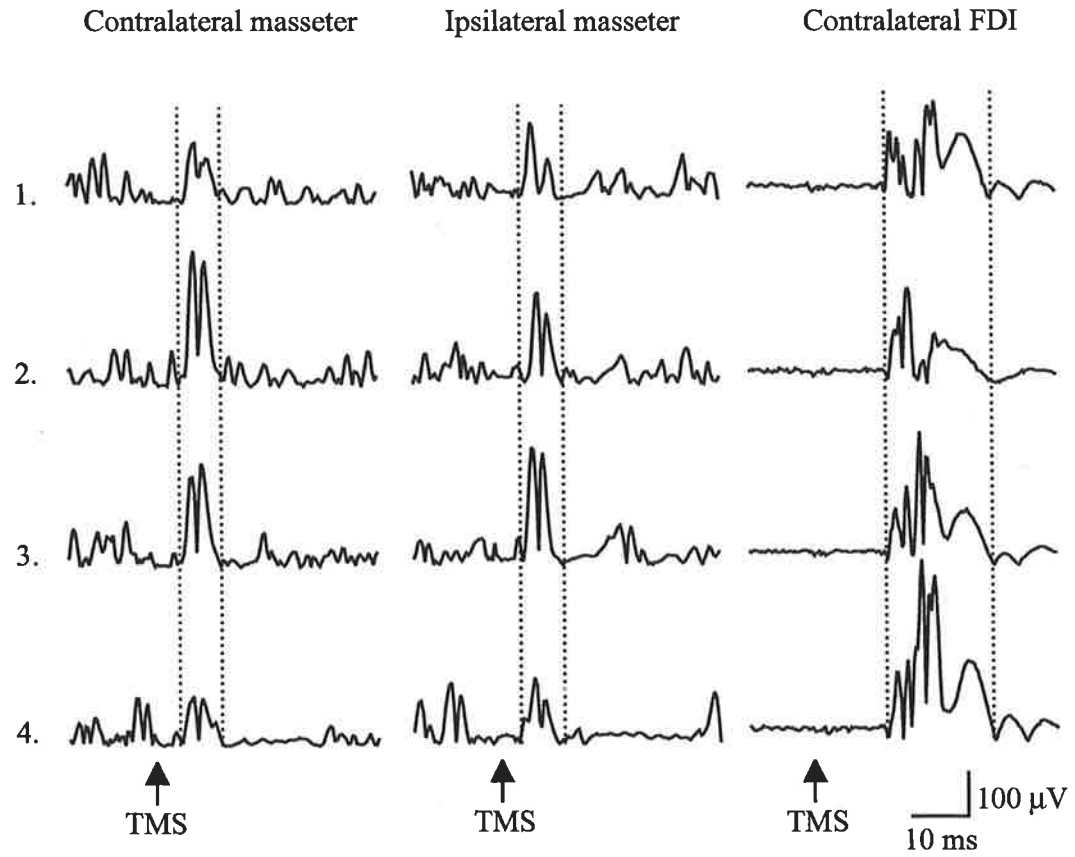

Figure 6.1

Examples of MEPs evoked by TMS in both masseter muscles and in the contralateral FDI in one subject. Responses to four consecutive magnetic stimuli are shown. The time of stimulation is indicated by the arrow. TMS intensity was 50% of maximum stimulator output. Note that the amplitude of the MEP varies with successive stimuli in all muscles. In this subject there was a positive correlation between the size of MEPs from left and right masseter (r^2=0.12, n=50, $p<0.05$), but the responses in FDI were not related to the responses in either masseter (contralateral masseter *vs.* contralateral FDI, r^2=0.002, n=50; ipsilateral masseter *vs.* contralateral FDI, r^2=0.03, n=50).

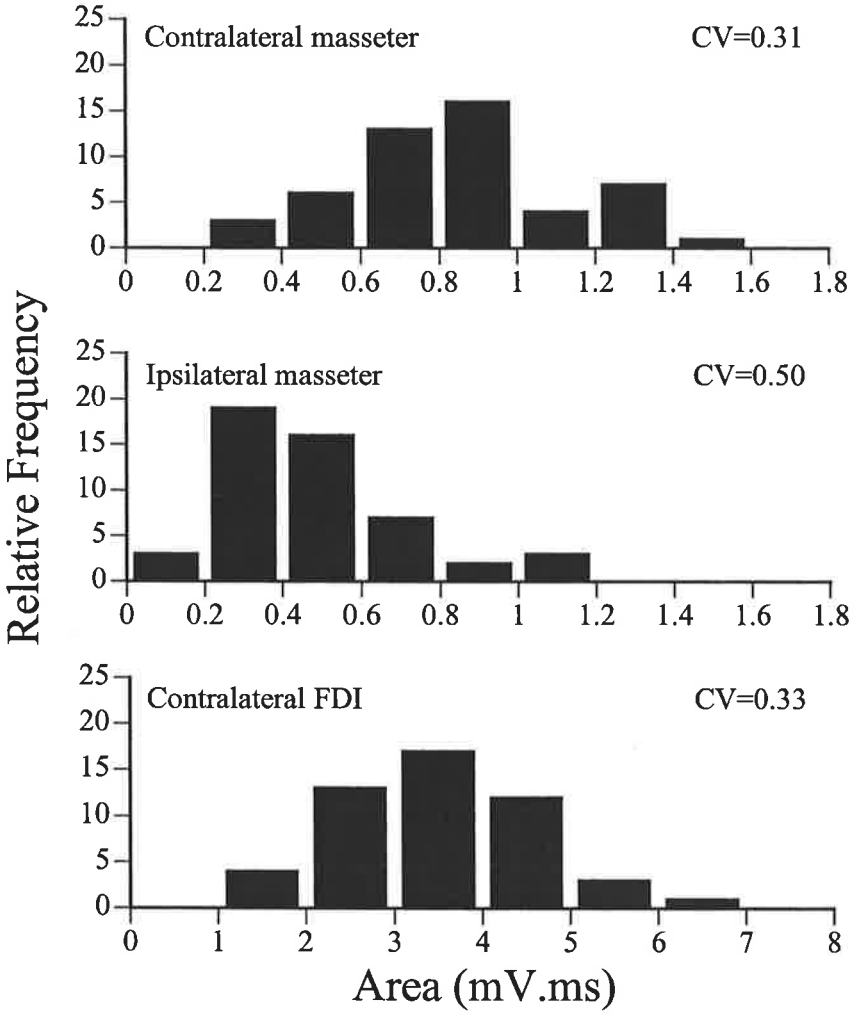

Figure 6.2

Frequency distributions of MEP size resulting from 50 TMS stimuli recorded in both masseter muscles and in the contralateral FDI (same subject as Figure 6.1). TMS intensity was 50% of maximal stimulator output. Both masseter muscles were active, while FDI was at rest. There was variation in the size of the MEPs obtained from the 50 trials in all three muscles. Coefficient of Variation was 0.31 in contralateral masseter, 0.50 in ipsilateral masseter and 0.33 in contralateral FDI.

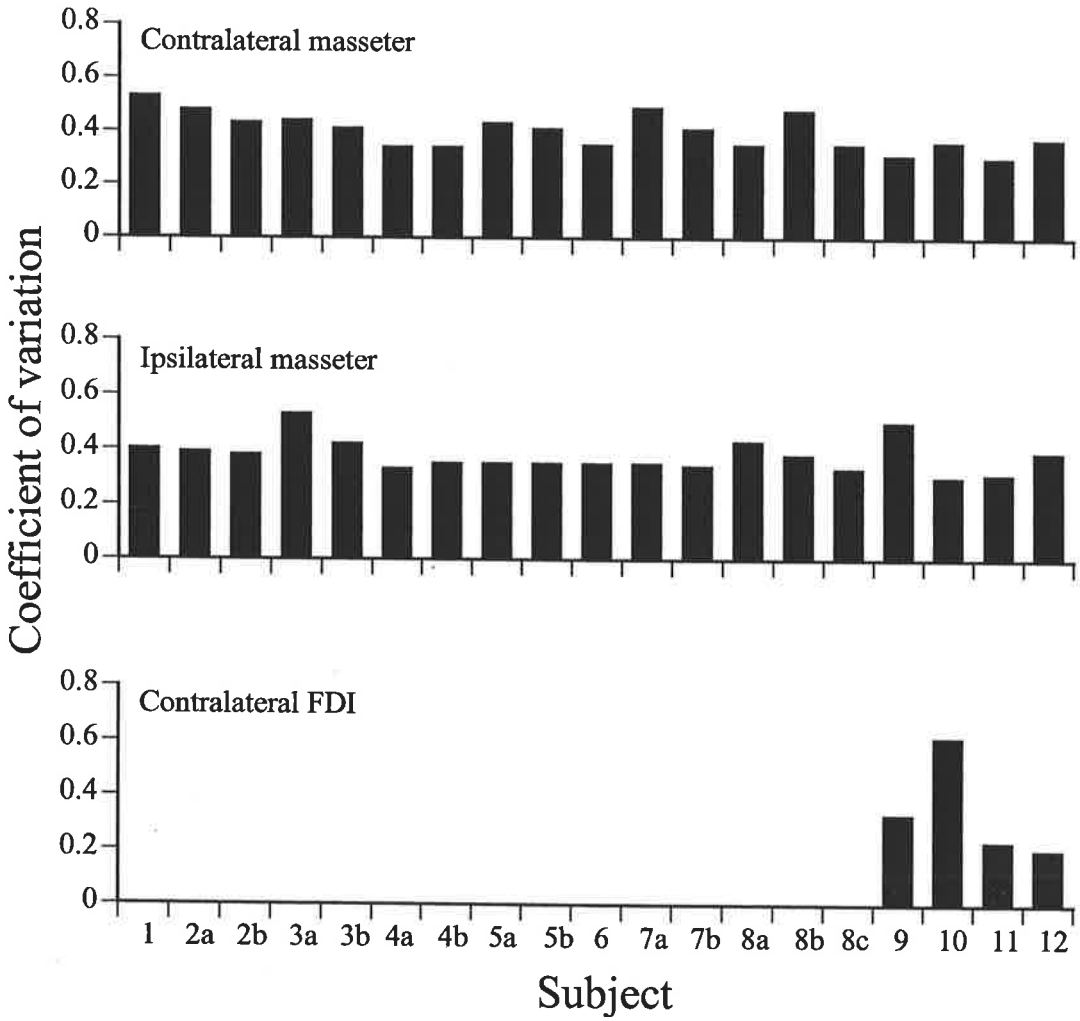

Figure 6.3

Variability (coefficient of variation) of MEP size in each of the three muscles in 12 subjects (19 trials). Data are derived from the MEPs evoked from TMS (n=50) delivered during the same block of trials in each subject. Each subject is numbered (some were tested at more than one stimulus intensity). There was no difference in the variability of MEP size between any of the muscles (ANOVA, $p>0.05$).

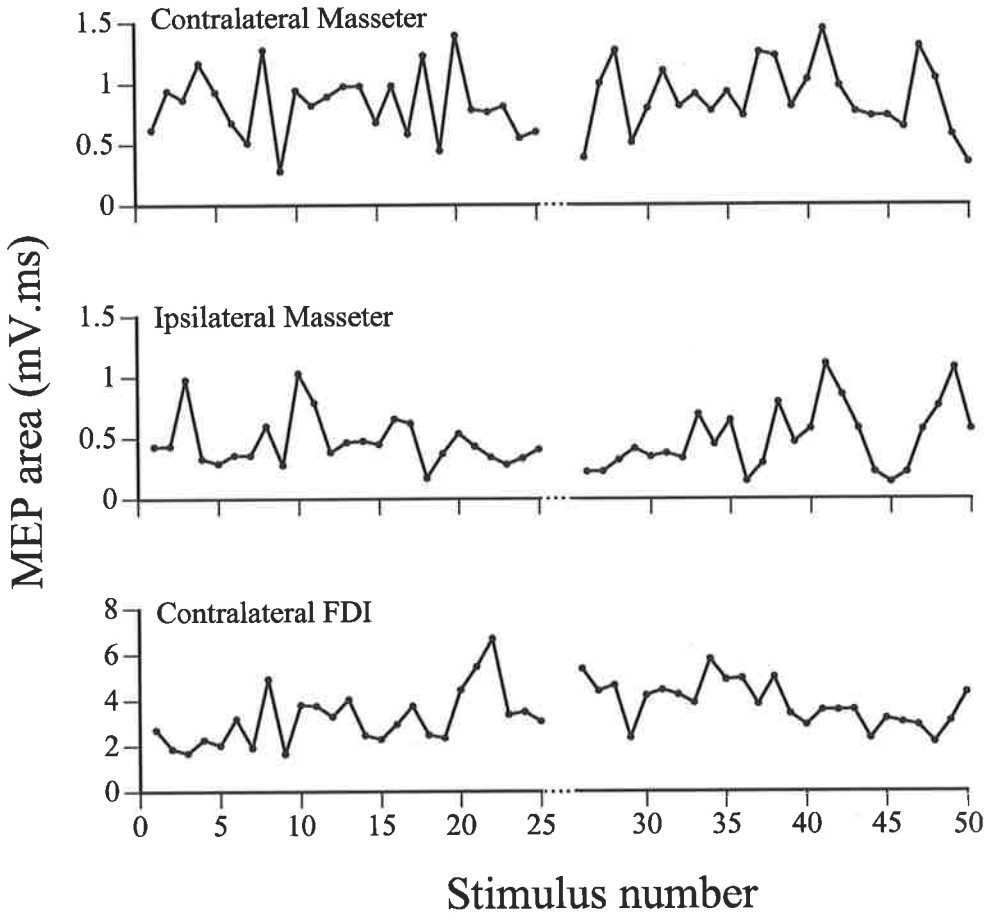

Figure 6.4

Data from one subject showing that there was no progressive change in MEP area over time. MEP area from each stimulus is shown as a function of stimulus order. TMS intensity in this subject was 50% of maximal stimulator output. There was no difference in mean MEP areas obtained from the first 25 stimuli with those obtained from the second 25 stimuli, for any muscle.

6.3.2 Trial-by-trial correlations in MEP size

Correlations in the size of MEPs elicited in different muscles by TMS was examined on a trial-by-trial basis as evidence of shared branched axon inputs from single corticobulbar neurons to both masseter motor pools. Data from subject 9 are shown in Figure 6.5. In this subject there was a significant positive correlation in the size of MEPs from the two masseter muscles over the 50 trials (Figure 6.5A; r^2= 0.12, $p<0.02$). There was no significant correlation between pre-stimulus EMG level and MEP size for the same 50 trials in either the contralateral (Figure 6.5B; r^2 = 0.002 or ipsilateral (Figure 6.5C; $r^2=1x10^{-5}$) masseter. There was no significant correlation in ipsilateral and contralateral pre-stimulus EMG fluctuations in this subject (data not shown, r^2=0.01). The significant correlation in Figure 6.5A is therefore not due to any influence on the MEP of parallel fluctuation in the pre-stimulus EMG level in both masseter muscles. The absence of a significant correlation between contralateral masseter and FDI MEPs (Figure 6.5D; r^2 = 0.002) suggests that non-specific fluctuations in motor cortex excitability during stimulation or changes in coil position were not responsible for the significant correlation seen between MEPs in left and right masseter.

In 6 of 19 comparisons (32%) in 12 subjects there was a significant positive correlation between the size of MEPs in contra- and ipsilateral masseter muscles for the 50 stimuli. These data are summarised in Table 6.1. The significant correlation coefficient (r^2) values ranged from 0.08 to 0.18 (p values between 0.0023 and 0.0402). The mean r^2 value for all 19 correlations was 0.05 ± 0.01. There was a significant positive correlation between the pre-stimulus masseter EMG levels in each side in 10 of 19 comparisons. This was the

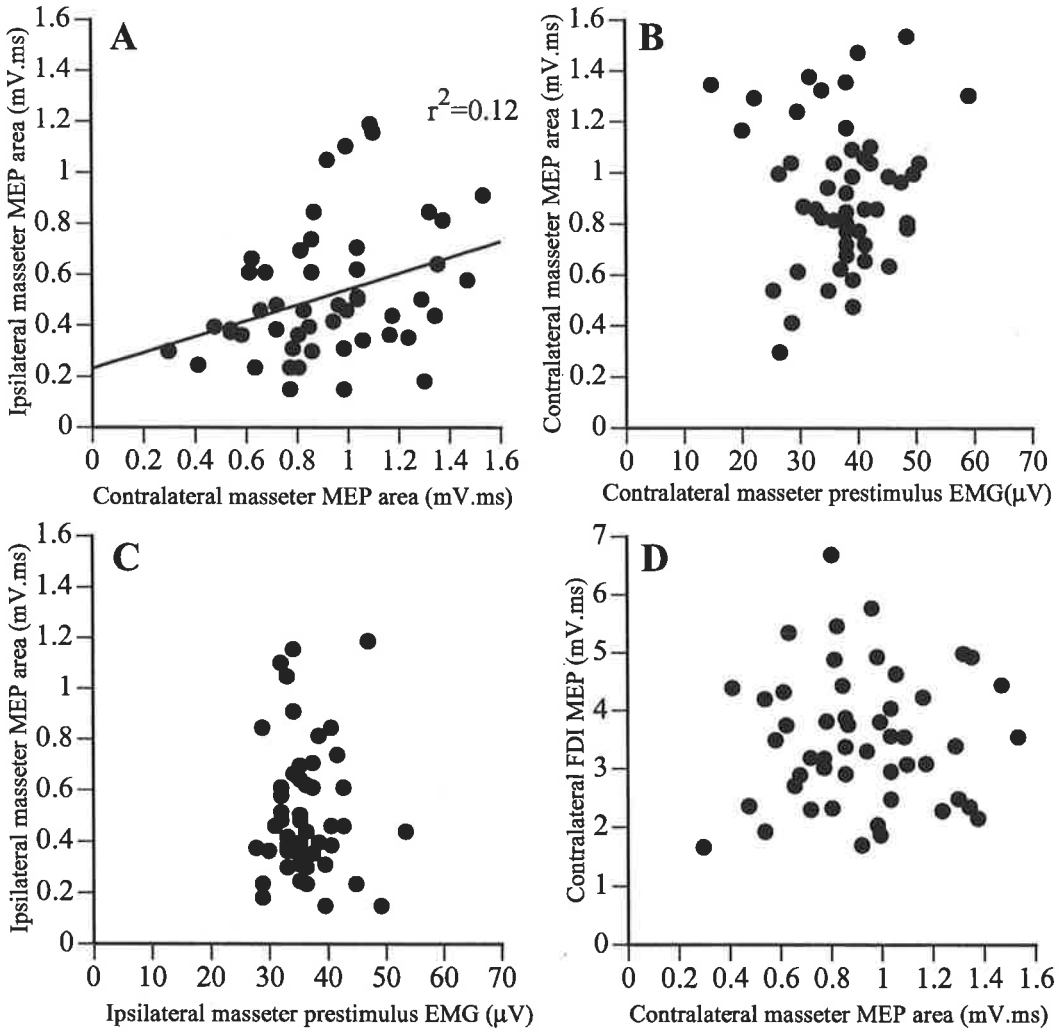

Figure 6.5

Correlation in size of MEPs elicited by focal TMS. Each plot consists of data from the same 50 stimuli in one subject, with a TMS intensity of 50% and delivered during a bilateral bite with the FDI muscle relaxed. **A**, contralateral masseter MEP area *vs.* ipsilateral masseter MEP area (r^2=0.12, p<0.02). **B**, contralateral masseter pre-stimulus EMG *vs.* contralateral masseter MEP area (r^2=0.002, p>0.05). **C**, ipsilateral masseter pre-stimulus EMG *vs.* ipsilateral masseter MEP area (r^2=0.00001, p>0.05). **D**, Contralateral masseter MEP *vs.* contralateral FDI MEP (r^2=0.002, p>0.05). The trial-by-trial correlations in masseter MEP size (A) are not explained by parallel changes in masseter activation levels (B,C). The absence of a correlation between masseter and FDI MEPs (D) suggests that global cortical excitability changes or coil movement are not responsible for the significant correlation in A.

SUBJECT	TMS INTENSITY (% MAX. OUTPUT)	VARIABLES FOR LINEAR REGRESSION COMPARISONS					
		Masseter MEP size	**Masseter pre-stimulus EMG levels**	**Masseter pre-stimulus EMG level and MEP size**		**Masseter and FDI MEP size**	
		Contralateral *vs.* Ipsilateral	Contralateral *vs.* Ipsilateral	Contralateral pre-stimulus EMG *vs.* Contralateral MEP	Ipsilateral pre-stimulus EMG *vs.* Ipsilateral MEP	Contralateral masseter *vs.* Contralateral FDI	Ipsilateral masseter *vs.* Contralateral FDI
1	67%	n.s.	n.s.	n.s.	n.s.	-	-
2	54%	n.s..	n.s.	n.s.	n.s.	-	-
	60%	n.s.	n.s.	n.s.	n.s.	-	-
3	48%	n.s.	n.s.	r^2=0.18**	n.s.	-	-
	54%	n.s.	r^2=0.10 *	n.s.	n.s.	-	-
4	62%	r^2=0.12*	r^2=0.56**	n.s.	n.s.	-	-
	65%	r^2=0.14**	r^2=0.28**	n.s.	n.s.	-	-
5	42%	n.s.	r^2=0.22 **	n.s.	n.s.	-	-
	46%	n.s.	r^2=0.11 *	n.s.	n.s.	-	-
6	50%	r^2=0.08 *	r^2=0.13 *	n.s.	n.s.	-	-
7	40%	n.s.	r^2=0.08 *	n.s.	n.s.	-	-
	45%	n.s.	r^2=0.08 *	n.s.	n.s.	-	-
8	50%	n.s.	n.s.	n.s.	n.s.	-	-
	50%	n.s.	n.s.	n.s.	n.s.	-	-
	57%	r^2=0.10 *	n.s.	n.s.	n.s.	-	-
9	45%	-	-	-	-	n.s.	-
	50%	r^2=0.12 *	n.s.	n.s.	n.s.	n.s.	n.s.
10	60%	n.s.	r^2=0.17 **	n.s.	n.s.	n.s.	n.s.
11	45%	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
12	55%	r^2=0.18	r^2=0.36**	n.s.	r^2=0.21**	n.s.	n.s.

Table 6.1

Summary of linear regression analysis of trial-by-trial fluctuations of MEP size and baseline EMG in masseter and FDI muscles. Values are the coefficient of determination (r^2) for each comparison. Data were obtained from 50 TMS stimuli delivered during a bilateral bite in 10 subjects. r^2 values are shown only for significant positive linear relationships (* $p<0.05$, **$p<0.01$). n.s. indicates that the correlations were not significant. '-' indicates that data were not available for that comparison. 6 of 19 (32%) comparisons showed a significant trial-by-trial correlation between MEP size in contralateral and ipsilateral masseter.

case for 4 of the 6 examples in which significant correlations were seen between masseter MEPs on each side. A significant positive correlation between pre-stimulus rectified EMG level and MEP size for the 50 trials was seen in only 2 of 38 comparisons, and in a further 2 comparisons there was a significant negative correlation between MEP size and pre-stimulus EMG level. This indicates that the masseter MEP size was relatively independent of the small fluctuations of background EMG levels in these trials. In the four subjects for whom FDI recordings were available (including two subjects who showed significant correlations between masseter MEPs), there were no significant correlations between contralateral masseter and contralateral FDI MEP size. Similarly there were no significant correlations between ipsilateral masseter and contralateral FDI MEP size.

6.4 Discussion

The present study involved the analysis of MEPs elicited concurrently in both masseter muscles on a trial by trial basis during bilateral biting. The evidence suggests that the synchronous fluctuations in MEP size in the two masseter muscles evident in 32% of comparisons arise from cortical mechanisms. The significant positive correlation of MEPs shows that the population of CM cells in one hemisphere providing short-latency excitation to masseter muscles on each side are not independent. The simultaneous co-variation in MEP size in the two masseter muscles must arise from either branched-axon projections to both motor pools from single CM cells, or highly correlated fluctuations in excitability of separate functional groups of CM cells which innervate one side or the other.

These experiments depended on the variability in MEP size resulting from TMS, and hence the coefficient of variation (CV) was recorded as a measure of variability. There was no difference in the CV between the active masseter muscles, or the inactive FDI (ANOVA $p>0.05$). The CV values were comparable to those recorded in previous studies in various hand muscles (Chapter 4, Kiers et al., 1993; Ellaway et al., 1998).

In 6 comparisons out of 19 (32%) there was a significant positive correlation between the size of MEPs in both masseter muscles on a trial by trial basis, but no significant correlations between MEP size in masseter and FDI. The correlation in MEP size between the two masseter muscles could be due to a number of factors, which are discussed below.

6.4.1 Fluctuations in MEP size and parallel fluctuations in activity of both masseter muscles

In theory parallel fluctuation in excitatory drive to the masseter motoneuron pools on each side could have contributed to significant MEP correlations because of the dependence of MEP size on background EMG levels (Rothwell et al., 1991). However, this does not appear to be the case since a significant correlation in masseter EMG levels between sides was seen in just over half of the comparisons, and in only 4 of these cases were the masseter MEP sizes also correlated between muscles.

Further analysis revealed that the small fluctuations in background EMG level had little effect on MEP size in the present study. A positive correlation between the pre-stimulus EMG activity and the size of the MEP in masseter was seen in only 2 of 36 comparisons.

This may seem surprising, since MEP size is known to increase with increasing EMG levels (Rothwell et al., 1991). In attempted constant force contractions of the triceps surae, however, the MEP was independent of fluctuations of background EMG levels in a number of subjects (Funase et al., 1999). Also, in a study of hand muscle MEPs Schieppati et al. (1996) showed that small fluctuations in pre-stimulus EMG level did not affect the MEP size. Lim and Yiannikas (1992) showed that the pre-stimulus EMG is correlated with MEP size only up to about 6% of maximum surface EMG. Subjects in the present study were attempting to maintain a steady contraction at a target level of 10% MVC, and so the fact that the small fluctuations in EMG level preceding the stimulus did not affect MEP size is consistent with the findings of Lim and Yiannikas (1992), Schieppati et al. (1996) and Funase et al. (1999).

Longer-term parallel changes in EMG activity can also not explain the correlations in masseter MEPs. Each trial was done in two parts, and it is theoretically possible that the subject may have changed the level of activation in the muscles from the first half to the second half of the trial. This was monitored carefully during the experiment and subjects were given a target EMG level for each muscle which was the same throughout the trial. In addition, the exclusion of data showing an EMG time effect (1 of 23 trials excluded) ensured that long-term changes in EMG were not contributing to masseter MEP correlations.

6.4.2 Fluctuations in MEP size and movement of the stimulating coil

Slight changes in the position and orientation of the stimulating coil will alter the number of cortical cells activated by a given stimulus, and therefore affect the resulting MEP.

However, movement of the coil between each stimulus is unlikely to account for the results presented here, since there were no correlations in the size of MEPs between masseter and FDI. The motor cortical representation for FDI and masseter are in close enough proximity that changes in the effectiveness of the stimulus due to coil position should be reflected in FDI as well as masseter. Careful positioning of the coil ensured no differences in its position between the two blocks of stimuli. To confirm that this was not an issue, trials in which both muscles showed a difference in the size of the MEP from the first 25 stimuli compared with those from the second 25 were excluded (MEP time effect, 3 of 23 trials excluded).

6.4.3 Fluctuations in MEP size reflect specific changes in the corticomotor region supplying masseter rather than global cortical excitability fluctuations.

Global cortical excitability changes would affect the MEP sizes in all muscles. Indeed, Ellaway et al (1998) has recently demonstrated under resting conditions a correlation in size of MEPs from left and right hand muscles elicited by bilateral TMS. These were presumably due to simultaneous fluctuations in the excitability of the hand areas of the motor cortex in each hemisphere. However, widespread cortical excitability changes are unlikely to account for the present results for two reasons. First, no correlation in the size of the MEPs from masseter and FDI was seen. If widespread fluctuations in cortical excitability were responsible for the correlations between left and right masseter, then one would expect to see significant correlations between FDI and masseter. Second, in related experiments I have demonstrated that the global cortical excitability changes responsible for MEP correlations in the left and right hand muscles disappear when a muscle is

activated (see Chapter 4), even though correlations between active synergistic muscles within the same hand remain. It is not possible to obtain MEPs in resting masseter, and so the correlations reported here were for active masseter muscles. Since the interhemispheric coupling of excitability of the hand corticomotor representation reported by Ellaway et al. (1998) disappears with voluntary activation of a muscle (Chapter 4), the mechanism producing it is not likely to be responsible for the simultaneous fluctuations in size of masseter MEPs.

6.4.4 There may be separate populations of contralaterally and ipsilaterally projecting corticobulbar neurons.

These experiments cannot completely exclude the possibility that there are separate populations of contralateral and ipsilateral corticobulbar projections with common fluctuations in excitability. In fact I have already shown in Chapter 2 that at least some of the corticobulbar cells projecting to masseter have exclusively contralateral excitatory projections. However, it is less likely that MEPs in left and right masseter would be correlated if they were completely innervated by different populations of corticobulbar cells, than if the two muscles actually shared some common corticobulbar input.

6.4.5 There may be a population of corticobulbar cells which branch to innervate the masseter motoneuron pools on both sides.

Branched axons to intrinsic hand muscle motoneurons supplying muscles of the same limb are known to exist in normal human subjects (see Porter and Lemon, 1993). In related experiments I have studied MEP variation in muscles that are known to share branched axon input (see Chapter 4, within-limb comparisons of MEP fluctuations, and

Chapter 5, within- and between-limb comparisons in a patient with mirror movements). Co-variation in MEP size in pairs of muscles sharing branched CM input was demonstrated. Similarly, in the present study I have shown that within a single hemisphere, excitability of cortical neurons providing short-latency excitation to the two masseter muscles is not independent, on a moment-to-moment basis.

The present results suggest the presence of a population of corticobulbar neurons with axons that branch to innervate the masseter motoneurons on both sides. Evidence for the existence of these cells has already been obtained in monkeys using intra-cortical micro-stimulation (Huang et al., 1988) and indirectly in humans using cross correlation of motor unit firing rates in left and right masseter (Carr et al., 1994). This arrangement of branched cortical cells is consistent with the way in which the motor cortex innervates synergistic hand muscles.

6.4.6 Conclusion

The present study has demonstrated a degree of co-fluctuation in size of MEPs elicited in left and right masseter muscles by TMS. This may arise from a number of different mechanisms, but the most likely is that there is a population of corticobulbar cells that arise from one hemisphere of the motor cortex and branch to activate masseter motoneurons on both sides of the body. The existence of these cells has important implications in the understanding of how the motor cortex controls movement of the jaw muscles during such finely controlled activities as speech and mastication. In Chapter 2 a model of how the motor cortex may control bilateral and unilateral activation of masseter

was presented. The results of this study provide further support for elements of this model.

CHAPTER 7

IS THE LONG LATENCY STRETCH REFLEX IN HUMAN MASSETER TRANSCORTICAL?

7.1 Introduction

The execution of co-ordinated muscle contraction depends upon sensory feedback from muscle and joint receptors. If a movement is interrupted by an unexpected change in muscle length then muscle spindle receptors are activated, eliciting compensatory reflex contractions. The response in the stretched muscle consists of at least two peaks of excitation. The first response is known as the short latency stretch reflex (SLSR) and is thought to be mediated by a monosynaptic pathway. At a longer latency there is a more sustained period of excitation that is known as the long latency stretch reflex (LLSR).

The LLSR is thought to be the most important physiological response to stretch since it accounts for most of the force of the response (Hammond, 1960). Its increased latency has led researchers to suggest that the LLSR in upper limb muscles involves the motor cortex (Hammond, 1960; Phillips, 1969; Matthews, 1991). An advantage of a long loop involving the motor cortex is the added flexibility that this would give the reflex response. Indeed, studies have demonstrated that the LLSR is flexible ("set-dependent"), and can be modulated by prior instruction to the subject (Calancie and Bawa, 1985), although more recent studies question this finding (Capaday et al., 1994).

Cheney and Fetz (1984) recorded from single cortical neurones projecting monosynaptically onto motoneurons (corticomotoneuronal (CM) cells). They showed that in conscious monkeys the motor cortex responds to muscle stretch at an appropriate time to mediate the LLSR. This finding has been confirmed in humans by indirectly assessing motor cortex excitability during stretch. Day *et al.* (1991) activated CM cells projecting to the hand using magnetic transcranial magnetic stimulation (TMS). The size of the motor evoked potential (MEP) observed in the rectified surface electromyograph (EMG) of flexor digitorum profundus was used as an index of CM cell excitability. It was found that the excitability of CM cells increased during the interval that would correspond with the passage of the stretch-evoked afferent volley from the muscles through the cortex. In contrast, there was no convergence of afferent signal onto the CM cells during the SLSR. This observation was later confirmed by Palmer and Ashby (1992b) who measured the MEP in motor units of flexor pollicis longus and found that the excitability of CM cells was facilitated during the LLSR.

While the stretch reflex mechanism in hand muscles has been studied extensively, the trigeminally-innervated jaw muscles have received little attention. The reflex pathways of the trigeminal motor system are different in several respects from the limb muscles. For example, the existence of Golgi tendon organs in the adult jaw muscles is controversial; the jaw opening muscles contain few, if any muscle spindles and there are no trigeminal equivalents of Renshaw cells or Ia inhibitory interneurons (see Luschei and Goldberg, 1981). Also, a single spindle Ia afferent projects to only 10 - 30% of homonymous motoneurons in masseter (Appenteng et al., 1978; Nozaki et al., 1985). This is a considerably smaller figure than the divergence of single Ia afferent projections to

motoneurons of limb muscles, where it approaches 100% (Mendell and Henneman, 1971; Watt et al., 1976).

Stretch reflexes are important in the masseter muscle since the load between the teeth can change both unpredictably and progressively as the food is broken down during chewing. The stretch reflex is also important during locomotion, where it is thought to be involved in the maintenance of mandibular position (Lund et al., 1984; Miles and Nordstrom, 2001). Until recently it was believed that stretch of the human masseter muscle resulted in a reflex response at short latency, but not at a longer latency. This was a frequently cited difference between masseter and limb muscles and raised important questions regarding the function of the LLSR in motor control. However, it has since been shown that slow, smooth stretch of the masseter muscles does evoke both a short latency and a long latency response (Poliakov and Miles, 1994). The CNS pathways mediating the masseter LLSR remains unknown.

The present study aimed to establish the role of the motor cortex in the LLSR of masseter in man. The cortical response to stretch of the masseter was assessed using TMS, following the protocol of Day et al. (1991). The ability to modify the LLSR by voluntary command ("set-dependence") was analysed by examining the effect of prior instruction to the subject, using the protocol of Calancie and Bawa (1985).

7.2 Methods

The experiments were conducted with the approval of the Human Research Ethics Committee at the University of Adelaide. Ten neurologically normal subjects (five males

and five females aged from 21 to 36 years) participated in fifteen experiments (two males and three females were tested twice). All subjects gave informed consent.

7.2.1 Apparatus and recording

The surface EMG of left (13 experiments) or right (2 experiments) masseter was recorded using self-adhesive bipolar silver/silver chloride electrodes placed along the line of the masseter muscle fibres. One electrode of the pair was placed at the level of the lower border of the mandible, and the other about 2.5 cm above this, close to the motor point. The subjects were grounded by a lip-clip electrode on the lower lip (Türker et al., 1988). This series of experiments was conducted with an EMG amplifier that did not have artefact suppression capability.

Subjects were instructed to bite isometrically, using the incisor teeth, onto the stainless steel bars of a purpose-built jaw muscle stretcher (see Miles et al., 1993). The baseline jaw separation, determined by the thickness of the jaw bars, was about 3 mm. Stretches were delivered to the masseter muscle by means of a servo-controlled electromagnetic vibrator, which imposed controlled displacements on the lower jaw (see Miles et al., 1993).

A strain gauge attached to the lower jaw bar was used to measure the jaw-closing force. Displacement and vertical acceleration of the lower jaw bar were measured with a length transducer and accelerometer mounted on the apparatus. Amplified (1000x), rectified and unrectified surface EMG signals (bandwidth 5-500 kHz), force, displacement and acceleration were digitised on-line (1 kHz sampling rate per channel), averaged, and

stored on computer. The masseter surface EMG and the jaw closing force were recorded on separate channels of a 22 kHz PCM data recorder (Vetter 400, A.R. Vetter Co., Pennsylvania, USA).

Transcranial magnetic stimulation (TMS) was achieved through a magnetic stimulator (Magstim model 200) with a peak maximal field strength of 2.0 T. A high-power 13 cm diameter circular stimulating coil was placed over the vertex of the subject's head and positioned so that a minimal stimulus strength produced an MEP in the active masseter muscle. The use of supportive head blocks helped to eliminate any movement between the coil and the scalp. In 13 experiments a clockwise current in the coil was used to preferentially activate the right side cortex (Rothwell et al., 1991), and hence the left side masseter. In the remaining 2 experiments, an anticlockwise current was used to preferentially stimulate the left side cortex and the right side masseter.

7.2.2 Protocol

Subjects were seated in front of an oscilloscope that provided visual feedback of the rectified and smoothed masseter EMG. The maximum voluntary contraction of masseter was recorded and used as a reference to determine 10% maximal biting capacity. Throughout the experiment subjects were required to maintain a constant biting force at this level.

Initially, various stretches of differing amplitude and duration were tested until one was found that evoked a monosynaptic and long latency reflex in the masseter muscle. This stretch was then used for the rest of the experiment. Various TMS intensities were tested

until a MEP which was clearly discernible above the background EMG activity, and was sub-maximal in size, was achieved in most trials.

7.2.2.1 Conditioning-testing with stretch and TMS.

The protocol involved a sequence of tests, each consisting of 25 stimuli and delivered at least four seconds apart. The tests were:

A) Stretch alone;

B) TMS alone;

C-D) Stretch (conditioning) and TMS (test) stimuli combined, with conditioning-testing intervals of 3 and 5 ms respectively, to test convergence of the stretch-evoked afferent activity onto the CM cells during the short-latency stretch reflex. These tests provided a control since the SLSR is a segmental reflex, and does not converge onto the CM cells. Three and 5 ms intervals were chosen, since it is known that the onset latency of the SLSR in masseter is 9-10 ms (Poliakov and Miles, 1994), and the time for the excitation induced by the magnetic stimulus to reach the muscle is 6-7 ms (Butler et al., 2001). The chosen intervals between stretch and TMS therefore ensured that the afferent volley induced by the TMS arrived at the masseter motoneuron pool at the beginning (3ms interval) and middle (5ms interval) of the short latency stretch reflex response.

E-K) Stretch (conditioning) and TMS (test) stimuli combined, with conditioning-testing intervals of 23, 26, 29, 32, 35, 38 and 41 ms respectively to test convergence of the afferents excited by stretch onto the CM cells during the

masseter long-latency stretch reflex. These intervals were chosen because the LLSR in masseter begins around 35 ms and lasts for 35 ms (Poliakov and Miles, 1994) and the efferent signal conducted from the motor cortex to the masseter takes around 6-7 ms (Cruccu et al., 1989; Butler et al., 2001). Based on these latencies, convergence at the CM cells would not be expected until an interstimulus interval of 29 ms, but to be certain, the shorter intervals of 23 and 26 ms were also tested.

Each conditioning-testing interval was examined in a minimum of nine experiments.

L) Test B was repeated at the end of the experiment to ensure that the MEP had not altered more than 15% from its size in test B. (The data from one experiment were not included in the analyses because the MEP was reduced in amplitude by the end of the experiment.)

7.2.2.2 Modulation of the masseter LLSR by prior instruction.

In eight of the subjects (five females and three males aged from 21 to 36 years) a further series of experiments was performed in which the effect of prior instruction on the masseter stretch reflex was examined. Blocks of 25 of the same stretch stimuli were delivered to the jaw at random intervals, with a minimum interstimulus interval of four seconds. Three conditions were examined in separate blocks of trials:

1. Control stretch; subject was not required to react to the stretch.

2. "Resist stretch" condition; subject was instructed to resist the stretch by increasing the biting force as soon as the stretch was perceived.
3. "Let-go" condition; subject was instructed to "let go", or reduce the biting force upon perception of the stretch.

The order of conditions was randomised.

7.2.3 Data Analysis

7.2.3.1 Conditioning-testing with masseter stretch and TMS.

Responses were quantified from the rectified surface EMG averages. The basis of the analysis was that if convergence onto CM cells occurred the response to tests C-K (stretch and TMS combined) would be larger than the algebraic sum of responses to tests A (stretch) and B (TMS). To test this, the following analysis was performed. The response from test A (stretch reflex alone) was subtracted from the responses to tests C-K (stretch and TMS combined), creating what will be referred to as "subtracted" EMG records. In doing so, the time-varying EMG activity caused by the stretch reflex response was removed, allowing quantification of the MEP response alone. The area of the MEP was calculated from the "subtracted" EMG records (i.e., the area exceeding the stretch reflex EMG activity) in the epoch 6-14 ms following TMS. The area of the MEP in trials where TMS was given alone (test B) was calculated as the area above the baseline EMG activity in the epoch 6-14 ms following the stimulus (i.e. the area of the MEP with the background EMG activity subtracted). MEP areas were normalised by expression as a percentage of the pre-stimulus baseline EMG value. The normalised values were pooled across subjects and the normalised MEP resulting from test B (TMS alone) was compared to the

normalised MEP area from tests C-K (stretch and TMS combined) using a one way analysis of variance (α= 0.05).

7.2.3.2 *Modulation of the masseter LLSR by prior instruction.*

The area of the SLSR (10-25 ms epoch) and the LLSR (30-70 ms epoch) was determined from the rectified surface EMG records, and normalised to pre-stimulus EMG activity. A one way analysis of variance (α = 0.05) was used to compare the size of the LLSR invoked during the control condition to the size when subjects were instructed to increase or decrease their biting force upon perception of the stretch. As a control, a separate one way analysis (α = 0.05) was performed on the SLSR during the different conditions.

7.3 Results

7.3.1 TMS and muscle stretch

In all subjects controlled displacement of the mandible resulted in a short latency response followed by a longer latency response in both masseter muscles. The latencies of these responses, measured in the masseter contralateral to the hemisphere preferentially stimulated using TMS in later trials, was 10.5 ± 0.2 ms and 34.0 ± 1.4 ms, respectively. Magnetic stimulation of the cortex resulted in excitation of the contralateral masseter at a latency of 7.0 ± 0.3 ms; ipsilateral masseter was also activated by stretch and TMS, but the response was usually obscured by the stimulus artefact. Examples of the masseteric stretch reflex and the contralateral masseter MEP response to TMS obtained in two subjects is shown in Figure 7.1.

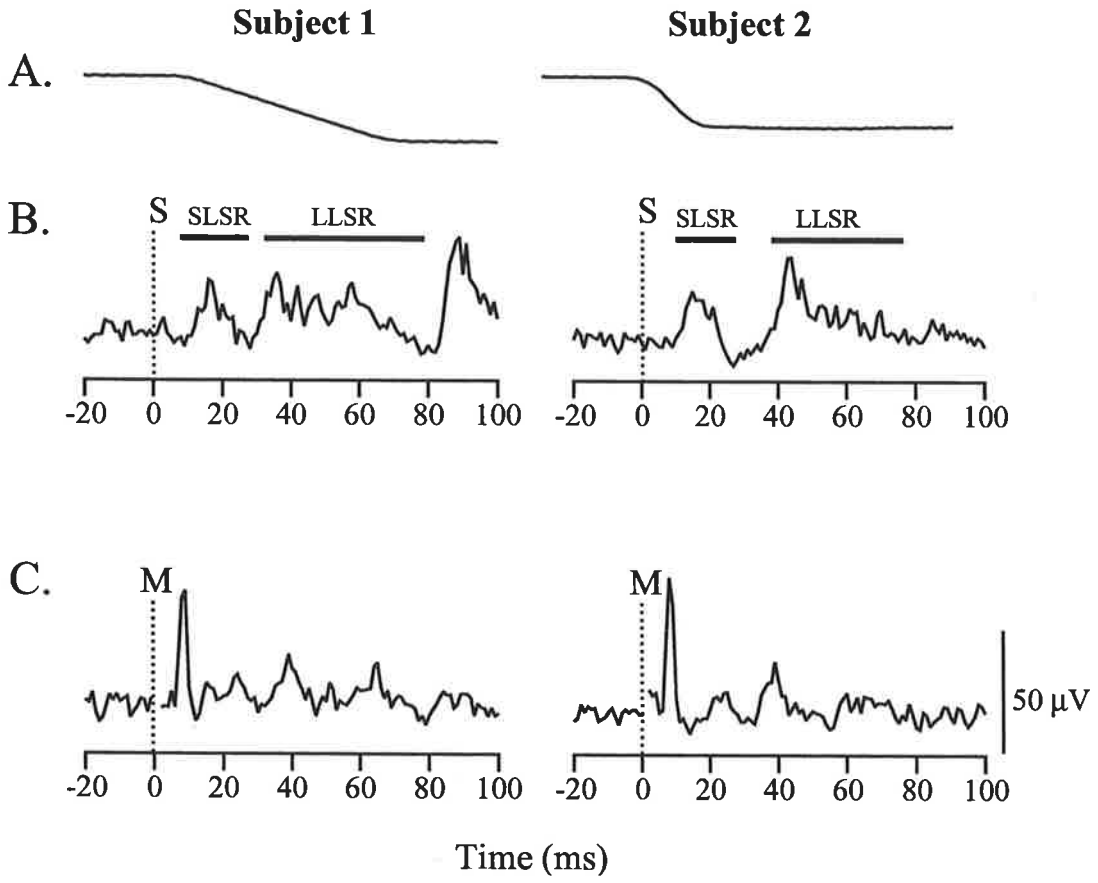

Figure 7.1

The masseter stretch reflex and response to TMS recorded from the left masseter in two subjects. Stimulus onset is shown by the vertical line. **A**, displacement of the jaw following the stretch. **B** and **C** are averaged (n=25) rectified EMG recorded from surface electrodes over left masseter. **B**, the masseter stretch reflex resulting from the jaw displacement shown in **A**. Two components of the stretch reflex can be observed. The short latency stretch reflex begins at around 10 ms, and the LLSR begins at ~30 ms. **C**, the masseter muscle evoked potential resulting from TMS. The stimulus artefact has been deleted from the EMG traces. The MEP occurred around 7 ms following the TMS.

Figure 7.2 shows the effect of combining the stretch with TMS at various conditioning-testing intervals. The masseter response to stretch alone and the response to TMS alone are shown in the top traces. The MEP recorded at the beginning of the experiment (B) was used as a reference to compare to the size of the MEP when superimposed onto the SLSR (C, D) and the LLSR (E-K). Figure 7.3 shows the same data as Figure 7.2, but the stretch reflex response (A in Figure 7.2) has been subtracted from the combined stretch/TMS trials. By removing the variable EMG baseline caused by the stretch reflex it was possible to compare the MEP size during the different C-T conditions. The MEP was not facilitated when TMS was delivered during the SLSR (C-D compared with B) or when delivered during the LLSR (E-J compared with B).

Figure 7.4 shows the summary of pooled data from 10 subjects. The area of the MEPs, with the stretch reflex response subtracted (or baseline EMG subtracted for the "TMS alone" condition), have been measured from the rectified EMG and normalised to the pre-stimulus EMG activity. With TMS alone, mean (± s.e.) MEP area above baseline was 56 ± 9%. This was no significant difference in the size of the response when conditioned with the muscle stretch at any C-T interval (One-way ANOVA, $p>0.05$).

In 8 of the 10 subjects the protocol was performed at several different TMS intensities. While the MEP induced by TMS was larger with increased stimulus intensity, MEPs were never facilitated when superimposed onto either the SLSR or the LLSR.

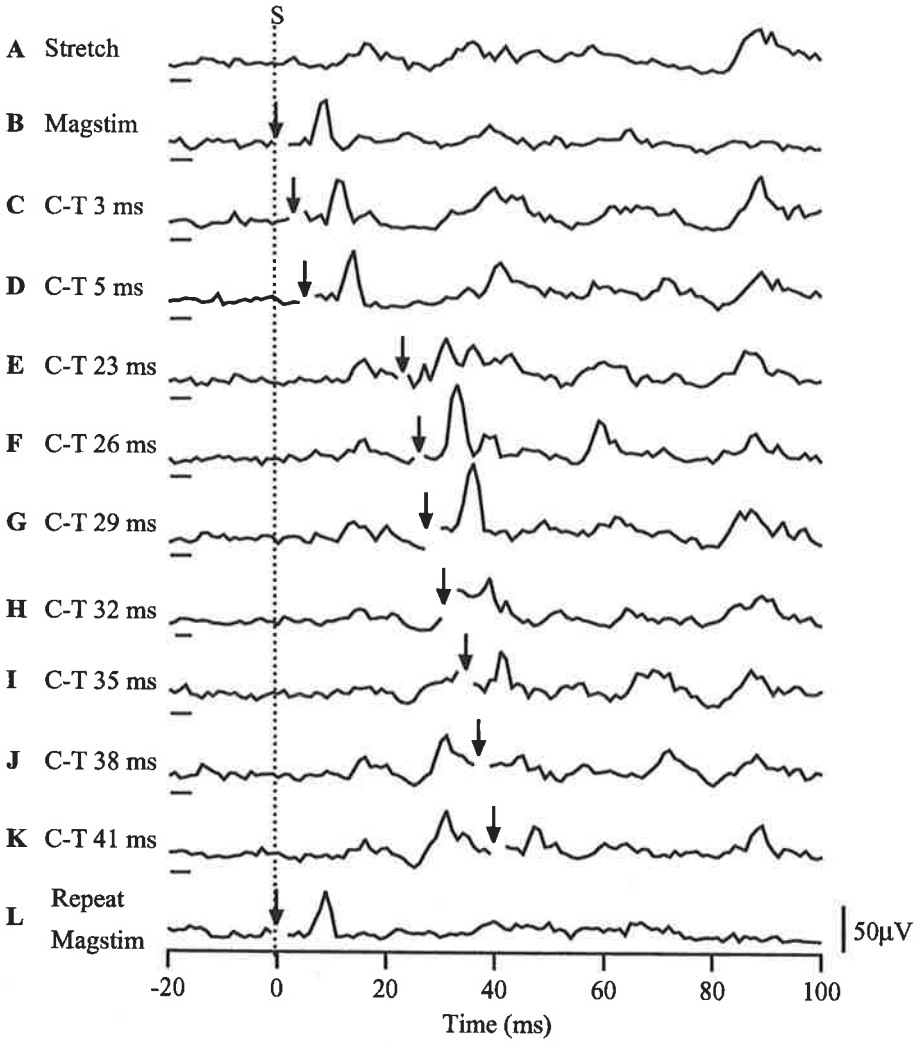

Figure 7.2

Records from one subject showing the effect of conditioning muscle stretch on the response to TMS in masseter. Traces are averaged (n=25) rectified EMG recorded from surface electrodes over masseter. The stimulus artefact resulting from the TMS has been deleted from the EMG traces. **A**, masseter stretch reflex response evoked by slow stretch of the jaw. SLSR begins at ~10 ms, LLSR starts at ~30 ms. **B**, masseter MEP evoked by TMS. Latency of response is ~ 6 ms. **C-K**, Stretch and TMS combined at various conditioning-testing (C-T) intervals. The onset of the stretch stimulus is indicated by the dotted line at time 0. TMS timing is shown by the arrows. **L**, repeat of test B (TMS alone) performed at the end of the experiment. Note that the size of the MEP did not change over the course of the experiment (compare B with L). Further analysis was done following subtraction of the stretch response (A) from the C-T traces (see Figure 7.3).

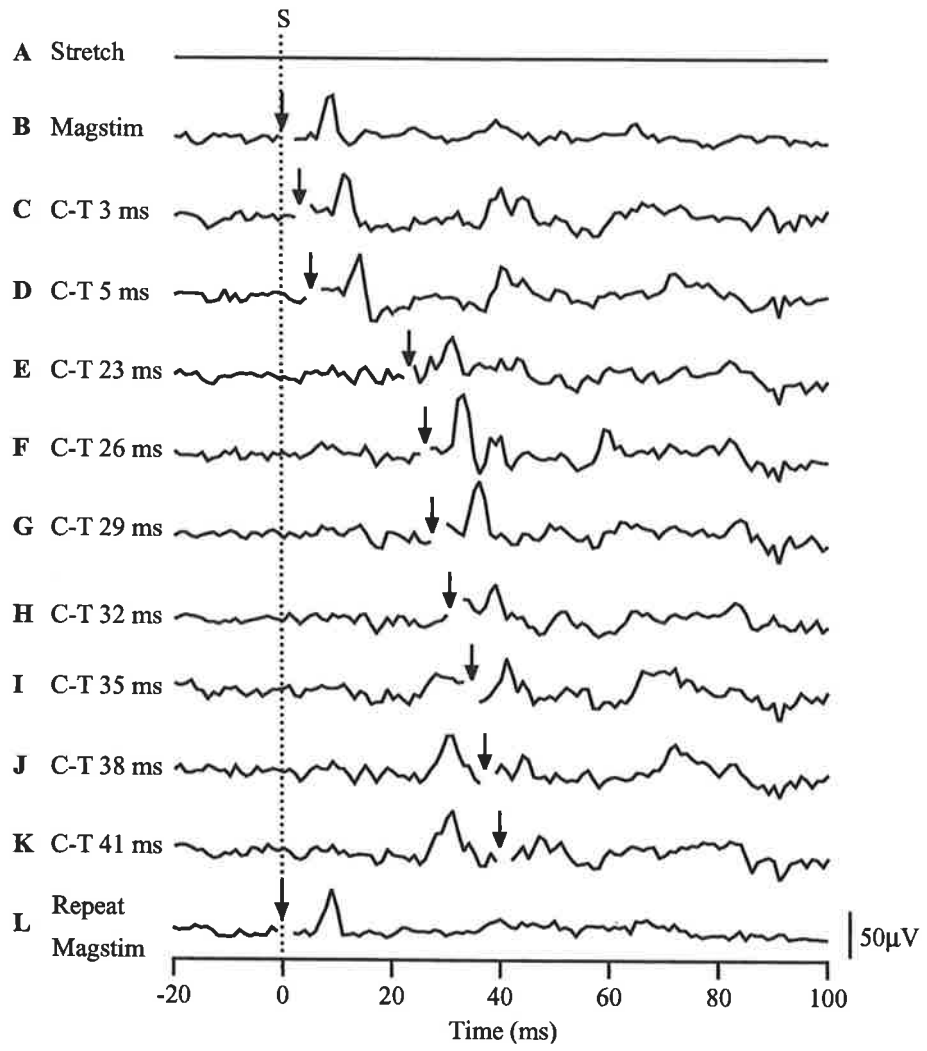

Figure 7.3

Records from one subject showing the same data as Figure 7.2, but with the stretch reflex response subtracted from the traces. Data arranged as in Figure 7.2. Traces are averaged (n=25) rectified EMG recorded from surface electrodes over masseter. The stimulus artefact resulting from the TMS has been deleted from the EMG traces. All but traces B and L (TMS only traces) have had the initial stretch reflex response (cf. Fig. 7.2A) subtracted (notice flat line in A). There was no facilitation of the MEP when it was superimposed on the SLSR (C-D) or the LLSR (E-K).

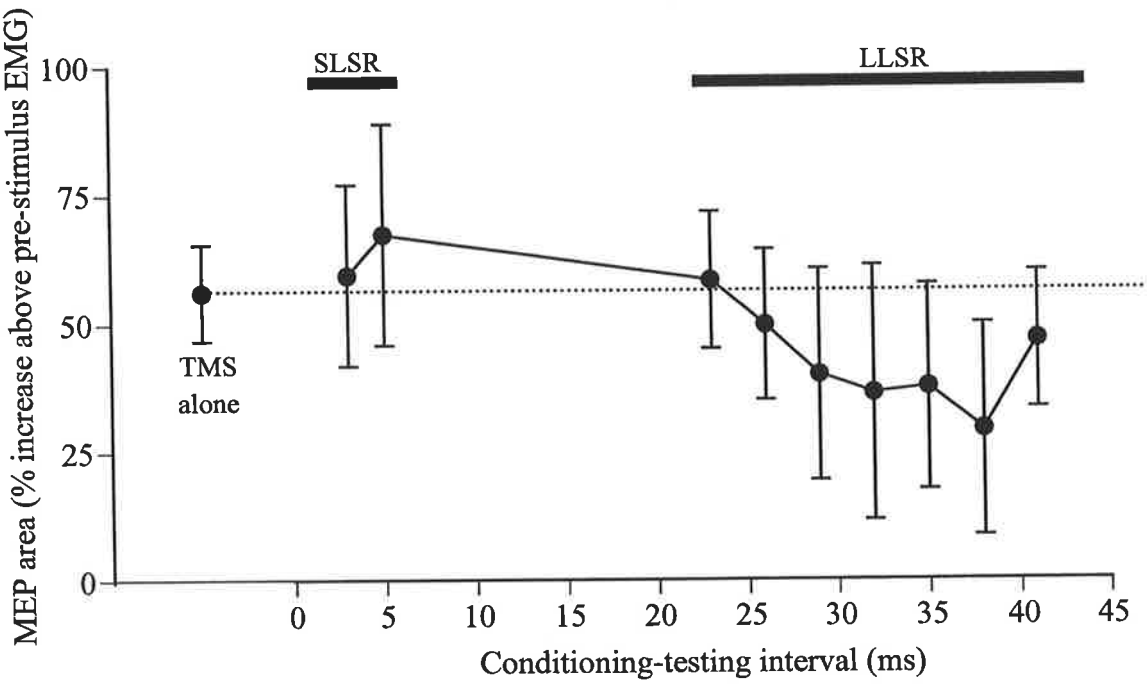

Figure 7.4

Pooled data from 10 subjects showing the effect of conditioning muscle stretch on masseter MEPs elicited by TMS. Data points are mean (± s.e.) MEP area expressed as a percentage increase above the EMG activity prior to the stretch. MEP area was measured from EMG traces which had the stretch reflex response subtracted, except for TMS alone trials, where pre-stimulus EMG activity was subtracted. The size of the masseter MEP to TMS alone is shown, and indicated by the dotted line. There was no difference in the size of the MEP when TMS was given alone, during the SLSR or during the LLSR (ANOVA, $p>0.05$).

7.3.2 Modification of LLSR with prior instruction

Neither the SLSR nor the LLSR in masseter was affected by prior instruction of the response required to the stretch ("motor set"). Representative data from one subject are shown in Figure 7.5. The size of the stretch reflex was the same between the control condition (A) and the conditions where the subject was instructed to resist the stretch (B) or relax the jaw when the stretch was perceived (C). Differences in EMG between the three conditions were only observed after 80 ms, which is beyond the subjects voluntary reaction time (see Brodin et al., 1993b). Figure 7.6 shows the pooled data from 8 subjects comparing the size of the SLSR and LLSR in the 3 different conditions. A one-way ANOVA revealed no significant differences in the size of the reflexes with differing prior instruction ($p>0.05$).

7.4 Discussion

The present study has examined the evidence for a transcortical pathway for the long latency stretch reflex (LLSR) in the masseter muscle, and the influence of motor set on the LLSR. The hypothesis that the masseter LLSR transverses the motor cortex was tested using transcranial magnetic stimulation (TMS) to examine the excitability of masseter CM cells at intervals throughout the reflex. No evidence for motor cortex involvement in the LLSR of masseter was found, and motor set did not influence the masseter SLSR or LLSR.

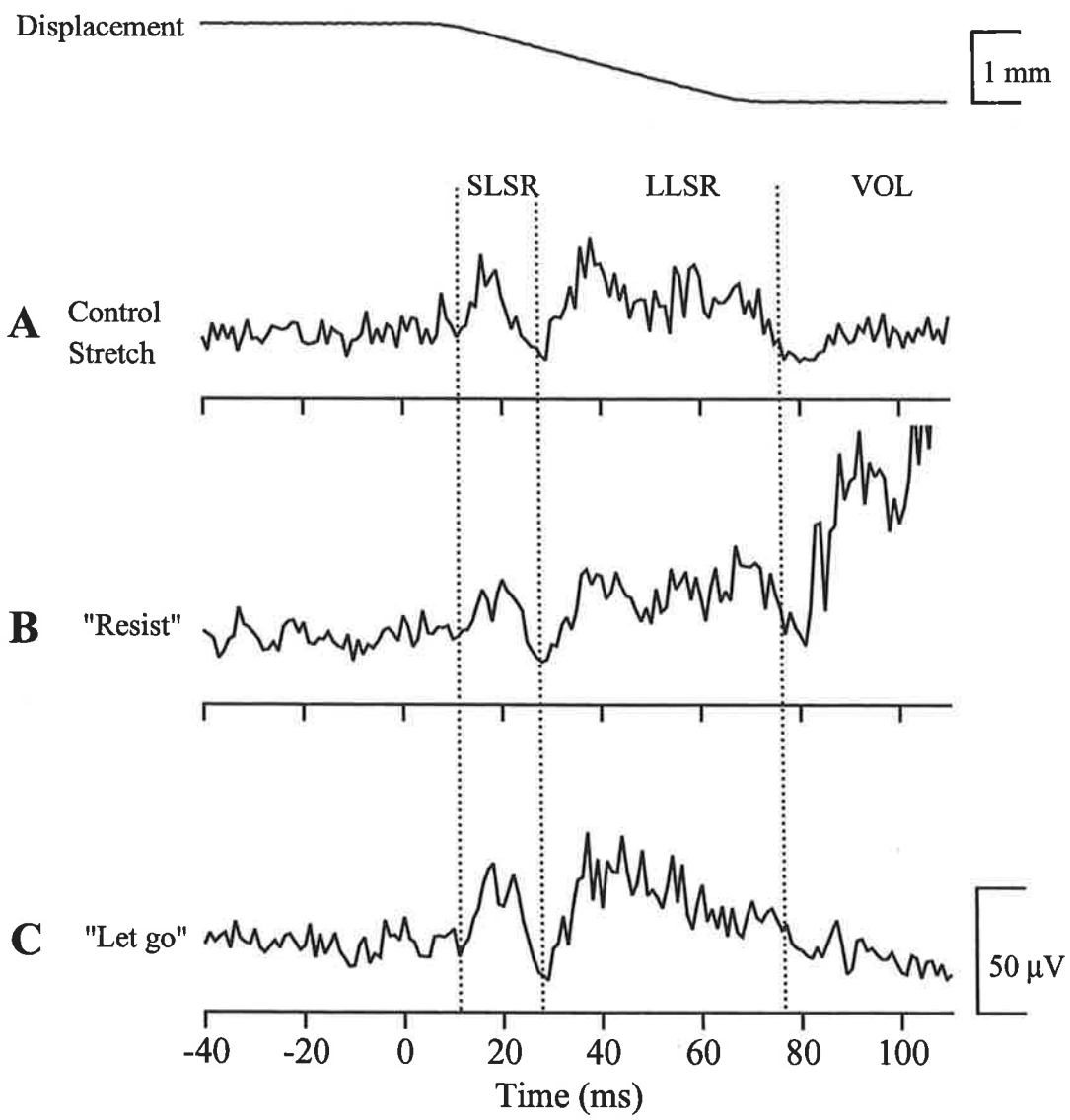

Figure 7.5

Records from one subject showing the effect of prior instruction on the size of the masseter stretch reflex. Top trace is the displacement of the jaw following the stretch. Bottom three traces are averaged (n=25) rectified EMG recorded from surface electrodes over masseter. Stretch stimulus was delivered at time 0. Data shown for when the subject was told not to react to the stretch (**A**), to resist the stretch (**B**) and to relax the jaw when the stretch was perceived (**C**). Dotted vertical lines separate the SLSR (~ 10-25 ms), the LLSR (~ 30-75 ms) and the subjects voluntary reaction period (> 80 ms). The subject's voluntary reaction to the stretch had no effect on the size of the SLSR or the LLSR.

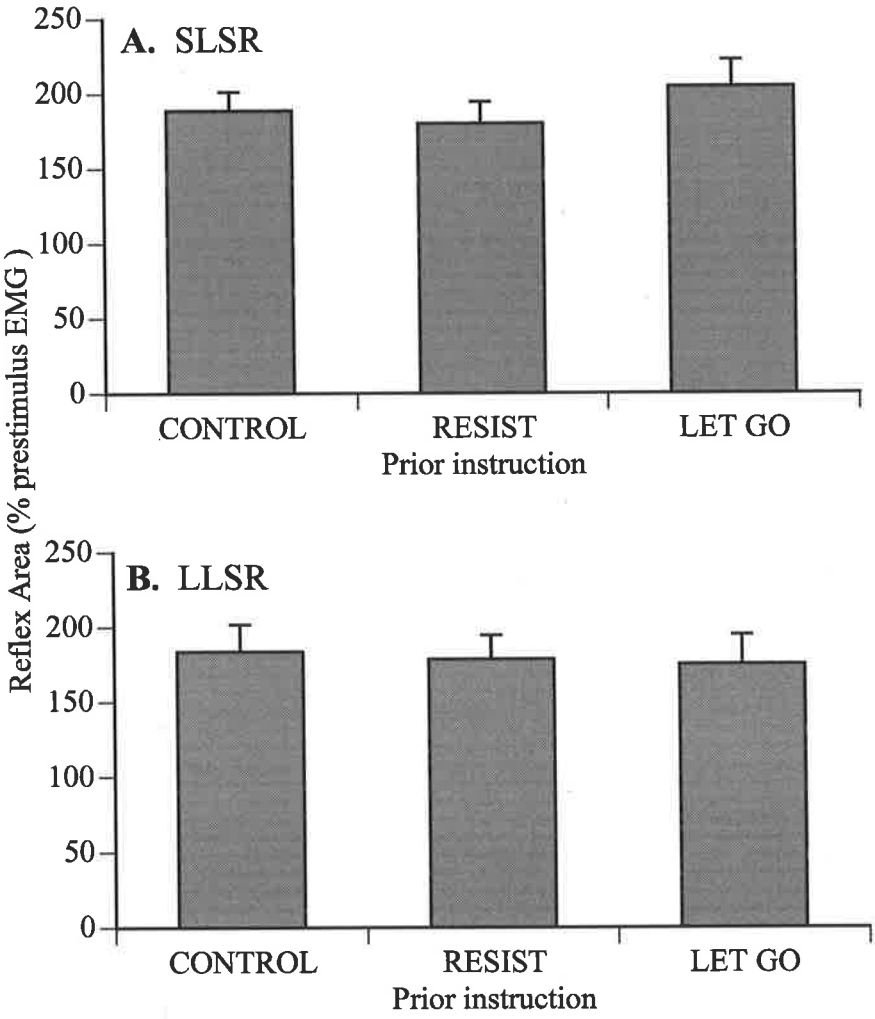

Figure 7.6

Pooled data from 8 subjects showing the effect of prior instruction on the size of the masseter SLSR and the LLSR. Bars are mean (± s.e.) reflex area normalised to pre-stimulus EMG activity. The subject's reaction to the stretch had no effect on the size of either the SLSR (**A**) or the LLSR (**B**) (ANOVA, $p>0.05$).

Until recently it was thought that the masseter did not have a LLSR, however the existence of this reflex has now been demonstrated in humans by Poliakov and Miles (1994). It is necessary to apply slow, smooth stretch to the masseter to elicit the LLSR, as faster stretch produces a strong SLSR with motoneuron discharge at short latency that leaves them hypo-excitable at the LLSR latency (Poliakov and Miles, 1994; Miles et al., 1995). The potential anatomical substrate for a transcortical LLSR has been identified in the motor cortex. Cheney and Fetz (1984) recorded from single CM cells projecting monosynaptically onto wrist motoneurons. They showed that in conscious monkeys the motor cortex neurons respond to muscle stretch at an appropriate time to mediate the LLSR. Similar studies have not been performed for the masseter LLSR, although Hoffmann and Luschei (1980) recorded from monkey precentral cortical cells and showed that small amplitude sinusoidal movements of the jaw applied during biting modulated the discharge rates of about 70% of the activated cells. In contrast Huang et al. (1989a) showed that only a small proportion of motor cortex neurons could be activated by the stretch of the orofacial muscles, and that most neurons only responded to cutaneous stimulation.

Using TMS to test for convergence of the stretch reflex onto cortical neurons assumes that the cortical cells activated by the TMS are those with direct projections to motoneurons. In the present study the response in masseter following TMS had an onset latency of 7.0 ± 0.3 ms. This is comparable with the onset latencies reported in Chapter 2 and is consistent with a monosynaptic connection from the motor cortex to the masseter motoneurons (see Chapters 2, 3 and Butler et al., 2001).

TMS activates either excitatory pre-synaptic inputs (Day et al., 1987a) or the initial segment of the cortical neurons (Edgley et al., 1997). The resultant MEP is therefore readily influenced by, and thus provides a good measurement of, cortical excitability. If the masseter LLSR involves a pathway that includes the cortical cells activated by the TMS, then motor cortex excitability will be enhanced following the stretch at a latency consistent with central conduction of the afferent signal, and the response to TMS during this time will therefore be amplified. This has been demonstrated following muscle stretch of flexor digitorum profundus (Day et al., 1991), flexor pollicis longus (Palmer and Ashby, 1992b; Wallace and Miles, 2001), and first dorsal interosseous (Macefield et al., 1996). In contrast to the results obtained in hand muscles in this laboratory and others, the present study using the same approach showed no change in MEP size when the TMS was timed to coincide with the cortical arrival of a putative afferent volley producing the LLSR. This was despite the fact that the MEP produced by the TMS was sub-maximal, and could be increased in size by increasing the TMS intensity. These results strongly suggest that the motor cortex is not involved in the long latency stretch reflex of human masseter.

7.4.1 Summation of Rectified EMG averages

The present study used averages of the rectified EMG so as to avoid the cancellation of positive and negative potentials that may occur in successive sweeps of the unrectified average. However, the interpretation of data in which rectified EMG averages are used to compare the response to two stimuli given together is not straightforward (Baker and Lemon, 1995). This is because averaging the rectified EMG can lead to non-linearities, which become particularly important when considering the summation of two

independent responses. When two stimuli with highly stereotyped response waveforms were delivered together, Baker and Lemon (1995) showed that the size of the response in the rectified EMG was greater than the linear sum of the responses to each of the stimuli delivered alone. Conversely they showed that when responses were highly variable from trial to trial, resulting in no consistent deflection being seen in the unrectified averaged EMG, the summed responses in rectified and averaged EMG were smaller than expected if the summation were linear.

In the present study the two responses being studied were the stretch reflex and the MEP, both of which have large stereotyped waveforms in the EMG, and therefore summing their responses in the rectified EMG would tend to result in a response greater than the algebraic sum of their individual responses. This supra-linear summation of EMG is an artefact that would make it difficult to attribute any increase in MEP size to increased cortical excitability during the reflex. Previous studies have overcome this problem in a number of different ways. Day et al. (1991) used superimposition of the MEP onto the SLSR as a control, since the issue of non-linear summation of rectified EMG averages would be present during both the SLSR and the LLSR. In addition, as a further control they analysed the rectified EMG average of the response to electrical brain stimulation superimposed onto the stretch reflex. Palmer and Ashby (1992b) studied non–linear summation of TMS and stretch in individual motoneurons, rather than surface EMG, thus overcoming the problems associated with rectified EMG. Baker and Lemon (1995) describe a method of analysis which allows prediction of the size of a response to two stimuli given together, when measured from averages of rectified EMG, on the assumption that they act independently. Wallace and Miles (2001) applied this technique

to the analysis of the LLSR in flexor pollicis longus and were still able to demonstrate a non-linear summation of the MEP and LLSR, confirming that the excitability of the motor cortex was increased as the stretch evoked afferent volley reached it.

If in the present study the response to TMS given during the LLSR had been greater than the sum of the two responses given individually, it may have been necessary to analyse the data further, using the methods described by Baker and Lemon (1995). However, the results of present study showed no difference in the size of the MEP when TMS was given alone and when it was superimposed on SLSR or the LLSR, so further analysis was not necessary.

7.4.2 Alternative mechanisms for the LLSR

The fact that the LLSR in masseter does not appear to involve the motor cortex may not be surprising when the latency of the response is considered. In hand muscles the onset latency of the LLSR is around 55 ms which is approximately the same as the time it takes for an afferent signal to reach the cortex, and for the efferent signal to reach the muscle (for a review see Deuschl and Lucking, 1990). In contrast, the latency of the LLSR in masseter is actually much longer than one may expect if it is delayed simply because of the time taken to traverse the motor cortex. Afferent signals from non-painful trigeminal stimuli take around 8 ms to reach the sensory cortex (Findler and Feinsod, 1982), and the efferent signal is conducted from the motor cortex to the masseter takes around 6-7 ms (Cruccu et al., 1989; Butler et al., 2001), giving a transcortical loop time of around 15 ms. Even allowing some time for temporal summation, this is considerably shorter than the 35 ms latency of the LLSR.

While the results of the present study suggest that the LLSR in masseter does not involve a transcortical pathway through the motor cortex, the actual cause of the delayed response to stretch remains to be elucidated. Brodin et al. (1993a) reported that pressure on an incisor tooth evokes a long latency excitatory reflex in human masseter. However this cannot account for the long-latency excitation in masseter following stretch, because it is not altered by local anaesthesia of the teeth (Poliakov and Miles, 1994), whereas this procedure abolished the pressure-evoked response (Brodin et al., 1993a).

Long latency stretch reflexes which do not involve the motor cortex have previously been reported in muscles of the leg (Thilmann et al., 1991) and proximal arm (Thilmann et al., 1991; Fellows et al., 1993). Two main alternative explanations to the transcortical theory of long latency stretch reflexes exist. The first is that muscle stretch produces distinct bursts of activity in the Ia spindle afferents which in turn, via the monosynaptic pathways, produce the various bursts of EMG activity (Hagbarth et al., 1980). This theory was rejected for the hand muscles since it cannot explain the occurrence of a LLSR without the presence of a SLSR (see Matthews, 1991). Similarly, it is not likely to be the cause of the LLSR in masseter, since some masseter motor units respond to stretch with long latency excitation, but not short latency excitation (Miles et al., 1995).

A second explanation for the LLSR was offered by Matthews (1984) as an alternative to the transcortical hypothesis in hand muscles. He argued that the LLSR was produced by the slower conducting group II muscle spindle afferents via a spinal cord circuit. Evidence for this came from the observation that the LLSR was absent, but the SLSR present following vibration of the flexor pollicis brevis. However, further experiments involving cooling of the arm subsequently resulted in Matthews withdrawing this

hypothesis to explain the LLSR in hand muscles (Matthews, 1989a, b). The fact that this theory has been discounted for muscles of the hand does not mean it cannot account for the LLSR in other muscles. Indeed, there is a mounting body of evidence that the longer latency stretch reflex in muscles of the lower limb is mediated via spindle group II afferents (Berger et al., 1984; Schieppati and Nardone, 1997).

7.4.3 Modulation of the LLSR

It has been suggested that a potential benefit of a long latency stretch reflex that traverses the motor cortex would be adaptive modifications of the reflex according to the requirements of the task (Phillips, 1969; Calancie and Bawa, 1985). In support of this, the LLSR in flexor carpi radialis was shown to be modified according to the intention of the subject on how to react to the stretch (Calancie and Bawa, 1985). The fact that in the present study the size of the masseter LLSR was unaltered by prior instruction seems to add support to the fact that the reflex does not involve the motor cortex in masseter. This evidence alone, however, is insufficient to draw conclusions about the nature of the LLSR, since a more recent study re-examining the effect of prior instruction on the long latency stretch reflex showed the LLSR could not be modified by intention (Capaday et al., 1994). This is despite the fact that there is considerable evidence that the LLSR in the muscle studied is transcortical (Capaday et al., 1994). The advantage of a LLSR that traverses the motor cortex therefore remains to be elucidated.

7.4.4 Conclusion

In conclusion, the present study has shown that the long latency stretch reflex elicited in masseter following slow stretch of the jaw does not result from a transcortical pathway. The reasons why the reflex involves the motor cortex in hand muscles but not in the jaw are not yet clear, but may reflect the fact that hand muscles depend more heavily on direct cortical control. This result confirms previous studies which have suggested that the long latency stretch reflex in different muscles of the human body may utilise different neural pathways (Thilmann et al., 1991). Further studies are required to determine the neural pathways responsible for the masseter LLSR.

CHAPTER 8

CONCLUDING REMARKS

This thesis has been concerned with the control of movement in human masseter muscles by the motor cortex. Much research has focussed on the role of corticomotoneuronal (CM) cells in the control of movement in hand muscles, which like masseter, require fine precision and co-ordination of movement. The representation of jaw muscles in the motor cortex is large, and previous work suggested that CM cells project to jaw muscles, indicating certain similarities between muscles of the jaw and hand. Unlike hand muscles, however, jaw muscles are involved in movement patterns characterised by bilateral activity, which means that it is not possible to extrapolate the role of CM cells in jaw muscle control from their role in hand muscle control. Direct demonstration of trigeminal CM cells is lacking from animal experiments, and the available evidence in humans is not definitive. Hence specific investigation of CM projections to human jaw muscles is required. The experiments presented in this thesis have therefore used transcranial magnetic stimulation (TMS) to examine the existence, nature and function of masseter CM projections in awake humans.

TMS activates CM cells that have a monosynaptic connection with masseter motoneurons. The latency of the response to TMS in the whole masseter muscle and in individual masseter motor units is consistent with conduction along a fast pathway and a monosynaptic connection. In addition, the brief duration of the peaks in masseter motor unit PSTHs following TMS is further evidence for the monosynaptic nature of the CM cells activated by TMS. The features of the PSTH peak suggest it is produced by direct

activation of the CM cells (D-wave) and subsequent monosynaptic compound EPSP in the masseter motoneurons. This is in contrast with the situation in hand muscles, where TMS usually activates the CM cells indirectly (I-waves), and produces multiple peaks of increased short latency firing probability in the PSTHs. Studies comparing the response of masseter motor units to electrical brain stimulation and TMS are required to determine unambiguously whether the response in masseter to TMS is a D-wave, but the available evidence is compelling that this is the case.

In addition to producing excitatory responses, TMS can evoke a period of inhibition. Most masseter motor units are inhibited by TMS of the ipsilateral motor cortex, and have a silent period that follows the excitation produced by contralateral TMS. The threshold for inducing inhibition is usually lower than for producing excitation with contralateral TMS. The experiments reported in this thesis do not allow me to conclude whether the silent period in masseter following TMS is due to segmental or cortical inhibition. Based on the results from previous studies in other muscles it seems likely that the latter part of the silent period has a cortical origin, but further study of the TMS evoked inhibition in masseter is required.

Each hemisphere of the motor cortex has CM projections that project to both masseter muscles. This was demonstrated when one hemisphere of the motor cortex was activated with focal TMS, and excitatory responses were obtained in the masseter muscles on both sides. Although bilateral, the projection is not symmetrical and is stronger to the contralateral masseter resulting in larger motor evoked potentials (MEPs) than in the ipsilateral muscle. This is most likely achieved by a population of CM cells with exclusively contralateral projections. This was confirmed at a motor unit level, where

most of the low threshold motor units were excited by TMS of the contralateral motor cortex, but not the ipsilateral motor cortex. The present studies were not able to identify the nature of projections to higher threshold masseter motoneurons, although it seems likely that these may receive a more bilateral input, thus accounting for the bilateral response seen in the surface EMG following focal TMS.

The motor cortex excitability varies with biting task in an asymmetric manner. In orofacial tasks requiring precision, such as during unilateral activation of masseter, the activity in the contralateral motor cortex is reduced, resulting in smaller MEPs than during bilateral biting. In contrast, there is no modulation of MEP size in ipsilateral masseter when it is activated for unilateral biting, suggesting that the activity in the ipsilateral cortex remains unchanged. Thus, it seems that the CM component of the command for unilateral biting originates from the contralateral hemisphere only. This may be accomplished in part by reduced activity of the population of CM neurons in the contralateral hemisphere with branched-axon projections to both masseter motor pools. Further study was required to provide evidence for the existence of branched CM projections, and the evidence obtained in these experiments is discussed later. At this stage it is not known whether intracortical inhibitory circuits are responsible for the selective activation of exclusively contralateral projections during unilateral biting, and this is an area of further study.

The size of the excitatory response to TMS fluctuates from stimulus to stimulus. This variation was correlated between left and right masseter in 32% of comparisons. The source of the correlation is relatively specific to masseter, since there is no correlation between the size of responses in masseter and responses in the hand. These results may

indicate the presence of a population of CM cells that branch to innervate the motoneurons on each side. However, another possible explanation for the result is that there are cortical oscillations that affect the excitability of separate ipsilateral and contralateral projecting CM cells. Indeed experiments comparing the MEP fluctuations in resting muscles of either hand show that the MEP variation in muscles on either side is correlated, a result clearly not explained by branched CM axons. However, I have shown that when one or both of the muscles in the hand are activated, the correlations in MEP size in muscles on either side disappear. In contrast, muscles that are known to share branched corticospinal input show correlations in MEP size, which are still present when the muscles are active. Therefore, the underlying neural process involved in producing MEP correlations in pairs of muscles is different in resting and active muscles. These experiments provide support for the hypothesis that the MEP correlations in the active masseter muscles are due to the presence of branched CM cells.

Previous studies have identified the importance of branched CM projections in control of muscles of the hand, and the experiments reported here suggest they may also play a role in the control of the masseter muscles on each side. In addition to being involved in normal motor control, it has been suggested that branched CM axons are responsible for the mirror movements seen in some patients suffering from infantile hemiplegia. In such a patient I demonstrated correlations in MEP variability in muscles of the upper limb and jaw, when muscles were at rest and when they were active. The correlations in MEP size were specific, and not seen between muscles of the jaw and hand. Together with the demonstration of synchronous firing of motor units in muscles in each hand, these results suggest that the mirror movements of the hands evident in this patient are the result of

intact corticospinal axons which branch to innervate homonymous motoneurons in the motoneuron pools innervating each side of the body. Correlation in MEP size from left and right masseter in the mirror movement patient is stronger than in normal subjects, probably reflecting the absence of exclusively contralateral projections originating from the damaged hemisphere.

Finally, the involvement of the motor cortex in the masseter long-latency stretch reflex was analysed by testing for convergence of afferent input onto masseter CM cells using TMS. Following slow stretch of the jaw, the excitability of CM cells was tested using TMS at a time that would coincide with the passage of the LLSR through the motor cortex. Similar studies in muscles of the hand have demonstrated a clear increase in CM excitability during the LLSR of these muscles. In contrast, no change in the excitability of the CM cells was found during the masseter LLSR. Furthermore, the LLSR in masseter could not be modulated by the subject. The origin of the masseter LLSR is not known, and further study is required to determine the afferent and efferent pathways involved.

In summary, the present series of experiments have provided a detailed examination of the nature and function of CM cells projecting to masseter. Numerous differences have been demonstrated between the motor cortex control of the jaw and what is known for the hand. First, masseter motoneurons receive input from both hemispheres of the motor cortex. Second, there may be a population of CM cells that branch to innervate masseter motoneurons on each side. Third, masseter CM cells appear to be activated directly by TMS, without the production of I-waves. And finally, the motor cortex does not appear to be involved in the masseter long-latency stretch reflex.

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