HUMAN MOTOR CORTEX PLASTICITY INDUCTION IS INFLUENCED BY MULTIPLE FACTORS

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Abstract

The primary motor cortex (M1) has the essential role of controlling voluntary movement, but is also a crucial site for learning new motor skills and recovery of motor function after injury. The development of non-invasive brain stimulation techniques, particularly transcranial magnetic stimulation, has significantly contributed to our understanding of human M1 and its ability to alter in structure and function (cortical plasticity). However, large within- and between-subject differences in the capacity for cortical plasticity exist in humans. This thesis examined factors capable of influencing human M1 plasticity and motor learning, focussing on the effects of exercise, ageing, hand preference and genetics.

Study 1 examined whether regular exercise influenced plasticity in human M1. Individuals with increased physical activity levels had increased M1 excitability and enhanced neuroplasticity. This was the first study to demonstrate that participation in regular physical activity offers a generalised neuroplastic enhancement within M1. Therefore, these results suggest that participation in regular physical activity may offer global benefits to human M1 function.

Study 2 addressed the influence of age (young and old adults) and hand preference (dominant and non-dominant) on human M1 plasticity and motor learning for a simple motor task. In contrast to previous studies, the extent of plasticity was not diminished in old compared with young adults for either hand following simple ballistic training. However, neuroplasticity was enhanced in the right hemisphere (left non-dominant hand) compared with the left hemisphere (right dominant hand) with training. This finding suggests that there is greater strengthening of corticomotor circuits for control of the left compared with the right hand during simple ballistic training. Subsequently, Study 3 examined the effect of advancing age on a complex task, which more likely engages M1 and increases attentional demand. Following training of complex visuomotor tracking, the extent of plasticity remained similar between young and old

adults, suggesting that a reduction in plasticity is not an obligatory consequence of the ageing process. The findings from Studies 2 and 3 demonstrate that older adults may have a similar neuroplastic capacity under some circumstances, and identifying factors for this maintenance may guide healthy ageing interventions aimed at promoting brain health across the life-span

In Study 4, it was found that the modulation of M1 excitability was strongly influenced by a common polymorphism in the *BDNF* gene, but the effect was dependent on the intervention used. The most pronounced differences in plasticity between *BDNF* genotypes were observed following the complex motor task, but this did not influence motor learning. Although there was no effect on motor performance and short-term motor learning in healthy young subjects, the differences in brain plasticity between *BDNF* genotypes may be more important for the recovery of motor function after neurological injury.

These findings suggest that sustained regular exercise, hand preference, and *BDNF* genotype contribute to the variability of M1 plasticity in healthy adults. Therefore, to further understand factors that influence M1 plasticity, future studies should assess these subject characteristics as potential confounding factors in the response. Furthermore, there may be potential to capitalise on these factors to optimise M1 plasticity and recovery of motor function following neurological injury.

Declaration

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Now that another chapter in life has almost been finalised, I look forward to seeing what the future will hold for the remaining chapters.

1. Literature Review

The human brain, including the primary motor cortex, has the potential to functionally and structurally reorganise following physiological (learning) and pathological (injury or disease) events. As a result, in addition to having the essential function of controlling voluntary movement, the primary motor cortex is a crucial site for learning new motor skills and recovery of motor function after injury.

The following review is intended to provide an overview of the literature regarding the involvement of the motor cortex in human motor function, transcranial magnetic stimulation, cortical plasticity in healthy human motor cortex, and several factors that influence the induction of cortical plasticity. Initially, the anatomy of the human motor cortex will be broadly outlined. The physiological mechanisms in controlling movement from the human motor cortex will then be described. The principles of cortical plasticity, with a focus on the human motor cortex, will follow movement control. How plastic change can be investigated in human motor cortex and methods (experimentally and use-dependent) for inducing plastic change will then be discussed. Finally, as there is substantial variability in the induction of cortical plasticity in neurologically normal adults, a number of factors capable of influencing plasticity will be described. As plasticity in motor cortex can be influenced by numerous factors, with many of these factors still unknown, additional understanding is beneficial in comprehending the influences of motor cortex plasticity in neurologically normal adults. In addition, and importantly, understanding of these determinants is critical for optimising therapeutic rehabilitation strategies following neurological injury or insult.

1.1. The human motor cortex

There are over 100 billion cells in the human brain (Azevedo *et al.*, 2009). The cerebral cortex contains between 17 and 26 billion neurons (Pakkenberg & Gundersen, 1997; Pelvig *et al.*, 2008; Azevedo *et al.*, 2009), with more than 1 billion of these neurons estimated to

comprise the human motor cortex (Gredal *et al.*, 2000; Toft *et al.*, 2005). The composition of human motor cortex includes a distinct number of cortical regions, namely primary motor cortex (Brodmann's area 4; M1) and non-primary motor cortices of supplementary motor area (lateral aspect of Brodmann's area 6), premotor cortex (medial aspect of Brodmann's area 6), and cingulate motor areas (Brodmann's areas 6c, 23c, 24c) (Picard & Strick, 1996; Chouinard & Paus, 2006).

The existence of an area associated with motor function in the cerebral cortex has been suggested for many centuries. For example, as early as the 1600s, Robert Boyle proposed this notion following his case report on a patient with depressed cranial fracture following a horse riding accident (Rengachary & Ashan, 2007). However, the notion of compartmentalisation was not realised until the work of John Hughlings Jackson demonstrated that epileptic seizures had an orderly representation (Jackson, 1873a, b, c). Physiological evidence following electrical stimulation in animals, such as cats and dogs, supported the clinical deduction by John Hughlings Jackson (Ferrier, 1874). However, it was not until the first detailed motor map of the primate cortex following electrical stimulation by Albert Leyton and Charles Sherrington that the true extent of the motor cortex was established (Leyton & Sherrington, 1917). Observations by Wilder Penfield from direct electrical stimulation of the exposed cortex in patients undergoing surgery for epilepsy was in agreement with the previous findings. From these recordings a topographical map, or "homunculus", was constructed for humans initially of the sensory cortex (Penfield & Boldrey, 1937) and later the motor cortex (Penfield, 1950; Penfield & Rasmussen, 1950; Penfield & Welch, 1951). The motor homunculus represents body parts organised in a general medial (lower limb) to lateral (upper limb, head and face) topography (Sanes & Donoghue, 2000).

Although partitions of the head, upper extremity, and lower extremity remain distinct, body parts organised within these regions are not as discrete as initially suggested (Rizzolatti *et al.*,

1998; Schieber, 2001). Many studies in primates and recently humans demonstrate that representation of movement, particularly in the hand, is widely distributed and overlaps (Gould *et al.*, 1986; Schieber & Hibbard, 1993; Rao *et al.*, 1995; Sanes *et al.*, 1995; Indovina & Sanes, 2001). However, despite distributed and overlapping representations, recent evidence suggests that adjacent representations in the motor cortex maintain a homuncular order (Kleinschmidt *et al.*, 1997; Beisteiner *et al.*, 2001; Dechent & Frahm, 2003; Kapreli *et al.*, 2007; Plow *et al.*, 2010). In addition, a number of studies demonstrate that neuronal populations encode a specific movement pattern rather than a particular muscle or muscle group (Buys *et al.*, 1986; Georgopoulos *et al.*, 1986). This recent information about the motor cortex indicates a highly flexible nature allowing the potential to reorganise functionally and structurally in response to physiological and pathological events.

The cellular architecture of the cortex, initially examined by Korbinan Brodmann in 1909 (Brodmann & Garey, 2006), demonstrates a complex yet flexible nature. Typically, there are six horizontal layers present in the cerebral cortex (Layer I, closest to the outer surface of the cortex, to layer VI, preceding the white matter). Each layer is primarily differentiated by the presence or absence of cell types. There are two main neuronal cell types in the cerebral cortex. These include the projection neurons (pyramidal cells) and interneurons (non-pyramidal or stellate cells), which can determine if a layer is mainly associated with receiving or sending information to other regions. For example, the size of layer IV, the main target of sensory information arriving from the thalamus, is significantly reduced in primary motor cortex given its main role as an output region of the cortex. Therefore, the formation of layers is dependent on the function of the region within the cerebral cortex.

The main output cells of the motor cortex are pyramidal cells. In humans, these cells are most prominently located in layers III, V, and VI and use the excitatory amino acid glutamate as their neurotransmitter (Kandel *et al.*, 2000). Dendrites of pyramidal cells extend horizontally

and vertically, and form extensive networks in layers II to IV (Porter & Lemon, 1993). The primary motor cortex contains large pyramidal cells which originate in layer V and terminate directly on motor neurons in the ventral horn of the spinal cord. These cells provide the most direct pathway for movement execution and are also referred as Betz cells (Kandel *et al.*, 2000). Traditionally it was thought that neurons forming a direct pathway with spinal motor neurons solely originated from the primary motor cortex and surrounding motor cortices sent their output to the primary motor cortex to execute voluntary movements. Recent information suggests that other motor cortices besides primary motor cortex have a significant number of direct projections to spinal motor neurons (Dum & Strick, 1991). However, the threshold to evoke movements from premotor areas is much higher than primary motor cortex (Dum & Strick, 2002). Therefore, although multiple areas in motor cortex have direct projections to spinal motor neurons, voluntary movement is predominantly executed by signals from primary motor cortex.

The stellate (non-pyramidal) cells comprise 25-30% of cortical neurons in the motor cortex (Sloper *et al.*, 1979). In contrast to pyramidal cells, the axons and dendrites of stellate cells do not project beyond the cortex. Stellate cells are divided into spiny and non-spiny cell types. Spiny stellate cells are mainly located in layer IV and use glutamate as their neurotransmitter (Jones, 1981). Non-spiny stellate cells are located in all layers and use the main inhibitory neurotransmitter γ-aminobutyric acid (GABA) to make inhibitory synapses with pyramidal cells (Jones, 1981). The excitatory and inhibitory cell distribution mediates the formation of different cortical layers, maintaining columnar organisation.

1.1.1. The corticospinal system

There are a number of descending neural pathways which influence the activity of the spinal cord (Brodal, 1969). These neural pathways originate from a wide variety of cortical areas and mainly descend through the corticospinal tract, which is composed of the lateral and

anterior systems. Corticospinal tract projections have a different pattern of termination within the spinal gray matter depending on their origin, suggesting that each cortical region has a different functional role (Lemon & Griffiths, 2005). The largest neural pathway is the pyramidal tract. In humans, the fibres of this tract predominantly (80% of all pyramidal tract fibres) originate in layer V of the primary motor cortex and supplementary motor area, but also originate in other motor cortical areas (such as premotor cortex) and sensory cortices (Kuypers, 1981). A majority (~75 %) of the fibres in the pyramidal tract decussate at the junction between the medulla and spinal cord before entering the lateral column of the spinal cord, forming the lateral corticospinal tract (see Chouinard & Paus, 2006). These fibres then descend the spinal cord and synapse with motor neurons and interneurons of muscles primarily responsible with limb movements. A further number of fibres (~15 %) also decussate in the spinal cord (see Chouinard & Paus, 2006). These remaining fibres that do not decussate descend ipsilaterally (forming the anterior corticospinal tract) and primarily innervate axial and proximal muscles associated with postural control (Canedo, 1997).

Several motor functions, including locomotion, are common to all mammalian species, but the ability to control fine movements (such as independent finger movements) is solely attributed to certain non-human primates (primarily great apes) and humans. The development of new aspects of motor behaviour in higher order primates is attributed to an increased dominant role of the motor cortex and corticospinal tract. The presence of direct, monosynaptic connections between the motor cortex and spinal motor neurons, called cortico-motoneuronal (CM) cells, is unique to primates and supports a greater influence from the cerebral cortex (Lemon, 2008). Interestingly, studies conducted on non-human primates indicate that direct CM cells are confined to the caudal region of M1 (Rathelot & Strick, 2006, 2009). Consequently, all corticospinal projections to spinal motoneurons in the rostral region of M1 are indirect, requiring at least a disynaptic pathway (Rathelot & Strick, 2009).

The result from this work proposes a subdivision of M1 organisation into old (rostral) and new (caudal) compartments in higher order primates (Rathelot & Strick, 2009). In hand muscles, evidence suggests that each spinal motor neuron receives input from many CM cells (Weber & Eisen, 2002). This direct, monosynaptic connection to the muscles of the hand from the cerebral cortex is functionally important in producing skilled, fractionated movements (see Lemon, 1993). Preliminary studies that established a direct, monosynaptic pathway in primates required anatomical post-mortem analysis (Kuypers, 1981). However, the recent introduction of transcranial magnetic stimulation (TMS) (see section 1.2.2.) allows this pathway to be assessed non-invasively in conscious humans.

The importance of the CM cells (which are excitatory) in producing precise and independent movements of the digits is without question. However, CM cells can synapse with motor neurons of multiple muscles (Shinoda *et al.*, 1981; Buys *et al.*, 1986). The facilitated muscles from a single CM cell are likely to have a close functional relationship, with activation from these muscles essential for optimal performance (Buys *et al.*, 1986). Nonetheless, it is evident that other mechanisms influencing the control of movement are critical for precise movements.

1.1.2. GABA and its physiological importance

Inhibition of neural activity in the motor cortex is an important component required to fine-tune precise movements. This type of inhibition to modulate control commonly occurs through GABAergic cortical neurons. I will review the physiology of GABA-mediated inhibition throughout this section, particularly its role in modulating selective hand muscle activation and neuroplasticity.

GABAergic neurons constitute ~25% of the total population of neurons in the cortex (Hendry *et al.*, 1987). In the motor cortex, GABA cells form 95-100% of the neuron population in layer I and 15-40% in layers II to IV, which progressively decreases toward the white matter

(Hendry *et al.*, 1987; Beaulieu *et al.*, 1992; Jones, 1993). There are three receptor classes ($GABA_A$, $GABA_B$, and $GABA_C$) which mediate the action of GABA. $GABA_A$ and $GABA_B$ receptors are widely distributed throughout the mammalian brain, whereas $GABA_C$ receptors (also known as $GABA_{A-\rho}$ receptors) are predominantly located in the vertebrate retina (Young & Chu, 1990; Bormann & Feigenspan, 1995).

The most widespread GABA receptor is $GABA_A$, which is found at most GABAergic synapses. The $GABA_A$ receptor directly acts on chloride membrane channels of the post-synaptic cell by increasing permeability. An influx of chloride occurs when GABA molecules bind to $GABA_A$ causing membrane hyperpolarisation, which results in an inhibitory post-synaptic potential (IPSP). The generation of an IPSP reduces the ability for the membrane potential of the initial axon to achieve threshold for spike generation (Krnjevic & Schwartz, 1967). The $GABA_B$ receptor is coupled to calcium and potassium channels through G-proteins and second messenger systems. $GABA_B$ receptors are extensively located on both pre- and post-synaptic cells (Misgeld *et al.*, 1995). Activation of $GABA_B$ receptors results in an IPSP through increased conductance of potassium or decreased conductance of calcium and is associated with slow synaptic inhibition (Kerr & Ong, 1995). Similar to $GABA_A$ receptors, $GABA_C$ ($GABA_{A-\rho}$) receptors are coupled to chloride ion channels (Bormann & Feigenspan, 1995). However, unlike other GABA receptors, $GABA_C$ receptors are insensitive to the plant alkaloid bicuculline ($GABA_A$ receptor antagonist) and the drug baclofen ($GABA_B$ receptor agonist) (Johnston *et al.*, 1975).

The GABAergic inhibitory system determines the task specificity of pyramidal tract neurons in the motor cortex. Removal of unwanted movements to perform precise motor tasks results from GABA hyperpolarising the post-synaptic membrane, generating an IPSP. Animals injected with the GABA antagonist bicuculline to block this form of inhibition demonstrate increased task-related activity of pyramidal tract neurons and total muscle activity resulting in

disrupted, dystonic-like movement (Chagnac-Amitai & Connors, 1989; Matsumura *et al.*, 1991; Matsumura *et al.*, 1992). The GABA antagonist bicuculline also results in rapid (within hours) modification in cortical maps, with the representation of neighbouring limb areas expanding into the injected area in the rat motor cortex (Jacobs & Donoghue, 1991). These rapid changes in motor maps are similar to those observed with nerve transaction, which is attributed to reduced GABA levels (Sanes *et al.*, 1988; Donoghue *et al.*, 1990). In humans, there is evidence that transient deafferentation induced by ischemic nerve block decreases cortical GABA (Levy *et al.*, 2002). Furthermore, similar to the GABA antagonist bicuculline in the rat motor cortex, transient deafferentation induced by ischemic nerve block in humans rapidly (within minutes) increases the motor cortical output to muscles proximal to the nerve block (Brasil-Neto *et al.*, 1992b; Brasil-Neto *et al.*, 1993; Ridding & Rothwell, 1995, 1997; Ziemann *et al.*, 1998a). These deafferentation-induced changes in the motor cortex are diminished when selectively activating a muscle (Ridding & Rothwell, 1995, 1997). This is not surprising, because the amount of GABAergic inhibition of corticospinal neurons is reduced when selectively activating the appropriate muscle for a particular movement (Ridding *et al.*, 1995b). Furthermore, Zoghi and colleagues (2003) proposed that GABAergic inhibitory neurons differentially modulate corticospinal neurons controlling selective fractionated contractions of hand muscles. Therefore, the precision of movements in a healthy population is dependent on the GABAergic inhibitory system.

GABAergic inhibition has an essential role in isolating movements, but it is also important in neuroplasticity (see section 1.3.1.). Animal studies provide strong evidence that a reduction in GABA-mediated inhibition is required to facilitate synaptic potentiation and changes in representational maps (Jacobs & Donoghue, 1991; Hess & Donoghue, 1994). In humans, there is evidence that enhanced neuroplasticity in the motor cortex following physiological (learning) and pathological (injury or disease) events require a reduction in inhibitory tone by

down-regulating GABAergic function (Ziemann *et al.*, 1998a, b; Ziemann *et al.*, 2001). Changes in cortical properties, such as a reduction in GABA-mediated inhibition, for neuroplastic changes can be both beneficial and maladaptive. For example, reduced GABA-mediated inhibition is important in improving the performance of a motor task with practice (Liepert *et al.*, 1998; Garry *et al.*, 2004; Perez *et al.*, 2004; Garry & Thomson, 2009). However, if GABA-mediated inhibition is reduced excessively, such as in focal dystonia (Ridding *et al.*, 1995a), abnormal enhancement of neuroplasticity occurs (Quartarone *et al.*, 2003). Therefore, the extent of neuroplasticity is strongly influenced by the amount of GABA-mediated inhibition, which is important in optimising motor task performance.

The ability to modulate GABA-mediated inhibition provides an essential role in executing fine movements and developing new motor skills and memories. Due to this role, modulation of GABA-mediated inhibition may also be important in recovery following neurological injury or disease. Throughout the experiments described in chapters 2, 3, and 4, I have quantified GABA-mediated inhibition in human motor cortex with TMS. I have assessed inhibition dependent on $GABA_A$ (chapters 2, 3, and 4) and $GABA_B$ (chapter 2) receptors and whether these neurophysiological measures influence the modulation of neuroplasticity in human motor cortex.

1.2. Non-invasive techniques used to assess human motor cortical function

Over the last few decades there have been a number of techniques developed and advanced for assessing brain function and structure. The ability to routinely use these non-invasive techniques in humans has also seen an increase in the number of neuroscience research studies conducted. Examples of non-invasive techniques to assess cortical function include functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), transcranial electric stimulation (TES), and transcranial magnetic stimulation (TMS). The techniques of fMRI and MEG are designed for neuroimaging, although each uses a different

approach. For example, fMRI identifies the hemodynamic response (changes in blood flow) and provides excellent spatial resolution, but relatively poor temporal resolution. Conversely, MEG records magnetic fields produced by electric currents in the brain and has excellent temporal resolution, but reduced spatial resolution. The combination of MEG and fMRI can also be used to optimise spatial and temporal resolutions (Nimsky *et al.*, 1999; see Liu *et al.*, 2006 for review). However, despite these imaging improvements, a limitation of these techniques is the inability to assess the neurophysiological mechanisms of brain function. Therefore, the changes observed simply reflect total neuronal activity and do not differentiate between excitatory or inhibitory inputs.

The mechanisms of brain function can be assessed non-invasively in humans using the techniques of TES and TMS. These techniques provide excellent temporal resolution and relatively good spatial resolution with the ability to assess both excitatory and inhibitory processes of superficial cortical structures. These non-invasive techniques and their role in human motor cortex stimulation will be discussed in more detail in the next section.

1.2.1. Transcranial electric stimulation

The initial method to stimulate the human motor cortex was to directly stimulate the exposed cortex with trains of stimuli, which was commonly performed on patients undergoing neurosurgery. Several attempts were made in the 1950's to advance this method by electrically stimulating the human motor cortex with trains of stimuli through the scalp non-invasively. These attempts yielded limited success and those that stimulated the contralateral limb most likely required anaesthesia as a result of the associated pain (Gualtierotti & Paterson, 1954; Merton, 1981; Rothwell *et al.*, 1991). Successful non-invasive stimulation was consistently achieved using transcranial electric stimulation (TES) (Merton & Morton, 1980). Unlike earlier forms of electrical stimulation that used trains of stimuli, the technique of TES involved a single discharge of a large current from electrodes placed on the scalp over

the motor cortex. The underlying corticospinal neurons activated by TES are believed to occur directly at the neuronal cell body, axon hillock, or node of Ranvier (Day *et al.*, 1987; Day *et al.*, 1989). However, a large current is required to activate these neurons as only a small fraction flows into the cortex due to the high resistance of the skull and scalp. As a result, the current causes local pain between the electrodes and muscle activation of the scalp. Although non-invasive stimulation of the human motor cortex was achieved, studies using TES were limited due to the discomfort and contraction of muscles surrounding the scalp.

1.2.2. Transcranial magnetic stimulation

The development of transcranial magnetic stimulation (TMS) overcame the limitations of TES for non-invasive brain stimulation (Barker *et al.*, 1985). TMS operates on the principle of electromagnetic induction. The magnetic stimulator produces a very large and brief electric current (a flow of 5000 A or more and rise time of 100 μs) in an insulted wire coil when a capacitor is discharged (Barker *et al.*, 1985). The current produces a magnetic field with lines of flux perpendicular to the coil. The design of stimulating coils can vary. A circular coil induces a diffuse field, whereas a figure-of-eight coil induces a weaker more focal area. The magnetic field from the stimulating coil passes largely unimpeded through the skull and scalp stimulating conductive structures as if they were being directly stimulated by electrodes. An electric field is then produced at right angles to the magnetic field providing a current to flow into underlying neural tissue. If the stimulus intensity induces a sufficient current, then corticocortical and corticospinal neurons are depolarised (Rothwell *et al.*, 1991).

Depolarisation of corticospinal neurons from TMS induces multiple descending volleys of action potentials. The corticospinal descending volleys activate spinal motor neurons and produce short-latency contractions of contralateral muscles that can be measured as a motor evoked potential (MEP). The MEP can be assessed using electromyography (EMG) and reflects the excitability of the sub-set of corticospinal and motor neurons responsible for the

movement of a particular muscle (Rothwell, 1997). Animal studies indicate that a descending volley generated from electrical current stimulation consists of a number of waves with an approximate interval of 1.5 ms (Patton & Amassian, 1954). The initial wave results from direct activation of corticospinal axons (termed direct- or D-waves). Later waves of the descending volley occur indirectly by excitatory synaptic activation of interneurons (termed indirect- or I-waves). Direct recording of descending volleys from the spinal cord in humans indicate that I-waves (indirect) are preferentially recruited by TMS at low TMS intensities (Day *et al.*, 1989; Di Lazzaro *et al.*, 1998a). At higher intensities of TMS, D-waves (direct) are also recruited (Di Lazzaro *et al.*, 1998a). Conversely, TES preferentially recruits D-waves at threshold and subsequently I-waves when the intensity is increased (Boyd *et al.*, 1986; Burke *et al.*, 1990). Therefore, TMS allows indirect assessment of human motor cortex activity by activating corticospinal neurons trans-synaptically, which produces I-wave activity in the pyramidal tract (Rothwell, 1997).

TMS can also be administered to other parts of the nervous system, such as the peripheral nerves. At the level of the peripheral nerve, TMS stimulates motor (and sensory) neurons directly on nerve axons. However, TMS of the motor cortex can stimulate both excitatory and inhibitory connections and evoke I- and D- waves (Rothwell *et al.*, 1991). Therefore, the ability to depolarise corticospinal neurons via synaptic mechanisms makes TMS a highly sensitive measure of cortical excitability (Day et al. 1989). As a result, I have used TMS throughout my studies to assess (Chapters 2, 3, 4, and 5) and also induce (Chapters 2 and 5) neuroplastic changes in human motor cortex.

Excitability of the human motor cortex using TMS can be assessed using several methods. For example, the representation of a specific muscle or group of muscles can be mapped by applying a number of stimuli at the same intensity over several scalp sites (usually 1cm x 1cm) until no response (MEP) is recorded (Wassermann *et al.*, 1992). The MEP indirectly

reflects the excitability of corticospinal neurons and can be assessed by measuring the MEP area or peak-to-peak amplitude elicited by TMS. Following this assessment, a number of key features result from cortical mapping. One of these features includes the location of the optimal spot on the scalp for eliciting a response (determined by the largest MEP). The optimal location for eliciting a response can also be used to assess cortical excitability. This can be achieved using a stimulus-response curve, which applies a range of intensities over the optimal spot. Although the two methods mentioned are performed differently, they both may reflect similar corticospinal changes after injury or during learning (Ridding & Rothwell, 1997). For this thesis, a single optimal location on the scalp to assess cortical excitability has been used throughout all studies. The next section will discuss in greater detail several measures of TMS using this method, with particular emphasis on the techniques used in the studies described in this thesis.

1.2.2.1. Motor threshold

The threshold for evoking a response in the target muscle is defined by the lowest TMS intensity to evoke a MEP (Kobayashi & Pascual-Leone, 2003). The threshold is thought to reflect the membrane excitability of corticospinal neurons and interneurons which project onto the corticospinal neurons (Rothwell *et al.*, 1991; Devanne *et al.*, 1997; Hallett, 2000). In addition, the motor threshold reflects the excitability of cortical synapses (neurons projecting onto corticospinal neurons) and synapses between the corticospinal neurons and muscle, such as spinal motor neurons (Rothwell *et al.*, 1991; Devanne *et al.*, 1997).

Cortical motor thresholds can be assessed when the target muscle is relaxed (resting motor threshold, RMT), or when performing a voluntary contraction (active motor threshold, AMT). A number of stimuli are applied to determine threshold as variability exists between subjects and between trials in the MEP amplitude (or area) evoked (Rossini *et al.*, 1994). The RMT is commonly defined as the lowest stimulus intensity that evokes a MEP of 50 μV in 5 out of 10

trials (Rossini *et al.*, 1994) or 3 out of 5 consecutive trials (Carroll *et al.*, 2001). Assessment of AMT is similar to that of RMT and requires a lower stimulus intensity to evoke a response as the voluntary activation (usually 5-10% maximum voluntary contraction) increases excitability of cortical and spinal neurons. Motor threshold, in particular RMT, has been assessed throughout all studies to determine whether there are any changes in cortical membrane excitability associated with various interventions of neuroplasticity.

1.2.2.2. MEP Size

A commonly assessed parameter following TMS is the size of the MEP (amplitude, area, or duration). MEP size indirectly reflects the number of corticospinal neurons activated. However, the characteristics of MEPs are also influenced by the number of recruited motor neurons in the spinal cord, the number of motor neurons discharging more than once in response to TMS, and the synchronisation of the TMS-induced motor neuron discharges (Rosler *et al.*, 2008). The peak-to-peak amplitude is most commonly used to assess the MEP. The MEP amplitude gives an indication of both the size and excitability of the corticospinal projection. The size of the MEP can be influenced by several factors including the stimulus intensity, shape and placement of the magnetic coil, and any tonic excitatory or inhibitory drive (such as muscle activation) (Brasil-Neto *et al.*, 1992a; Kiers *et al.*, 1993). Frequently, the size of the MEP evoked by a specific stimulus (usually a stimulus that evokes a ~1 mV amplitude before an intervention in hand muscles) is used as a marker of plastic changes following experimental interventions (Stefan *et al.*, 2000; Rosenkranz & Rothwell, 2006). Throughout all my studies I have recorded MEPs from a constant test stimulus before and after experimentally-induced or physiological (learning) interventions to assess neuroplastic changes.

1.2.2.3. Input-Output Curve

The input-output curve involves a range of stimulus intensities (usually a percentage of RMT or test intensity) delivered over the optimal location of the motor cortex for eliciting a response (MEP) in the target muscle. The MEP amplitude is then assessed over a range of stimulus intensities. For hand muscles, the input-output curve is sigmoidal involving a steep slope from threshold levels and plateau towards the maximum MEP amplitude (Devanne *et al.*, 1997; Carroll *et al.*, 2001; Pitcher *et al.*, 2003). The balance between the inhibitory and excitatory inputs within the corticospinal pathway and the spatial distribution of inputs in the motor cortex under the stimulating coil reflect the slope of the input-output curve (Devanne *et al.*, 1997; Siebner & Rothwell, 2003). As mentioned previously, excitability changes in input-output curves reflect changes in cortical mapping. This is due to the inability to distinguish changes in the area of a motor output zone of constant excitability from changes in excitability of a zone of constant area (Ridding & Rothwell, 1997). For example, increased excitability of corticospinal projections is evident in greater MEP amplitudes evoked by a given stimulus, an increase in the slope of the input-output curve, and a greater area of a motor representation for a specific muscle (Ridding & Rothwell, 1997). However, asymmetric changes in spatial distribution can only be assessed by cortical mapping. Nonetheless, a change in the slope of the input-output curve is used as a marker of plasticity. I have used input-output curve slopes, in addition to test intensity, in chapters 2 and 3 as a marker of neuroplasticity following experimentally-induced (chapter 2) and physiological (chapter 3) interventions.

1.2.2.4. Paired-pulse stimulation

All TMS measures described so far are single-pulse paradigms which assess excitability of corticospinal projections. However, TMS can also be used to assess inhibitory and excitatory mechanisms in corticospinal neurons. The paired-pulse protocol, first developed by Kujirai

and colleagues (1993), is commonly used to investigate inhibitory and excitatory circuits in the human motor cortex. I will next discuss the paired-pulse TMS protocols of short-interval intracortical inhibition (SICI) and facilitation (SICF), with more detail on SICI as I have used this technique throughout my studies (Chapters 2, 3, and 4).

Paired-pulse stimulation consists of one TMS pulse termed the conditioning stimulus and a second TMS pulse termed the test stimulus. The conditioning stimulus is often set below MEP threshold aimed at activating intracortical circuits which project onto corticospinal neurons without activating the corticospinal projections. The test stimulus is predominantly set at a suprathreshold level as determined by a single-pulse TMS. The effect of the conditioning stimulus on the test stimulus is commonly quantified by expressing the MEP amplitude of paired-pulse stimulation (conditioned MEP) as a ratio of single-pulse test stimulation (unconditioned MEP). The time between the paired stimuli (inter-stimulus interval) and intensity of the preceding conditioning stimulus determine whether there is an inhibitory or excitatory effect on corticospinal neurons. Several studies have investigated a number of inter-stimulus intervals (ISIs) between the two TMS pulses. Following short ISIs of 1 to 6 ms the conditioning pulse suppresses the test pulse and at the longer ISIs of 8 to 15 ms the test pulse is facilitated by the preceding conditioning pulse (Kujirai *et al.*, 1993; Ziemann *et al.*, 1996b).

The inhibition resulting from short ISIs is termed short-interval intracortical inhibition (SICI). Evidence suggests that SICI is mediated by $GABA_A$ receptors (Ziemann *et al.*, 1996a; Werhahn *et al.*, 1999; Ilic *et al.*, 2002). However, GABAergic inhibitory networks are mediated only at ISIs of 2 to 5 ms (Fisher *et al.*, 2002; Hanajima *et al.*, 2003). Inhibition observed with an ISI of 1 ms is thought to reflect the relative refractory period of the target cells or the conditioning pulse colliding with the target pulse (Hanajima *et al.*, 2003). The most common protocols that test SICI are with an ISI of 2-3 ms. Recent information suggests

the use of an ISI of ~2 ms for SICI as ISIs of ~2.5-3 ms may be contaminated by facilitatory mechanisms (Peurala *et al.*, 2008). However, differences in net inhibition only occur when a conditioning stimulus exceeds facilitation threshold (greater than 100% AMT) (Peurala *et al.*, 2008).

A number of factors have an influence on SICI. Although many of these are a result of individual anatomical and physiological differences (intrinsic factors), which cannot be controlled, other extrinsic factors can be controlled to minimise the influence on SICI. For example, SICI interacts with later I-waves (particularly I3 waves) and not the earlier I1 waves or D-waves (Di Lazzaro *et al.*, 1998a, b). Therefore, the size of the test stimulus can alter SICI. Specifically, a test stimulus that does not recruit later I-waves is not altered by the conditioning stimulus and a test stimulus which recruits significant D-waves exhibits a reduced amount of inhibition by the conditioning stimulus. Often a test stimulus which elicits a ~1 mV MEP amplitude is used, although the change in the amount of inhibition with a stronger stimulus (4 mV) is limited (Sanger *et al.*, 2001; Roshan *et al.*, 2003). Voluntary activation of the muscle is also another extrinsic factor which can influence SICI. A number of studies have shown that activating the muscle at ~10-20% of maximum force results in a decrease in inhibition when performing SICI measurements (Ridding *et al.*, 1995b; Zoghi *et al.*, 2003; Zoghi & Nordstrom, 2007). Although all I-waves are facilitated with voluntary activation (Di Lazzaro *et al.*, 1998a), an increased contribution of I1 waves to the MEP has been observed during muscle activity (Hanajima *et al.*, 1998). As a result, I1 waves have a greater influence on the MEP than later I-waves during voluntary activation, which reduces the magnitude of SICI compared with the relaxed muscle (Di Lazzaro *et al.*, 1998a, b; Zoghi *et al.*, 2003). Therefore, controlling the influence of factors such as test stimulus and muscle activation when performing SICI minimises extrinsic influence and allows for other influences (intrinsic or extrinsic) to be assessed.

Using the paired-pulse technique with longer ISIs (8 to 15 ms) produces MEP facilitation. It has been proposed that MEP facilitation at longer ISIs results from a decrease of ongoing profound inhibitory activity impinging on the same cell, as in the mechanism proposed for SICI at shorter ISIs (Werhahn *et al.*, 1999). However, it seems that separate neuronal populations invoke intracortical inhibitory and excitatory effects. The facilitatory effect seen at longer ISIs is thought to reflect the activation of cortico-cortically projecting pyramidal cells, located in superficial cortical layers, by the conditioning stimulus (Ziemann *et al.*, 1996b).

The paired-pulse technique also has the ability to induce facilitatory effects at short ISIs. This facilitation is termed short-interval intracortical facilitation (SICF) and is achieved when a suprathreshold conditioning stimulus is followed by a subthreshold test stimulus. Facilitation of the MEP occurs when the ISI is given at I-wave intervals of 1.5 ms (Hanajima *et al.*, 2002). This characteristic of SICF suggests that facilitation occurs by the subthreshold test pulse directly activating excitatory intracortical interneurons in the motor cortex, which were made hyperexcitable by excitatory post-synaptic potentials (EPSPs) elicited by the suprathreshold conditioning pulse (Hanajima *et al.*, 2002).

1.2.2.5. Cortical Silent Period

Inhibitory mechanisms can also be investigated using single-pulse TMS. This is assessed by a suprathreshold single-pulse TMS delivered over the optimal location of the motor cortex for eliciting a response (MEP) in the target muscle while it is tonically activated (commonly between 10% and 20% of MVC), which results in a period of EMG inactivity following the MEP. This inactivity is termed the cortical silent period (CSP) and its duration can persist for up to 300 ms in hand muscles (Inghilleri *et al.*, 1993). The duration of the CSP is dependent on the TMS intensity, but is not correlated with the size of the MEP amplitude or the level of muscle activation (Inghilleri *et al.*, 1993). EMG inactivity during the CSP is a result of

cortical and spinal inhibitory mechanisms (Ho *et al.*, 1998). However, the late part of the CSP is predominantly a result of cortical mechanisms (Chen *et al.*, 1999). It is thought that the $GABA_B$ receptor-mediated component of the inhibitory post-synaptic potential is involved in CSP duration (Siebner *et al.*, 1998; Werhahn *et al.*, 1999), although this is not a consistent finding (Inghilleri *et al.*, 1996; Ziemann *et al.*, 1996b; McDonnell *et al.*, 2006). Studies that have observed an increased CSP duration following $GABA_B$ agonist administration did not assess spinal inhibition (Siebner *et al.*, 1998; Werhahn *et al.*, 1999). Therefore, as the CSP has a spinal contribution, it is unclear whether the duration of the CSP results from $GABA_B$ receptor mediated-inhibition in the cortex, spinal cord, or both (McDonnell *et al.*, 2006).

1.3. Cortical plasticity

Experimentally, neuronal plasticity may be defined as any functional change within the nervous system that outlasts a manipulation. This can occur as a result of any lasting morphological or structural change in the cortex in response to normal or abnormal experiences. A change in the nervous system was initially believed to only occur in the very young during nervous system development. However, it is now known that cortical plasticity is an essential feature of the adult central nervous system, which continually adapts from experience by modifying connections between neurons to optimise performance. The potential of the brain to functionally and structurally reorganise not only occurs following physiological events (learning from experience), but also following pathological (injury or disease) events (Sanes & Donoghue, 2000). The progressive knowledge of neural substrates and mechanisms involved with cortical plasticity assist in the difficult task of understanding the physiological basis of learning and memory. In addition, the ability to induce or optimise cortical plasticity artificially or experimentally may be used to develop more effective treatment protocols for various neurological disorders (Ridding & Rothwell, 2007). The

purpose of the experiments conducted in this thesis was aimed to improve understanding of factors which have the capability to influence cortical plasticity in the human motor cortex.

Cortical plasticity has been demonstrated not only in a number of animal models, such as rodents, but also in humans. Plastic changes in the human brain have been demonstrated in a number of cortical regions including the sensory (Wu *et al.*, 2005), motor (Donoghue, 1995; Karni *et al.*, 1995), and auditory (Jancke *et al.*, 2001) cortices. The primary motor cortex (M1) has the essential function of controlling voluntary movements. Recent evidence also suggests that M1 is involved in higher order functions of movement (Donoghue & Sanes, 1994), and is a crucial site for motor learning (Pascual-Leone *et al.*, 1995; Sanes & Donoghue, 1997; Classen *et al.*, 1998; Kleim *et al.*, 1998; Kleim *et al.*, 2004). The M1 region was studied in the experiments conducted in this thesis because of its involvement in short-term motor learning and the ability to assess and induce changes in M1 using TMS.

1.3.1. Mechanisms of cortical plasticity

Cortical plasticity mechanisms can be broadly divided into two categories. First, those involved during rapid plastic changes, and second, those required for long-term structural reorganisation of cortical circuits. Rapid plastic changes in the cortex may involve the unmasking of pre-existing connections and activation of silent synapses (Jacobs & Donoghue, 1991; Malinow *et al.*, 2000), excitability changes of post-synaptic neurons (Woody *et al.*, 1991), or activity dependent change in synaptic strength (Donoghue *et al.*, 1996).

Unmasking of pre-existing silent synaptic connections in M1 can induce rapid plastic change in response to motor inputs (Donoghue *et al.*, 1990; Jacobs & Donoghue, 1991; Donoghue *et al.*, 1996). Silent synapses are characterised as connections between neurons that exhibit no α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) responses (Isaac *et al.*, 1995; Liao *et al.*, 1995). Activation of silent synapses can occur rapidly by the insertion of postsynaptic AMPA receptors (Gomperts *et al.*, 1998; Liao *et al.*, 1999), an increased release

of excitatory neurotransmitter or, most likely, a reduction in tonic inhibition (Kaas, 1991; Chen *et al.*, 2002).

The importance of GABA-mediated inhibition in the modulation of cortical plasticity has been demonstrated by a number of studies. For example, administration of the GABA antagonist bicuculline in rodents results in rapid changes of the size and distribution of motor cortical representation (Jacobs & Donoghue, 1991). Studies conducted in humans also demonstrate that a reduction in GABA-mediated inhibition induced by ischemic nerve block facilitates motor cortical plasticity (Ziemann *et al.*, 2001). Furthermore, an increase in GABA-mediated inhibition with the $GABA_A$ receptor agonist lorazepam depresses plasticity in the human motor cortex (Ziemann *et al.*, 2001). These findings suggest that the unmasking of silent synapses is influenced by GABA-mediated inhibition and provides means for rapid modulation of human motor cortical outputs.

Modulation of activity-dependent synapses has been a mechanism of much interest regarding rapid changes in cortical plasticity. A change in synaptic efficacy was initially demonstrated by Bliss and Lomo (1973) in the rabbit hippocampus. High frequency repetitive stimulation delivered to the perforant path in the dentate area of the hippocampus produced enhanced EPSPs that persisted for up to several hours following stimulation (Bliss & Lomo, 1973). This increase in synaptic efficacy resulting from high frequency repetitive stimulation is termed long-term potentiation (LTP) (Bliss & Lomo, 1973). Expression of LTP observed in inhibitory interneurons suggests that LTP is not limited simply to excitatory neurons (Kullmann & Lamsa, 2007). Repetitive stimulation at lower frequencies can also alter synaptic efficacy. However, unlike the enhanced EPSPs demonstrated with high frequency stimulation, lower frequencies reduce synaptic efficacy and is termed long-term depression (LTD) (Dudek & Bear, 1992). Although a large number of studies have been conducted in the hippocampal region because of its cognitive function, LTP and LTD has been

demonstrated in a number of cortical regions, including the motor cortex (Iriki *et al.*, 1989; Keller *et al.*, 1990; Hess & Donoghue, 1994, 1996).

Activity-dependent synaptic potentiation through LTP can be divided into two principal categories of N-methyl –D-aspartate (NMDA) receptor-dependent processes or NMDA-receptor-independent processes (Bliss & Collingridge, 1993). Mechanisms of synaptic modification are commonly studied with slice preparations (a synapse or neuronal circuit isolated from the intact nervous system). In the motor cortex, such studies show that activity-dependent synaptic potentiation exists within the intrinsic horizontal connections (Jacobs & Donoghue, 1991; Hess & Donoghue, 1994; Hess *et al.*, 1996). Pharmacological manipulations in slice preparations indicate that horizontal excitatory connections are primarily glutamatergic (Keller, 1993; Hess & Donoghue, 1994). Furthermore, activation of NMDA receptors requires the binding of glutamate (Collingridge & Bliss, 1987). Therefore, most forms of LTP depend on the activation of voltage-dependent NMDA receptors.

The reliance of the NMDA receptor results in a number of properties that characterise activity-dependent synaptic potentiation. Of particular importance are the properties of cooperativity, associativity, and input-specificity. However, unlike the hippocampus, high frequency stimulation alone does not induce LTP in the rodent motor cortex (Hess *et al.*, 1996). LTP was successfully induced in the adult motor cortex with the same stimulation pattern following a transient reduction in inhibition by applying the GABA antagonist bicuculline (Chen *et al.*, 1994; Hess *et al.*, 1996). Similarly, in humans, a TMS intervention normally ineffective in facilitating motor cortical excitability became effective when GABA-mediated inhibition was reduced by ischemic nerve block prior to the intervention (Ziemann *et al.*, 1998a). These findings suggest that GABA-mediated inhibition is important in regulating the potential for LTP induction in the motor cortex.

Cooperativity refers to the intensity threshold for induction of LTP (Bliss & Collingridge, 1993), which is a function of stimulus intensity and stimulation pattern (Bliss & Lomo, 1973). Therefore, depolarisation of the NMDA receptor on the post-synaptic membrane is achieved when the level of magnesium channel block is reduced allowing calcium influx to reach threshold (Bliss & Collingridge, 1993). Associativity refers to the induction of LTP in the sense that a weak input can be potentiated when it is coactive with a strong convergent input (Bliss & Collingridge, 1993; Bi & Poo, 2001), or when there is synchronous activity of another input to the same post-synaptic cell (Buonomano & Merzenich, 1998). The strong input achieves threshold by expelling magnesium from the NMDA receptor, which then allows it to respond to the weaker input (Murphy *et al.*, 1997). The property of associativity, often referred as Hebbian plasticity, is consistent with Hebb's postulate which proposes that synapses connecting two cells are strengthened if the two cells are active at the same time (Hebb, 1949). LTP is input-specific because it is only the inputs which are active along the pathway at the time of stimulation that are potentiated (Andersen *et al.*, 1977). Threshold is achieved as the concentration of glutamate released pre-synaptically is sufficient to activate adequate NMDA receptors to potentiate the synapse (Bliss & Collingridge, 1993). Compared with LTP, the role of LTD in memory and learning is less clear, although the reorganisation of cortical representations and maps also rely on LTD-like mechanisms (Buonomano & Merzenich, 1998).

Changes required for long-term reorganisation of cortical circuits involve a number of mechanisms in addition to LTP. These include morphological changes such as neurogenesis, synaptogenesis, and synaptic remodelling. Neurogenesis refers to the generation of new neurons. This process was believed not to be possible in the mammalian brain after early development. However, recent information in adult rodents has reported neurogenesis in brain areas important for cognitive function following an associative learning task (Gould *et*

al., 1999). Synaptogenesis is the formation of new synapses, and has previously been shown to occur in a number of cortical areas, including M1 (Klintsova & Greenough, 1999). Much of this information has come as a result of animals housed in an enriched environment, which may be classified as any addition to the standard living environment, such as objects, tunnels, running wheels, and other animals (van Praag *et al.*, 2000). More specific motor skill learning paradigms in animals also show synaptogenesis in M1 (Black *et al.*, 1990; Kleim *et al.*, 1996; Kleim *et al.*, 1997). Following skilled motor training, alterations of existing synapses are present in the mammalian M1 (Nudo *et al.*, 1996a; Kleim *et al.*, 1998). In humans, LTP-like mechanisms in M1 are also present after short-term motor practice (Bütefisch *et al.*, 2000; Muellbacher *et al.*, 2002; Sawaki *et al.*, 2002). However, whether LTP during rapid plastic change induces synaptogenesis or synaptic remodelling is unclear. In rodents, co-occurrence of functional and structural plasticity within the same cortical regions of M1 suggests that synapse formation plays a role in supporting learning dependent changes in cortical function (Kleim *et al.*, 2004). In humans, improvement or maintenance of a motor task with sustained practice does not result in LTP-like mechanisms (Rosenkranz *et al.*, 2007a). These changes in performance are likely a result of increased synaptogenesis, leading to enhanced corticospinal and intracortical recruitment (Rosenkranz *et al.*, 2007a). Therefore, it is possible that LTP-like mechanisms used to improve motor performance following short-term practice induce or act in concert with synaptogenesis in humans.

1.3.2. Learning and use-dependent plasticity

A central role for M1 in the control of voluntary movement has long been established. More recently, evidence suggests the functional organisation of M1 is modified by use (Pascual-Leone *et al.*, 1995; Classen *et al.*, 1998; Kleim *et al.*, 1998; Muellbacher *et al.*, 2001). The mechanisms underlying use-dependent plasticity in human M1 were recently assessed through pharmacological manipulations. Use-dependent plasticity in human motor cortex was

substantially reduced when premedicated with drugs which block the NMDA receptor (dextromethorphan) and enhance modulation of the $GABA_A$ receptor (lorazepam) (Bütefisch *et al.*, 2000). In contrast, downregulation of GABAergic inhibition by ischemic nerve block facilitated subsequent use-dependent plasticity (Ziemann *et al.*, 2001). Therefore, LTP-like mechanisms in use-dependent plasticity exist in the motor system of animals and humans (Sanes & Donoghue, 2000).

Motor learning in humans has evidently resulted in use-dependent plasticity. A number of motor learning studies have demonstrated an association between changes in motor cortex organisation and long-term skill acquisition. For example, motor representation of digits for the skilled hand involved in Braille reading (Pascual-Leone *et al.*, 1993) and musical instruments (Elbert *et al.*, 1995) are enlarged. For both studies, there was increased cortical representation of digits in the hemisphere contralateral to the skilled hand. In the other cerebral hemisphere (ipsilateral to the skilled hand) or in control (untrained) individuals no changes in cortical representation were observed. In addition to distal hand muscles, similar expansions in the representation of proximal muscles of the dominant side have been observed in professional badminton (Pearce *et al.*, 2000) and volleyball (Tyĉ *et al.*, 2005) players. Therefore, motor cortex reorganisation resulting from long-term specialised use is a widespread occurrence.

Changes in motor cortex organisation are not only observed following years of motor training, but also following short-term (minutes) motor training. A number of studies have demonstrated motor cortex reorganisation after a short (less than 60 minutes) repetitive ballistic thumb movement task (Classen *et al.*, 1998; Muellbacher *et al.*, 2001). From the perspective of the motor system, the ballistic task requires new information regarding agonist and antagonist motor unit activation to optimise the movement, and such training is considered a simple form of motor learning. Changes after training in these studies include an

increase in peak thumb acceleration (Muellbacher *et al.*, 2001) or TMS-evoked thumb movements in the trained (opposite) direction (Classen *et al.*, 1998) and an increase in MEP amplitude. These changes in motor cortex organisation occurred only for the trained muscle and are localised in motor cortex (Muellbacher *et al.*, 2001). In addition, these cortical excitability changes were not seen with TES (Classen *et al.*, 1998) or reduced substantially when GABAergic inhibition is enhanced (Bütefisch *et al.*, 2000). These findings suggest an involvement of M1 in use-dependent plasticity at a cellular level.

Changes in motor cortex organisation have also been demonstrated following more complex motor tasks. For example, training of a one-handed, five-finger exercise daily for 2 hours on a piano over 5 days progressively enlarged cortical representation of the involved hand muscles (Pascual-Leone *et al.*, 1995). In addition, these changes in cortical representation were not observed (or significantly reduced) in the untrained hand, were not observed in subjects who did not practice the task, and were also not observed in subjects who played the piano daily for 2 hours over five days with no specific sequence practiced (Pascual-Leone *et al.*, 1995). These outcomes indicate that repetitive movement practice with a clear performance goal results in increased motor cortex excitability.

Reorganisation of motor cortex is not limited to short- or long-term training of a motor task. In animals, it has been previously demonstrated that the adult motor cortex can reorganise rapidly (within minutes to hours) in response to peripheral lesions (Merzenich *et al.*, 1983; Sanes *et al.*, 1988; Donoghue *et al.*, 1990; Kolarik *et al.*, 1994; Silva *et al.*, 1996). A rapid motor cortical reorganisation in response to a lesion is also observed in humans. For example, transient deafferentation (ischemic nerve block) reduced intracortical inhibition (SICI) and increased cortical excitability and expansion of muscles proximal to the lesion (Chen *et al.*, 2002). The suggested mechanism resulting in these rapid changes is the removal of GABAergic inhibition (Ziemann *et al.*, 1998a; Levy *et al.*, 2002). Similar findings of

increased cortical excitability and representation of muscles proximal to the affected region have been demonstrated in amputees (Hall *et al.*, 1990; Cohen *et al.*, 1991).

There are a number of models developed for the induction of cortical plasticity and those mentioned above mainly include examples of training-induced plasticity. There have also been a number of models developed experimentally with the ability of inducing neuroplastic changes. In the next section I will discuss a number of these experimentally-induced plasticity methods.

1.3.3. Methods of experimentally-inducing plasticity

The ability to experimentally modify cortical organisation in humans provides an opportunity to improve functional outcomes following neurological injury and disease. A number of techniques inducing neuroplastic change have been developed. Models targeting human motor cortex using non-invasive brain stimulation will be discussed in this section. Specifically, I will focus on repetitive cortical stimulation of motor cortex and peripheral stimulation paired with cortical stimulation.

Several repetitive cortical stimulation models have been developed for inducing neuroplastic changes in motor cortex. One commonly applied non-invasive method is repetitive TMS (rTMS). The technique of rTMS (trains of TMS pulses delivered at regular or varying intervals) can either enhance or depress cortical excitability depending on the frequency. Following testing of frequencies from 0.1 to 25 Hz, low-frequency stimulation (1 Hz) depresses cortical excitability (Chen *et al.*, 1997; Muellbacher *et al.*, 2000), whereas high-frequency stimulation ($\geq$ 5 Hz) enhances cortical excitability (Berardelli *et al.*, 1998; Pascual-Leone *et al.*, 1998). In addition to frequency, the number and intensity of TMS pulses delivered are important factors in determining whether rTMS is facilitatory or depressive. For example, while high-frequency stimulation has been shown to enhance cortical excitability, high-frequency stimulation applied at low intensities (Todd *et al.*, 2006) or over a short

duration (≤ 13 TMS pulses) (Modugno *et al.*, 2001) result in decreased cortical excitability. Despite an MEP amplitude increase following high-frequency rTMS and decrease following low-frequency rTMS, the effects of rTMS on inhibitory and excitatory measures (including SICI, CSP, and ICF) are highly variable between studies (Fitzgerald *et al.*, 2006). Nonetheless, it is proposed that LTP/LTD-like mechanisms are involved in rTMS. For example, cortical excitability following rTMS is depressed when pre-treated with the NMDA receptor antagonist dextromethorphan (Ziemann *et al.*, 1998b; Fitzgerald *et al.*, 2005) or GABA agonist lorazepam (Ziemann *et al.*, 2001; Fitzgerald *et al.*, 2005) and enhanced when GABAergic inhibition is reduced with ischemic nerve block (Ziemann *et al.*, 1998a). Furthermore, evidence also suggests that changes in cortical excitability occur in the motor cortex (Quartarone *et al.*, 2005). However, following prolonged periods of rTMS, changes occur throughout the corticospinal output system, including spinal motoneurons (Quartarone *et al.*, 2005).

Several novel paradigms altering motor cortex excitability have derived from the initial rTMS technique. In particular, studies have focussed on protocols which induce neuroplastic changes by applying short high-frequency trains (bursts of stimulation) at a predefined repetition rate. The most common example is theta burst stimulation (TBS) (Huang *et al.*, 2005). The TBS protocol involves three-magnetic pulses at a frequency of 50 Hz repeated in intervals of 200 ms (5 Hz, theta rhythm) (Huang *et al.*, 2005). An increase or decrease in cortical excitability follows TBS depending on the temporal pattern of the stimuli. Depressed cortical excitability results from continuous TBS (cTBS) for a period of up to 60 minutes following the intervention (Huang *et al.*, 2005). Enhanced cortical excitability results from intermittent TBS (iTBS) for a period of up to 15 minutes following the intervention (Huang *et al.*, 2005). The cortical changes after TBS outlast the stimulation period (manipulation). In addition, indirect evidence also demonstrates that TBS is cortical in origin and is NMDA-

receptor dependent (Huang *et al.*, 2007). Therefore, neuroplastic changes induced by TBS are thought to be a result of LTP/LTD-like mechanisms.

Neuroplastic change induced by repetitive cortical stimulation is not limited solely to non-invasive stimulation over the cortex. Changes in cortical excitability occur following repetitive pairing of a peripheral electrical stimulus with single-pulse TMS over the contralateral motor cortex. This method is adapted from associative plasticity techniques used in animal neocortical slices (Markram *et al.*, 1997). Termed paired associative stimulation (PAS), the induction of neuroplastic changes in human motor cortex following PAS were first demonstrated over a decade ago (Stefan *et al.*, 2000). In the initial study, PAS consisted of electrical stimulation of the median nerve (motor innervation for the target abductor pollicis brevis, APB, muscle) at the wrist followed by TMS of the contralateral motor cortical representation of APB (Stefan *et al.*, 2000). Electrical peripheral nerve stimulation was set at three times perceptual sensory threshold (300%). The proceeding TMS intensity was suprathreshold to result in action potentials in corticospinal neurons and was set at 130% RMT to evoke a MEP amplitude of approximately 1 mV (Stefan *et al.*, 2000). PAS, consisting of 90 paired peripheral and cortical stimuli delivered over 30 minutes (frequency of 0.05 Hz), enhanced MEPs at an ISI (the timing of single-pulse TMS with reference to median nerve stimulation) of 25 ms for up to 60 minutes following the intervention (Stefan *et al.*, 2000). Increased cortical excitability was focal to APB as cortical excitability of other muscles not innervated by the median nerve did not change (Stefan *et al.*, 2000). In addition, the longer ISIs examined (100, 525, and 5000 ms) did not affect the MEP following PAS (Stefan *et al.*, 2000). Neuroplastic changes following PAS only at an ISI of 25 ms signify a dependence on synchronous arrival. It is estimated that the peripheral median nerve stimulation at the wrist arrives in somatosensory cortex at around 20 ms (Allison *et al.*, 1991) and an additional few milliseconds is then required for the signal to relay to motor cortex.

Therefore, the afferent signal evoked by peripheral stimulation arrives in motor cortex near synchronous to depolarisation of corticospinal neurons by the TMS pulse at an ISI of 25 ms.

Subsequent studies of PAS have further explored the notion of PAS-induced plasticity in motor cortex. For example, the influence of a number of varying ISIs (-10 to 50 ms) on PAS-induced plasticity were examined (Wolters *et al.*, 2003). An enhanced MEP following PAS was confirmed at an ISI of 25 ms (Wolters *et al.*, 2003). However, the authors also observed a depressed MEP following PAS with an ISI of 10 ms (Wolters *et al.*, 2003). The finding that PAS-induced changes are linked with associativity of inputs suggests a strict temporal Hebbian rule governs PAS. For LTP-like effects the pre-synaptic input (peripheral nerve stimulation) slightly precedes or synchronously arrives with depolarisation of the post-synaptic output (TMS). In contrast, for LTD-like effects the depolarisation of corticospinal neurons by TMS precedes the input from peripheral nerve stimulation (Wolters *et al.*, 2003). However, the timing of LTP/LTD-like PAS induced changes is highly specific (Wolters *et al.*, 2003). A number of studies have also demonstrated PAS-induced changes with an increased number and rate of paired stimuli (Ziemann *et al.*, 2004). In addition, the facilitatory PAS-induced changes have been shown to be dependent on the number of paired stimuli (Nitsche *et al.*, 2007).

The change in cortical excitability induced by PAS largely reflects a change in motor cortex function. Although some evidence exists of a contribution from within spinal cord circuits (Meunier *et al.*, 2007) several lines of evidence suggests that PAS is cortical in origin. First, F-waves (an index of spinal motor neuron excitability) remained unchanged following increased cortical excitability resulting from PAS (Stefan *et al.*, 2000). Second, assessment of corticospinal axons directly using electrical brainstem stimulation (Ugawa *et al.*, 1991) was not affected following PAS (Stefan *et al.*, 2000). Third, PAS prolonged the cortical silent period, which, as described earlier, is in part a result of cortical mechanisms and are $GABA_B$

receptor-mediated (Stefan *et al.*, 2000). Fourth, descending corticospinal activity evoked by TMS assessed from epidural recordings showed that PAS increased the amplitude of later descending waves (Di Lazzaro *et al.*, 2009a, b). The later descending waves evoked by TMS reflect intracortical trans-synaptic activation of pyramidal neurons (Ziemann & Rothwell, 2000; Di Lazzaro *et al.*, 2004). Five, reversing the direction of the induced current in the brain (posterior-anterior to anterior-posterior, preferentially activating later descending waves) allows a subthreshold TMS intensity to result in facilitation that is similar to a suprathreshold TMS intensity following PAS (Kujirai *et al.*, 2006). Last, PAS results in a specific interference of preparatory volitional motor cortical activity of movements for those targeted by PAS (Lu *et al.*, 2009).

There is evidence from examples mentioned above to suggest that PAS-induced changes in motor cortex are a result of LTP-like mechanisms. This includes a rapid onset, is long lasting (outlasts manipulation), reversible (returns to baseline), and is muscle (input) specific. Pharmacological studies involving PAS also suggest LTP/LTD-like mechanisms. Facilitatory (ISI 25 ms) and depressive (ISI 10 ms) effects following PAS were blocked when subjects were pre-treated with a NMDA receptor antagonist (dextromethorphan) (Stefan *et al.*, 2002; Wolters *et al.*, 2003). These findings coincide with previously mentioned studies which demonstrate that LTP and LTD induction is dependent on NMDA receptor activation (Bi & Poo, 1998). In addition, PAS-induced LTD-like plasticity is blocked by an L-type voltage-gated calcium channel antagonist (nimodipine) (Wolters *et al.*, 2003). This finding is important as activation of voltage-gated calcium channels for post-synaptic calcium influx is critical for the induction of LTD (Bi & Poo, 1998).

The neuronal circuits mediated by PAS-induced LTP/LTD-like mechanisms are also functionally relevant. For example, learning resulting from repeated fastest possible thumb abduction movement (an increase in maximum peak acceleration) or isometric thumb

abduction movement (an increase of successful force production) blocked subsequent PAS-induced LTP-like plasticity (Ziemann *et al.*, 2004; Stefan *et al.*, 2006) or resulted in LTD-like plasticity (Rosenkranz *et al.*, 2007b). In contrast, PAS-induced LTD-like plasticity was enhanced (Ziemann *et al.*, 2004; Rosenkranz *et al.*, 2007b) or unchanged (Stefan *et al.*, 2006) following motor training. Furthermore, when motor training was performed 90 minutes after PAS, PAS-induced LTD-like plasticity facilitated motor learning, whereas PAS-induced LTP-like plasticity depressed motor learning (Jung & Ziemann, 2009). These outcomes imply that, in the human motor cortex, primarily homeostatic mechanisms interface with plasticity mechanisms to ensure that neural activity remains at a stable level (Davis, 2006). However, LTP- and LTD-like PAS both facilitated motor learning (more after LTD-like PAS) when immediately preceded by PAS, suggesting non-homeostatic mechanisms (such as LTP-induced blockade of LTD and non-saturated LTP-induced facilitation of motor learning) may also contribute when there is no delay between interventions (Jung & Ziemann, 2009).

One important concept of homeostatic plasticity in controlling neural activity is the modulation of membrane excitability depending on the previous activity of the post-synaptic neuron (Bienenstock *et al.*, 1982). This concept suggests that there is a sliding threshold for synaptic plasticity. For example, low-levels of post-synaptic activity decrease membrane excitability, which increases the probability of LTP induction and decreases that of LTD-induction (Bienenstock *et al.*, 1982). In contrast, high levels of post-synaptic activity increase membrane excitability, which decreases the likelihood of LTP induction, but increases that of LTD induction (Bienenstock *et al.*, 1982). However, previously mentioned studies proposing a homeostatic mechanism in the human motor cortex used two different experimental manipulations (motor learning and PAS), which makes it difficult to determine whether homeostatic interactions occur within the same or different cortical circuits. The findings that PAS-induced LTP-like plasticity was suppressed if preceded by PAS-induced LTP-like

plasticity and was facilitated if preceded by PAS-induced LTD-like plasticity are in support of homeostatic mechanisms regulating human motor cortex plasticity (Müller *et al.*, 2007).

For this thesis, PAS was used to experimentally induce plasticity in Chapters 2 and 5. The reasons for choosing PAS, as outlined above, include that it is associative, primarily cortical in origin, LTP/LTD-like dependent, and tests functionally relevant neuronal circuits. In the next section I will discuss factors which influence neuroplasticity in human motor cortex, particularly those central to the studies I have conducted.

1.4. Factors influencing cortical plasticity in human motor cortex

Disease and injury to the brain are central factors that influence the magnitude of plasticity following experimentally-induced and use-dependent paradigms. However, there is also large variability in human motor cortex response to experimentally-induced and use-dependent plasticity paradigms in neurologically normal adults. This variability is complex in nature and the causes are likely to be multifactorial. Although much of the variability remains unknown, a number of factors which can influence the induction of plasticity in neurologically normal adults have been identified. Several of these factors have received much attention. In particular, it is well accepted that the history of synaptic activity influences motor cortex plasticity (Iyer *et al.*, 2003; Ziemann *et al.*, 2004; Müller *et al.*, 2007; Jung & Ziemann, 2009). In addition, sex (Inghilleri *et al.*, 2004; Tecchio *et al.*, 2008), attention (Hazeltine *et al.*, 1997; Stefan *et al.*, 2004), and time of day (Sale *et al.*, 2007, 2008) have an influence on the induction of plasticity in motor cortex. My PhD studies aim to provide a better understanding of several factors that have limited knowledge of their influence on human motor cortex plasticity. An increased understanding of these determinants is not only beneficial in comprehending the influences of motor cortex plasticity in neurologically normal adults, but also critical for optimising therapeutic rehabilitation

strategies following neurological insult. In the following sections I will describe the factors that I have assessed in relation to their impact on motor cortex plasticity.

1.4.1 Aerobic exercise and brain function

It has been known for several decades that regular exercise has an impact on most physiological systems. More recently, a growing body of evidence has suggested that participation in physical activity and exercise is beneficial to brain health and function. Further information suggests that physical activity may confer health-protective benefits for several neurological diseases including Parkinson's disease, Alzheimer's dementia and ischaemic stroke (see Kramer & Erickson, 2007), and may even slow functional decline during the neurodegeneration process (Heyn *et al.*, 2004). Recent evidence indicates that regular physical activity and exercise can increase brain plasticity (see Cotman & Berchtold, 2002; Colcombe *et al.*, 2004). Aged populations demonstrate the most noticeable effects of exercise. For example, constant exercise participation enhances learning and memory, improves executive function, counteracts age-related mental decline, and protects against age-related brain atrophy (Kramer *et al.*, 1999; see Colcombe & Kramer, 2003 for review). These studies suggest that neuroprotective and neuroplastic benefits to the brain may result from regular physical activity and exercise. In addition, participation in physical activity and exercise may assist to improve memory and learning in humans (Kramer *et al.*, 1999; Colcombe & Kramer, 2003).

Recent evidence in the animal model suggests that some of the beneficial aspects of exercise act directly on specific aspects of brain function. Several studies have related exercise induced changes in the number of genes that regulate synapses with promotion of brain plasticity (Cotman & Engesser-Cesar, 2002). In particular, brain-derived neurotrophic factor (BDNF) expression has been extensively studied. BDNF is important in the maintenance and survival of existing neurons and promotes growth and differentiation of new neurons and

synapses believed to be essential for neuronal plasticity (Cotman & Berchtold, 2002; Cotman & Engesser-Cesar, 2002; Lu, 2003; Klintsova *et al.*, 2004) and spatial learning and memory (Gomez-Pinilla *et al.*, 2001; Vaynman *et al.*, 2003, 2004). The hippocampus, a region of the brain important in learning and memory, exhibits the most immediate changes in BDNF expression after exercise and also the earliest and most prominent changes in plasticity in response to exercise (Cotman & Berchtold, 2002).

A beneficial effect of exercise on brain plasticity in the hippocampus has also been reported in humans (Cotman & Engesser-Cesar, 2002), particularly in older adults (Colcombe *et al.*, 2004; Kramer *et al.*, 2006). For example, Kramer and colleagues (1999) have shown a positive effect of exercise on memory and cognitive tasks. In this study, sedentary adults (aged 60 to 75 years) were randomly placed into either an aerobic (walking) or anaerobic (stretching and toning) exercise group for a period of six months. Performing neuropsychological measures of executive control, the aerobic group significantly improved neurocognitive function compared to the anaerobic group (Kramer *et al.*, 1999). Furthermore, a retrospective study from surveys has suggested that individuals with a more active lifestyle during middle adulthood were less likely to develop Alzheimer's disease (Friedland *et al.*, 2001). Therefore, physical activity and exercise is suggested to promote increased neuroplasticity in the human hippocampus that is associated with improved memory and cognition.

In contrast to the cognitive aspects, much less is known about how plasticity in human motor cortex (a critical site for motor learning) is influenced by regular exercise. It is well established that experience-specific alterations in cortical aspects of movement result from different types of training (see Adkins *et al.*, 2006 for review). However, only the limbs involved in the training have been examined for changes in motor cortex function. Furthermore, molecular mechanisms believed responsible for improved cognition with

exercise, such as BDNF, have also been observed in several other brain regions besides the hippocampus, including the cerebellum and motor cortex (Ding *et al.*, 2004; Klintsova *et al.*, 2004; see Vaynman & Gomez-Pinilla, 2005 for review). This widespread increase of molecular mechanisms with exercise may suggest a more general effect on brain and motor function. Therefore, for the first series of experiments, I was interested in whether, like for neurocognitive function, the benefits of regular endurance (aerobic) exercise would offer widespread benefits to human motor cortex function that extends beyond the neural boundaries responsible for control of the exercising limbs. This question was addressed in Chapter 2.

1.4.2. Ageing

The ageing process affects all body systems. The CNS is no exception, with progressive losses in function across multiple systems, including sensation, cognition, memory, motor control, and affect (Mahncke *et al.*, 2006). Advances in neuroimaging techniques have significantly contributed to understanding age-related changes in the brain. For example, recent evidence demonstrates that the reduced brain volume accompanying ageing mainly occurs from a decline in the number and size of synapses rather than simply a decreased number of neurons (see Fjell & Walhovd, 2010 for review). Age-related changes in the brain have predominantly focussed on impaired cognitive function, and evidence suggests that structural and functional plasticity is reduced in cognitive systems with progression into old age (Hedden & Gabrieli, 2004). In addition, ageing is also associated with an altered capacity for processes important for synaptic plasticity, such as LTP (see Barnes, 2003 for review). The literature for changes in motor cortical areas is not as extensive as that for cognitive function, but there are parallels in ageing between the motor and cognitive systems, such as increased cortical activation in older adults to optimise task performance (Calautti *et al.*,

2001; Mattay *et al.*, 2002; Ward & Frackowiak, 2003; Heuninckx *et al.*, 2005; Heuninckx *et al.*, 2008).

Much work focussing on motor control has established that performance is diminished with advancing age, particularly as task difficulty is increased (Light & Spirduso, 1990; Smith *et al.*, 1999a). However, a reduced capacity for learning new motor skills in older adults is not always a consistent finding (Voelcker-Rehage, 2008). Recent information demonstrates that sustained practice of a new motor skill induces structural changes (such as synaptogenesis) in the mammalian adult M1 (Nudo *et al.*, 1996a; Kleim *et al.*, 1998). In humans, Boyke and colleagues (2008) have demonstrated that the ability to alter cortical structure is maintained in older adults when learning a novel motor skill. However, the extent of structural plasticity of cortical gray matter in older adults was reduced when compared with a young population learning the same motor task (Boyke *et al.*, 2008).

Functional plasticity changes in the adult motor cortex are also present when learning a new motor skill (Bütefisch *et al.*, 2000; Muellbacher *et al.*, 2002; Sawaki *et al.*, 2002). Information on whether the ageing process influences functional plasticity is also limited. A number of recent TMS studies have used PAS to investigate the influence of age on human motor cortex plasticity induction. The facilitation in MEP amplitude accompanying LTP-like PAS in young adults was not observed in older adults, supporting an age-related decrease in plasticity of cortical circuits (Müller-Dahlhaus *et al.*, 2008; Tecchio *et al.*, 2008; Fathi *et al.*, 2010). A reduction in motor cortex plasticity with advancing ageing is not limited to PAS. Following an inhibitory rTMS protocol, LTD-like changes in synaptic efficacy were shown to be reduced in the ageing brain (Todd *et al.*, 2010). Furthermore, reduced use-dependent plasticity in the ageing motor cortex has been demonstrated by impaired encoding of a novel elementary motor memory (Sawaki *et al.*, 2003) and the absence of a facilitated MEP following repetitive ballistic thumb training in older adults (Rogasch *et al.*, 2009).

Although ageing is a factor which might modulate plasticity induction in human motor cortex, the effects of ageing have been inadequately examined. In particular, the mechanisms underlying reduced motor cortex plasticity are poorly understood, although it is thought that an age-related decline in LTP (Burke & Barnes, 2006), neurotransmitters (Luo & Roth, 2000; Clayton *et al.*, 2002), or gene expression (Pang *et al.*, 2004) important for synaptic plasticity may be partly responsible. Studies conducted in humans examining use-dependent plasticity in older adults are limited. The studies that have focussed on use-dependent plasticity in older adults have all been restricted to the right hand (left hemisphere). However, structural and functional differences exist in the human motor system between left and right hands that may influence plasticity (see section 1.4.3. below). In addition, lateralisation (of hands) is reduced with progression into old age (see section 1.4.3. below). Therefore, Chapter 3 addresses whether asymmetries in the motor system influence use-dependent plasticity in older adults. Furthermore, all studies examining use-dependent plasticity in older adults were performed with simple motor tasks (repetitive ballistic motions). However, complex motor tasks are more likely to engage the motor cortex (as well as other cortical and higher order brain structures) and influence the extent of plasticity (see section 1.4.4. below). Understanding use-dependent plasticity following complex motor tasks in older adults is functionally important. This was addressed in Chapter 4. Therefore, the use-dependent plasticity studies in older adults I have conducted focussing on motor system asymmetries (Chapter 3) and a more complex motor task (Chapter 4) will help inform and model future studies investigating the ageing system.

1.4.3. Hemispheric asymmetries

The most characteristic feature of the healthy human brain when viewed intact is the division into two halves, the right and left hemisphere, which are approximate mirror images of each other. However, pioneering work by Broca (1864) and Wernicke (1874) demonstrating the

lateralised function of language indicate that the two hemispheres are structurally and functionally asymmetric. The development of neuroimaging techniques, such as fMRI, not only support lateralisation between hemispheres, but also provides detailed information on the structural and functional asymmetries (Binder *et al.*, 1997; Serrien *et al.*, 2006).

An important feature in motor control relates to hand preference. Although the left and right hands almost anatomically mirror each other, there are clear asymmetries in motor function. For example, when highly specialised movements required for fine motor control are required, one hand (generally the right) is preferred to perform the task over the other (Barnsley & Rabinovitch, 1970). Surprisingly, from the number of studies conducted on motor cortical asymmetry, few have directly focussed on hemispheric features and hand preference. Evidence from these studies suggests that the motor system of the left and right brain hemispheres is not symmetrical, with anatomical and physiological differences between sides potentially contributing to asymmetries in hand performance. For example, right-handed individuals have a greater sulcal surface (White *et al.*, 1994) and a more pronounced length of the posterior wall of the precentral gyrus bordering the central sulcus (measure of the size of primary motor cortex) (Amunts *et al.*, 1996) in the left hemisphere (right hand) compared with the right hemisphere (left hand). The opposite was true for left-handed individuals (Amunts *et al.*, 1996). In addition, MEG studies demonstrate increased neuronal activity is present in the preferred compared with non-preferred hand during simple hand and finger movements (Volkmann *et al.*, 1998). The use of fMRI has also revealed more extensive connections from primary motor cortex to pyramidal tracts, premotor areas, parietal cortices, thalamus, and cerebellum in the left compared with right hemisphere of right-handed adults (Guye *et al.*, 2003). These asymmetries between brain hemispheres suggest that more cortical circuitry is devoted to the representation of the preferred hand.

The use of TMS has also been used to demonstrate asymmetries in the motor system. For example, the threshold for eliciting MEPs in the right hand (left hemisphere) was reduced compared with the left hand (right hemisphere) for right-handed adults (Macdonell *et al.*, 1991; Triggs *et al.*, 1997; Ilic *et al.*, 2004). In contrast, for left-handed adults, the threshold for eliciting MEPs was reduced in the left hand compared with the right (Triggs *et al.*, 1997). Although altered thresholds between hemispheres is not a consistent finding (Brouwer *et al.*, 2001; Garry *et al.*, 2004; Ridding & Flavel, 2006; Gallasch *et al.*, 2009), an increased cortical motor representation (increased number of scalp stimulation sites eliciting MEPs) for the right compared with left hand suggests asymmetry in cortical motor representation (Triggs *et al.*, 1999). Furthermore, intracortical inhibition, assessed using SICI, was less in the left compared with right hemisphere in right-handed adults (Ilic *et al.*, 2004; Ridding & Flavel, 2006). Ilic and colleagues (2004) suggest that, as a result, the right hand (left hemisphere) is controlled by less inhibitory tone than the left hand (right hemisphere), which may be advantageous for voluntary activation and use-dependent plasticity.

Despite these hemispheric differences in the motor system, very few studies have examined the extent of motor cortex plasticity in left and right hemispheres and whether this has implications for improved motor performance. Motor cortex plasticity induced experimentally by PAS in the left and right hemispheres (on separate occasions) of right-handed individuals increased motor cortex excitability similarly in both hemispheres (Ridding & Flavel, 2006). Additionally, performance improvement following simple repetitive ballistic movements was greater in the left compared with right hand, although baseline performance was significantly greater in the right hand (Ridding & Flavel, 2006). The overall findings by Ridding and Flavel (2006) led them to conclude that decreased SICI observed in the left hemisphere does not enhance use-dependent plasticity of the right hand. Use-dependent plasticity studies involving hand muscles generally show no hemispheric

differences in young subjects (Garry *et al.*, 2004; Gallasch *et al.*, 2009). However, repeat performance of a goal-directed movement task suggests a more sustained effect in the left hand (Gallasch *et al.*, 2009). The functional implications of any hemispheric differences in use-dependent plasticity for motor skill learning are unknown.

The human hand undergoes many physiological and anatomical changes over a lifetime (Carmeli *et al.*, 2003). One noticeable change in older adults is a reduction in motor performance (Smith *et al.*, 1999a). In young adults, performance of motor tasks requiring a more precise action is greater in the preferred hand than the non-preferred (Kabbash *et al.*, 1993). This clear difference in performance between hands is substantially reduced with advancing age, resulting from a more pronounced age-related decline in performance of the preferred hand (Kalisch *et al.*, 2006). Furthermore, M1 activation in older adults becomes less lateralised following simple motor tasks with a significant increase in ipsilateral M1 activity present (Naccarato *et al.*, 2006). Despite less lateralisation in older adults, previous ageing studies examining use-dependent plasticity have all been performed on the right (preferred) hand (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009). In addition, all previous studies that have assessed hemispheric differences in plasticity have only involved young subjects. Therefore, the next set of experiments (Chapter 3) compared use-dependent plasticity in the left and right hands of young and old adults.

1.4.4. Task complexity

Each day humans perform a number of fine motor skills. The difficulty of motor tasks can vary from picking up a pen to using it to write a sentence. Numerous studies indicate that movement complexity influences motor performance. A decline in motor performance results from an increased task complexity, particularly in older adults (Light & Spirduso, 1990; Smith *et al.*, 1999a). In addition to influences on motor performance, task complexity also varies the amount of cortical activation required to optimally execute the motor task. For

example, using neuroimaging techniques such as fMRI, young subjects were observed to require activation of other cortical and subcortical areas in addition to M1 as task complexity increased (Rao *et al.*, 1993; Wexler *et al.*, 1997). This suggests that more complex tasks involve a number of brain areas to plan and execute the movement. Furthermore, older adults are known to activate a wider network of brain regions within the motor system compared with young adults (Ward, 2006). For example, additional cortical and subcortical areas are also activated in older adults when performing simple motor tasks (Calautti *et al.*, 2001; Ward & Frackowiak, 2003; Heuninckx *et al.*, 2005; Naccarato *et al.*, 2006; Heuninckx *et al.*, 2008). Although it is not conclusive, it is possible that increased cortical activation in older adults when executing motor tasks is a compensatory mechanism for functional deficits in the ageing brain (Ward, 2006).

The ability of the motor system to reorganise has been observed following simple and complex tasks. Although long-term changes in cortical reorganisation are observed following complex tasks, such as playing a musical instrument (Elbert *et al.*, 1995), short-term changes in motor cortex occur following both simple (Classen *et al.*, 1998; Muellbacher *et al.*, 2001) and complex training (Pascual-Leone *et al.*, 1995). Recent TMS studies have demonstrated that use-dependent plasticity is reduced in older adults (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009). However, these studies have been restricted to simple motor tasks. Despite increased activation of brain regions within the motor system to execute simple tasks with advancing age (Ward, 2006), it is also evident that complex tasks are more likely to involve M1 (Flament *et al.*, 1993; Rao *et al.*, 1993; Wexler *et al.*, 1997) and increase attentional demand, which is known to influence plasticity induction in human motor cortex (McNevin *et al.*, 2000; Stefan *et al.*, 2004). Furthermore, reduced short-term motor learning in old compared with young adults is commonly more pronounced with increased task difficulty (Light & Spirduso, 1990; Smith *et al.*, 1999a; Voelcker-Rehage, 2008). Therefore, I was interested in

better understanding the neuroplastic capacity of older adults. To investigate this, I conducted experiments examining how learning a more complex visuomotor tracking task, where the improvement in tracking performance is dependent on changes in motor cortex (Paz *et al.*, 2003; Roche & O'Mara, 2003; Perez *et al.*, 2004), alters use-dependent plasticity in young and old adults.

1.4.5. Genetic variation

There are several neurotrophins expressed throughout the CNS, with brain-derived neurotrophic factor (BDNF) the most abundant growth factor in the brain (Pearson-Fuhrhop *et al.*, 2009). BDNF is crucial for the proliferation, differentiation, and survival of neurons, but is also important in the adult CNS by enhancing synaptic transmission, facilitating long-term potentiation (LTP), and mediating use-dependent plasticity (Schinder & Poo, 2000; Gottmann *et al.*, 2009). In addition, BDNF is thought to have a role in regulating the homeostatic process of Hebbian plasticity (Turrigiano, 1999). The variability in plasticity observed in neurologically normal adults has recently been attributed with a significant genetic contribution (Missitzi *et al.*, 2011). In humans, a naturally occurring single nucleotide polymorphism in the *BDNF* gene produces a valine (Val) to methionine (Met) substitution at codon 66 (*BDNF* Val66Met). This *BDNF* single nucleotide polymorphism can be present in one or both alleles and results in an 18% (Val/Met heterozygotes) to 30% (Met/Met homozygotes) reduction in BDNF release in cortical neurons (Egan *et al.*, 2003). The incidence of the *BDNF* polymorphism is relatively common, occurring in 30-50% in the general population (Shimizu *et al.*, 2004). However, the prevalence of Met allele carriers is dependent on ethnicity, with the highest prevalence in south-east Asian countries, such as Japan and Korea (Shimizu *et al.*, 2004; Pivac *et al.*, 2009). A number of previous studies have demonstrated that individuals carrying the Met allele have altered cortical morphology compared with homozygotes for the Val allele (Val/Val). The morphological changes are

predominantly linked with a reduced hippocampal volume in people with the Met allele (Pezawas *et al.*, 2004; Szeszko *et al.*, 2005). Previous studies also show functional deficits in people with the *BDNF* polymorphism. These functional deficits are primarily related to hippocampal function, with poorer episodic and working memory in Met allele carriers (Egan *et al.*, 2003). The Met allele has also been associated with altered susceptibility to some neurological and psychiatric disorders (see Bath & Lee, 2006), and may influence CNS repair and functionally beneficial neuroplasticity after neurological injury (Pearson-Fuhrhop *et al.*, 2009).

The functional organisation of M1 can be modified experimentally or by use. The response to use-dependent plasticity in M1 with TMS has recently been shown to be significantly influenced by the *BDNF* polymorphism. For example, less motor map reorganisation and reduced changes in M1 excitability following a 30 minute training period involving index finger tapping and pinch-grip strength were exhibited by individuals with the *BDNF* Met allele (Kleim *et al.*, 2006). Neuroimaging techniques also show similar observations, with index finger training resulting in a reduced activation volume of the sensorimotor cortex in people with the Met allele (McHughen *et al.*, 2010). However, reduced use-dependent plasticity in Met allele carriers is not consistent, with similar MEP facilitation observed between different *BDNF* genotypes following a 40 minute training period of index finger abduction acceleration (Li Voti *et al.*, 2011). The *BDNF* polymorphism has also been shown to reduce the response to experimentally-induced plasticity in M1. For example, changes in MEPs following TBS and PAS that are evident in homozygotes for the Val allele were absent in Met allele carriers (Cheeran *et al.*, 2008; Antal *et al.*, 2011; Missitzi *et al.*, 2011).

Limited information is available about the influence of the rare Met homozygote *BDNF* genotype (5-15% of the general population, Shimizu *et al.*, 2004). In cortical areas involved with planning and higher order cognitive functioning, Met homozygotes display decreased

activation volume (Pezawas *et al.*, 2004). In M1, only one study to date has assessed plasticity in Met homozygotes. Use-dependent plasticity in M1 was assessed following simple motor training and, although M1 excitability was reduced compared with Val homozygotes, there were no differences in corticospinal output and motor map area between Met/Met and Val/Met *BDNF* genotypes (Kleim *et al.*, 2006). However, evidence in the rodent M1 suggests that BDNF expression is more likely to be modified by complex motor tasks (Klintsova *et al.*, 2004). Therefore, it remains unclear whether plasticity in M1 following more demanding tasks is related to the putative reduction in activity-dependent BDNF release from cortical neurons in Met allele carriers. This was investigated in Chapter 5.

1.5. Summary

Initial studies involving M1 quickly established its vital role in controlling voluntary movement. However, it is now recognised that M1 is implicated in learning new motor skills and in the recovery of motor function after injury. The development of non-invasive stimulation techniques, particularly transcranial magnetic stimulation (TMS), has significantly contributed to understanding the capability of human motor cortex to structurally and functionally reorganise (cortical plasticity). Although the ability to alter human motor cortex in response to physiological and pathological influences is of critical importance, studies have also revealed that several factors can influence the induction of plasticity in motor cortex.

Using experimentally-induced and use-dependent plasticity methods in human motor cortex, the following chapters describe experiments I have conducted investigating a number of factors capable of influencing motor cortex plasticity. Chapter 2 examines whether the benefits of regular endurance (aerobic) exercise, performed with the lower limbs, influences the induction of plasticity (paired associative stimulation, PAS) in human motor cortex for control of hand muscles. The influence of age on motor cortex plasticity is assessed in

chapters 3 and 4. Chapter 3 examines whether hemispheric function can influence the induction of plasticity in human motor cortex following simple repetitive movement in young and old adults. Chapter 4 examines whether an increase in task complexity (visuomotor tracking) alters plasticity induction in older adults. The final experimental chapter (chapter 5) investigates how a common single nucleotide polymorphism on one or both alleles of the *BDNF* gene influences plasticity following PAS, simple repetitive movement, and complex visuomotor tracking.

The factors of physical activity, ageing, hand preference, task complexity, and *BDNF* genotype provide strong evidence of influencing plasticity. However, how these factors influence M1 plasticity and motor learning in humans are poorly understood. Despite the discovery of several factors capable of influencing the capacity for M1 plasticity in humans there still remain large within- and between-subject differences. Therefore, to help minimise variability between subjects and assess a more homogeneous population of subjects, it is important to understand the contribution of other determinants capable of influencing M1 plasticity. In addition, understanding the effect of these determinants in M1 plasticity may be critical for optimising therapeutic rehabilitation strategies following neurological injury or insult.

CHAPTER II

MOTOR CORTEX PLASTICITY INDUCED BY PAIRED ASSOCIATIVE STIMULATION IS ENHANCED IN PHYSICALLY ACTIVE INDIVIDUALS

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CIRILLO, J. (Candidate)

Experimental design, subject recruitment, data collection and analysis on all samples, interpretation of data, and wrote manuscript.

I hereby certify that the statement of contribution is accurate.

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Helped with collection of data and manuscript evaluation.

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Verified experimental design, supervised development of work, helped with data interpretation, critical manuscript evaluation, and acted as corresponding author.

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2. Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals

2.1. Abstract

Recent evidence indicates that regular physical activity enhances brain plasticity (i.e. the ability to reorganise neural connections) and improves neurocognitive function. However, the effect of regular physical activity on human motor cortex function is unknown. The purpose of this study was to examine motor cortex plasticity for a small hand muscle in highly active and sedentary individuals. Electromyographic recordings were obtained from the left abductor pollicis brevis (APB) muscle of 14 active and 14 sedentary subjects (aged 18-38 yrs). The extent of physical activity was assessed by questionnaire, where the physically active subjects performed >150 min/day moderate-to-vigorous aerobic activity on at least 5 days/week, whereas the sedentary group performed <20 min/day of physical activity on no more than 3 days/week. Transcranial magnetic stimulation (TMS) of the right hemisphere was used to assess changes in APB motor-evoked potentials (MEPs), input-output curve (IO curve), short-interval intracortical inhibition (SICI) and cortical silent period (CSP). Neuroplastic changes were induced using paired-associative stimulation (PAS), which consisted of 90 paired stimuli (0.05 Hz for 30 mins) of median nerve electrical stimulation at the wrist followed 25 ms later by TMS to the hand area of motor cortex. The IO curve slope was 35% steeper in individuals with increased physical activity (combined before and after PAS, $P < 0.05$), suggesting increased motor cortex excitability, although there was no difference in SICI or CSP between groups. PAS induced an increase in MEP amplitude in the physically active subjects (54% increase compared with before, $P<0.01$), but no significant facilitation in the sedentary subjects. We conclude that participation in regular physical activity may offer global benefits to motor cortex function that enhances neuroplasticity, which could improve motor learning and neurorehabilitation in physically active individuals.

2.2. Introduction

There is converging evidence at the molecular, cellular, systems and behavioural levels that participation in physical activity and exercise is beneficial to brain health and function. Within the last decade, epidemiological evidence has accumulated to suggest that physical activity may confer health-protective benefits for several neurological diseases including Parkinson's disease, Alzheimer's dementia and ischaemic stroke (see Kramer & Erickson, 2007 for review), and may even slow functional decline during the neurodegeneration process (Heyn *et al.*, 2004). Furthermore, exciting new evidence has emerged indicating that regular physical activity and exercise can increase brain plasticity (see Cotman & Berchtold, 2002; Colcombe *et al.*, 2004), which is believed to be instrumental in the process of memory and learning. In humans, robust effects of exercise have been most clearly demonstrated in ageing populations, where sustained exercise participation enhances learning and memory, improves executive function, counteracts age-related mental decline, and protects against age-related brain atrophy (Kramer *et al.*, 1999; see Colcombe & Kramer, 2003 for review). These studies suggest that regular physical activity and exercise provides both neuroprotective and neuroplastic benefits to the brain, and may serve to improve memory and learning in humans.

In contrast to the cognitive aspects, much less is known about how regular exercise influences plasticity in human primary motor cortex (M1), which plays a fundamental role in learning new motor skills (Sanes & Donoghue, 2000). It is well established that different types of exercise produce experience-specific alterations in cortical aspects of movement (see Adkins *et al.*, 2006 for review), but these changes in M1 function have usually been examined only for the limbs involved in the exercise. Furthermore, it has been shown that the molecular mechanisms believed responsible for improved cognition with exercise occur in several brain regions including the hippocampus, cerebellum, and M1 (Ding *et al.*, 2004; Klintsova *et al.*, 2004; see Vaynman & Gomez-Pinilla, 2005 for review) suggesting that exercise may have a more general effect on brain and motor function. We were particularly interested in whether,

like for neurocognitive function, the benefits of regular exercise would extend beyond the neural boundaries responsible for control of the exercising limbs, and offer more global benefits for M1 control of muscles not specifically related to the exercise. To address this issue, we have used transcranial magnetic stimulation (TMS) applied in single and paired-pulse paradigms to test the excitability of corticospinal projections and extent of intracortical inhibition to a small hand muscle in physically active and sedentary subjects.

To experimentally induce neuroplasticity, the technique of paired associative stimulation (PAS) was used, as it has been deliberately adapted from similar protocols used in brain slices and neuronal cultures, which demonstrate bidirectional spike-timing dependent synaptic plasticity (Dan & Poo, 2004; Caporale & Dan, 2008). A major strength of this technique is that it shares many physiological properties of synaptic plasticity obtained at the cellular level in animal preparations, such as rapid onset, duration, specificity, associativity, and NMDA-receptor dependence (see Ziemann *et al.*, 2008 for review). PAS in humans involves a stimulus to the median nerve followed by a single TMS pulse applied over the hand area of the motor cortex (Stefan *et al.*, 2000). When appropriately timed, PAS induces a lasting increase in corticospinal excitability which is interpreted as a marker of plasticity within M1 (Di Lazzaro *et al.*, 2009a), with LTP-like processes thought to play a major role (Stefan *et al.*, 2002). An interaction between PAS and motor training suggests that this technique tests functionally relevant neuronal circuits (Ziemann *et al.*, 2004; Stefan *et al.*, 2006; Jung & Ziemann, 2009), and there is strong evidence that altered neuroplasticity (assessed with PAS) may be related to impaired motor learning in some clinical conditions, such as Parkinson's disease (Ueki *et al.*, 2006) and schizophrenia (Frantseva *et al.*, 2008). Taken together, these characteristics of PAS suggest that LTP-like plasticity can be tested at the systems level of the human motor cortex, and the circuits tested are functionally relevant and clinically important (Ziemann *et al.*, 2008).

The purpose of this study was to examine M1 plasticity in highly active and sedentary young subjects. The exercise routine of the active individuals consisted largely of endurance (aerobic) exercise involving lower limb muscles such as running and cycling. Subjects reported no specialised use of their hand muscles such as playing a musical instrument, as this is known to influence hand muscle excitability and plasticity (Rosenkranz *et al.*, 2007b). Because exercise is able to improve overall brain health and function in several brain areas including M1, we hypothesise that there will be increased plasticity in the motor cortical projection to a small hand muscle in physically active compared with sedentary subjects. Such a finding might suggest that regular exercise may offer global benefits to human M1 function, which may improve the acquisition of new motor skills and be beneficial for recovery of function following brain injury.

2.3. Materials and Methods

Subjects. Experiments were performed on the left hand of 28 young subjects (13 women, 15 men; mean ± SD, 24 ± 4 yrs; range 18-38 yrs) with no known history of peripheral or neurological impairment. All subjects were right handed (median LQ = 0.77, range 0.5-1.0) as assessed by the Edinburgh Handedness Questionnaire (Oldfield, 1971) and were free of any cognitive mental state disorders as assessed by the mini-mental state examination (MMSE) (Folstein *et al.*, 1975). Subjects were categorised into active (5 women and 9 men) and sedentary (8 women and 6 men) classifications using the International Physical Activity Questionnaire (IPAQ). The long version of the IPAQ was used, consisting of 31 items describing the extent of leisure time physical activity involving aerobic exercises such as running, cycling and walking (Craig *et al.*, 2003; Fogelholm *et al.*, 2006). This questionnaire has been shown to produce reliable and repeatable measures of physical activity, and is comparable to objective assessment by accelerometer (Craig *et al.*, 2003). To more accurately equate the self reported IPAQ score to physical (cardiorespiratory) fitness, subjects were asked to focus on leisure-time physical activity, with an emphasis on vigorous physical

activity such as running and cycling (Fogelholm *et al.*, 2006). All experiments were performed in the afternoon or evening to minimise variations in circulating cortisol and its effect on plasticity induction (Sale *et al.*, 2008), and subjects were asked to refrain from physical activity prior to the experiment (on that day), but could perform exercise after the experiment was completed. Furthermore, no subjects reported long term specialised use of the hands, such as playing a musical instrument (Rosenkranz *et al.*, 2007b). All subjects gave written informed consent prior to participation in the study, which was approved by the University of Adelaide Human Research Ethics Committee and were conducted in accordance to the standards established by the Declaration of Helsinki.

Experimental arrangements. Subjects were seated comfortably in an experimental chair with their left shoulder abducted approximately 45° to allow the hand and arm to rest on a manipulandum. The hand was positioned with the palm facing down and the proximal phalanx of the thumb was placed in a metal ring attached to a load cell (LC 1205-K100, A&D Co., Ltd., Japan) to facilitate measurement of thumb abduction force. Thumb abduction force was displayed on an oscilloscope to provide visual feedback to the subject, and was also digitised online (2 kHz) via a CED 1401 interface (Cambridge Electronic Design, UK) and stored on computer for offline analysis.

Surface electromyography (EMG) was recorded from the abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscles of the left hand using Ag-AgCl electrodes placed 2 cm apart. The EMG signals were amplified (x 1000), filtered (13 Hz – 1000 Hz), digitised online (2 kHz/channel) via a CED 1401 interface, and stored on computer for offline analysis. The EMG signals of both muscles were displayed on an oscilloscope to assist the subject in maintaining EMG silence when required.

TMS. Transcranial Magnetic Stimulation (TMS) was applied using a figure-of-eight coil (outer coil diameter 70 mm) with two Magstim 200 magnetic stimulators connected with a

Magstim Bistim unit (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. The coil was positioned at the optimal scalp position over the right hemisphere for eliciting a motor evoked potential (MEP) in the relaxed left APB muscle. The optimal scalp position was marked for reference, and the coil position was continually checked throughout the experiment.

PAS. Paired associative stimulation (PAS) was performed as described previously by Stefan *et al.* (2000). The PAS protocol consisted of percutaneous electrical stimulation of the median nerve at the left wrist (300% of perceptual threshold) followed by suprathreshold TMS (130% resting motor threshold) 25 ms later over the right motor cortex. The intervention consisted of 90 paired stimuli delivered at 0.05 Hz with the procedure lasting for 30 min. Electrical stimuli were applied to the median nerve at the wrist using a constant current stimulator (DS7A stimulator, Digitimer Co. Ltd., Hertfordshire, UK) with bipolar surface electrodes, separated by 30 mm, and with the cathode proximal. Stimuli were square wave pulses with a pulse width of 200 μs.

The attentional focus of the subject has been shown to be an important factor influencing PAS effectiveness (Stefan *et al.*, 2004). Therefore, subjects were instructed to direct their attention on the stimulated (left) hand and count the peripheral stimuli they perceived during the PAS intervention (total of 90 stimuli).

Experimental Parameters. At the beginning of each experiment, abduction force exerted by the left thumb during a maximum voluntary contraction (MVC) was measured. Visual feedback of thumb abduction force was displayed on an oscilloscope to aid the subject. Three MVC trials were performed, with a minimum of 30-s rest between trials, and the MVC with the largest thumb abduction force was used for the assessment of muscle strength.

Measures of motor cortical excitability using TMS included resting motor threshold (RMT), active motor threshold (AMT), MEP amplitude, input-output curve (IO curve), short-interval intracortical inhibition (SICI) and cortical silent period (CSP) duration. All measures were performed before and after PAS, with the exception of AMT, which was only recorded before PAS.

Resting motor threshold was determined as the minimum stimulus intensity required to produce a MEP in the relaxed APB of at least 50 μV in 3 out of 5 consecutive trials. Active motor threshold was defined as the minimum stimulus intensity required to produce a MEP in the APB muscle of at least 200 μV in 3 out of 5 consecutive trials during a low-level voluntary thumb abduction (10% MVC). The stimulus intensity was altered in 1% increments of maximum stimulator output (MSO) throughout this process.

The stimulus intensity that produced a MEP amplitude of approximately 1 mV in resting APB was determined before PAS. Using this stimulator intensity, ten trials were recorded to investigate resting MEP amplitude before PAS, 5 min after PAS (After 5), and 30-40 min following PAS (After 30). The mean amplitude was calculated from each trial at each time point.

The intensities used to construct the IO curves were determined for each individual according to their RMT before PAS. Eight trials at 90, 100, 110, 120, 130, and 140 % of RMT were recorded for each subject at rest. The order of presentation of the six conditions was pseudorandomised throughout the trials and stimuli were given every 5 s. Amplitudes were measured for each trial to calculate the mean MEP amplitude for each TMS intensity.

Short-interval intracortical inhibition (SICI) was investigated using a paired-pulse TMS paradigm consisting of a subthreshold conditioning stimulus that preceded a suprathreshold test stimulus by 3 ms (Kujirai *et al.*, 1993). The intensity of the conditioning stimulus was

randomised as 70, 80, or 90 % of AMT, and the test stimulus intensity was that used to produce a MEP amplitude of approximately 1 mV in resting APB. The test stimulus intensity in paired-pulse trials was adjusted following PAS, if required, so that test MEP amplitudes were equivalent before and after PAS (APB MEP amplitude of approximately 1 mV). Each data block consisted of twelve trials for each of two conditions; test stimulus alone and SICI (conditioning and test stimulus ISI = 3 ms). The order of presentation of the two conditions was randomised throughout the trials and stimuli were given every 5 s. The conditioned MEP amplitude was expressed as a percentage of the unconditioned test MEP amplitude to calculate the influence of the conditioning stimulus.

Measurements of CSP duration were made during a low-level voluntary contraction of APB (10% MVC) before and after PAS. Subjects were provided with visual feedback of thumb abduction force displayed on an oscilloscope. TMS intensity was 130% RMT and ten stimuli were given at a frequency of 0.2 Hz. CSP duration was analysed using a modified cumulative sum (CUSUM) method (Brinkworth & Turker, 2003). The EMG signal was rectified and CSP duration was assessed from the point of TMS until the EMG crossed the pre-stimulus mean (pre-stimulus period of 200 ms) following the MEP. All measurements were made off-line on individual trials and averaged for the ten trials.

Data Analysis and statistics. Unpaired *t*-tests were used to compare differences in physical activity levels (IPAQ), age, handedness, cognitive mental state (MMSE), attention, RMT, and 1 mV TMS intensity before PAS. MEP amplitudes were measured peak-to-peak in each individual trial. The slopes of the IO curve were quantified by a linear regression analysis for all data points between 110 and 140 % RMT and a two-way analysis of variance (ANOVA) was used to compare differences between groups (sedentary, active) and time (before, after 5, after 30). These points were used as they form the linear portion of the IO curve (see Rosenkranz *et al.*, 2007b). Two-way repeated measures ANOVA (Group, Time) was also

used to analyse RMT, 1 mV MEP amplitude TMS intensity, and CSP duration and three-way repeated measures ANOVA (Group, Time, Intensity) was employed for analysis of IO curve and SICI. A Fisher's LSD post-hoc test that performed all possible comparisons was used to analyse significant main effects and interactions. All dependent variables were tested for non-sphericity using Mauchly's Test. The only dependent variable not meeting the assumption of sphericity is the MEP input-output curve data, which was adjusted using the Greenhouse-Geisser correction. The significance level was set at $P < 0.05$ for all comparisons and all group data are provided as mean ± SEM.

2.4. Results

All subjects were comfortable with the TMS procedure and no side effects were reported. There was no difference for age, handedness, cognitive mental state, attention to the intervention, and TMS thresholds between the two groups (Table 2.1). The one separating characteristic between the two groups was the level of physical activity, as quantified by the IPAQ. The active group had leisure-time physical activity levels that were 11-fold greater than the sedentary group (Table 2.1). On a weekly average, individuals in the active group performed four sessions of vigorous intensity activity (predominantly running and cycling) for a period of 60 min each session, and five sessions of moderate intensity activity (such as jogging and walking) for 90 min each session. Two of the active individuals were semi-professional athletes and performed vigorous running or cycling exercise for 120 min six times a week, along with moderate intensity exercise (jogging, swimming, or walking) for 90 min sessions three times a week. In contrast, individuals in the sedentary group performed, on average, no greater than three sessions of walking for 20 min each session during their leisure-time.

Table 2.1 *Description of subject characteristics and baseline excitability measures before PAS.*

	Physical Activity Level	
	Sedentary (Mean ± SD)	Active (Mean ± SD)
Age (years)	24 ± 4	24 ± 5
Sex	6 M, 8 F	9 M, 5 F
Physical Activity (IPAQ, MET-mins)	491 ± 308	5572 ± 2075*
Handedness (-1 to 1)	0.81 ± 0.16	0.73 ± 0.23
MMSE (Total of 30)	29.4 ± 0.5	29.4 ± 0.9
Attention (Total of 90)	89.5 ± 2.1	87.1 ± 4.4
MVC (N)	38.0 ± 15.8	47.4 ± 17.8
RMT (% MSO)	50.8 ± 10.8	50.3 ± 10.2
1 mV TMS intensity (% MSO)	62.1 ± 14.3	59.0 ± 11.7
AMT (% MSO)	40.8 ± 7.4	39.1 ± 7.1

IPAQ, International Physical Activity Questionnaire. *MMSE*, Mini-Mental State Examination. *MVC*, Thumb abduction Maximum Voluntary Contraction. *RMT*, APB Resting Motor Threshold. *AMT*, APB Active Motor Threshold. *MSO*, Maximum Stimulator Output. * $P < 0.001$ compared with sedentary subjects.

Figure 2.1 shows original recordings from one sedentary and one active subject before PAS, 5 min following PAS (After 5), and ~30 min later (After 30). In the sedentary subject, there were minimal changes in the MEPs recorded in the left APB after PAS. In contrast, there was significant MEP facilitation following PAS at both time-points in the active subject. The group data in Figure 2.2A shows the mean MEP amplitude in the target muscle (APB) before and after PAS. No difference in TMS intensity was required to evoke a ~1 mV response in resting APB between the two groups before PAS ($P = 0.54$; Table 1), and this intensity was used to quantify the MEP response after PAS as a marker of neuroplasticity. A two-way repeated measures ANOVA revealed that there was no difference in MEP amplitude between Physical Activity groups ($F = 2.1$, $P = 0.16$), but there was a significant difference between time points ($F = 5.3$, $P < 0.01$) and a Physical Activity x Time interaction ($F = 2.7$, $P = 0.04$). Post hoc analysis indicated that APB MEP amplitude in the active group was 54% larger 5

minutes after PAS (P = 0.01) and 34% larger 30 mins later (P = 0.03) compared with before PAS. There was also a significant difference in APB MEP amplitude in the sedentary and active groups 5 minutes after PAS (P = 0.02). There was no change in the control muscle FDI MEP amplitude after PAS in both groups. Despite these striking group differences in MEP facilitation after PAS, the individual subject responses revealed substantial variability within each subject group (Figure 2.2B,C). Although most subjects in the active group showed marked MEP facilitation after PAS, there was only moderate facilitation in some sedentary subjects, whereas 4 sedentary subjects showed substantial PAS-induced MEP depression.

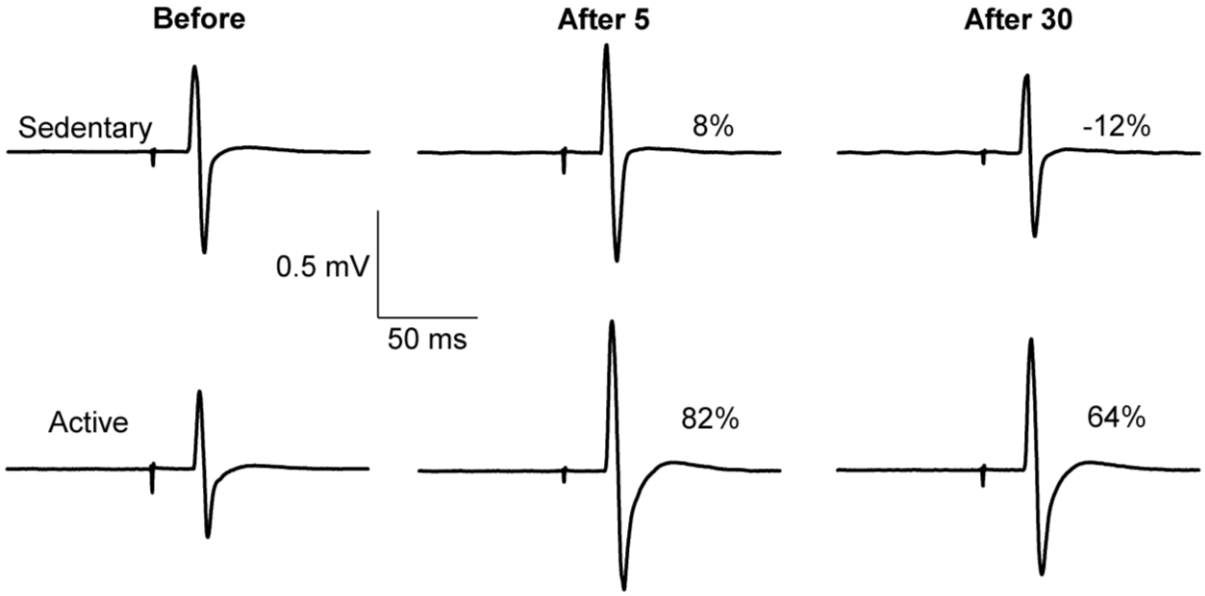

Figure 2.1 *Average MEP recordings from the resting APB of one sedentary (upper panel) and one active subject (lower panel) before the onset of PAS (Before), 5 min after PAS (After 5), and 30-40 min after PAS (After 30). At both time points after PAS, the MEP amplitudes were substantially larger in the active subject, but only small changes were observed in the sedentary subject. Both subjects participated in the experiment in the afternoon and had similar characteristics of RMT before and after PAS (Before: Sedentary = 45% MSO, Active =52 % MSO; After: Sedentary = 44% MSO, Active = 50% MSO), attention (Total of 90: Sedentary = 87, Active = 84), handedness (LQ: Sedentary = 0.7, Active = 0.9), and cognitive mental state (MMSE Total of 30: Sedentary = 29, Active = 30). The difference between the two individuals was the level of physical activity (IPAQ: Sedentary = 460 MET-min, Active = 6900 MET-min). Numbers indicate the percent change in MEP amplitude following PAS.*

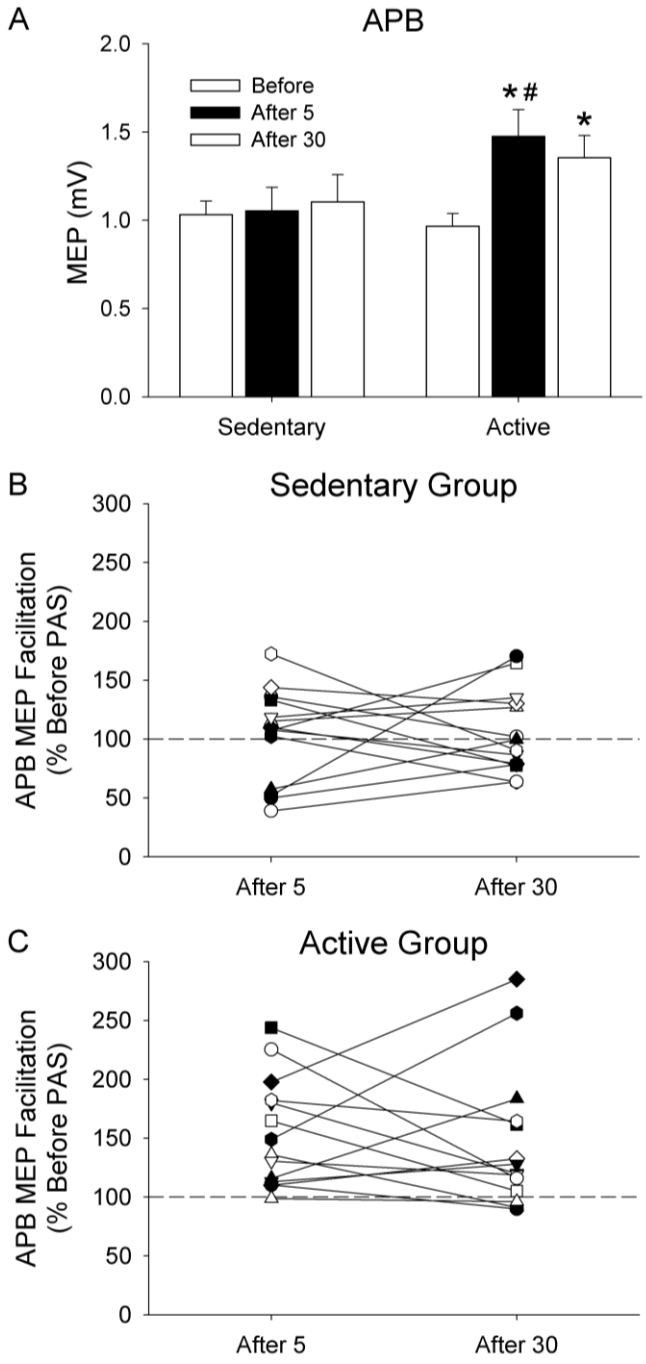

Figure 2.2 *A, Mean (± SEM) APB MEP amplitudes in sedentary and active subjects before, 5 minutes after (After 5) and 30 minutes after PAS (After 30). B, C, Individual subject responses showing the percent change in MEP amplitude after PAS relative to before PAS in sedentary (B) and active (C) subjects. A value of 100% represents the amplitude of the response before PAS. * P< 0.05 compared with before PAS. # P < 0.05 compared with the same time point in sedentary subjects.*

The effect of PAS on the IO curves of the relaxed APB in active and sedentary subjects is shown in Figure 2.3. A three-way repeated measures ANOVA (with Greenhouse-Geisser correction for sphericity) indicated an increase in the size of the APB MEP amplitude with increasing stimulus intensity in the sedentary and active groups (Intensity effect, $F = 88.5$, $P < 0.01$) and MEP amplitude was greater after PAS (Time effect, $F = 4.2$, $P < 0.05$). There were no significant differences between Groups ($F = 1.2$, $P = 0.28$) or Interactions. To quantify the change in the IO curve, the slopes were calculated for the linear portion of the curve (between 110 and 140% RMT) and a two-way ANOVA was performed to compare the differences in slope between physical activity groups before and after PAS. This analysis revealed that the IO curve slopes were 35% steeper for the active compared with the sedentary group for both time points combined (Physical Activity effect, $F = 4.1$, $P < 0.05$) (Figure 2.3C inset), but there was no change in the IO curve slopes following PAS (Time effect, $F = 0.01$, $P = 0.92$) and no significant Physical Activity x Time interactions ($F = 0.18$, $P = 0.68$). Although not statistically significant, the IO curve slopes were 27% greater in active subjects before PAS and 44% greater in active subjects after PAS compared with sedentary subjects. The RMT did not change after PAS ($P = 0.18$), and was 50.4 ± 10.0% MSO in sedentary subjects and 49.9 ± 9.8% MSO in active subjects after PAS (compare with before PAS in Table 2.1).

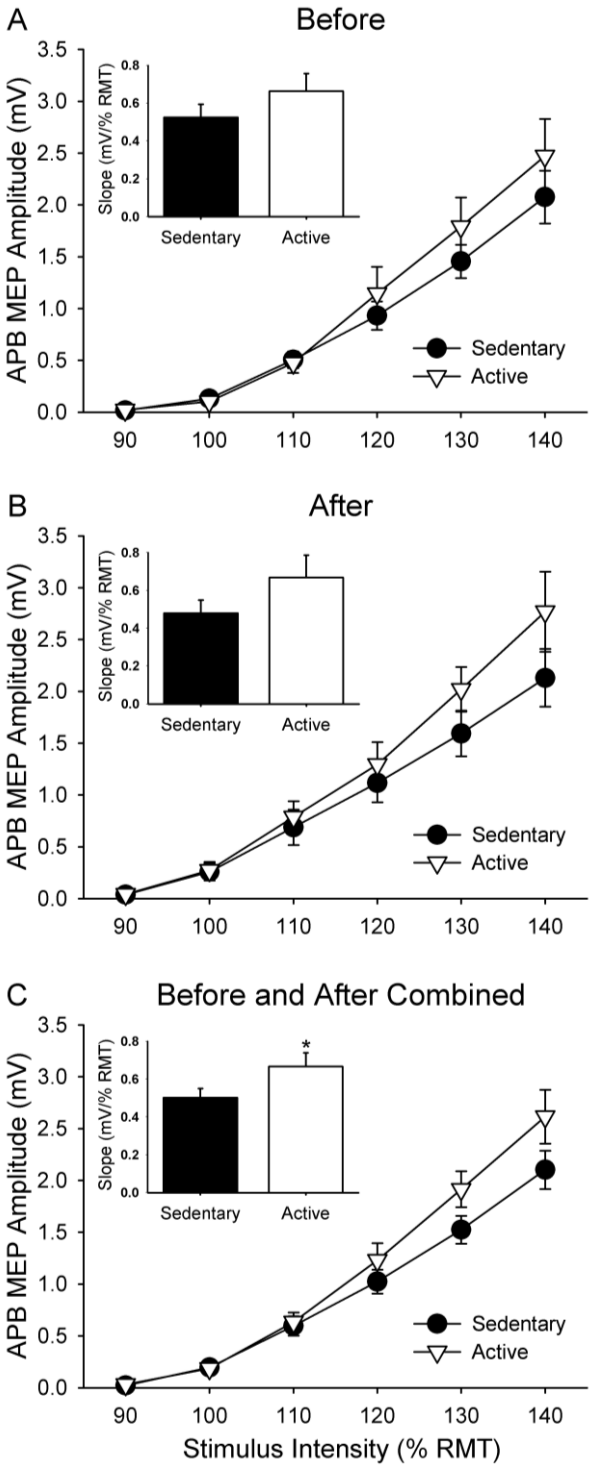

Figure 2.3 *Mean (± SEM) MEP amplitude data for APB IO curves in sedentary and active subjects shown before (A) and after PAS (B) and for before and after combined (C). The slope of the curve has been calculated between 110 and 140% RMT and is shown in the inset of each graph. The slope of the IO curve was significantly steeper in the active subjects compared with the sedentary subjects when data were combined before and after PAS (* $P < 0.05$).*

SICI was assessed using a paired-pulse paradigm that utilised a ~1 mV test pulse that was preceded by a subthreshold conditioning pulse (70, 80, or 90% AMT) at 3 ms (SICI). If necessary, the TMS intensity for the ~1 mV test pulse was adjusted after PAS, which resulted in a significant reduction in the ~1 mV TMS intensity after PAS ($P = 0.004$), where it was 61.6 ± 14.8% MSO in sedentary subjects and 57.6 ± 12.4% MSO in active subjects after PAS (compare with before PAS in Table 2.1). Data showing the extent of SICI in each group before and after PAS is shown in Figure 2.4. Increasing the intensity of the conditioning stimulus increased the amount of SICI in both groups ($F = 43.6$, $P < 0.001$). However, SICI was not different between physical activity groups ($F = 0.2$, $P = 0.65$), and there was no difference in SICI before and after PAS ($F = 3.7$, $P = 0.07$). Furthermore, there were no significant Group x Time ($F = 0.6$, $P = 0.45$), Group x Intensity ($F = 0.4$, $P = 0.69$) or Group x Time x Intensity interactions ($F = 0.1$, $P = 0.91$).

The duration of the CSP before and after PAS for the sedentary and active groups is shown in Figure 2.4C. There was no difference in CSP duration between groups (Physical Activity effect, $F = 0.8$, $P = 0.38$). However, the CSP was 13 ms longer after PAS in both groups (Time effect; $F = 12.3$, $P < 0.01$). The longer CSP duration after PAS was largely due to a longer CSP in the sedentary group, although this interaction just failed to reach statistical significance (Physical Activity x Time interaction, $F = 3.7$, $P = 0.07$).

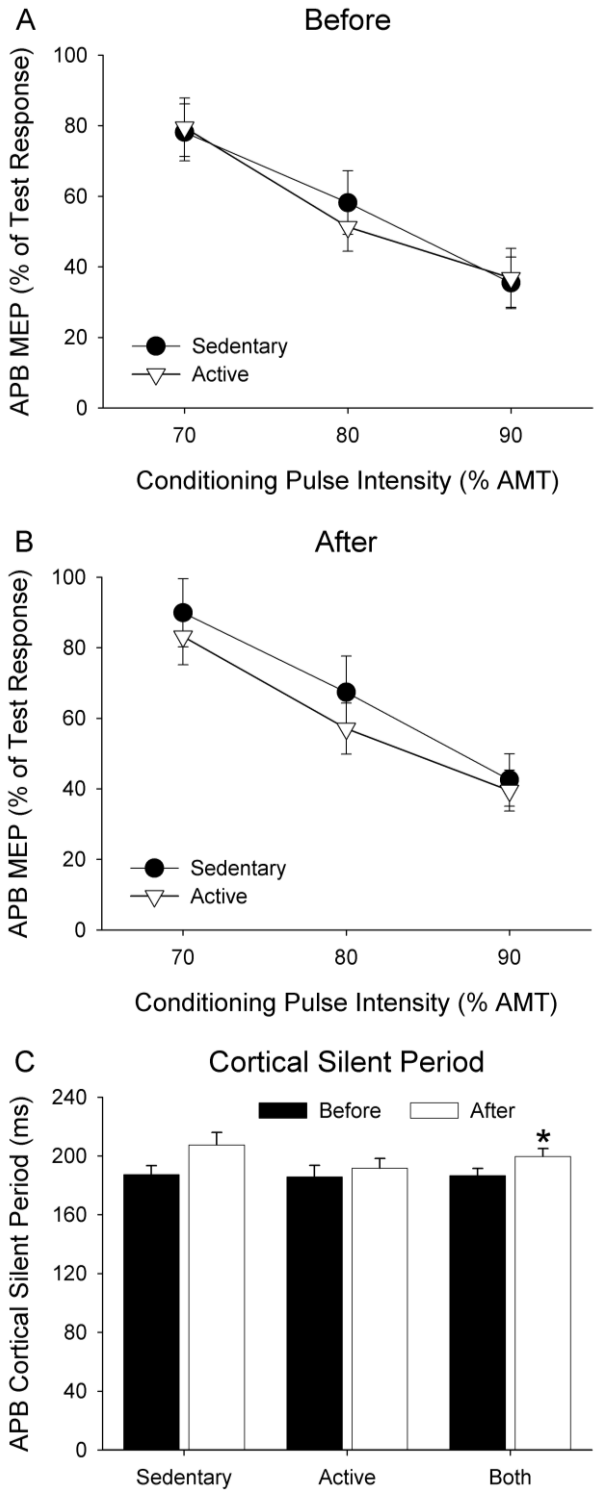

Figure 2.4 *Data represents mean (± SEM) SICI (A, B) and CSP duration (C) shown before and after PAS in active and sedentary subjects. SICI was obtained with a conditioning TMS intensity of 70, 80, and 90% AMT expressed as a percentage of the APB MEP amplitude evoked by the test stimulus alone (100%). The extent of SICI was influenced by conditioning TMS intensity before and after PAS, but was not different between sedentary and active subjects. There was a significant increase in CSP duration after PAS from pooled data in both groups (* P = 0.002), which was largely influenced by the sedentary subjects.*

2.5. Discussion

There were two main findings in the present study that investigated specific features of the corticospinal pathway in highly active and sedentary young subjects. First, the IO curve for a small hand muscle (APB) was similar in sedentary and active subjects before PAS, but was steeper in active subjects when pooled before and after PAS. Second, PAS induced significantly more facilitation of APB MEP amplitudes in the active compared with the sedentary subjects, indicating heightened synaptic plasticity in the motor system of physically active individuals. Because the main distinguishing characteristic between these subject groups was the difference in physical activity levels, we suggest that these features of corticospinal excitability and plasticity arise from enhanced regular physical activity involving endurance (aerobic) exercise.

Subjects in the sedentary and active groups were well matched for age, sex, handedness and cognitive mental state (see Table 2.1). However, substantial differences existed in the extent of physical activity in the two groups as reported by questionnaire (IPAQ, Craig *et al.*, 2003). To obtain a clearly distinct group of active and sedentary subjects, individuals classified as highly active from the IPAQ had to perform at least 3 sessions/week of vigorous physical activity to be included in the study. This stringent selection criterion resulted in a marked (11-fold) difference in physical activity levels between active and sedentary subjects. Based on the IPAQ scores and physical fitness assessments from a large population of subjects (Fogelholm *et al.*, 2006), we estimate that the physical activity levels of our subject groups lie within the top and bottom 20% of a healthy young population.

2.5.1. Corticospinal excitability in physically active and sedentary individuals

As expected, increasing TMS intensity resulted in an increase in MEP amplitude in physically active and sedentary subjects, reflecting increased activation of a population of cortico-cortical, corticospinal and spinal motor neurons activated by TMS. The TMS intensities for

this IO curve were expressed relative to threshold intensity required to produce MEPs in resting APB muscle, which were similar for the sedentary and active groups, along with a similar IO curve slope between groups before PAS. However, the IO curve slope was 35% steeper in physically active subjects when the data before and after PAS were combined (Figure 2.3C), suggesting an increased strength of the corticospinal connections that are activated with higher TMS intensities in physically active individuals (Ridding & Rothwell, 1997). Several studies have shown that chronic physical activity can produce functional adaptations in corticospinal and spinal motor neurons in humans. For example, there are increased cortical representations and MEP amplitudes to the involved muscles in highly skilled racquet players (Pearce *et al.*, 2000) and steeper IO curves in musicians (Rosenkranz *et al.*, 2007b). Furthermore, examinations of spinal cord circuitry indicate that endurance-trained individuals have enhanced H and stretch reflexes (see Koceja *et al.*, 2004 for review), with increasing amplitude of H reflexes in more active individuals (Nielsen *et al.*, 1993). However, these changes in excitability have all been assessed in the muscle groups involved in the training, reflecting a likely task-specific adaptation for the M1 representation of muscles in the exercising limb. For the physically active subjects in the present study, we have found increased excitability in the corticospinal pathway to muscles not directly involved in the exercise, but only when the data were combined before and after PAS. Although this adaptation could conceivably occur within the spinal cord, there is no change in spinal (H) reflexes to upper limb muscles after lower limb exercise (Motl & Dishman, 2003), suggesting that the increased slope of the IO curve in physically active individuals may occur, at least in part, through changes in M1 function.

The input-output properties of M1 can also be influenced by inhibitory interneurons that use γ-aminobutyric acid (GABA) as their transmitter (Sanes & Donoghue, 2000), which constitute approximately 25-30% of neurons in primate neocortex (Jones, 1993). These GABAergic inhibitory systems within human M1 are studied with paired-pulse TMS to assess SICI, or

with suprathreshold single-pulse TMS that suppresses voluntary activation for up to 300 ms (CSP). Cortical mechanisms are believed to contribute to SICI (Di Lazzaro *et al.*, 1998b) and the later stages (>60 ms) of the silent period (Inghilleri *et al.*, 1993). SICI is mediated by $GABA_A$ receptors while CSP is mediated by $GABA_B$ receptors, and the cortical neurons mediating these two forms of intracortical inhibition appear to be distinct (Chen, 2004). Increasing evidence suggests that GABAergic systems responsible for the CSP (Classen *et al.*, 1997) and SICI (Zoghi *et al.*, 2003) play an important role in motor performance, and alterations in GABAergic inhibition have been associated with both enhanced (Rosenkranz *et al.*, 2007b) and impaired motor skills (Ridding *et al.*, 1995a; Sale & Semmler, 2005). Using these two markers of intracortical inhibition, we found no differences in SICI or CSP inhibition between physically active and sedentary subjects before or after PAS, suggesting that the threshold and/or distribution of GABAergic inhibitory interneurons in M1 is not influenced by regular physical activity.

In contrast to motor skill training, short term (<1 month) endurance exercise does not seem to result in alterations in synaptic connectivity within M1, although there can be substantial changes in cerebral vasculature and blood flow (see Adkins *et al.*, 2006 for review). For example, Kleim *et al*, (2002) have shown that 30 days of exercise does not alter motor cortical representation but increases angiogenesis in rat M1. Furthermore, animals given free access to a running wheel for 30 days show increased angiogenesis that is specific to M1, but was not evident in other frontal or subcortical areas (Swain *et al.*, 2003). These increases in regional cerebral blood flow are not only elevated during activity, but could also be enhanced in trained individuals in the resting state (Xiong *et al.*, 2009). Several studies have shown that this increased blood flow to M1 with exercise is accompanied by increased neurotrophic factors that facilitate the survival and differentiation of neurons (Klintsova *et al.*, 2004; Vaynman & Gomez-Pinilla, 2005), providing a more supportive neural environment (see Adkins *et al.*, 2006). It is therefore possible that this improved cortical environment may

promote neural survival and increased neural density in M1 neurons with longer-term exercise, resulting in changes in corticospinal function and excitability in individuals who have been physically active over a period of several years. This idea is supported by the finding of reduced age-related loss of brain tissue in older adults with heightened aerobic fitness (Colcombe *et al.*, 2004).

2.5.2. Increased synaptic plasticity in physically active individuals

PAS is a common procedure used in neurophysiological studies to experimentally-induce neural plasticity in humans. The conventional PAS approach combines low-frequency, percutaneous electrical stimulation of the median nerve at the wrist paired with TMS over the contralateral hand area of M1 (Stefan *et al.*, 2000). The TMS is timed to coincide with the arrival at the cortex of the afferent volley evoked 25 ms earlier by the peripheral stimulus. This protocol results in substantial increases in the amplitude of hand muscle MEPs, which is interpreted as a marker of neuroplasticity. The increase in corticospinal excitability following PAS is long-lasting, being elevated for 30-60 minutes in most subjects (Stefan *et al.*, 2000). Despite some evidence of a contribution from within spinal cord circuits (Meunier *et al.*, 2007), the increased excitability is thought largely to reflect a change in M1 function, as there is no change in spinal excitability measured with F-waves and electrical brainstem stimulation (Stefan *et al.*, 2000). More recently, direct evidence from epidural recordings of corticospinal descending volleys have shown that PAS enhances responses of later descending (I or indirect) waves (Di Lazzaro *et al.*, 2009a), providing strong evidence of a cortical origin in the changes induced by PAS. The increased excitability is thought to occur through LTP-like effects (Stefan *et al.*, 2002), and an interaction between PAS and motor training suggests that this technique involves functionally relevant neuronal circuits (Ziemann *et al.*, 2004; Jung & Ziemann, 2009).

Several factors are known to influence the extent of MEP facilitation induced with a PAS intervention, including subject age (Tecchio *et al.*, 2008), attention to the procedure (Stefan *et al.*, 2004), and time of day the experiments were performed (Sale *et al.*, 2008). Each of these factors were similar between the sedentary and active subjects in the present study. Despite these similarities, we found striking differences in PAS-induced neuroplasticity in a hand muscle of the physically active compared with sedentary subjects in the present study, which resulted in a 40% larger MEP in the active compared with sedentary subjects after PAS. It is likely that part of this difference can be attributed to the 27% steeper (although non-significant) IO curve slope in the active subjects before PAS, making these individuals more susceptible to PAS effects (see Rosenkranz *et al.*, 2007b). Using the theoretical observations described by (Rosenkranz *et al.*, 2007b) to estimate the change in MEP amplitude based on the different IO slopes observed in the present study, an increase in the test TMS pulse by 10% (equivalent to the increase in MEP observed after PAS with the 1 mV TMS intensity) would produce an increase in MEP of 52% (1.52 mV) in the sedentary subjects and 66% (1.66 mV) in the active subjects. This represents only a 9% (relative) difference in MEP amplitude after PAS that can be explained by the baseline difference in slopes of the IO curves in each group. However, we report a 40% larger MEP (1 mV TMS intensity) in physically active compared with sedentary subjects after PAS, which suggests that a large proportion (~75%) of the increase in MEP in physically active subjects is due to increased PAS-induced plasticity in these individuals. An additional confounding factor is that a fixed TMS intensity (130% RMT) was used in the present study (Stefan *et al.*, 2000), which potentially provided a stronger activation of the corticospinal system during PAS in physically active subjects. However, there was no significant difference in MEP amplitudes at this TMS intensity between the two groups before or during PAS, suggesting that this is unlikely to be a major contributor to the increased 1 mV MEP after PAS in physically active subjects. We therefore suggest that an additional lifestyle factor that can contribute to increased neuroplasticity after

PAS is the physical activity status of the subjects under investigation. However, there was still substantial variability in the extent of facilitation within subject groups, indicating that other factors are also likely to contribute to this effect.

Despite greater differences in the slope of the IO curve between physically active and sedentary subjects after PAS (27% difference before PAS, 44% difference after PAS), there was some mismatch between the effect of PAS on the 1 mV MEP compared with the IO curve. There are at least three methodological differences between these two measures that might explain this effect. First, MEP 1 mV is based on absolute MEP amplitude resulting in different TMS intensities relative to resting threshold in each subject, whereas the MEP IO curve is expressed relative to the subjects own resting threshold. Second, these two measures were obtained at different times before and after PAS. The MEP 1 mV was assessed 5-10 minutes following PAS whereas the IO curve was assessed an additional 10 minutes later (15-20 minutes following PAS), and it is not known how time may affect cortical excitability (MEP amplitude) following PAS in physically active individuals. Third, for logistical reasons, each method was assessed with a different number of trials, with more trials included in the analysis of MEP 1 mV (10 trials at 1 mV TMS intensity) compared with the MEP IO curve (8 trials for each TMS intensity of 90-140 % RMT). The substantial trial-to-trial fluctuations in MEP amplitude could therefore influence the mean and variability of the MEP differently between the two measures (see McDonnell *et al.*, 2004).

Several studies have shown that alterations in GABAergic inhibition play a fundamental role in cortical reorganisation and plasticity. For example, studies in rat cortical slice preparations have shown that LTP is only induced in the presence of a GABA antagonist (Hess & Donoghue, 1994), which disinhibits the cortex. In humans, pharmacological studies in which levels of GABA is enhanced through the use of a GABA agonist (lorazepam) or reduced through ischaemic nerve block have clearly demonstrated that a reduction of GABA-mediated

inhibition facilitates cortical plasticity (Ziemann *et al.*, 2001). In support of this, SICI is deficient in focal task-specific dystonia (Ridding *et al.*, 1995a), whereas the response to PAS is exaggerated in these individuals (Quartarone *et al.*, 2003). In line with previous studies (Ridding & Taylor, 2001; Stefan *et al.*, 2002; Sale *et al.*, 2007), we found no change in SICI before and after PAS in both subject groups, indicating that changes in the operation of $GABA_A$ inhibitory circuits cannot be responsible for the increased PAS-induced plasticity in physically active individuals. In contrast, PAS is known to produce a significant increase in CSP duration (Stefan *et al.*, 2000; Sale *et al.*, 2007), which was also observed in the present study, indicating that PAS increases the effectiveness of $GABA_B$ mediated inhibitory cortical circuits that are activated by TMS during voluntary contraction. However, the change in CSP after PAS was much smaller in the active subjects, indicating that these circuits are less susceptible to modulation from the PAS intervention in physically active individuals.

2.5.3. Factors influencing PAS-induced plasticity in physically active individuals

Despite the subject groups being well matched on a number of baseline characteristics (see Table 2.1), the cross-sectional design of this study allows several confounding factors to potentially contribute to the increased PAS-induced plasticity in physically active subjects. First, we cannot exclude the possibility that the increased plasticity in M1 of physically active individuals represents a genetic trait, which makes these individuals more susceptible to participation in regular physical activity. However, the only published genetic influence on PAS-induced plasticity is the presence of a BDNF polymorphism, which limits the extent of M1 plasticity in these individuals (Cheeran *et al.*, 2008). This polymorphism is present in ~30% of the normal population (Bath & Lee, 2006), and the probability that all 14 sedentary subjects have a BDNF polymorphism, whereas all 14 active subjects do not, is therefore extremely low. Second, we do not know what effect the recent history of (or withdrawal from) physical exercise has on PAS-induced M1 plasticity in these subjects. It is well established that prior motor learning causes a homeostatic interaction with subsequent PAS-

induced plasticity when focussed on the same muscle group (Ziemann *et al.*, 2004; Stefan *et al.*, 2006), but it is not known whether regular exercise (or withdrawal) performed with the lower limbs would produce a homeostatic interaction with PAS performed on upper limb muscles. Third, it is known that corticospinal excitability and plasticity in women is dependent on the menstrual cycle (e.g. Smith *et al.*, 1999b; Inghilleri *et al.*, 2004). However, we found no difference between men and women in the response to PAS, and removal of the female subjects (8 sedentary, 5 active) from the analysis did not influence the main findings of the study, as there was only a 20% increase in 1 mV MEP response in the 6 sedentary male subjects, but a 50% increase in 1 mV MEP in the 9 active male subjects. Although we cannot rule out these factors, we suggest that their contribution is likely to be minimal under the current experimental conditions, and conclude that at least some of the changes in plasticity are a consequence of regular physical activity.

In summary, we have used TMS to examine motor cortex excitability and PAS-induced plasticity in a small hand muscle of sedentary and active young subjects. We found that regular physical activity, primarily involving lower limb muscles, was accompanied by increased M1 plasticity in a small hand muscle compared with sedentary subjects. These findings indicate that regular exercise may offer benefits to M1 function that extend beyond the neural boundaries for control of the exercising limb, which has important implications for developing improved strategies for motor learning and rehabilitation following injury to the motor system.

CHAPTER III

HEMISPHERIC DIFFERENCES IN USE-DEPENDENT CORTICOMOTOR PLASTICITY IN YOUNG AND OLD ADULTS

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ROGASCH, N.C.

Helped with collection of data and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

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Verified experimental design, supervised development of work, helped with data interpretation, critical manuscript evaluation, and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

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3. Hemispheric differences in use-dependent corticomotor plasticity in young and old adults

3.1. Abstract

The aim of this study was to examine corticomotor excitability and plasticity following repetitive thumb abduction training in left and right hands of young and old adults. Electromyographic recordings were obtained from the abductor pollicis brevis (APB) muscle of 12 young (aged 18-27 years) and 14 old (aged 63-75 years) adults. Motor training consisted of 300 ballistic abductions of the thumb to maximise peak abduction acceleration, with each hand tested in a separate session. Transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) was used to assess changes in contralateral APB motor-evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) before and after training. For young and old adults, APB MEP amplitude increased for both hands after training, which is indicative of use-dependent plasticity. However, the increase in MEP amplitude was 21% ($P = 0.04$) greater in the left (non-dominant) hand compared with the right (dominant) hand. This occurred despite a 40% greater improvement in peak thumb abduction acceleration (motor learning) for the right hand in young subjects compared with the left hand in young subjects ($P < 0.04$), and the right hand in old subjects ($P < 0.01$). Furthermore, no difference in use-dependent plasticity was observed between young and old adults, and SICI remained unchanged following ballistic training for both hands in all subjects. These findings suggest that there is greater strengthening of corticomotor circuits for control of the left compared with the right hand during simple ballistic thumb training, and that an age-related decline in motor learning was observed only in the dominant hand. In contrast to previous studies, these data also indicate that young and old adults can demonstrate similar use-dependent corticomotor plasticity during this simple thumb-training task.

3.2. Introduction

The human brain, in particular the primary motor cortex (M1), has the potential to functionally and structurally reorganise following physiological (learning) and pathological (injury or disease) events (see Sanes & Donoghue, 2000 for review). As a result, in addition to having the essential function of controlling voluntary movement, M1 is a crucial site for use-dependent plasticity, such as learning new motor skills and recovery of motor function after injury (Pascual-Leone *et al.*, 1995; Nudo *et al.*, 1996a). In the context of motor learning, use-dependent plasticity can be assessed in humans by examining the change in the muscle-evoked potential (MEP) from electromyography (EMG) recordings following transcranial magnetic stimulation (TMS) of M1. An increase in the MEP after motor training reflects the increased excitability of corticospinal and spinal motor neurons (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001), which is thought to occur through long-term potentiation (LTP)-like mechanisms (Bütefisch *et al.*, 2000). Although several factors are known to influence motor system plasticity (see Ridding & Ziemann, 2010 for review), limited information exists on differences in use-dependent plasticity with hand preference and advancing age. These factors could contribute to differences in use-dependent plasticity in a diverse subject population and have important implications for therapeutic interventions in neurological patients.

The motor system between left and right brain hemispheres is not symmetrical, with anatomical and physiological differences between sides potentially contributing to asymmetries in hand performance (Amunts *et al.*, 1996; Triggs *et al.*, 1997; Volkmann *et al.*, 1998; Guye *et al.*, 2003; Ilic *et al.*, 2004). However, few studies have examined the extent of corticomotor plasticity in left and right hemispheres and whether this has implications for improved motor performance. Studies involving artificially induced (Ridding & Flavel, 2006) and use-dependent plasticity (Garry *et al.*, 2004; Gallasch *et al.*, 2009) have generally shown no hemispheric differences in MEP facilitation in young subjects, although repeat

performance of a goal-directed movement task results in more sustained facilitation in the left hand (Gallasch *et al.*, 2009). Recent evidence suggests that artificially induced and use-dependent plasticity involve overlapping and functionally relevant cortical circuits (Ziemann *et al.*, 2004; Stefan *et al.*, 2006), but the functional implications of any hemispheric differences in use-dependent plasticity for motor skill learning are unknown.

Both structural and functional plasticity in response to motor training are altered with advancing age. For example, there is reduced structural plasticity of cortical gray matter in older adults when learning a novel motor skill (Boyke *et al.*, 2008), and there is an age-related decrease in the ability of M1 to reorganise in response to motor training (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009). More recently, we have shown reduced use-dependent plasticity and motor learning following 30 minutes of ballistic thumb abduction training in older adults, with these differences unrelated to the extent of improvement in motor performance in young or old subjects (Rogasch *et al.*, 2009). However, these previous studies have focused on the right (preferred) hand to assess use-dependent plasticity with advancing age, and there are no studies that have compared use-dependent plasticity in left and right hands of older adults.

The aim of this study was to examine the extent of use-dependent plasticity following repetitive thumb abduction training for M1 control of the right (dominant) and left (non-dominant) hands of young and old adults. The repetitive ballistic thumb abduction task was selected as it is commonly used to induce use-dependent plasticity in young subjects (e.g. Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001), and there is recent evidence from TMS studies indicating improvements in ballistic motor performance are dependent on adaptations in M1 (Muellbacher *et al.*, 2001; Muellbacher *et al.*, 2002; Carroll *et al.*, 2008). Using the ballistic thumb training task, we hypothesise that use-dependent corticomotor plasticity and motor learning will be reduced with advancing age, and there will be greater use-dependent

plasticity in the right (non-dominant hand) hemisphere compared with the left (dominant hand) hemisphere in all subjects.

3.3. Materials and Methods

Experiments were performed on the right and left hand of 27 subjects with no known history of peripheral or neurological impairment. Data were excluded from one young male subject as he displayed excessive thumb flexion throughout the training task (standard deviation 3x greater than the mean of all subjects), suggesting that it was not performed correctly. Therefore, data were analysed from twelve young (7 women, 5 men; 22 ± 2 yrs; range 18-27 years) and fourteen older subjects (7 women, 7 men; 67 ± 4 yrs; range 63-75 years). All subjects were strongly right handed (Laterality Quotient (LQ); Young: median LQ = 0.82, range 0.6-1.0; Old: median LQ = 0.92, range 0.6-1.0) as assessed by the Edinburgh Handedness Questionnaire (Oldfield, 1971) and no subjects reported long term skilled use of the hands, such as playing a musical instrument (Rosenkranz *et al.*, 2007b). Subjects also completed the long version of the International Physical Activity Questionnaire (IPAQ), consisting of 31 items describing the extent of leisure time physical activity involving aerobic exercises such as running, cycling and walking (Craig *et al.*, 2003; Fogelholm *et al.*, 2006). All subjects gave written informed consent prior to participation in the study, which was approved by the University of Adelaide Human Research Ethics Committee.

3.3.1. Experimental arrangement

The experimental setup used for these studies has been described previously (Rogasch *et al.*, 2009). Subjects were seated comfortably with either their right or left shoulder abducted approximately 45° to allow the hand and arm to rest on a manipulandum. Experiments were conducted in the afternoon and each hand was tested on a different day separated by at least 2 weeks, with the hand examined first selected randomly. For all measures other than training, the forearm was pronated and the palm was facing down on the manipulandum. Surface

EMG was recorded from the abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles throughout the experiment using bipolar surface electrodes (Ag-AgCl, 4mm diameter) placed ~ 2 cm apart using a muscle belly-tendon configuration. A grounding strap placed around the elbow was used as a common reference for all electrodes. The EMG signals were amplified (× 100-1000), bandpass filtered (high pass at 13 Hz, low pass at 1000 Hz), digitised online at 2 kHz with a CED interface system (Cambridge Electronic Design Co. LTD, UK) and recorded onto computer for offline analysis. The EMG signals of both muscles were displayed on an oscilloscope to assist the subject in maintaining EMG silence when required.

3.3.2. Experimental procedures

At the beginning of each experiment, maximal thumb abduction force (maximum voluntary contractions; MVCs) was measured and resting and active motor thresholds were determined using TMS (see below). To examine the extent of corticomotor plasticity in left and right hands of young and old adults, corticomotor excitability (input-output curves; IO curves) and short-interval intracortical inhibition (SICI curves) were assessed using TMS before and after a motor training task. To examine training-induced changes in peripheral neuromuscular processes, maximum compound muscle action potentials (M-waves) were also recorded before and after training in the majority of these subjects. The order of these measurements for all subjects was M-waves, IO curves and SICI before training, whereas it was M-waves, SICI (5 mins after training) and IO curve (10 mins) after training. This order was selected to obtain SICI measurements as close as possible to the training, as SICI can return to baseline levels within 15 minutes (see Garry *et al.*, 2004), whereas MEP amplitude can remain elevated for up to an hour after the intervention (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001).

MVC. The hand was positioned with the palm facing down and the proximal phalanx of the thumb was placed in a metal ring attached to a load cell (LC1205-K020, A&D Mercury Pty Ltd, Australia) to facilitate measurement of thumb abduction force. Maximum thumb abduction force was exerted by the subject for three seconds against the force transducer with verbal encouragement provided by the experimenters. Several MVC trials were performed, with a minimum of 30-s rest between trials, until the peak force from two trials were within 10% of each other, and the MVC with the largest thumb abduction force was used for the assessment of muscle strength. Visual feedback of thumb abduction force was displayed on an oscilloscope, and the subject was monitored in each trial to ensure that proximal limb muscles were not contributing to the thumb abduction force. Force signals were amplified (× 1000), digitised online (2 kHz) via a CED 1401 interface (Cambridge Electronic Design, UK) and stored on computer for offline analysis.

TMS. TMS was applied using a figure-of-eight coil (external wing diameter 90 mm) with two Magstim 200 magnetic stimulators connected with a Magstim Bistim unit (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. With this coil orientation, current flow within the cortex was induced in a posterior-anterior direction. The coil was positioned at the optimal scalp position over the appropriate hemisphere for eliciting a MEP in the relaxed APB muscle. The optimal scalp position was then marked for reference. TMS was delivered at 0.2 Hz for all conditions and optimal coil position was continually monitored throughout the experiment.

RMT and AMT. Resting motor threshold was determined as the minimum stimulus intensity required to elicit a MEP in the relaxed APB of at least 50 μV in 3 out of 5 consecutive trials. Active motor threshold was defined as the minimum stimulus intensity required to elicit a MEP in the APB muscle of at least 200 μV in 3 out of 5 consecutive trials during a low-level

voluntary thumb abduction (10% MVC). Both RMT and AMT are expressed relative to 100% maximum stimulator output (MSO) and the stimulus intensity was altered in 1% increments of MSO throughout this process until the appropriate threshold level was achieved.

IO curve. The intensities used to construct the TMS IO curves were determined for each individual according to their RMT before ballistic thumb training. Seven TMS intensities of 100, 110, 120, 130, 140, 160, and 180 % of RMT were recorded for each subject at rest. TMS pulses that included obvious EMG during the pre-stimulus period (100 ms before TMS) were discarded, and the TMS pulse repeated at the end of the IO curve block. A single IO curve block consisted of 56 stimuli (8 stimuli at each intensity) with the order of presentation of the seven conditions pseudorandomised throughout the trial. MEP amplitudes were measured for each TMS pulse to calculate the mean MEP amplitude for each TMS intensity.

SICI. Short-interval intracortical inhibition (SICI) was assessed using a paired-pulse TMS paradigm consisting of a subthreshold conditioning stimulus that preceded a suprathreshold test stimulus by 3 ms (Kujirai *et al.*, 1993). The test stimulus intensity was set to produce a MEP amplitude of ~1 mV in resting APB before training, whereas the conditioning stimulus was randomised as 70, 80, or 90% of AMT. The test stimulus intensity remained constant before and after training. Each data block consisted of ten trials for each of four conditions; test stimulus alone and SICI at 70, 80 and 90% AMT, with the order of presentation randomised throughout the trials (40 trials total). The conditioned MEP amplitude was expressed as a percentage of the unconditioned test MEP amplitude to calculate the influence of the conditioning stimulus on SICI circuits.

M-waves. Maximal M-waves from the APB muscle were recorded from both left and right hands in 10 young and 10 old subjects. Supramaximal electrical stimulation was administered to the median nerve at the wrist using a constant current stimulator (DS7A, Digitimer, UK)

via bipolar surface electrodes, separated by 20 mm, with the cathode proximal. Stimuli were square wave pulses with 100 μs pulse duration. Stimulator intensity was set at 120% of that required to elicit a maximal M-wave response from APB. The M-wave responses from five stimuli were recorded before and immediately after training in these subjects.

3.3.3. Motor training task

The motor training task was similar to that described previously (Rogasch *et al.*, 2009), which required the subject to maximise peak thumb abduction acceleration (TAAcc) during ballistic movement of the thumb. Subjects sat with their forearm placed in a custom designed splint and their arm abducted at the shoulder and bent at approximately 90° at the elbow. The splint was designed to have the forearm placed in a neutral position (between pronation and supination) with the thumb free to move while the other digits were immobilised. Two blocks of 150 ballistic thumb abduction movements (total of 300 trials) paced at 0.5 Hz by an audible tone from a metronome were performed in each session. Subjects rested their thumb for 30 seconds after ten trials and for five minutes between the first and second blocks to avoid fatigue. A bi-axial accelerometer (sensitivity ± 6g, LIS3L06AL, STMicroelectronics, Switzerland) placed over the interphalangeal joint of the thumb was used to assess thumb acceleration in the abduction-adduction plane. Thumb acceleration > +3 $m.s^{-2}$ in the abduction-adduction plane triggered a recording sweep of ± 500 ms, and each movement recorded acceleration data in the abduction-adduction plane, along with EMG from the APB and ADM muscles. Continual verbal encouragement and visual feedback of thumb abduction acceleration displayed on a computer screen was provided to the subject throughout training to improve and maximise thumb acceleration. Acceleration signals were digitised online at 2 kHz using a CED interface system and recorded on computer for offline analysis.

3.3.4. Data analysis

All MEP and M-wave trials that contained any pre-stimulus EMG (100 ms before stimulation) were discarded from analysis. MEP and M-wave amplitudes were measured peak-to-peak in each individual trial and averaged for each condition. For paired-pulse TMS, conditioned MEPs were expressed as a percentage of the mean test alone MEP in each block to quantify the effectiveness of SICI. Maximum force was calculated during the MVC and maximum APB EMG was assessed as the mean rectified EMG 500 ms before and after peak force.

For each movement trial, a baseline period from 400 to 200 ms before abduction acceleration was used to calculate the mean baseline acceleration in the abduction-adduction plane. The mean acceleration over this baseline period was subtracted so that baseline acceleration equalled 0 $m.s^{-2}$, and the peak TAAcc was then assessed. Abduction acceleration for each block of 150 ballistic thumb abduction movements was subdivided into blocks of 50 trials representing a start, middle and end (six segments of 50 trials in total) for detailed analysis. To assess improvement in peak TAAcc (motor learning), each block of 50 trials was normalised to the first 10 contractions. APB EMG was quantified for one in ten movement trials by obtaining the average rectified EMG from movement onset to peak TAAcc. Movement onset was defined as the time when EMG increased by more than 3 standard deviations above baseline (-400 to 200 ms before movement).

3.3.5. Statistical analysis

A paired *t*-test was performed to compare baseline TAAcc between hands and an unpaired *t*-test was performed to compare baseline TAAcc and IPAQ scores between age groups. A Mann-Whitney U test was used to compare non-parametric handedness scores between young and old adults. Two-way ANOVA (Hand, Age) was used to analyse subject characteristics of maximum thumb abduction force, maximum M-wave amplitude, RMT, AMT, and MEP test

alone TMS intensity before training. A three-way ANOVA (Hand, Age, Time) was used to examine M-wave amplitude and test MEP amplitude from SICI data. A three-way ANOVA (Hand, Age, Time) was also used to examine TAAcc improvement and mean rectified EMG during acceleration. A four-way ANOVA was used to analyse IO curve (Hand, Age, Time, TMS Intensity) and SICI (Hand, Age, Time, Conditioning Intensity). A Fisher's LSD post-hoc test that performed all possible comparisons was used to analyse significant main effects and interactions where necessary. The significance level was set at $P < 0.05$ for all comparisons and all group data are provided as mean ± SEM.

3.4. Results

All subjects were comfortable with the TMS and training task procedures and no side effects were reported. Age and hand differences in subject characteristics before training are displayed in Table 3.1. There was no difference between left and right hands in maximum thumb abduction force or APB EMG during MVC, TMS thresholds, and M-Waves before training. However, when both hands were combined, older adults had reduced APB EMG during MVC and M-Wave amplitude compared with young adults (Table 3.1). In addition, there were no differences in physical activity levels assessed by the IPAQ between young and old adults (Old = 3492 ± 777 MET-min; Young = 4117 ± 1296 MET-min, $P = 0.67$), although older adults were more strongly right handed than young adults (Old: median LQ = 0.92 ± 0.12; Young: median LQ = 0.82 ± 0.13, $P < 0.04$).

Table 3.1 *Group data before training in the left and right hand of young and old adults.*

	Young			Old			All		
	Left	Right	Both	Left	Right	Both	Left	Right	Both
MVC (N)	32.5 (18.9)	27.4 (9.7)	30.0 (14.9)	32.8 (12.7)	33.8 (19.8)	33.3 (16.3)	32.6 (15.5)	30.8 (16.0)	31.7 (15.6)
Maximum EMG (mV)	0.86 (0.26)	0.84 (0.34)	0.85 (0.29)	0.61 (0.28)	0.61 (0.24)	0.61* (0.26)	0.73 (0.29)	0.72 (0.31)	0.72 (0.30)
RMT (% MSO)	43.4 (8.9)	45.1 (8.6)	44.3 (8.6)	46.3 (7.8)	44.9 (6.7)	45.6 (7.3)	45.0 (8.3)	45.0 (7.5)	45.0 (7.9)
AMT (% MSO)	35.2 (8.5)	37.3 (8.9)	36.8 (8.7)	38.1 (7.8)	37.6 (7.5)	37.8 (7.6)	36.7 (8.1)	37.9 (8.1)	37.3 (8.0)
Test Intensity (% MSO)	56.3 (13.5)	61.7 (13.5)	59.0 (13.5)	59.9 (16.8)	60.9 (16.9)	60.4 (16.5)	58.2 (15.2)	61.3 (15.1)	59.8 (15.1)
M-Wave (mV)	15.8 (2.3)	15.5 (3.4)	15.7 (2.9)	11.9 (4.4)	11.8 (4.7)	11.8* (4.4)	13.8 (4.0)	13.6 (4.4)	13.7 (4.2)

Values are mean (SD). *MVC,* APB maximum voluntary contraction; *EMG,* APB electromyography; *RMT,* APB resting motor threshold; *AMT,* APB active motor threshold; *MSO,* maximum stimulator output. M-Wave data obtained from 10 young and 10 older adults. * $P < 0.02$ compared with young adults.

3.4.1. Effect of age and hand on motor learning

To quantify motor learning, the improvement in peak TAAcc for each 50 contractions was normalised to the first 10 movement trials for each session. For the first 10 movement trials (baseline), peak TAAcc was greater in the left compared with the right hand in all subjects (paired *t*-test, left = 21.15 ± 2.21 $m.s^{-2}$; right = 16.04 ± 1.50 $m.s^{-2}$, P = 0.02), but there was no difference between peak TAAcc in young and old adults (unpaired *t*-test, young = 17.86 ± 2.17 $m.s^{-2}$; old = 19.22 ± 1.76 $m.s^{-2}$, P = 0.63). Improvement in peak TAAcc in the left and right hand of young and old adults throughout training is shown in Figure 3.1. A three-way repeated measures ANOVA indicated that normalised TAAcc improved over training blocks (Time effect, P < 0.01), and was greater for the right hand compared with the left hand (Hand effect, P = 0.03), but was not different between young and old adults (Age effects, P = 0.47). A significant Hand x Time x Age interaction (P < 0.01) showed that the improvement in peak TAAcc for the last 100 movement trials (201-250 and 251-300) was ~40% greater in the right hand in young adults compared with the left hand in young adults (P < 0.04) and the right hand in old adults (P < 0.01). No difference between hands was observed in normalised TAAcc in old adults, and no age-related difference was observed in normalised TAAcc for the left hand.

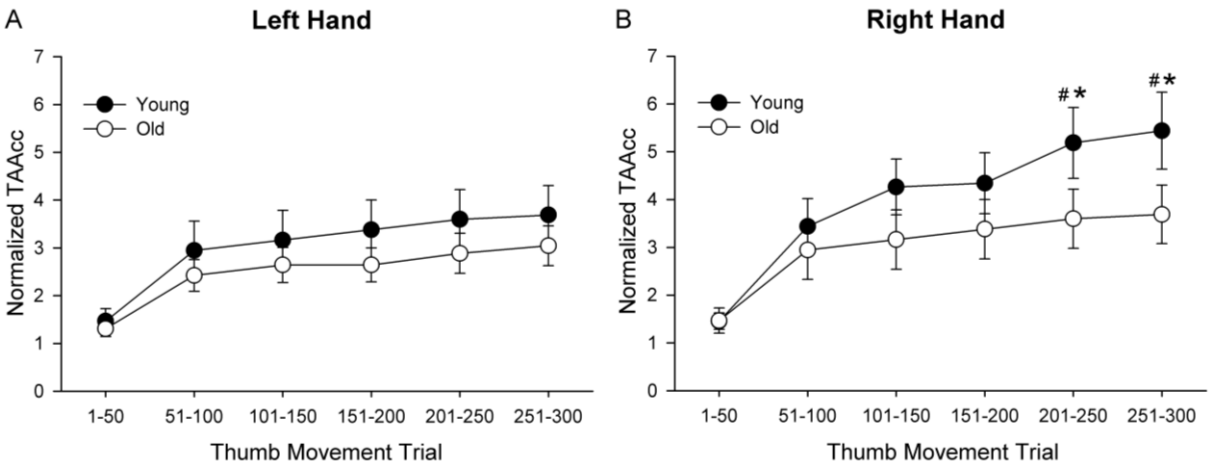

Figure 3.1 *Improvement in peak thumb abduction acceleration (TAAcc) for all 300 thumb movement trials for young and old adults in the left (A) and right (B) hand. Each symbol represents the mean of 50 movement trials that have been normalised to the first 10 movements in that session. * P < 0.05 compared with older adults. # P < 0.05 compared with the same time point in the left hand.*

For the mean rectified APB EMG throughout training, a three-way repeated measures ANOVA revealed that EMG was different between training blocks (Time effect, $P = 0.01$), EMG was greater in young subjects (Age effect, $P = 0.02$), but there were no differences between left and right hands (Hand effect, $P = 0.59$). A Time x Age interaction ($P < 0.01$) and subsequent post-hoc analysis showed that EMG was similar in young and old adults for training block 1 ($P = 0.12$), but was on average 32% greater in young subjects in training blocks 2-6 (all P values < 0.02). No significant differences in APB EMG were observed throughout training in young or old adults. Furthermore, the significant Age effect was removed ($P = 0.8$) when the EMG was normalised to the MVC EMG in each subject.

3.4.2. Effect of age and hand on training-dependent corticomotor excitability

The effect of motor training on the IO curves (assessed over TMS intensities of 100-180 % RMT) of the relaxed APB in left and right hands of young and old adults are shown in Figure 3.2. A four-way repeated measures ANOVA indicated that there was an increase in the size of the APB MEP amplitude with increasing stimulus intensity in both hands of all subjects (Intensity effect, $P < 0.0001$), MEP amplitude was greater following training (Time effect, $P < 0.0001$), and MEP amplitude was greater in the left compared with the right hand (Hand effect, $P = 0.04$). However, no difference between young and old adults (Age effect, $P = 0.75$) was observed. A Hand x Time x Intensity interaction ($P = 0.02$) showed that MEP amplitude increased after training for the left hand at TMS intensities of 160% ($P = 0.04$) and 180% ($P = 0.01$) RMT for young and old adults combined. No differences in MEP amplitude were observed in the right hand after training at any TMS intensity (all comparisons, $P > 0.28$). For the ADM IO curve, one young and one old subject were excluded due to contamination by consistent pre-stimulus EMG activity. As for APB, MEP amplitude increased with increasing stimulator output for the control ADM muscle (Intensity effect, $P < 0.0001$). However, there was no significant Time ($P = 0.64$), Hand ($P = 0.41$), or Age ($P = 0.30$) effects, and no significant interactions (all interactions, $P > 0.18$). These data show that

training did not influence MEPs in ADM, and that the training-related increase in APB MEP amplitudes was specific to the muscles involved in the task.

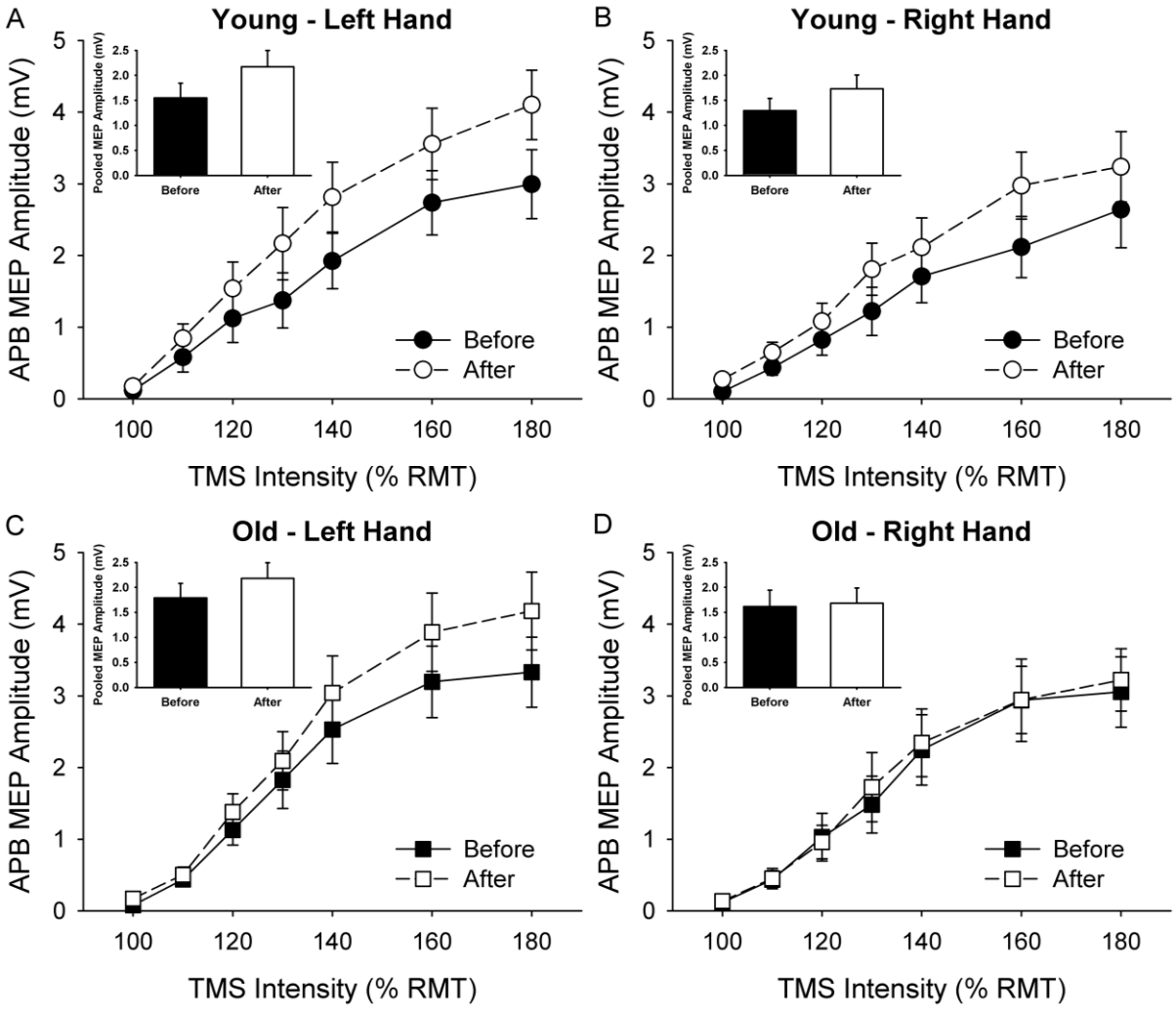

Figure 3.2 *Mean MEP amplitudes before and after training in the left (A, C) and right (B, D) APB muscle of young (A, B) and old (B, D) adults. Data are shown at increasing TMS intensities expressed relative to resting motor threshold (RMT). For young and old adults combined, MEP amplitude increased after training for the left hand at TMS intensities of 160% (P=0.04) and 180% (P=0.01) RMT. Inset shows MEP amplitudes before and after training that have been pooled across TMS intensities.*

M-wave amplitudes were 34% greater in young compared with old adults ($P < 0.01$). However, there were no differences in M-wave amplitude between hands (left = 13.94 ± 0.63 mV, right = 14.09 ± 0.74 mV, $P = 0.72$), no changes after training (before = 13.89 ± 0.68 mV, after = 14.14 ± 0.70 mV, $P = 0.28$), and no significant interactions (all interactions, $P > 0.17$).

Using linear regression of data from individual subjects, we examined whether the training-related change in APB MEP amplitude was associated with the improvement in peak TAAcc. There was no significant association between the change in APB MEP amplitude and the improvement in TAAcc for young ($r^2 = 0.04$, $P = 0.38$) or old ($r^2 = 0.06$, $P = 0.23$) adults, and for left ($r^2 = 0.05$, $P = 0.29$) or right ($r^2 = 0.12$, $P = 0.08$) hands.

SICI was assessed using a paired-pulse paradigm that utilised an ~1 mV test pulse (before training) that was preceded by a subthreshold conditioning pulse (70, 80, or 90% AMT) at 3 ms. Data showing the changes in test alone APB MEP amplitude and the extent of SICI in left and right hands of young and old adults are shown in Figure 3.3. There was an increase of 41% in test APB MEP amplitude after training (Time effect, $P < 0.0001$), but no difference between left and right hands (Hand effect, $P = 0.12$) or young and old adults (Age effect, $P = 0.62$). A significant Hand x Time interaction ($P = 0.04$) and post hoc analysis indicated that test MEP amplitude after training for the left hand increased by 55% ($P < 0.0001$) and for the right hand increased by 25% ($P = 0.05$) compared with before training. In addition, test MEP amplitude was 21% greater in the left hand compared with the right hand after training ($P = 0.04$). This increase in MEP amplitude after training was specific to the muscle used in the task (APB) as there was no change in MEP amplitude (one young subject excluded due to pre-stimulus EMG activity) of the ADM in either left or right hands of young and old adults after training. For SICI, increasing the intensity of the conditioning stimulus increased the amount of SICI in APB for both hands in all groups (Intensity effect, $P < 0.0001$). However, there were no significant Time ($P = 0.60$), Hand ($P = 0.69$) or Age ($P = 0.16$) effects, and no significant interactions (all interactions, $P > 0.16$).

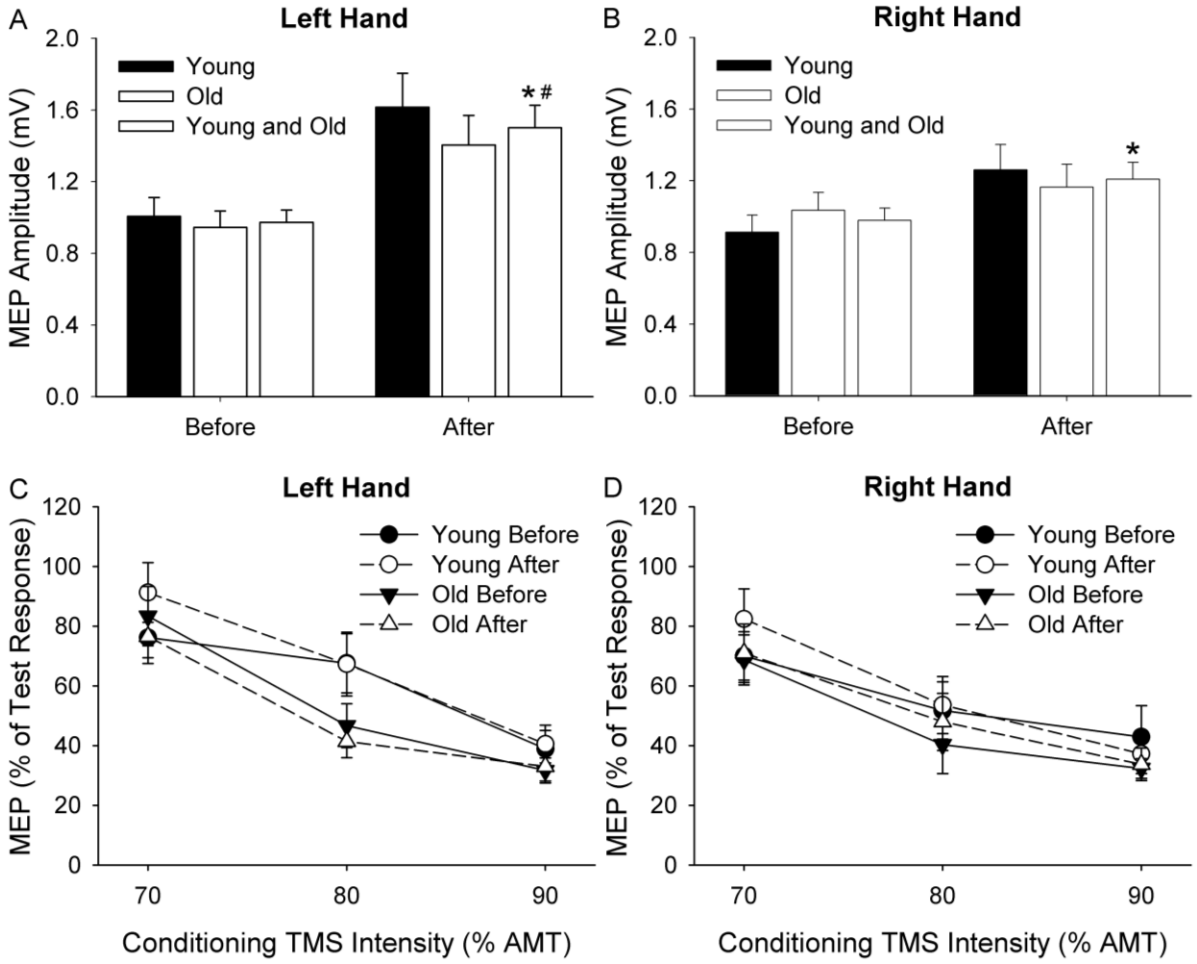

Figure 3.3 *Mean test MEP amplitude (A, B) and the extent of SICI (C, D) in left and right hands of young and old adults. Test MEP amplitude increased after training for both the left (A) and right (B) hand and this increase was greater in the left hand. The extent of SICI was influenced by conditioning TMS intensity, but was not different between left and right hands. * P < 0.05 compared with before training. # P < 0.05 compared with the same time point in the right hand.*

3.5. Discussion

The purpose of this study was to examine hemispheric differences in use-dependent corticomotor plasticity and motor learning following repetitive thumb abduction training in young and old adults. There were several new findings in this study. First, use-dependent corticomotor plasticity following repetitive thumb abduction training was greater in the left (non-dominant) hand, although there were no differences between young and old adults. Second, the extent of motor learning was greater for the right hand in young adults compared with the left hand in young adults and the right hand of old adults. Furthermore, SICI was not

altered by training in either hand for young or old adults, suggesting that the increased use-dependent corticomotor plasticity in the right hemisphere (left hand) was not due to hemispheric differences in GABAergic intracortical inhibition in M1.

3.5.1. Increased corticomotor plasticity for control of the left hand

A number of studies using TMS have demonstrated corticomotor plasticity following motor learning, motor practice, or training. These studies have shown that increased MEP amplitude in the target muscle can last for up to an hour after the motor intervention (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001), although substantial variability in the response exists between studies. One factor that could be responsible for this variability is whether the dominant or non-dominant hand is used for the training. Similar to previous performance based training intervention studies, we demonstrate that MEP amplitudes in the target muscle (APB) of both left and right hands were significantly facilitated following training, but the changes were larger when training was performed with the left (non-dominant) hand. This increased MEP following an intervention is believed to reflect use-dependent plasticity (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001), with pharmacological (Bütefisch *et al.*, 2000; Sawaki *et al.*, 2002) and physiological evidence (Muellbacher *et al.*, 2002) suggesting that this occurs through LTP-like mechanisms. It is conceivable that the greater use-dependent plasticity in the right hemisphere (controlling the left hand) could take effect at cortical and/or spinal levels, but several lines of evidence suggest that at least some of the changes with this ballistic task are occurring at the cortical level. For example, there is a reduced change in spinal excitability as assessed with transcranial electrical stimulation (Classen *et al.*, 1998), and there is impaired motor learning following administration of pharmacologically induced neuromodulators (Meintzschel & Ziemann, 2006). Furthermore, learning of a brisk motor task is disrupted with repetitive TMS over M1, but not when administered over other brain areas (Muellbacher *et al.*, 2002).

Despite known anatomical and physiological asymmetries between left and right motor regions of the brain (see Hammond, 2002 for review), no previous studies have examined use-dependent plasticity for control of the left and right hands in older adults. Previous studies in young adults have found no differences in MEP facilitation between hands following the performance of complex sensorimotor tasks (Garry *et al.*, 2004; Gallasch *et al.*, 2009). However, greater training-related MEP facilitation has been observed for the right hemisphere after multiple training sessions, suggesting a more sustained MEP facilitation with goal-directed visuomotor tasks for the left hand (Gallasch *et al.*, 2009). In support of this, we found a larger increase in MEPs following performance of a ballistic thumb-training task in the left hand. These findings suggest that the hand used for training is an important feature in determining the magnitude of use-dependent plasticity for simple ballistic tasks performed by hand muscles, and that the factors responsible for the increased MEP with training are more effective in the right hemisphere. Alternatively, the time course of the training-induced MEP facilitation could be different between hands, with a more rapid facilitation in the skilled (right) hand followed by over learning of the task, which results in a return of MEP amplitude to baseline (Muellbacher *et al.*, 2001; Rosenkranz *et al.*, 2007a). Furthermore, the increased training-related MEP facilitation for control of the left hand may be due to the greater complexity of the task when performed with the non-dominant (unskilled) hand (Semmler & Nordstrom, 1998), with an increased MEP evident during more demanding tasks (Datta *et al.*, 1989).

The increased MEP amplitude in the left hand cannot simply be explained by greater motor learning, as the improvement in peak TAAcc was greater for the right hand of young adults, and there was no association between MEP facilitation and improvement in motor performance in individual subjects. These findings suggest that the extent of training-related MEP facilitation is not an important determinant of the magnitude of the behavioural improvement. It may be that factors other than the magnitude of motor learning, such as

attentional focus (McNevin *et al.*, 2000) are more important in mediating changes in corticomotor excitability. Nonetheless, differences in hand performance and learning between left and right sides are inconsistent, and may depend on the details of the task performed. For example, some studies have shown that complex tasks involving the precise manipulation of objects are performed better with the right than the left hand (Bryden & Roy, 1999; Garry *et al.*, 2004). In contrast, the improvement in motor performance associated with learning new motor skills is achieved to a similar extent in both hands during complex motor tasks (Garry *et al.*, 2004; Gallasch *et al.*, 2009), but not for simple ballistic tasks (Ridding & Flavel, 2006). In the present study, we found that baseline motor performance was greater in the left hand during the thumb abduction task, which may be a confounding factor in the reduced improvement in performance for this hand in young subjects.

Several studies have examined SICI in the left and right hemispheres in resting hand muscles, but the results have been inconsistent. For example, the left hemisphere can show increased (Smith *et al.*, 2009), decreased (Ilic *et al.*, 2004; Ridding & Flavel, 2006) and no difference (Garry *et al.*, 2004; Baümer *et al.*, 2007; Gallasch *et al.*, 2009) in SICI compared with the right hemisphere, with these divergent findings likely due to the details of the experimental procedures used (muscle, stimulus parameters) and the subject population tested (extent of laterality, hand use etc). Furthermore, one important technical difference between many SICI studies involving training is whether the test stimulus is kept constant, or whether it is adjusted so that the MEPs are matched before and after training. We tested SICI with a constant test TMS intensity as previous studies have shown that measures of SICI are sensitive to test TMS intensity (Zoghi *et al.*, 2003) and are unrelated to cortical excitability state and MEP size (Garry & Thomson, 2009). When maintaining a constant test TMS intensity, we found no difference in resting SICI between hemispheres in young or old adults, suggesting that differences in resting SICI were not responsible for the differences in use-dependent plasticity between left and right sides.

3.5.2. Age-related changes in corticomotor plasticity and motor learning

Several previous studies involving TMS have shown that artificially-induced and use-dependent plasticity are diminished in older adults (Sawaki *et al.*, 2003; Tecchio *et al.*, 2008; Rogasch *et al.*, 2009). However, a reduction in neural plasticity in older adults is not always a consistent finding, as previous studies provide evidence that older adults are able to retain a high capacity for learning new motor skills (McNay & Willingham, 1998; Smith *et al.*, 1999a; Wu & Hallett, 2005; Voelcker-Rehage, 2008). In support of this, we found that the extent of use-dependent plasticity was similar in young and older adults during ballistic thumb abduction training. This finding was unexpected, given that we had previously observed a lack of training-induced MEP facilitation in older adults during the same task when testing the left hemisphere during right hand performance (Rogasch *et al.*, 2009). As similar experimental techniques were used in the two studies, it can only be assumed that differences in the subject population may have contributed to these disparate findings. For example, it is possible that the older subjects tested in the present study were more physically active than those in our previous study. In general, physical activity levels are reduced with increasing age (Ravussin & Bogardus, 1989), but there were no differences in physical activity levels (assessed by questionnaire) between young and old adults in the present study, although this was not measured in our previous study (Rogasch *et al.*, 2009). It is now well accepted that regular physical activity and exercise provides neuroprotective and neuroplastic benefits to the ageing brain, and may serve to reduce biological senescence in humans (see Cotman & Berchtold, 2002). We have recently shown that artificially induced motor cortex plasticity is greater in physically active individuals (Cirillo *et al.*, 2009), and participation in regular exercise in older adults may have contributed to the similar extent of use-dependent plasticity observed in the present study. Other factors specific to the subject population that could contribute to alterations in corticomotor excitability and plasticity include the extent of skilled hand use (Rosenkranz *et al.*, 2007b), prior history of synaptic activity (see Ridding &

Ziemann, 2010), attentional focus (McNevin *et al.*, 2000) and emotional state of the subjects (Tormos *et al.*, 1997).

Age-related changes in the central and peripheral neuromotor system are believed to be responsible for reduced motor function with advancing age. Recent studies on central nervous system changes with ageing have attributed the decline in motor function to reduced brain volume and decreased cerebral gray and white matter in older adults (Courchesne *et al.*, 2000; Jernigan *et al.*, 2001). Furthermore, there are substantial neuromuscular changes with advancing age (see Vandervoort, 2002), including a decline in the proportion of muscle occupied by fast twitch (type II) fibres (Klein *et al.*, 2003), which is likely to influence performance on ballistic tasks. However, we found diminished motor learning in older adults compared to young adults, but only for the right (dominant) hand. These findings suggest an age-related decline in asymmetry of motor learning. One possible reason for an age-related reduction in motor learning in the right hand is a reduced need to learn new motor skills with advancing age, as shown by a more balanced use of left and right hands in everyday tasks performed by older adults (Kalisch *et al.*, 2006), resulting in an age-related modification of the mechanisms important for use-dependent plasticity. Furthermore, several neuroimaging studies have shown increased recruitment of cortical and subcortical areas during movement tasks in older adults (Mattay *et al.*, 2002), including increased bilateral cortical activation (Naccarato *et al.*, 2006), which are usually interpreted as compensatory changes in the ageing brain (Ward, 2006). The possibility exists that these compensatory mechanisms lead to less cortical lateralisation in older adults, which result in similar motor learning capabilities for both hands in the elderly (Kalisch *et al.*, 2006).

Several studies have shown that modulation of SICI plays an important role in skilled hand movement (Stinear & Byblow, 2003; Zoghi *et al.*, 2003) and removal of SICI plays a critical role in use-dependent plasticity (Ziemann *et al.*, 2001). It is therefore possible that age-

related differences in intracortical inhibition may contribute to impaired hand performance (see Sale & Semmler, 2005) and reduced M1 plasticity (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009) that is generally observed in older adults. Some studies have shown a decrease (Peinemann *et al.*, 2001) or an increase (Kossev *et al.*, 2002; Smith *et al.*, 2009) in SICI in older adults, but a larger number of studies have reported no age-related change (Wassermann, 2002; Oliviero *et al.*, 2006; Rogasch *et al.*, 2009), which was supported by the present study. Furthermore, as reported previously (Rogasch *et al.*, 2009) there was no change in SICI in young or old adults after training with the ballistic thumb abduction task, suggesting that the modulation of SICI that sometimes accompanies training may require a more demanding task involving selective muscle activation (e.g. Liepert *et al.*, 1998).

In conclusion, we have examined corticomotor plasticity and motor learning following repetitive thumb abduction training in left and right hands of young and old adults. Despite greater task improvement for the right (dominant) hand, there was increased use-dependent corticomotor plasticity in the right hemisphere controlling the left (non-dominant) hand, suggesting that the factors responsible for the increased MEP with training are more effective in the right hemisphere. Although an age-related decline in motor learning occurred for the right (dominant) hand, use-dependent corticomotor plasticity was not altered with advancing age during this task. We therefore suggest that some older adults are able to retain similar use-dependent plasticity for both hands during a ballistic motor training task. The factors that promote this age-related maintenance in use-dependent plasticity remains to be determined.

CHAPTER IV

CORTICOMOTOR EXCITABILITY AND PLASTICITY FOLLOWING COMPLEX VISUOMOTOR TRAINING IN YOUNG AND OLD ADULTS

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STATEMENT OF AUTHORSHIP

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Experimental design, subject recruitment, data collection and analysis on all samples, interpretation of data, and wrote manuscript.

I hereby certify that the statement of contribution is accurate.

Signed ..... *Date* 2/4/12

TODD, G.

Aided with experimental design, data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... *Date* 28/3/12

SEMMLER, J.G.

Verified experimental design, supervised development of work, helped with data interpretation, critical manuscript evaluation, and acted as corresponding author.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

Signed ..... *Date* 2/4/12

4. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults

4.1. Abstract

Previous studies with transcranial magnetic stimulation (TMS) have shown that advancing age may influence plasticity induction in human motor cortex (M1), but these changes have been assessed with TMS-induced paradigms or simple motor tasks. The aim of this study was to examine changes in corticospinal excitability and intracortical inhibition as markers of corticomotor plasticity following complex motor training in young and old adults. Electromyographic recordings were obtained from the right first dorsal interosseous (FDI) muscle of 16 young (20-35 yrs) and 16 older adults (aged 60-75 yrs) before and after motor skill training. Motor training consisted of three six-minute blocks of a complex visuomotor task that required matching the metacarpophalangeal (MCP) joint angle of the index finger using abduction-adduction movements. Single- and paired-pulse TMS over the left M1 was used to assess changes in right FDI motor-evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) before and after each training block. Visuomotor tracking performance was diminished in old compared with young adults throughout training. However, improvement in tracking error was similar for young and old adults (7-24% increase in each training block). For young and old adults, motor training increased FDI MEP amplitude (≥20%) and reduced the magnitude of SICI (≥19%) after each visuomotor training block, reflecting use-dependent plasticity. However, no difference in corticomotor plasticity (change in MEP or SICI) was observed between young and old adults. Further studies are needed to identify the experimental or behavioural factors that might contribute to the maintenance of corticomotor plasticity in older adults.

4.2. Introduction

Progression into old age is typically accompanied by a reduced ability for central nervous system (CNS) reorganisation and plasticity. In the context of the motor system, plasticity within motor cortex and spinal motor neurons can be quantified by changes in the motor-evoked potential (MEP) elicited by transcranial magnetic stimulation (TMS). The increase in MEP amplitude after short-term motor training is thought to reflect long-term potentiation (LTP)-like changes in synaptic efficacy (Bütefisch *et al.*, 2000) that occur predominantly in cortical circuits (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001). Recent TMS studies show reduced TMS-induced (Müller-Dahlhaus *et al.*, 2008; Tecchio *et al.*, 2008; Todd *et al.*, 2010) and practice-dependent plasticity (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009) in cortical circuits in older adults, although this is not always a consistent finding (e.g. Cirillo *et al.*, 2010). Nonetheless, these studies have been restricted to simple motor tasks less likely to engage motor cortex for optimal task performance (Flament *et al.*, 1993) and examining more complex motor tasks are likely to increase the attentional demand required, which is known to influence plasticity induction in human motor cortex (see Ridding & Ziemann, 2010 for review).

In the present study, we will use TMS to examine how learning a complex visuomotor tracking task alters corticomotor plasticity, as assessed by changes in corticospinal excitability and intracortical inhibition in young and old adults. We have defined motor learning as the short-term (single session) acquisition of a visuomotor task resulting in improved motor performance beyond pre-existing levels (Muellbacher *et al.*, 2001; Rosenkranz *et al.*, 2007a). Given reduced practice-dependent motor cortex plasticity in older adults (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009), we hypothesise that corticomotor plasticity with learning of a complex visuomotor tracking task will be reduced with advancing age. Furthermore, numerous studies indicate that movement complexity influences motor performance. As motor performance declines with increasing task difficulty, particularly in older adults (Light & Spirduso, 1990;

Smith *et al.*, 1999a), we therefore predict that motor performance will be impaired in older adults during a complex visuomotor tracking task.

One advantage of TMS over other neuroimaging techniques is its ability to assess excitatory and inhibitory mechanisms in primary motor cortex (M1) circuits and their effects on corticospinal neurons. In particular, paired-pulse TMS can be used to assess $GABA_A$-mediated short-interval intracortical inhibition (SICI) in human motor cortex (Kujirai *et al.*, 1993). Following a period of motor training, several studies show reduced SICI for young adults (Liepert *et al.*, 1998; Garry *et al.*, 2004; Perez *et al.*, 2004; Gallasch *et al.*, 2009), with this change considered important in promoting cortical plasticity with training. However, assessment of SICI following simple ballistic movements in old adults show no change in SICI after training (Rogasch *et al.*, 2009; Cirillo *et al.*, 2010), although a reduction in SICI has recently been reported in young and old adults (Hinder *et al.*, 2011). Previous studies in older adults have not matched the test pulse to normalise SICI, which may confound quantification if, as expected, the MEP increases after training (Chen *et al.*, 1998; Roshan *et al.*, 2003). As such, we have matched the test pulse before and after training to assess SICI in young and old adults.

4.3. Materials and Methods

Experiments were performed on the right hand of sixteen young (9 women, 7 men; 23 ± 3 yrs; range 20-35 years) and sixteen older subjects (9 women, 7 men; 67 ± 5 yrs; range 60-75 years) with no known history of peripheral or neurological impairment. All subjects were right handed (Laterality Quotient (LQ); Young: median LQ = 0.83, range 0.5-1.0; Old: median LQ = 0.86, range 0.5-1.0) as assessed by the Edinburgh Handedness Questionnaire (Oldfield, 1971) and screened with the Mini-Mental State Examination (MMSE) (Folstein *et al.*, 1975) for any advanced signs of cognitive impairment. No subjects reported long term specialised use of the hands such as playing a musical instrument, as motor cortex excitability

and plasticity is enhanced in musicians (Rosenkranz *et al.*, 2007b), which may influence motor learning. All experiments were conducted in the afternoon. Subjects were also required to have a minimum visual capability (with corrective lenses if required) of 20/40 vision assessed using a Snellen Eye Chart. All subjects also completed a TMS safety screen questionnaire before participation (Rossi *et al.*, 2009). A number of older adults were identified with medical conditions that were being controlled with medication. The conditions involved abnormalities in reflux (two), blood pressure (five), cholesterol (four), inflammation associated with knee or shoulder arthritis (four), osteoporosis (two), and thyroxine (two). The prescribed drugs do not influence cortical excitability or suggest any involvement in influencing motor learning and medicated subjects were allowed to participate in the study. It is also important to note that arthritis was not present in the target hand and thus did not affect performance on the visuomotor task. In addition, subjects completed the long version of the International Physical Activity Questionnaire (IPAQ), consisting of 31 items describing the extent of leisure time physical activity involving aerobic exercises such as running, cycling and walking (Craig *et al.*, 2003; Fogelholm *et al.*, 2006). All subjects gave written informed consent prior to participation in the study, which was approved by the University of Adelaide Human Research Ethics Committee. All experiments we conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

4.3.1. Experimental arrangement

Subjects were seated comfortably with their right shoulder abducted approximately 45° to allow the hand and arm to rest on a manipulandum, with the forearm pronated and the palm facing down. A potentiometer was attached to the right index finger at the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles throughout the experiment using bipolar surface electrodes (Ag-AgCl, 8 mm diameter) placed ~2 cm apart on each muscle using a belly-tendon montage.

A grounding strap placed around the elbow was used as a common reference for all electrodes. The EMG signals were amplified (× 100-1000), bandpass filtered (high pass at 13 Hz, low pass at 1000 Hz), digitised online at 2 kHz with a CED interface system (Cambridge Electronic Design Co. LTD, UK) and recorded onto computer for offline analysis. The EMG signals of both muscles were displayed on an oscilloscope to assist the subject in maintaining EMG silence when required.

4.3.2. Experimental procedures

The timeline for the experimental procedures are shown in Figure 4.1. Maximum index finger abduction force was measured during maximum voluntary contractions (MVCs) performed at the beginning and end of each experiment. The hand was positioned with the palm facing down and the middle phalanx of the index finger placed alongside a load cell (LC1205-K020, A&D Mercury Pty Ltd, Australia) to facilitate measurement of index finger abduction force. Maximum index finger abduction force was exerted by the subject for three seconds against the force transducer with verbal encouragement provided by the experimenters. Several MVC trials were performed, with a minimum of 30-s rest between trials, until the peak force from two trials were within 10% of each other. The MVC with the largest index finger abduction force was used for the assessment of muscle strength. Visual feedback of index finger abduction force was displayed on an oscilloscope, and the subject was monitored in each trial to ensure that proximal limb muscles were not contributing to the force. Force signals were amplified (× 1000), digitised online (2 kHz) via a CED 1401 interface (Cambridge Electronic Design, UK) and stored on computer for offline analysis.

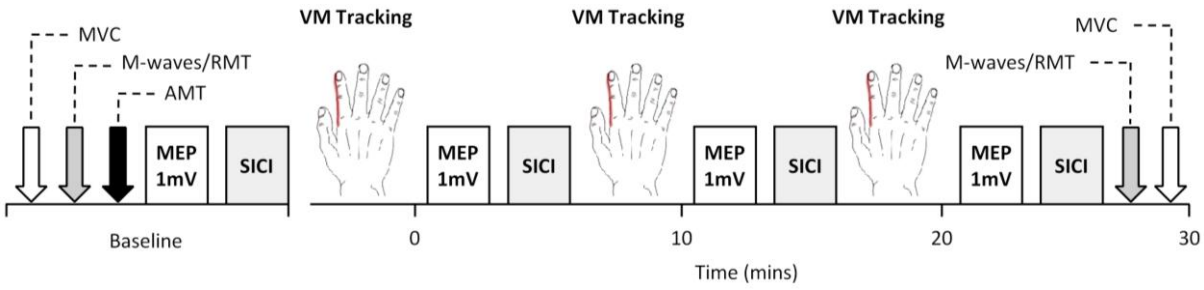

Figure 4.1 *Schematic representation of the experimental protocol with measures obtained before and after each visuomotor training block. Baseline measures include assessment of maximum voluntary contraction (MVC), maximal muscle compound action potential (M-wave), resting motor threshold (RMT), and active motor threshold (AMT). Ten single-pulse transcranial magnetic stimulation (TMS) trials which resulted in a motor evoked potential (MEP) of ~1mV before motor training were recorded. Short-interval intracortical inhibition (SICI) involved 20 single- and paired-pulse TMS trials (unconditioned MEP always ~1mV, conditioned MEP set to evoke 50% of unconditioned MEP before motor training). Motor training consisted of three six-minute blocks of a complex visuomotor tracking task that required matching the index finger metacarpophalangeal (MCP) joint angle to a target line using abduction-adduction movements for 18-unique 10-s frames repeated twice in each block.*

TMS was applied using a figure-of-eight coil (external wing diameter 90 mm) with two Magstim 200^2 magnetic stimulators connected with a Magstim Bistim Module (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. With this coil orientation, current flow within the cortex was induced in a posterior-anterior direction. The coil was placed at the optimal scalp position over the left hemisphere for eliciting a MEP in the relaxed right FDI muscle. The optimal scalp position was then marked on the scalp with an indelible ink pen for reference. TMS was delivered at 0.2 Hz for all conditions and optimal coil position was continually monitored by checking scalp position throughout the experiment.

Resting motor threshold (RMT) was determined as the minimum stimulus intensity required to elicit a MEP in the relaxed FDI of at least 50 μV in amplitude in 3 out of 5 consecutive trials. Active motor threshold (AMT) was defined as the minimum stimulus intensity required to elicit a MEP in the FDI muscle of at least 200 μV in amplitude in 3 out of 5 consecutive trials during a low-level voluntary index finger abduction (10% MVC) (Rothwell

et al., 1999). Both RMT and AMT are expressed relative to maximum stimulator output (MSO) and the stimulus intensity was altered in 1% increments of MSO throughout this process until the appropriate threshold level was achieved.

Test Intensity. The stimulus intensity that produced a MEP of approximately 1 mV in resting FDI was determined before training. Using this TMS intensity, ten trials were recorded to investigate resting MEP amplitude before and after each of three 6-minute blocks of visuomotor training (see below).

Input-Output curve. Five TMS intensities of 70, 90, 100, 110, and 130% of test intensity (~1 mV before training) were recorded for each subject at rest. A single input-output curve block consisted of 50 stimuli (10 stimuli at each intensity) with the order of presentation of the five conditions pseudorandomised throughout the trial. Responses from input-output curves were recorded before and immediately after completion of all visuomotor tracking tasks.

SICI. Short-interval intracortical inhibition (SICI) was assessed using a paired-pulse TMS paradigm consisting of a subthreshold conditioning stimulus that preceded a suprathreshold test stimulus by 3 ms (Kujirai *et al.*, 1993). The test stimulus intensity was set to produce a MEP of ~1 mV in amplitude in the resting FDI, whereas the intensity of the conditioning stimulus was set to produce approximately 50% inhibition of the MEP amplitude before training (Rosenkranz & Rothwell, 2006). The test stimulus intensity in paired-pulse trials was adjusted following each block of training, if required, so that test MEP amplitudes were equivalent before and after training (~1 mV). Each data block consisted of ten trials for each of two conditions; test stimulus alone and paired-pulse TMS with the conditioning pulse set to evoke 50% inhibition of the test stimulus (obtained before training), with the order of presentation randomised throughout the trials (20 trials total).

Maximal compound muscle action potential. Supramaximal electrical stimulation was administered to the ulnar nerve at the wrist using a constant current stimulator (DS7A, Digitimer, UK) and bipolar surface electrodes, separated by 20 mm, with the cathode placed proximally. Stimuli were square wave pulses with 100 μs pulse duration. Stimulator intensity was set at 120% of that required to elicit a maximal compound muscle action potential (M_{max}) from FDI. Five stimuli were delivered before and immediately after completion of all visuomotor tracking tasks. M_{max} was not obtained in 2 older subjects because the maximum stimulator output (100 mA in DS7A) was not sufficient to evoke a maximal response, presumably due to neuroanatomical variations in these individuals.

4.3.3. Training protocol

A visuomotor tracking task was used to examine use-dependent plasticity and motor skill learning, as described previously (Todd *et al.*, 2009). The task required subjects to match, as accurately as possible, MCP joint angle of the right index finger with a moving target on a computer screen (for example see Figure 4.2). The moving target consisted of 18 unique 10-s frames that moved automatically down the screen while making unpredictable left and right movements. Left movement of the target corresponded to abduction of the index finger and right movement of the target corresponded to adduction. The maximum MCP joint angle movement was ± 10° from neutral. Training consisted of three 6-minute blocks where each frame was repeated twice and the order of the frames was the same for each block. In order to avoid fatigue, there was a 4-minute rest period between each block (total training time was 26 mins). Visual feedback of the MCP joint angle relative to the target was provided to subjects along with continual verbal instructions to follow the moving target as closely as possible. Target and tracking lines were amplified (× 3000), digitised online at 2 kHz, and recorded onto computer for offline analysis.

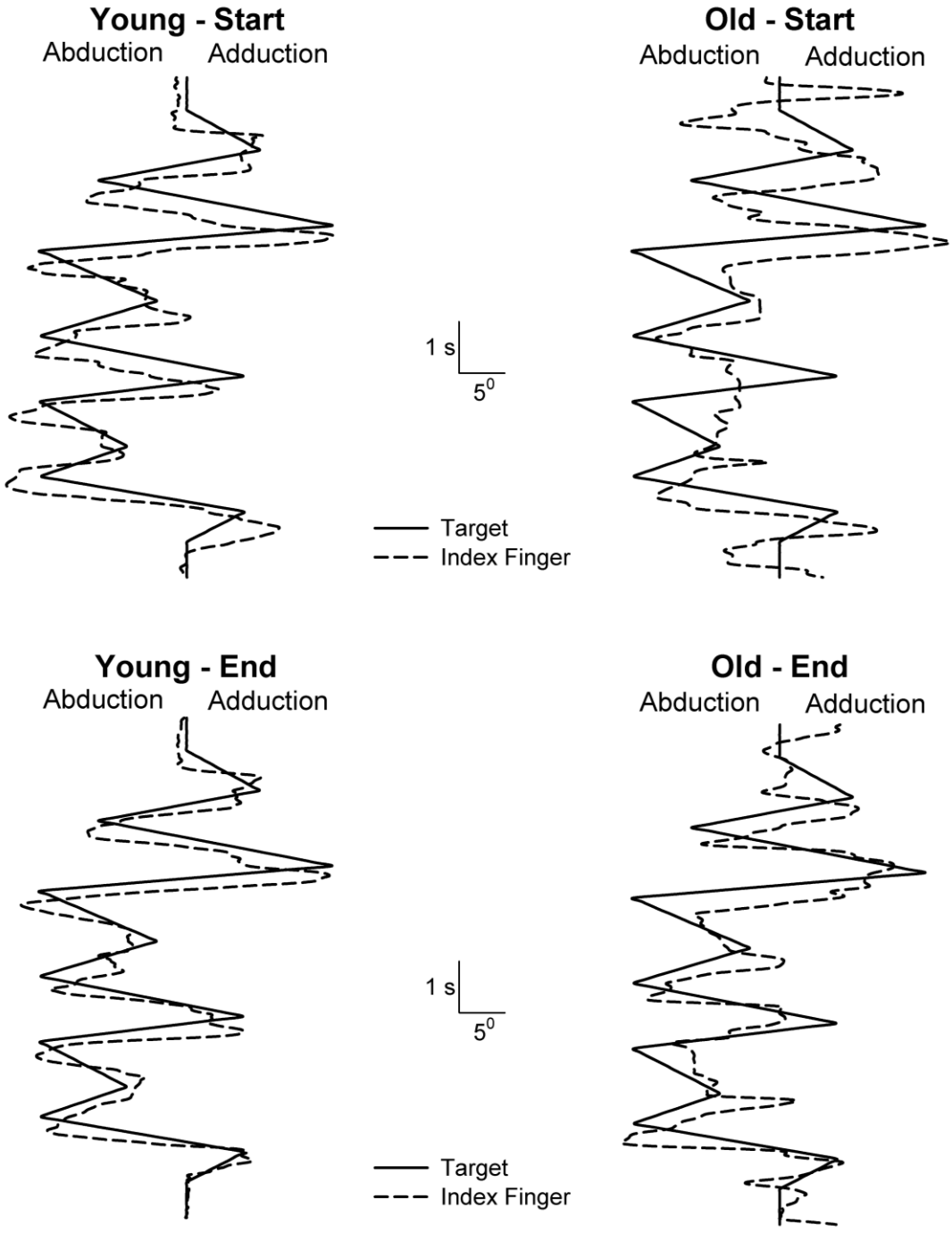

Figure 4.2 *Original recordings of visuomotor tracking for the same 10-s frame at the beginning (training block 1, upper panel) and the end (training block 3, lower panel) of motor learning from one young (left) and one old (right) adult. The solid line represents the target line and the dashed line is the position of the index finger. Movement to the left of the starting position (top of trace) is resultant from index finger abduction, whereas movement to the right results from adduction. Tracking error of the visuomotor task was greater in the old subject compared with the young subject at the beginning (35%) and end (39%) of motor training. However, there was a similar level of improvement in the task between the young (20%) and old (16%) adult.*

4.3.4. Data analysis

All MEP and M_{max} trials that contained pre-stimulus EMG activity (100 ms before stimulation) were discarded from the analysis, and repeated at the appropriate intensity following the data block. MEP and M_{max} amplitudes were measured peak-to-peak in each individual trial and averaged for each condition. For paired-pulse TMS, conditioned MEPs were expressed as a percentage of the mean unconditioned MEP in each block for quantification of SICI. Maximum force was calculated during the MVC and maximum FDI EMG was measured as the mean rectified EMG over 1 s (500 ms before and after peak force).

Performance during the visuomotor tracking task was assessed for each individual trial over the entire training block. For each block, the maximum cross-correlation coefficient and the lag time between the actual finger position and the target were calculated. Tracking error was calculated by subtracting the MCP joint angle (finger position) from the target line, with the mean absolute tracking error for each training block reported. Tracking error improvement was calculated by expressing the mean tracking error in each block relative to the mean tracking error obtained in the first minute of block 1 (6 trials).

4.3.5. Statistical analysis

An unpaired *t*-test was used to analyse subject characteristics, maximum index finger abduction force, maximum FDI EMG, M_{max} amplitude, RMT, AMT, test TMS intensity before training, conditioning stimulus for SICI, physical activity (IPAQ), and cognitive mental state (MMSE) between young and old adults. A Mann-Whitney U test was used to compare non-parametric handedness scores between young and old adults. Two-way repeated measures ANOVA was used to examine the effect of age (young, old; between-subject factor) and time (baseline, 6 mins, 18 mins, 24 mins; within-subject factor) on maximum index finger abduction force, maximum FDI EMG, M-wave amplitude, RMT, test intensity, test MEP amplitude, SICI, visuomotor tracking error, maximum cross-correlation

coefficient, and lag time. Three-way repeated measures ANOVA was used to analyse the effect of age (young, old; between-subject factor), time (baseline, 6 mins, 18 mins, 24 mins; within-subject factor), and TMS intensity (70%, 90%, 100%, 110%, 130% RMT; within-subject factor) on MEP amplitude during the input-output curve. A Fisher's LSD post-hoc test that performed all possible comparisons was used to analyse significant main effects and interactions. The significance level was set at $P < 0.05$ for all comparisons and all group data are provided as mean ± standard error of the mean (SEM).

4.4. Results

Age-related differences in subject characteristics before training are displayed in Table 4.1. There was no significant difference between young and old adults in maximum index finger abduction force or FDI EMG during MVC, and TMS thresholds before training. However, older adults had reduced M_{max} amplitude compared with young adults (Table 4.1). In addition, there were no differences in laterality, cognitive mental state, and physical activity between young and old adults (see Table 4.1).

4.4.1. Age-related differences in motor performance following visuomotor tracking

Figure 4.2 shows original recordings of visuomotor tracking for the same 10-s frame at the beginning (training block 1) and the end (training block 3) of motor learning from one young and one older adult. Performance of visuomotor tracking was diminished in the older adult compared with the young adult at the beginning and end of motor training for error (Block 1: Young = 3.03°, Old = 4.64°; Block 3: Young = 2.41°; Old = 3.92°) and maximum cross-correlation coefficient (ρ, Block 1: Young, 0.94; Old, 0.64; Block 3: Young, 0.94; Old, 0.70). Despite the difference in visuomotor tracking performance, there was a similar level of improvement in the task for the young (20%) and old (16%) adult.

Table 4.1 *Group data before visuomotor tracking of young and old adults.*

	Young	Old
Handedness (LQ)	0.83 (0.18)	0.86 (0.19)
Cognitive Mental State (MMSE)	29.6 (0.8)	29.5 (0.6)
Physical Activity (IPAQ)	4971 (4042)	4008 (3978)
MVC (N)	26.0 (8.1)	25.3 (6.5)
Maximum EMG (mV)	0.50 (0.19)	0.42 (0.17)
MMSE (Total of 30)	29.6 (0.2)	29.5 (0.2)
RMT (% MSO)	41.6 (10.0)	47.4 (8.2)
AMT (% MSO)	33.7 (8.4)	38.6 (7.0)
Test Intensity (% MSO)	53.3 (13.5)	61.8 (14.5)
CS Intensity (% MSO)	26.3 (1.5)	28.1 (1.3)
M-Wave (mV)	15.3 (3.6)	11.8 (5.1)*

Values are mean (SD). *LQ*, laterality quotient; *MMSE*, mini-mental state examination; *IPAQ*, international physical activity questionnaire; *MVC*, FDI maximum voluntary contraction; *EMG*, FDI electromyography; *MMSE*, mini mental state examination; *RMT*, FDI resting motor threshold; *AMT*, FDI active motor threshold; CS, conditioning stimulus required for short-interval intracortical inhibition; *MSO*, maximum stimulator output. M-Wave data obtained from 16 young and 14 older adults. * $P < 0.05$ compared with young adults.

The group data for performance and improvement of the visuomotor tracking task for young and old adults is shown in Figure 4.3. Performance of visuomotor tracking assessed by tracking error (Figure 4.3A) was better in young compared with older adults (Age Effect, $F_{1,30} = 29.81$, $P < 0.01$) and improved across training blocks (Time effect, $F_{2,60} = 121.65$, $P < 0.01$). A significant Age x Time interaction ($F_{2,60} = 3.37$, $P = 0.04$) showed a significant reduction in error between training blocks 1 and 2 ($P = 0.02$), and 1 and 3 ($P < 0.01$) for older adults, but only between blocks 1 and 3 ($P = 0.02$) for young adults. To quantify motor learning, visuomotor tracking error was normalised to the first minute of Block 1 (Figure 4.3B). A two-way repeated measures ANOVA indicated that normalised tracking error improved for each subsequent training block (Time effect, $F_{2,60} = 159.34$, $P < 0.001$). Despite diminished tracking error performance in old compared with young adults, the improvement in tracking

error was similar for both young and old adults (Age effect, $F_{1,30} = 0.31$, $P = 0.86$), and ranged from 7% improvement after the first block to 24% improvement after the third block. A two-way repeated measures ANOVA was performed on the maximum cross-correlation coefficient between the target line and index finger position as a reflection of tracking accuracy (Figure 4.3C). Maximum cross-correlation coefficient was greater in young compared with older adults (Age effect, $F_{1,30} = 31.28$, $P < 0.01$) and improved for each training block (Time effect, $F_{2,60} = 75.36$, $P < 0.01$). A significant Age x Time interaction ($F_{2,60} = 34.02$, $P < 0.01$) revealed that the maximum cross-correlation coefficient was greater for young compared with older adults for all training blocks (all interactions $P < 0.01$) and that maximum cross-correlation coefficient did not significantly change between training blocks for young adults (all interactions $P > 0.54$), but significantly increased between training blocks 1 and 2 ($P = 0.02$), and 1 and 3 ($P < 0.01$), but not 2 and 3 ($P = 0.58$) for older adults. The lag time to achieve maximum cross-correlation coefficient (data not shown) was significantly reduced between training blocks (Block 1 = 250 ± 9 ms, Block 2 = 203 ± 9 ms, Block 3 = 186 ± 8 ms; Time effect, $F_{2,60} = 48.99$, $P < 0.01$), but was similar between young and older adults (Age effect, $F_{1,30} < 0.01$, $P = 0.98$).

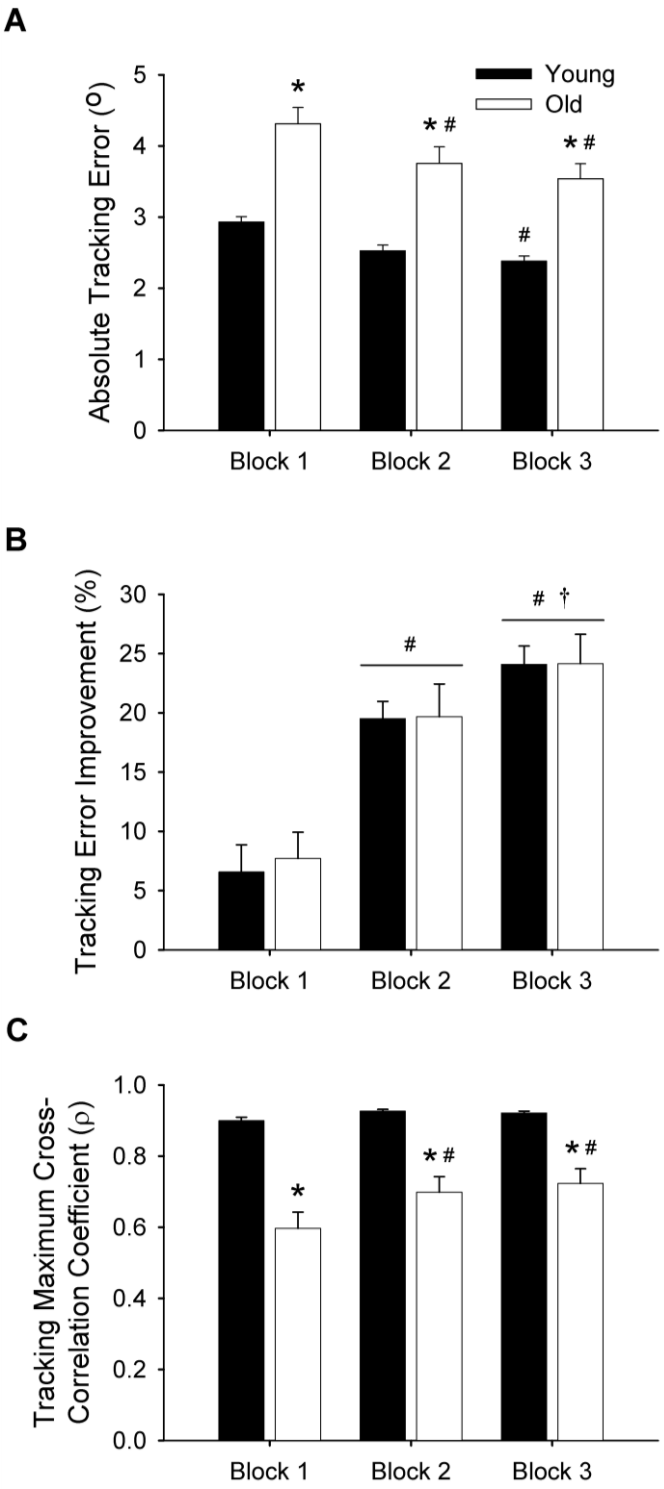

Figure 4.3 *Group changes in visuomotor tracking error for all three blocks of visuomotor training (A), tracking error improvement compared with the first minute of block 1 (B), and maximum cross-correlation coefficient for all three blocks of visuomotor training (C) in young and old adults. * $P < 0.05$ compared with young adults. # $P < 0.05$ compared with Block 1. † $P < 0.001$ compared with training Block 2.*

4.4.2. Age-related differences in corticomotor excitability following visuomotor tracking

RMT was not significantly different between young and old adults (Age effect, $F_{1,30} = 3.21$, $P = 0.08$) and did not change after visuomotor training (Time effect, $F_{1,30} = 1.65$, $P = 0.21$). Furthermore, M_{max} amplitude did not change after training ($F_{1,28} = 2.42$, $P = 0.13$).

The effect of visuomotor training on input-output curves in relaxed FDI of young and older subjects is shown in Figure 4.4. A three-way repeated measures ANOVA indicated that there was an increase in the size of the FDI MEP amplitude with increasing stimulus intensity for all subjects (Intensity effect, $F_{4,120} = 96.06$, $P < 0.01$), and MEP amplitude was greater following training (Time effect, $F_{1,30} = 9.71$, $P < 0.01$), but there were no significant differences in MEP amplitude between young and older adults (Age effect, $F_{1,30} = 0.25$, $P = 0.62$). There was a significant Intensity x Time interaction ($F_{4,120} = 6.53$, $P < 0.01$) which showed that FDI MEP amplitude was greater at the highest TMS intensity (130% of ~1mV before training) after visuomotor training in young and old adults ($P < 0.01$).

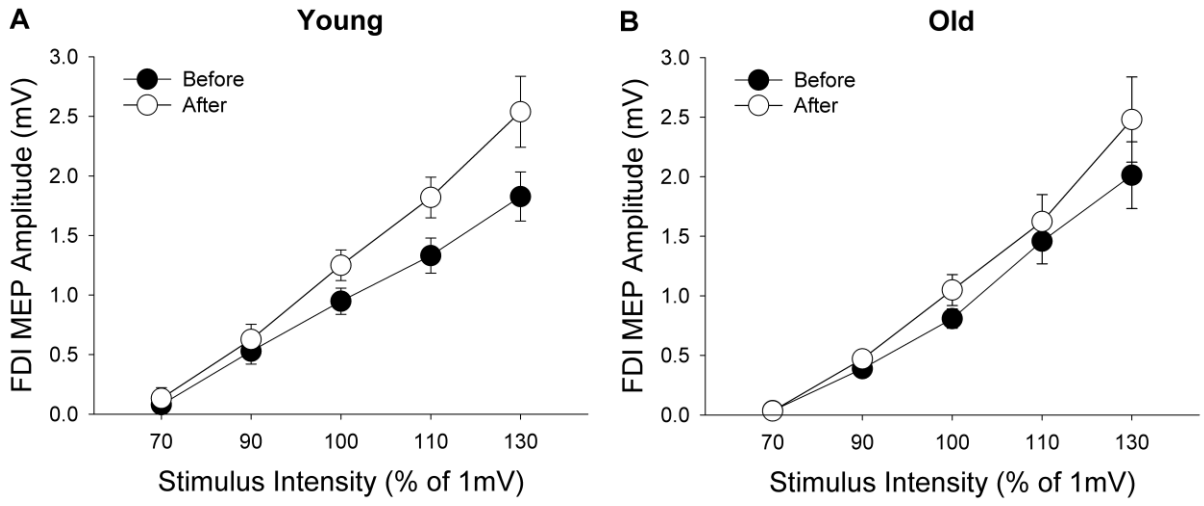

Figure 4.4 *Group data showing mean MEP amplitudes before and after visuomotor training in the relaxed FDI muscle of young (A) and old (B) adults. MEP amplitude assessed at the highest TMS intensity (130% of ~1mV before training) was increased after training in young and old adults combined ($P < 0.01$).*

Figure 4.5 shows group data for changes in test MEP amplitude and extent of SICI before and after each block of visuomotor tracking. The TMS intensity used to evoke a ~1 mV MEP in resting FDI was similar between young and older adults before motor training ($F_{1,30}$ = 2.95, P = 0.10, Table 4.1), and this intensity was used to quantify the change in MEP response after motor training. A two-way repeated measures ANOVA showed that FDI MEP amplitude increased after visuomotor tracking in young and old adults (Figure 4.5A), with increases of 25% after training block 1 (P < 0.01), 21% after training block 2 (P < 0.02), and 25% after training block 3 (P < 0.01) compared with before visuomotor tracking (Time effect, $F_{3,90}$ = 3.93, P = 0.01). However, there was no significant difference in FDI MEP amplitude between young and old adults (Age effect, $F_{1,30}$ = 0.16, P = 0.69) and no Age x Time interaction ($F_{3,90}$ = 0.87, P = 0.46).

To assess SICI, the intensity of the test pulse was adjusted after each training block to maintain a constant test MEP amplitude of ~1 mV in resting FDI (Figure 4.5B). For all subjects, the test TMS intensity was reduced over time (P = 0.01), with a difference in test TMS intensity after Block 1 (57.4 ± 2.6% MSO) compared with before training (58.3 ± 2.6% MSO). There was no difference between young and old adults (Age effect, P = 0.09) or Age x Time interaction (P = 0.27) for the adjusted TMS intensity. The adjusted test MEP amplitude did not differ between young and old adults (Age effect, $F_{1,30}$ = 0.13, P = 0.72) nor across training blocks (Time effect, $F_{3,90}$ = 0.48, P = 0.70). When the test MEP remained constant, a two-way repeated measures ANOVA showed that the magnitude of SICI was reduced after visuomotor tracking in young and old adults (Figure 4.5C), with a 30% versus 21% reduction after training block 1 (P < 0.01), a 38% versus 20% reduction after training block 2 (P < 0.01), and a 34% versus 7% reduction after training block 3 (P < 0.05), for young and old adults, respectively, compared with before visuomotor tracking (Time effect, $F_{3,90}$ = 3.73, P = 0.01). However, there was no significant difference in the extent of SICI between

young and old adults (Age effect, $F_{1,30} = 0.65$, $P = 0.43$), and no Age x Time interaction ($F_{3,90} = 0.62$, $P = 0.60$).

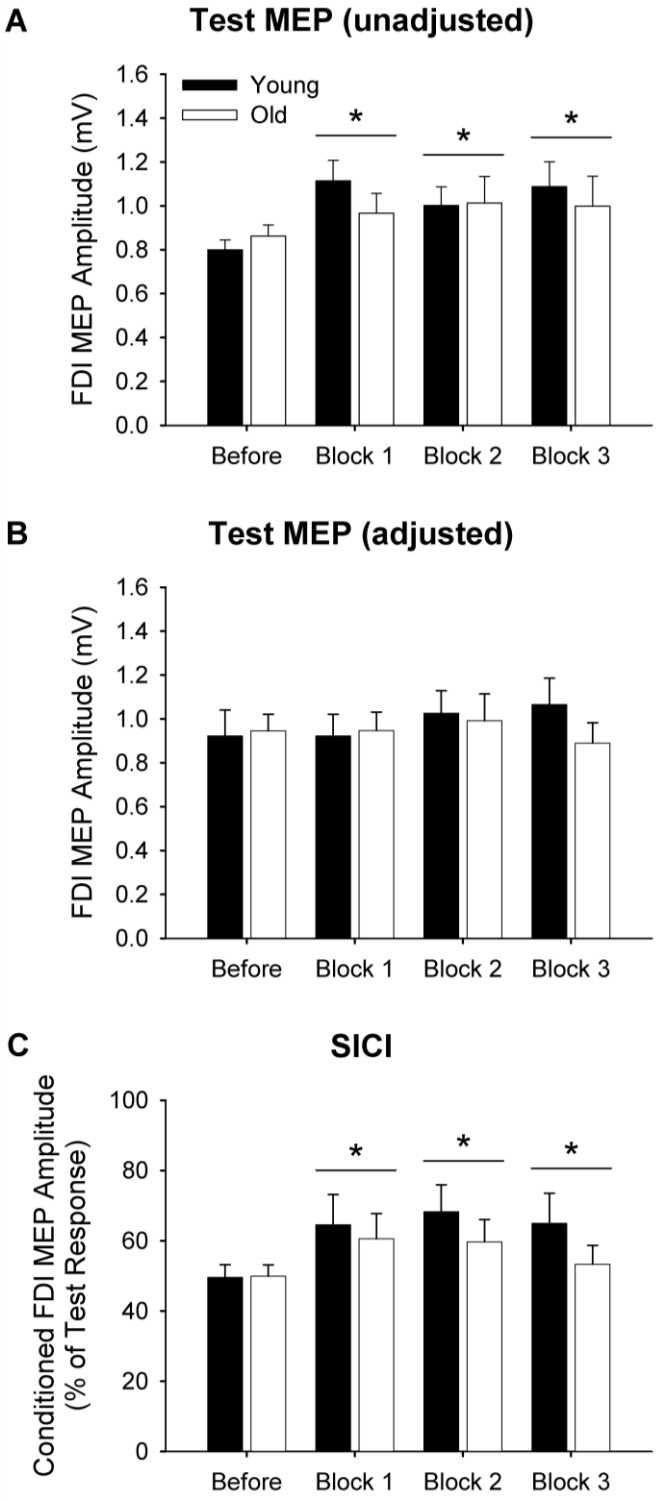

Figure 4.5 *Group data showing mean MEP amplitudes unadjusted (A), MEP amplitudes adjusted to evoke a test response of ~1 mV (B), and extent of SICI (C) before and after each training block in the relaxed FDI muscle of young and old adults. MEP amplitude when unadjusted increased after visuomotor tracking and the extent of SICI was reduced with*

*adjusted test TMS intensity following each training block. * P < 0.05 compared with before training.*

Using linear regression of data from individual subjects, we examined whether the training-related changes in plasticity (MEP amplitude and SICI) were associated with the extent of motor learning (improvement in tracking error). There was no significant association between the change in MEP amplitude and motor learning for young (block 1, $r^2 < 0.01$, P = 0.78; block 2, $r^2 < 0.01$, P = 0.89; block 3, $r^2 = 0.07$, P = 0.32) or old (block 1, $r^2 = 0.12$, P = 0.10; block 2, $r^2 = 0.03$, P = 0.55; block 3, $r^2 = 0.05$, P = 0.40) adults. There was also no significant association between the change in SICI and motor learning for young (block 1, $r^2 = 0.05$, P = 0.43; block 2, $r^2 < 0.01$, P = 0.95; block 3, $r^2 < 0.01$, P = 0.74) or old (block 1, $r^2 = 0.02$, P = 0.16; block 2, $r^2 < 0.01$, P = 0.73; block 3, $r^2 = 0.11$, P = 0.40) subjects.

4.5. Discussion

The purpose of this study was to examine the change in corticomotor excitability and intracortical inhibition, as an index of corticomotor plasticity, following complex visuomotor tracking in young and old adults. There were several important findings in this study. First, the ability of older adults to perform the visuomotor tracking task with the index finger was reduced compared with young adults. Second, the extent of motor learning was not different between young and old adults following complex visuomotor tracking. Third, the extent of the change in MEP amplitude and SICI in a hand muscle (FDI) were not different between young and old adults. Lastly, there was no association between the change in MEP amplitude and SICI (thought to reflect corticomotor plasticity) and the magnitude of motor learning for the visuomotor tracking task in young or old adults.

4.5.1. Reduced motor performance in old adults

In support of other previous studies using complex motor tasks (Light & Spirduso, 1990; Smith *et al.*, 1999a), we find that visuomotor tracking is performed with reduced precision

and accuracy in old compared with young adults. Alterations in the central and peripheral nervous systems are thought to be responsible for these age-related changes in motor function. Recent studies on the CNS have associated an age-related decline in motor function with reduced brain volume and decreased cerebral gray and white matter (Courchesne *et al.*, 2000; Jernigan *et al.*, 2001). Neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have also shown that more complex motor tasks require activation of not only motor cortex, but also other cortical and subcortical regions (Rao *et al.*, 1993; Wexler *et al.*, 1997). Additionally, a decline in vision in older adults may contribute to decreased motor performance. Although visual acuity (assessed with corrective lenses if required) was similar between young and older adults, it is possible that deficits are present in other visual processing areas involving the visual cortex (Ofori *et al.*, 2010). Although previous studies indicate that older adults can demonstrate compensatory changes in response to age-related changes in motor function, these putative changes do not appear to compensate for impairments in the performance of a complex visuomotor tracking task in older adults.

4.5.2. Motor skill learning in young and old adults with visuomotor tracking

In a neurophysiological context, we have defined motor learning as the short-term (single session) acquisition of the task resulting in new movement strategies that enhance performance beyond pre-existing levels, which was quantified as a within-session change in motor performance (e.g. Muellbacher *et al.*, 2001; Rosenkranz *et al.*, 2007a). Previous studies have indicated that differences in motor learning between young and older adults become more pronounced with increasing task difficulty (Light & Spirduso, 1990; Smith *et al.*, 1999a). However, other studies using either simple or complex motor tasks show that short-term motor learning can remain similar between young and older adults (see Voelcker-Rehage, 2008; Brown *et al.*, 2009; Cirillo *et al.*, 2010). Despite diminished performance of the motor task in older adults in the present study, we found that the extent of learning with the visuomotor tracking task was similar between young and old adults. One caveat to this

interpretation is that performance increases in the young subjects may have been influenced by a ceiling effect, where further increases in performance may have been limited by the maximum capabilities of the neuromuscular system. For example, young subjects began training with tracking-target cross-correlation values of ~0.9 (compared with ~0.6 in older adults), which limits the potential for improvements in performance with training in young subjects. Similarly, the magnitude of tracking error was reduced at the start of training in young compared with old subjects, with improvements in performance decreasing tracking error toward zero, but it is not possible to achieve a perfect match due to the non-physiological tracking template provided to the subjects (involving a series of interconnected straight lines). Although we found no age-related differences in motor learning in the present study, we suggest that it is not possible to make appropriate conclusions about age-related changes in motor learning unless both subject groups begin the task with similar performance levels. As motor performance generally declines with advancing age (Grabiner & Enoka, 1995), the selection of an appropriate task to address this question remains problematic, particularly when examining the magnitude of motor learning with complex motor tasks that are likely to accentuate baseline differences in motor performance in young and old adults.

4.5.3. Change in MEP amplitude after visuomotor tracking in young and old adults

An increase in MEP amplitude of the target muscle for up to one hour following a simple motor-training intervention is thought to reflect use-dependent plasticity (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001), via LTP-like mechanisms (Bütefisch *et al.*, 2000; Muellbacher *et al.*, 2002; Sawaki *et al.*, 2002) in cortical circuits (Classen *et al.*, 1998; Muellbacher *et al.*, 2002; Meintzschel & Ziemann, 2006). A similar increase in MEP amplitude has been observed following visuomotor tracking in young subjects (Perez *et al.*, 2004; Todd *et al.*, 2009), with these complex tasks expected to rely more heavily on processing within cortical circuits (e.g. Flament *et al.*, 1993) and demand more attentional focus for accurate performance (McNevin *et al.*, 2000) compared with simple tasks. However, the capacity for

motor cortex reorganisation and plasticity is believed to decrease with advancing age. Several previous TMS studies involving experimentally-induced plasticity (Müller-Dahlhaus *et al.*, 2008; Tecchio *et al.*, 2008; Fathi *et al.*, 2010; Todd *et al.*, 2010; Freitas *et al.*, 2011) and simple motor training interventions (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009) support an age-related reduction in motor cortex plasticity, evidenced by a reduced change in MEP amplitude. However, this finding is not always consistent because we have observed a similar magnitude of MEP change, and thus use-dependent plasticity, in young and older adults after simple ballistic thumb abduction (Cirillo *et al.*, 2010) and after more complex visuomotor tracking (current study).

There are at least three possibilities that could help explain the similar magnitude of MEP change in young and old adults observed in the present study. First, the increased task complexity and attentional demand may require increased activation of corticospinal neurons that have direct projections to motor neurons in older adults, with the performance of more simple tasks relegated to less direct pathways that are not amenable to study with TMS. The CNS has the capacity to change the balance of the descending command between direct and indirect pathways depending on the requirements of the task, with direct recording of neurons in motor cortex providing several examples of corticospinal neurons that are more active in tasks requiring precise voluntary control of muscle activation than less skilled tasks (Cheney & Fetz, 1980; Muir & Lemon, 1983). Second, it is possible that factors specific to the subject population may have contributed to the similar magnitude of MEP change in young and old adults, such as the amount of physical activity performed (Cirillo *et al.*, 2009), extent of skilled hand use (Rosenkranz *et al.*, 2007b), prior history of synaptic activity (see Ridding & Ziemann, 2010), attentional focus (McNevin *et al.*, 2000) and emotional state of the subjects (Tormos *et al.*, 1997). All subjects reported no specialised use of their hands, such as playing a musical instrument, or displayed advanced signs of cognitive impairment with similar MMSE scores for young and old adults. However, physical activity levels generally decline

with advancing age (Ravussin & Bogardus, 1989), but there was no difference in self reported physical activity levels between young and old adults in the present study, which potentially contributes to the maintenance of use-dependent plasticity in the older adults (see Cotman & Berchtold, 2002). Finally, it is now known that genetic factors make substantial contributions to the motor system plasticity response (Missitzi *et al.*, 2011). Several studies have shown that a single nucleotide polymorphism in the brain derived neurotrophic factor gene, which is present in ~30% of healthy people (see Bath & Lee, 2006), has recently been linked with different capabilities for motor learning and plasticity within motor cortex (Kleim *et al.*, 2006; Cheeran *et al.*, 2008; Fritsch *et al.*, 2010; McHughen *et al.*, 2010). The possibility therefore exists that these genetic factors may be misrepresented in either the young or older population tested in the present study, contributing to the similar use-dependent plasticity.

Based on the experimental design, a number of technical features may have potentially contributed to our findings. For example, it is possible that the TMS parameters used between training blocks (0.2 Hz, n=30 pulses) may have influenced cortical excitability and motor learning in young or old adults. However, several lines of evidence suggest that this is unlikely. For instance, the number and frequency of TMS are too low to produce any changes in motor cortex excitability (Chen *et al.*, 1997; see Hoogendam *et al.*, 2010), and most studies have shown that TMS-induced motor cortex plasticity is reduced in older adults (Müller-Dahlhaus *et al.*, 2008; Tecchio *et al.*, 2008; Todd *et al.*, 2010), suggesting that any potential effect of TMS between blocks on motor cortex excitability and motor learning would be less in older adults. Furthermore, the greatest improvements in motor performance in young and old adults occurred *within* the first training block (data not shown), with no improvement in performance *within* the second and third training blocks. An additional factor that could influence our findings in young and old adults is a difference in the within-subject MEP variability. We chose to use 10 TMS pulses in order to minimise the number of stimuli given between training blocks, and for consistency between each measure of corticospinal

excitability (test MEP, MEP input-output curve, SICI). The within-subject MEP variability (measured by the coefficient of variation for the test response) was similar in young and old adults, suggesting that the number of TMS pulses, or their variability, did not influence the findings. Finally, it is well known that healthy ageing is associated with a loss of muscle mass and a decrease in the number of motor neurons (see Doherty *et al.*, 1993 for review). This results in a 20-30% reduction in M-wave amplitude in small hand muscles of older adults (Sale & Semmler, 2005; Rogasch *et al.*, 2009; Cirillo *et al.*, 2010), which was observed in the present study. This suggests that the 1 mV test response in young and old adults is likely to activate a larger proportion of motor neurons (due to a smaller M-Wave) in the older subjects. Although it is not known how this may influence the present findings, the ability to activate corticospinal neurons was similar in young and old adults, as shown by no age-related difference in resting or active motor threshold (Table 4.1), and MEP input-output curves (Figure 4.4). Furthermore, when we normalised the MEP data to maximum M-wave in young and old adults it did not change the statistical interpretation of the data (other than creating the expected Age effect), which supports findings from our previous study (Rogasch *et al.*, 2009).

4.5.4. SICI in young and old adults after complex visuomotor tracking

Modulation of SICI is important in selective hand muscle activation (Stinear & Byblow, 2003; Zoghi *et al.*, 2003), suggesting that the modulation of intracortical inhibition is crucial for accurate motor performance. However, the contribution of SICI to the reduced motor performance in older adults is unresolved. Studies have showed increased (Kossev *et al.*, 2002; McGinley *et al.*, 2010) or decreased SICI (Peinemann *et al.*, 2001) in older adults, or no age-related difference (Wassermann, 2002; Oliviero *et al.*, 2006; Rogasch *et al.*, 2009; Cirillo *et al.*, 2010). These studies have typically quantified the magnitude of SICI by using a set conditioning intensity relative to motor threshold (resting or active) in each subject. In contrast, we used a conditioning intensity that produced ~50% inhibition in all subjects at

baseline, which does not rely on measures of resting or active threshold. Using this protocol, we provide further evidence showing no difference in baseline SICI between young and old adults, as the conditioning intensity required to produce 50% inhibition was similar in young and old adults (Table 4.1). Thus, it is unlikely that the diminished visuomotor tracking performance at the start of training in old adults is due to alterations in $GABA_A$-mediated intracortical inhibition.

Many studies report a reduction in SICI after learning of simple and complex motor tasks in young subjects (Liepert *et al.*, 1998; Garry *et al.*, 2004; Perez *et al.*, 2004; Gallasch *et al.*, 2009), suggesting that the removal of intracortical inhibition is an important substrate for optimal motor learning and motor cortex plasticity (Ziemann *et al.*, 2001). However, it is not yet clear whether the ageing process maintains the relevant physiological mechanisms to modulate intracortical inhibition to maximise motor learning. We have recently reported no change in SICI in older adults after the performance of simple ballistic tasks (Rogasch *et al.*, 2009; Cirillo *et al.*, 2010), but the lack of change with training in the young control groups suggested that the task used may not be optimal to induce SICI modulation with training. Furthermore, we used a constant test TMS intensity before and after training to assess SICI in young and old adults, which may have confounded the estimate of SICI if, as expected, there is an increased MEP after motor skill learning (see Chen *et al.*, 1998; Roshan *et al.*, 2003). When we adjusted the test TMS intensity after visuomotor tracking in the present study, we found a reduction in SICI after training, but there was no difference between young and old adults (Figure 4.4). This finding supports recent work in young and old adults showing reduced SICI after simple ballistic abduction training of the index finger (Hinder *et al.*, 2011), although this study used a single conditioning intensity (70% of resting threshold) and a test intensity that was not adjusted after training, and there was a tendency for reduced SICI in older adults at baseline. Nonetheless, the similar modulation of SICI after simple ballistic (Hinder *et al.*, 2011) and complex visuomotor tasks (present study) in young and old adults

suggests that older adults maintain the capacity to modulate GABAergic intracortical inhibition following motor skill training.

4.5.5. Association between corticomotor plasticity and motor learning

Despite the commonly accepted role of an increased MEP amplitude and decreased SICI in the plasticity response that usually accompanies motor skill learning, on an individual subject level these plastic changes within the motor cortex are not typically associated with the magnitude of the behavioural improvement (e.g. Li Voti *et al.*, 2011; see also Classen & Cohen, 2003). We have shown in a previous study that the change in MEP and SICI with training in individual subjects was not associated with the extent of motor learning during a simple ballistic thumb movement in young or old adults (Rogasch *et al.*, 2009). When using a more complex visuomotor tracking task in the present study, we also found no association between the magnitude of change in MEP and SICI and the improvement in visuomotor tracking performance. This lack of association is likely due to many factors influencing the plasticity response that may not influence motor learning, such as the attentional demand required for the task, the history of prior synaptic activity, and genetic effects (see Ridding & Ziemann, 2010 for review). Furthermore, it is important to note that the change in MEP and SICI are measured at rest after the task has been performed, and it is possible that the MEP response varies with time, and could be contraction-dependent or task-specific. Nonetheless, it is likely that measures other than basic motor performance as assessed in the present study are more important for the plasticity response when measured with TMS after motor learning.

In conclusion, previous studies with TMS have shown that advancing age is associated with diminished use-dependent plasticity during simple motor tasks. We examined use-dependent plasticity and motor learning in young and old adults during a complex visuomotor task that is expected to have an increased attentional demand and skill requirement compared with a simple motor task. Despite an age-related decline in performance of visuomotor tracking, we

found that the extent of corticomotor plasticity (change in MEP and SICI) and motor learning (short-term task improvement) was similar between young and old adults, which was contrary to expectations. Further studies are needed to identify whether increased task-complexity is responsible for the similar use-dependent plasticity observed in young and old adults in the present study, or whether specific features of the older subject population (e.g. physical activity levels, attentional focus, prior history of activity) may have contributed to the findings.

CHAPTER V

DIFFERENTIAL MODULATION OF MOTOR CORTEX EXCITABILITY IN *BDNF* MET ALLELE CARRIERS FOLLOWING EXPERIMENTALLY-INDUCED AND USE-DEPENDENT PLASTICITY

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Experimental design, subject recruitment, data collection and analysis on all samples, interpretation of data, and wrote manuscript.

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Verified experimental design, supervised development of work, helped with data interpretation, critical manuscript evaluation, and acted as corresponding author.

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Signed ..... *Date* 2/4/12

5. Differential modulation of motor cortex excitability in BDNF met allele carriers following experimentally-induced and use-dependent plasticity

5.1. Abstract

The purpose of this study was to investigate how people with one of three variants of the brain-derived neurotrophic factor (*BDNF*) gene modulate motor cortex excitability following experimentally-induced and use-dependent plasticity interventions. Electromyographic recordings were obtained from the right first dorsal interosseous (FDI) muscle of 12 Val/Val, 10 Val/Met, and 7 Met/Met genotypes (aged 18-39 years). Transcranial magnetic stimulation (TMS) of the left hemisphere was used to assess changes in FDI motor-evoked potentials (MEPs) following three separate interventions involving paired associative stimulation, a simple ballistic task, and complex visuomotor tracking task using the index finger. Val/Val subjects increased FDI MEPs following all interventions ($\geq$ 25%, $P < 0.01$), whereas the Met allele carriers only showed increased MEPs after the simple motor task ($\geq$ 26%, $P < 0.01$). In contrast to the simple motor task, there was no significant change in MEPs for the Val/Met subjects (7%, $P = 0.50$) and a reduction in MEPs for the Met/Met group (-38%, $P < 0.01$) following the complex motor task. Despite these differences in use-dependent plasticity, the performance of both motor tasks was not different between *BDNF* genotypes. We conclude that modulation of motor cortex excitability is strongly influenced by the *BDNF* polymorphism, with the greatest differences observed for the complex motor task. We also found unique motor cortex plasticity in the rarest form of the BDNF polymorphism (Met/Met subjects), which may have implications for functional recovery after disease or injury to the nervous system in these individuals.

5.2. Introduction

Brain-derived neurotrophic factor (BDNF) is a growth factor that is highly expressed throughout the central nervous system (CNS: Pearson-Fuhrhop *et al.*, 2009). BDNF is crucial

for development but is also important in adulthood, by facilitating long-term potentiation (LTP) and mediating use-dependent plasticity (Schinder & Poo, 2000; Gottmann *et al.*, 2009). In humans, a single nucleotide polymorphism of the *BDNF* gene (*BDNF* Val66Met) results in reduced BDNF release in cortical neurons (Egan *et al.*, 2003), which has been associated with altered cortical morphology (Pezawas *et al.*, 2004) and behavioural deficits primarily related to hippocampal functions (Egan *et al.*, 2003). Furthermore, the Met allele has been associated with altered susceptibility to some neurological and psychiatric disorders (Bath & Lee, 2006), and may influence CNS repair and functionally beneficial neuroplasticity after neurological injury (Pearson-Fuhrhop *et al.*, 2009). The *BDNF* polymorphism is relatively common (30-50% in the general population; Bath & Lee, 2006), indicating that any physiological differences might be used to guide features of therapy following neurological injury in these individuals.

Motor skill learning and recovery from brain injury requires plasticity in many areas of the brain, including primary motor cortex (M1: Sanes & Donoghue, 2000). At a systems level, recent studies with transcranial magnetic stimulation (TMS) have shown reduced experimentally-induced and use-dependent M1 plasticity in people with the BDNF polymorphism (Kleim *et al.*, 2006; Cheeran *et al.*, 2008). Similar observations were found using neuroimaging techniques, with a greater reduction of brain activation volume in the Met allele carriers after index finger training (McHughen *et al.*, 2010). However, the impact on M1 plasticity is not always consistent, with some protocols failing to reveal differences in M1 plasticity in people with different *BDNF* genotypes (Li Voti *et al.*, 2011; Nakamura *et al.*, 2011; Witte *et al.*, 2012). We expect that these inconsistent effects are related to important inter-study differences in the intervention used and its interaction with BDNF to induce M1 plasticity.

The purpose of this study was to examine experimentally-induced and use-dependent plasticity in people with different *BDNF* genotypes. Three interventions of similar duration (12-16 mins) were used, as BDNF effects are activity-dependent and require at least 6 minutes of neuronal discharge for BDNF to be released and to modify cellular function (Poo, 2001; Balkowiec & Katz, 2002; Tanaka *et al.*, 2008). Experimentally-induced plasticity was performed with paired associative stimulation (PAS), as it shares many physiological properties of synaptic plasticity in animal preparations (Ziemann *et al.*, 2008), with the PAS response shown previously to be influenced by *BDNF* genotype (Cheeran *et al.*, 2008; Missitzi *et al.*, 2011). Use-dependent plasticity was assessed using two motor tasks; simple ballistic movement and complex visuomotor tracking. These tasks were chosen because they differ in the amount of skill required to achieve the task, with complex motor tasks more likely to modify the expression of BDNF in M1 (Klintsova *et al.*, 2004). We therefore expect greater differences in M1 plasticity between different *BDNF* genotypes for the complex visuomotor tracking task compared with that induced by the simple ballistic task and PAS.

5.3. Materials and Methods

Experiments were performed on the right hand of 29 subjects (12 women, 17 men; mean ± SD, 24 ± 4 yrs; range 18-39 yrs) with no known history of peripheral or neurological impairment. All subjects were right handed (Laterality Quotient (LQ); median LQ = 0.83 ± 0.18, range 0.5-1.0) as assessed by the Edinburgh Handedness Questionnaire (Oldfield, 1971). No subjects reported long term specialised use of the hands, such as playing a musical instrument, as this may influence cortical plasticity (Rosenkranz *et al.*, 2007b). All experiments were performed in the afternoon or evening to minimise variations in circulating cortisol and its effect on plasticity induction (Sale *et al.*, 2008). A minimum visual capability (with corrective lenses if required) of 20/40 vision assessed using a Snellen Eye Chart was required for participation. Subjects also completed the long version of the International Physical Activity Questionnaire (IPAQ), consisting of 31 items describing the extent of

leisure time physical activity involving aerobic exercises such as running, cycling and walking (Craig *et al.*, 2003; Fogelholm *et al.*, 2006). All subjects gave written informed consent prior to participation in the study, which was approved by the University of Adelaide Human Research Ethics Committee and was conducted in accordance to the standards established by the *Declaration of Helsinki*. Following informed consent, subjects provided a buccal swab for genotyping of the *BDNF* val^{66}met polymorphism (see below). Initially 27 subjects were recruited and provided a buccal swab sample. From this pool, 12 subjects were identified with the Val/Val genotype, 10 with the Val/Met genotype, and 5 with the Met/Met genotype. Two additional subjects that were identified as Met/Met genotype from our other ongoing studies were recruited to selectively increase the sample size in this rare population. These subjects provided a buccal sample to verify that they possessed the *BDNF* Met/Met genotype. Therefore, a total of 7 *BDNF* Met/Met subjects participated in this study. Experimenters were blind to the *BDNF* genotype of all subjects, with the exception of the last two *BDNF* Met/Met subjects.

5.3.1. Genotyping

Genomic DNA was prepared from buccal swabs using an Isohelix DNA Isolation Kit (Cell Projects, Kent, UK). PCR was performed to amplify a 197bp product with the Val66Met polymorphism, when present, located at 73bp. Primers (actctggagagcgtgaatgg / agaagaggaggctccaaagg) were designed using Primer 3 software (Rozen & Skaletsky, 2000). PCR reaction conditions were: denaturation at 95°C for 2 minutes, 35 cycles of 95°C 15 seconds, 60°C 15 seconds, 72°C 30 seconds with a final extension at 72°C for 5 minutes. PCR products were purified using a Qiaquick PCR Purification Kit (Qiagen) and digested with *Eco*721. Restriction digests were resolved on a 2% agarose gel and since the Val66Met polymorphism destroys the *Eco*721 site, the samples could be classified as Val/Val, Val/Met or Met/Met based on the observed banding pattern. Control digests using *Taq*1 were

performed to ensure purified DNA was in a digestible form. Every sample was genotyped from two independent PCR reactions to ensure fidelity.

5.3.2. Experimental arrangement

Each subject participated in three experimental sessions, each involving a different intervention (see below), separated by at least 24 hours with the order of intervention selected randomly. For each experiment, subjects were seated comfortably with their right shoulder abducted approximately 45° to allow the hand and arm to rest on a manipulandum, with the forearm pronated and the palm facing down. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles throughout the experiment using bipolar surface electrodes (Ag-AgCl, 8 mm diameter) placed ~2 cm apart with a muscle belly-tendon montage. A grounding strap placed around the wrist was used as a common reference for all electrodes. The EMG signals were amplified (× 100-1000), bandpass filtered (high pass at 13 Hz, low pass at 1000 Hz), digitised online at 2 kHz with a CED interface system (Cambridge Electronic Design Co. Ltd, UK) and recorded onto computer for offline analysis. The EMG signals of both muscles were displayed on an oscilloscope to assist the subject in maintaining EMG silence when required.

5.3.3. Experimental procedures

The timeline for the experimental procedures are shown in Figure 5.1. All of the measurements were the same in each of the three sessions, with all measurements except for active motor threshold repeated after each of the three interventions. Maximum index finger abduction force (maximum voluntary contractions, MVCs) was assessed at the start and end of each experiment. The hand was positioned with the palm facing down and the middle phalanx of the index finger placed alongside a load cell (LC1205-K020, A&D Mercury Pty Ltd, Australia) to facilitate measurement of index finger abduction force. Maximum index finger abduction force was exerted by the subject for three seconds against the force

transducer with verbal encouragement provided by the experimenter. Several MVC trials were performed, with a minimum of 30-s rest between trials, until the peak force from two trials were within 10% of each other. The MVC with the largest index finger abduction force from these two trials was used for the assessment of muscle strength. Visual feedback of thumb abduction force was displayed on an oscilloscope, and the subject was monitored in each trial to ensure that proximal limb muscles were not contributing to the force. Force signals were amplified (× 1000), digitised online (2 kHz) via a CED 1401 interface (Cambridge Electronic Design, UK) and stored on computer for offline analysis.

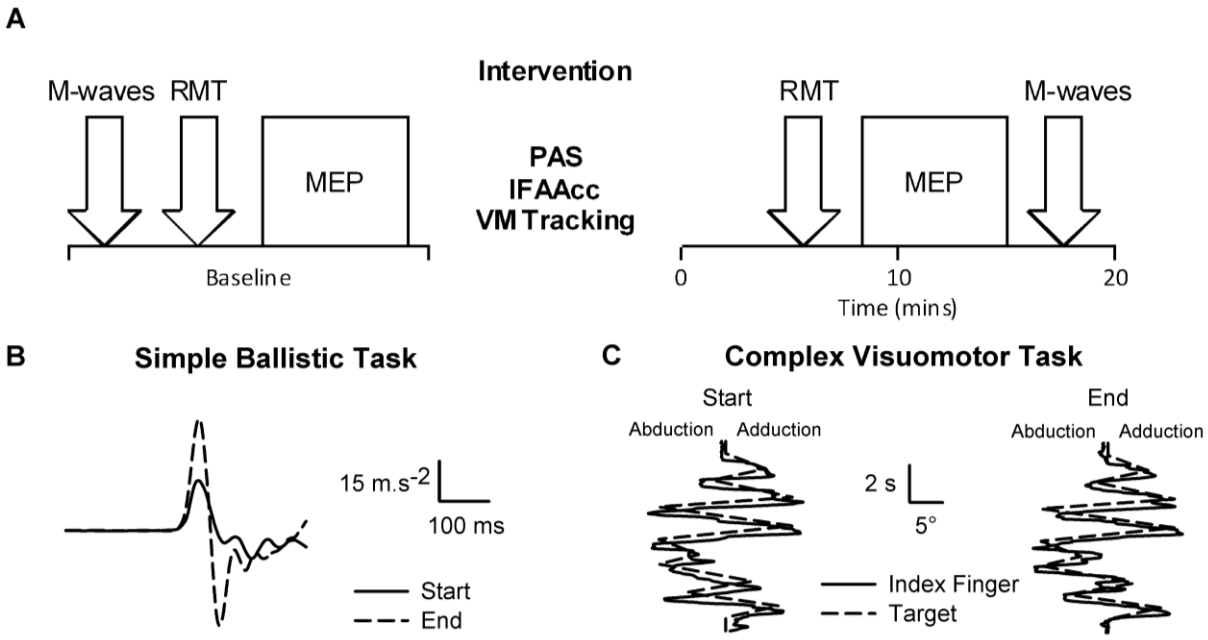

Figure 5.1 *Description of experimental procedures and examples of motor performance before and after training. A, Schematic representation of the experimental protocol with measures obtained before and after each intervention. Baseline measures include assessment of maximum voluntary contraction (MVC), maximal compound muscle action potential (M-wave), resting motor threshold (RMT), and active motor threshold (AMT). Motor evoked potentials (MEPs) at ~10% M_{max} for FDI at rest and active conditions were obtained from 15 single-pulse transcranial magnetic stimulation (TMS) trials. The intervention consisted of experimentally induced plasticity with paired associative stimulation, or an assessment of use-dependent plasticity by examining changes in maximal index finger abduction acceleration (IFAAcc) or visuomotor (VM) tracking performance. B, example of maximal index finger abduction in one subject at the start and end of training. C, example of visuomotor tracking performance in the same subject (Met/Met) at the start and end of the visuomotor tracking task.*

Maximal compound muscle action potential. Supramaximal electrical stimulation was administered to the ulnar nerve at the wrist using a constant current stimulator (DS7AH, Digitimer, UK) and bipolar surface electrodes (separated by 20 mm) with the cathode distal. Stimuli were square wave pulses with 100 μs pulse duration. Stimulator intensity was set at 120% of that required to elicit a maximal compound muscle action potential (M_{max}) from FDI. Five stimuli were delivered before and after the intervention.

Transcranial Magnetic Stimulation. TMS was applied using a figure-of-eight coil (external wing diameter 90 mm) with two Magstim 200^2 magnetic stimulators connected with a Magstim Bistim Module (Magstim, Whitland, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. With this coil orientation, current flow within the cortex was induced in a posterior-anterior direction. The coil was placed at the optimal scalp position over the left hemisphere for eliciting a motor-evoked potential (MEP) in the relaxed right FDI muscle. The optimal position was then marked on the scalp with a pen for reference. TMS was delivered at 0.2 Hz for all conditions (unless stated otherwise) and optimal coil position was continually monitored by checking scalp position throughout the experiment.

Threshold. Resting motor threshold (RMT) was determined as the minimum stimulus intensity required to elicit a MEP in the relaxed FDI of at least 50 μV in amplitude in 3 out of 5 consecutive trials. Active motor threshold (AMT) was defined as the minimum stimulus intensity required to elicit a MEP in the FDI muscle of at least 200 μV in amplitude in 3 out of 5 consecutive trials during a low-level voluntary index finger abduction (10% MVC). Both RMT and AMT are expressed relative to maximum stimulator output (MSO) and the stimulus intensity was altered in 1% increments of MSO throughout this process until the appropriate threshold level was achieved.

Test Intensity. The stimulus intensity that produced a MEP of approximately 10% M_{max} during resting and low-level (10% MVC) voluntary index finger abduction (active) in FDI was determined before training. Using this TMS intensity, 15 trials were recorded to investigate MEP amplitude before and after each intervention at rest and during muscle activation.

5.3.4. Interventions

Three different protocols to induce plasticity, one experimentally-induced with TMS and two that were based on motor training interventions (use-dependent), were tested. Paired associative stimulation (PAS) was selected to experimentally-induce plasticity in cortical circuits (Stefan *et al.*, 2000), with previous studies showing changes induced by PAS may be different in Val/Val and Val/Met subjects (Cheeran *et al.*, 2008; Missitzi *et al.*, 2011). For the motor training interventions, a simple index finger abduction task and a more complex visuomotor tracking task were used to compare use-dependent plasticity and motor learning between different *BDNF* genotypes. The different interventions used lasted for a similar duration (12-16 mins).

Paired Associative Stimulation. PAS was performed as described previously by Rosenkranz *et al.* (2007b). The PAS protocol consisted of percutaneous electrical stimulation of the ulnar nerve at the right wrist (300% of perceptual threshold) followed by suprathreshold TMS (10% M_{max}) 25 ms later over the optimal scalp position for the index finger of the left motor cortex. The interstimulus interval of 25 ms between the peripheral and TMS pulse has previously been shown to induce a LTP-like MEP increase (Stefan *et al.*, 2000; Stefan *et al.*, 2002). The intervention consisted of 200-paired stimuli delivered at 0.25 Hz. Electrical stimuli were applied to the ulnar nerve at the wrist using a constant current stimulator (DS7A stimulator, Digitimer Co. Ltd., Hertfordshire, UK) with bipolar surface electrodes, separated by 30 mm,

and with the cathode proximal. Stimuli were square wave pulses with a pulse width of 200 μs.

The attentional focus of the subject has been shown to be an important factor influencing PAS effectiveness (Stefan *et al.*, 2004). To quantify this, subjects received a total of 80 intermittent weak (200% perceptual threshold) electrical stimuli to their right index finger via ring electrodes (Stefan et al., 2004). Subjects were instructed to count and report the number of index finger stimuli they received. The level of attention was then assessed as the absolute error in the number of stimuli counted during PAS. The index finger stimulus was always delivered at the mid-point of the interval between successive paired stimuli in the PAS protocol.

Index Finger Abduction Acceleration. The simple motor training task was similar to that described previously (Rogasch *et al.*, 2009; Cirillo *et al.*, 2010), requiring the subject to maximise peak index finger abduction acceleration (IFAAcc) during ballistic movement of the right index finger. Subjects sat with their forearm placed in a custom designed splint and their arm abducted at the shoulder and bent at approximately 90° at the elbow. The thumb was restricted and all other digits (other than the index finger) were immobilised. This allowed the index finger to move freely for abduction-adduction movements. IFAAcc consisted of 150 ballistic index finger abduction movements paced at 0.5 Hz by an audible tone from a metronome. Subjects rested their index finger for 30 seconds after ten trials to avoid fatigue. A bi-axial accelerometer (sensitivity ± 6g, LIS3L06AL, STMicroelectronics, Switzerland) placed over the interphalangeal joint of the index finger was used to assess index finger acceleration in the abduction-adduction and flexion-extension planes. Index finger acceleration > +3 $m.s^{-2}$ in the abduction-adduction plane triggered a recording sweep of ± 500 ms, and each movement recorded acceleration data in the abduction-adduction and flexion-extension planes. Continual verbal encouragement and visual feedback of IFAAcc displayed

on a computer screen was provided to the subject throughout training to improve and maximise index finger acceleration. Acceleration signals were digitised online at 2 kHz using a CED interface system and recorded on computer for offline analysis.

Visuomotor Tracking. A visuomotor tracking task was used as a more complex motor training task requiring an increased attentional demand to achieve accurate task performance. The experimental arrangement for this task has been described previously (Todd *et al.*, 2009; Cirillo *et al.*, 2011). Briefly, a potentiometer was attached to the right index finger at the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints. The task required subjects to match, as accurately as possible, MCP joint angle of the index finger with a moving target on a computer screen (for example see Figure 5.2). The moving target consisted of 18 unique 10-s frames that moved automatically down the screen while making unpredictable left and right movements. A left movement of the target corresponded to abduction of the index finger and a right movement of the target corresponded to adduction. The maximum MCP joint angle movement was ± 10° from neutral. Training consisted of two 6-minute blocks where each frame was repeated twice and the order of the frames was the same for each block. In order to avoid fatigue, there was a 4-minute rest period between blocks. Visual feedback of the MCP joint angle relative to the target was provided to subjects along with continual verbal instructions to follow the moving target as closely as possible. Target and tracking lines were amplified (× 3000), digitised online at 2 kHz, and recorded onto computer for offline analysis.

5.3.5. Data analysis.

All MEP and M_{max} trials that contained pre-stimulus EMG activity (100 ms before stimulation) during rest conditions were discarded from the analysis, and repeated at the appropriate intensity following the data block. MEP and M_{max} amplitudes (expressed in mV) were measured peak-to-peak in each individual trial. The MEP size was then expressed as a

percentage of the peak-to-peak amplitude of M_{max}. Maximum force was calculated during the MVC and maximum FDI EMG was measured as the mean rectified EMG over 1 s (500 ms before and after peak force) expressed as a percentage of the peak-to-peak amplitude of M_{max}.

For each IFAAcc movement trial, a baseline period from 400 to 200 ms before abduction acceleration was used to calculate the mean baseline acceleration in the abduction-adduction and flexion-extension planes. The mean acceleration over this baseline period was subtracted so that baseline acceleration equalled 0 $m.s^{-2}$ in both planes. Peak IFAAcc magnitude was then assessed, along with flexion-extension acceleration magnitude at the time of peak abduction acceleration. Abduction acceleration for the 150 ballistic index finger abduction movements was subdivided into blocks of 50 trials representing a start, middle and end (three blocks of 50 trials in total) for detailed analysis.

Performance during the visuomotor tracking task was assessed for each individual trial over the entire training block. For each block, the maximum cross-correlation coefficient and the lag time between the actual finger position and the target were calculated (see Cirillo *et al.*, 2011 for details). Tracking error was calculated by subtracting the MCP joint angle (finger position) from the target line, with the mean absolute tracking error for each training block reported.

5.3.6. Statistical analysis.

A one-way ANOVA was used to examine the effect of *BDNF* genotype (Val/Val, Val/Met, Met/Met: between-subject factor) on subject characteristics; maximum index finger abduction force, maximum FDI EMG, M_{max} amplitude, RMT, AMT, test TMS intensity (before training), attention during PAS, physical activity (IPAQ), and cognitive mental state (MMSE). A Kruskal-Wallis one-way ANOVA was used to compare non-parametric handedness scores between genotypes. Two-way repeated measures ANOVA was used to examine the effect of genotype and training (Blocks 1, 2, 3: within-subject factor) on

maximum IFAAcc, visuomotor tracking error, maximum cross-correlation coefficient, and lag time. Measures of M_{max} amplitude and TMS were separately analysed into experimentally-induced (PAS) and motor training (IFAAcc and visuomotor tracking) interventions. Two-way repeated measures ANOVA was used to analyse the effect of genotype and time (before, after: within-subject factor) on M_{max} amplitude, RMT, test MEP amplitude, and PAS. Three-way repeated measures ANOVA was used to analyse the effect of genotype, time, and task (IFAAcc and visuomotor tracking: within-subject factor) on M_{max} amplitude, RMT, and test MEP amplitude. A Fisher's LSD post-hoc test that performed all possible comparisons was used to analyse significant main effects and interactions. The significance level was set at $P < 0.05$ for all comparisons and all group data are provided as mean ± standard error of the mean (SEM).

5.4. Results

The subject characteristics before training for each *BDNF* genotype are provided in Table 5.1. There was no significant difference between the *BDNF* Val/Val, Val/Met, and Met/Met genotypes in their physical characteristics (handedness, physical activity levels), voluntary and electrically induced muscle responses (maximal strength, EMG, M-waves), or baseline responses to TMS (TMS thresholds and 10% maximal M-wave test intensity). However, there was a tendency for RMT and active test TMS intensity to be ~10% of maximal stimulator output higher in Met/Met subjects compared with the other two genotypes. Additional Met/Met subjects would be necessary to statistically confirm this finding.

Table 5.1 *Group characteristics for different BDNF genotypes.*

	Val/Val (12)	Val/Met (10)	Met/Met (7)	*P*-value
Handedness (LQ)	0.85 (0.19)	0.82 (0.17)	0.82 (0.22)	0.60
Physical Activity (IPAQ)	3689 (4540)	4396 (4037)	2821 (2935)	0.86
Gender	6 M, 6 F	6 M, 4 F	4 M, 3 F	
M-Wave (mV)	16.2 (4.0)	17.5 (4.2)	19.7 (4.4)	0.23
MVC (N)	42.1 (15.2)	39.4 (8.5)	35.1 (12.7)	0.52
Maximum EMG (% M-wave)	3.64 (0.50)	3.63 (1.08)	3.03 (0.80)	0.24
RMT (% MSO)	40.8 (8.0)	41.5 (8.5)	50.4 (11.0)	0.07
AMT (% MSO)	31.3 (5.5)	31.1 (6.7)	37.0 (6.8)	0.19
Test Intensity - Rest (% MSO)	61.9 (19.1)	64.2 (18.5)	71.5 (20.6)	0.58
Test Intensity - Active (% MSO)	37.3 (8.1)	37.1 (8.3)	46.7 (11.7)	0.07

Values are mean (SD). *LQ*, laterality quotient; *IPAQ*, international physical activity questionnaire; *MVC*, FDI maximum voluntary contraction; *EMG*, FDI electromyography; *RMT*, FDI resting motor threshold; *AMT*, FDI active motor threshold; *MSO*, maximum stimulator output.

There was a significant difference between muscles (FDI, ADM; $F_{1,26} = 20.30$, $P < 0.001$) for all data examining maximal M-waves. Therefore, maximal M-wave data were averaged for interventions (PAS, simple ballistic, complex visuomotor; $F_{2,52} = 0.21$, $P = 0.81$) and time (before, after; $F_{1,26} = 0.51$, $P = 0.48$) with analysis focused on genotype effects separated between muscles. Maximal M-waves did not significantly differ between *BDNF* genotypes for FDI (genotype effect, $F_{2,26} = 1.58$, $P = 0.23$) or ADM (genotype effect, $F_{2,26} = 1.58$, $P = 0.22$).

For all data examining MEP amplitudes, there was a significant difference between interventions (PAS, simple ballistic, complex visuomotor, $F_{2,52} = 3.35$, $P = 0.04$), Muscles (FDI, ADM; $F_{1,26} = 73.96$, $P < 0.001$) and Conditions (rest, active; $F_{1,26} = 24.30$, $P < 0.001$). Therefore, all subsequent analyses of MEP amplitudes focused on the Genotype and Time effects, which were separated between interventions, muscles, and rest and active conditions.

5.4.1. PAS and BDNF genotype

Figure 5.2A shows original MEP recordings in relaxed FDI from one Val/Val and one Met/Met subject before and after PAS. Both subjects participated in experiments in the afternoon and had similar baseline characteristics of handedness (LQ: Val/Val = 0.8, Met/Met = 1), physical activity (IPAQ: Val/Val = 2183 MET-min, Met/Met = 1575 MET-min), maximal M-wave amplitude (Val/Val = 19.2 mV, Met/Met = 17.4 mV), RMT (Val/Val = 45% MSO, Met/Met = 46% MSO), AMT (Val/Val = 29% MSO, Met/Met = 32% MSO), test TMS intensity at rest (Val/Val = 72% MSO, Met/Met = 74% MSO), and active test TMS intensity (Val/Val = 39% MSO, Met/Met = 37% MSO). The original recordings from FDI in Figure 2A show that there was a significant MEP increase following PAS in the Val/Val subject (45% increase), whereas there was no significant change in MEP amplitude after PAS for the Met/Met subject (6% increase).

Figure 5.2B and 5.2C show the mean MEP responses before and after PAS in FDI (target) and ADM (control) muscles in subjects with different *BDNF* genotypes. The TMS intensity used to quantify the change in MEP response was set to evoke a MEP amplitude of 10% M_{max} in resting FDI before PAS. Although there were no main effects between groups (Genotype effect, $F_{2,26} = 0.79$, P = 0.46) and following PAS (Time effect, $F_{1,26} = 2.32$, P = 0.14), there was a significant group x time interaction ($F_{2,26} = 4.87$, P < 0.02). *Post-hoc* analysis indicated that the FDI MEP amplitude was significantly greater in the Val/Val subjects after PAS (P = 0.003), but there was no effect of PAS in the Val/Met (P = 0.79) or Met/Met subjects (P = 0.81). For the non-target ADM muscle, there was a significant increase in MEP amplitude for all subjects combined (Time effect, $F_{1,26} = 6.29$, P = 0.02), but no difference between groups (Genotype effect, $F_{2,26} = 0.19$, P = 0.83) and no group x time interaction ($F_{2,26} = 1.30$, P = 0.29).

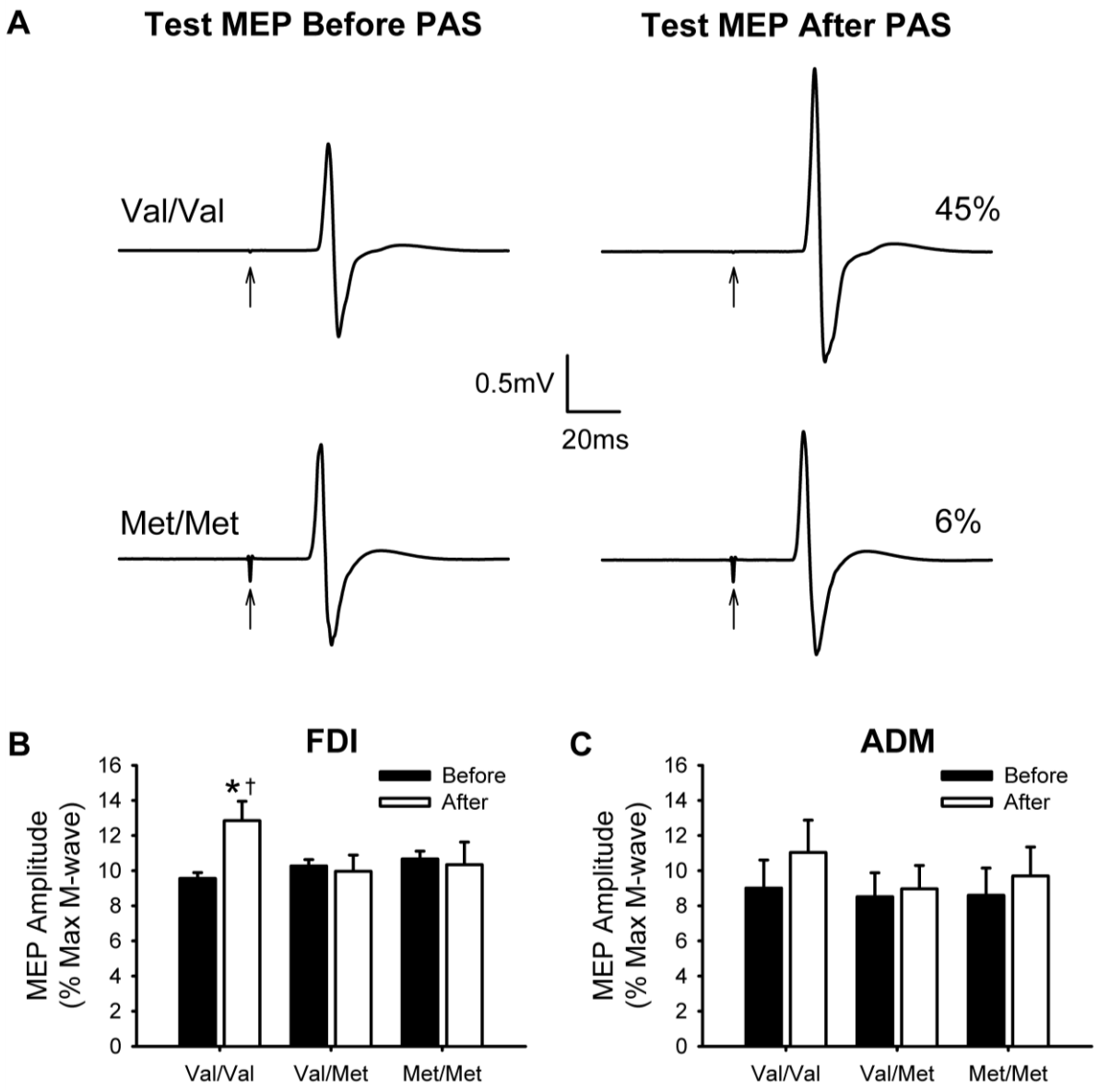

Figure 5.2 *Changes in MEPs before and after PAS in people with different BDNF genotypes. A, mean MEP in resting FDI from one Val/Val (upper panel) and one Met/Met (lower panel) subject before (left) and after (right) PAS. B and C show mean MEPs at rest in 12 Val/Val, 10 Val/Met and 7 Met/Met subjects for the FDI and ADM muscles. Baseline MEPs were obtained at ~10% of maximal M-wave for the target FDI muscle. * P < 0.05 compared with Before. † P < 0.05 compared with Val/Met and Met/Met genotypes.*

Changes in MEP amplitude before and after PAS were also assessed for FDI and ADM muscles during low-level (10% MVC) voluntary index finger abduction (data not shown). A two-way repeated measures ANOVA showed an increased FDI MEP amplitude after PAS during muscle activation (Time effect, $F_{1,26}$ = 4.87, P = 0.04), but no significant difference between *BDNF* genotypes (Genotype effect, $F_{2,26}$ = 0.03, P = 0.98) and no genotype x time

interaction ($F_{2,26}$ = 0.17, P = 0.84). A two-way repeated measures ANOVA showed that ADM MEP amplitude was significantly increased in the Met/Met group compared with the Val/Val and Val/Met groups (Genotype effect, $F_{2,26}$ = 4.03, P = 0.03), but did not change following PAS (Time effect, $F_{1,26}$ = 0.90, P = 0.35) and there was no significant genotype x time interaction ($F_{2,26}$ = 2.65, P = 0.09).

The level of attention during PAS was assessed as the absolute error in the number of attention stimuli (total of 80) counted during PAS. The mean error score was not significantly different between Val/Val (2.7 ± 0.7), Val/Met (2.5 ± 0.7), and Met/Met (2.9 ± 0.9) subjects (Genotype effect, $F_{2,26}$ = 0.05, P = 0.95). Linear regression analysis indicated that there was no significant correlation between FDI MEP facilitation induced by PAS and attention-related errors (r^2 < 0.01, P = 0.80). Furthermore, RMT for FDI did not significantly change following PAS (Time effect, $F_{1,26}$ = 6.75, P = 0.07) and remained similar between *BDNF* genotypes (Genotype x Time interaction, $F_{2,26}$ = 0.46, P = 0.64).

5.4.2. Motor performance and motor learning in different BDNF genotypes

Motor performance was assessed during a simple ballistic index finger task and a complex visuomotor tracking task in all subjects, and these data are shown in Figure 5.3. For the simple ballistic task (Figure 5.3A), there was a significant increase in peak IFAAcc throughout the training period (Time effect, $F_{2,52}$ = 30.22, P < 0.001), but there was no difference between *BDNF* genotypes ($F_{2,26}$ = 0.40, P = 0.68) and no genotype x time interaction ($F_{4,52}$ = 0.84, P = 0.51). Furthermore, the improvement in motor performance (i.e. motor learning) from the start (1st minute of block 1) to the end (block 3) of training was similar between the different *BDNF* genotypes (Val/Val, 131%; Val/Met, 138%; Met/Met, 138%). For the complex visuomotor task (Figure 5.3B), there was a decrease in visuomotor tacking error (improved performance) across training blocks (Time effect, $F_{1,26}$ = 111.88, P < 0.001), but this was not different between *BDNF* genotypes (Genotype effect, $F_{2,26}$ = 0.95, P =

0.40) and there was no genotype x time interaction ($F_{2,26}$ = 1.12, P = 0.34). The improvement in motor performance (block 2 performance normalised to the first minute of block 1) was similar for all *BDNF* genotypes (Val/Val, 11%; Val/Met, 11%; Met/Met, 13%). For this task, additional assessments of tracking accuracy were obtained by calculating the cross-correlation coefficient between the target line and index finger position, and identifying the maximum cross-correlation coefficient and its associated lag time (Table 5.2). Although there were significant improvements with training in maximum cross-correlation coefficient (Time effect, $F_{1,26}$ = 27.51, P < 0.001) and lag time ($F_{1,26}$ = 23.10, P < 0.001), these measures were not influenced by *BDNF* genotype.

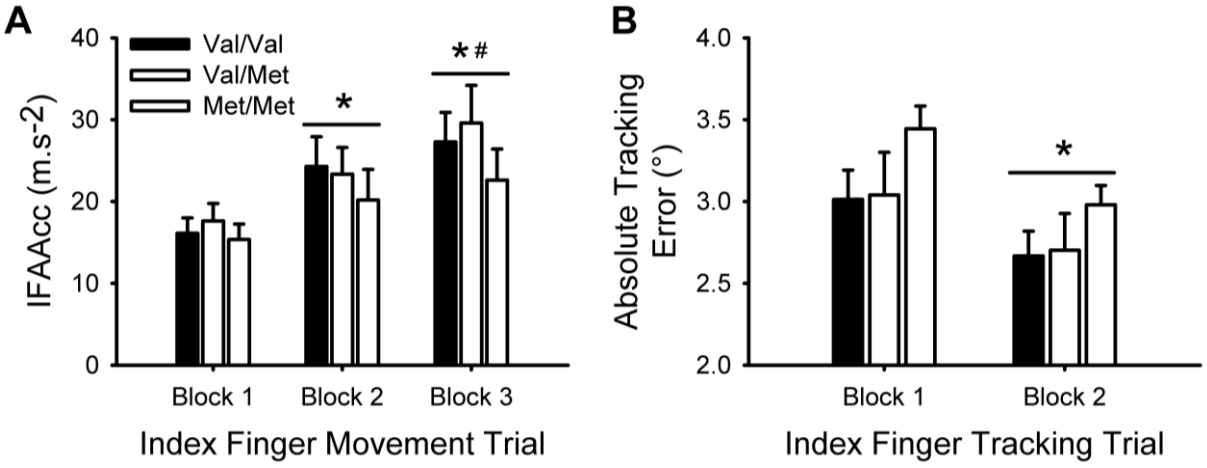

Figure 5.3 *Assessment of motor performance for simple ballistic (A) and complex visuomotor tasks (B) in different BDNF genotypes. A, Group changes in peak index finger abduction acceleration (IFAAcc) for all three training blocks. B, visuomotor tracking error for all two training blocks. * P < 0.05 compared with Block 1. # P < 0.05 compared with Block 2.*

Table 5.2 *Group visuomotor tracking data for different BDNF genotypes.*

	Maximum Cross-Correlation (ρ)		Lag Time (ms)	
Genotype	Block 1	Block 2	Block 1	Block 2
Val/Val	0.88 (0.07)	0.91 (0.06)	228 (46)	208 (42)
Val/Met	0.87 (0.11)	0.90 (0.07)	234 (57)	210 (50)
Met/Met	0.85 (0.08)	0.91 (0.05)	261 (19)	242 (23)

Values are mean (SD).

5.4.3. Use-dependent plasticity in different BDNF genotypes

The change in FDI MEP amplitude after motor training was used as a marker of use-dependent plasticity. Figure 5.4 shows mean resting FDI MEP amplitude before and after a simple ballistic (Figure 5.4A) and complex visuomotor (Figure 5.4B) task in people with different *BDNF* genotypes. For the simple ballistic task, there was an increase in MEP amplitude in all *BDNF* genotypes after training (Time effect, $F_{1,26} = 20.77$, P = 0.001), with a 38% increase in Val/Val subjects, 26% increase in Val/Met subjects, and a 39% increase in Met/Met subjects. There was no significant difference between genotypes ($F_{2,26} = 0.53$, P = 0.6) and no genotype x time interaction ($F_{2,26} = 0.15$, P = 0.9). In contrast, for the complex visuomotor tracking task there were clear differences in MEP amplitude between genotypes ($F_{2,26} = 6.42$, P = 0.005) and a significant genotype x time interaction ($F_{1,26} = 9.77$, P < 0.001). Post hoc analysis showed that there was a 26% increase in MEP amplitude after training in the Val/Val group (P = 0.008), no change in MEP in the Val/Met group (P = 0.5) and a 38% decrease in MEP amplitude in the Met/Met group (P = 0.002).

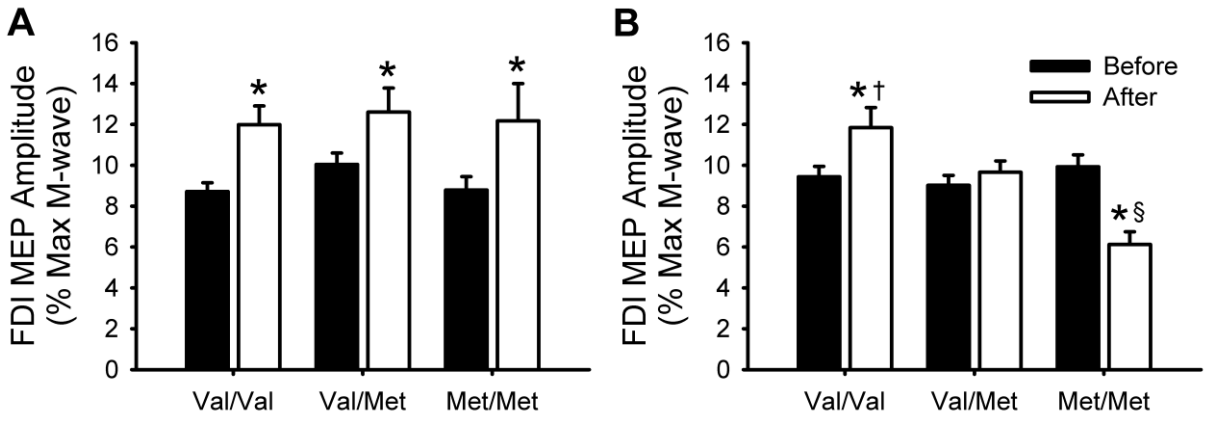

Figure 5.4 *Resting FDI MEP amplitude in different BDNF genotypes obtained before and after simple ballistic (A) and complex visuomotor training (B). Baseline MEP amplitudes were approximately 10% of maximal M-wave before training in each subject. * P < 0.05 compared with before training. † P < 0.05 compared with Val/Met and Met/Met genotypes. § P < 0.05 compared with Val/Val and Val/Met genotypes.*

Changes in FDI MEP amplitude before and after training were also assessed during low-level (10% MVC) voluntary index finger abduction with a TMS intensity set to evoke a MEP amplitude of 10% M_{max} in active FDI before the motor task. In the active FDI muscle with a lower TMS intensity (see Table 1), there was no significant difference between genotypes ($F_{2,26}$ = 0.54, P = 0.59), no change in the MEP amplitude after the simple ballistic task ($F_{1,26}$ = 3.13, P = 0.09), and no genotype x time interaction ($F_{2,26}$ = 0.19, P = 0.83). Similarly, for the complex visuomotor tracking task there was no difference between genotypes ($F_{2,26}$ = 0.85, P = 0.44), no genotype x time interaction ($F_{2,26}$ = 1.85, P = 0.18), but there was a significant increase in MEP amplitude after training ($F_{1,26}$ = 10.11, P = 0.004).

Changes in MEP amplitude before and after motor tasks were assessed for the control ADM muscle during relaxed and voluntary index finger abduction activation (10% MVC contraction). As expected, there were no genotype-related differences in MEP amplitude for the ADM muscle under any condition ($F_{2,26}$ values from 0.36 to 1.58, P values from 0.23 to 0.70) and no genotype x time interactions ($F_{2,26}$ = values from 0.25 to 1.95, P values from 0.16 to 0.78). However, there was a significant increase in ADM MEP amplitude after the simple ballistic task for the rest ($F_{1,26}$ = 4.91, P = 0.04) and active conditions ($F_{1,26}$ = 5.34, P = 0.03), but no change after the complex visuomotor task.

Using linear regression of data from individual subjects, we examined whether the training-related changes in excitability (MEP amplitude) in relaxed FDI were associated with the extent of motor learning (improvement in motor task performance). For the simple ballistic task, there was no association between the change in MEP amplitude and motor learning for Val/Val (r^2 = 0.03, P = 0.62), Val/Met (r^2 = 0.06, P = 0.50), or Met/Met (r^2 = 0.02, P = 0.78) subjects. Similarly, there was no association between the change in MEP amplitude and motor learning for Val/Val (r^2 < 0.01, P = 0.81), Val/Met (r^2 = 0.03, P = 0.63), or Met/Met (r^2 < 0.01, P = 0.96) subjects with the complex visuomotor task. We also correlated PAS-

induced changes with behavioural gains in the motor tasks. For the different *BDNF* genotypes, there was no association between the change in PAS MEP amplitude and motor learning of the simple ballistic task (Val/Val, $r^2 = 0.03$, $P = 0.62$; Val/Met, $r^2 = 0.11$, $P = 0.34$; Met/Met, $r^2 = 0.02$, $P = 0.78$) or the complex visuomotor task (Val/Val, $r^2 < 0.01$, $P = 0.94$; Val/Met, $r^2 = 0.17$, $P = 0.24$; Met/Met, $r^2 = 0.08$, $P = 0.53$).

5.5. Discussion

The present study investigated how people with one of three different *BDNF* genotypes modulate M1 excitability following experimentally-induced (PAS) and use-dependent (simple ballistic movement and complex visuomotor tracking) plasticity interventions. The main finding of this study was that the modulation of motor cortex excitability in people with different *BDNF* genotypes is dependent on the intervention used, with Val/Val subjects showing increased MEPs for all interventions, whereas the Met allele carriers only showed increased MEPs after the simple ballistic task. Furthermore, we provide new evidence showing that M1 plasticity in the rare Met homozygotes (Met/Met) differs to Met heterozygotes (Val/Met) following complex visuomotor training.

In the present study, the different subject groups were well matched for gender (Inghilleri *et al.*, 2004), hand dominance (Cirillo *et al.*, 2010), and physical activity levels (Cirillo *et al.*, 2009), so these factors are unlikely to contribute to differences in experimentally-induced and use-dependent plasticity between *BDNF* genotypes (see Ridding & Ziemann, 2010). Furthermore, all studies were conducted in the afternoon, which removes any influence of the circadian rhythm on the assessment of cortical plasticity with PAS (Sale *et al.*, 2007). However, there was a tendency for RMT to be ~10% MSO greater in Met/Met carriers compared with Val/Met and Val/Val subjects. This is in contrast to a previous study, which found no difference in RMT between the three different *BDNF* genotype groups (Kleim *et al.*,

2006). Testing additional BDNF Met/Met subjects to improve statistical power may help to determine whether corticospinal excitability is altered in *BDNF* Met/Met subjects.

In animal models, BDNF has been shown to play an important role in the survival, health and functioning of glutamatergic neurons (Mattson, 2008), and is crucial in modulating synaptic plasticity by LTP and long-term depression (LTD) (Patterson *et al.*, 1996; Ikegaya *et al.*, 2002; Gottmann *et al.*, 2009). However, the role of BDNF in modulating human cortical plasticity is less well established, presumably because it is not possible to obtain measures of cortical BDNF in humans. Furthermore, measures of BDNF in the blood (serum or plasma) may not reflect those in the brain, because BDNF is also produced in various peripheral tissues and only a small amount of this moderately sized protein crosses the blood-brain barrier (see Nagahara & Tuszynski, 2011). To circumvent these problems in humans, we are able to use TMS to examine the role of BDNF on motor cortex plasticity by comparing people with different *BDNF* genotypes, as it is known that there is an 18 (Val/Met subjects) to 30% (Met/Met subjects) reduction in activity-dependent secretion of cortical BDNF in Met allele carriers (Egan *et al.*, 2003). Along with abnormal cortical morphology and hippocampal function (Egan *et al.*, 2003; Pezawas *et al.*, 2004), more recent studies have suggested that the *BDNF* Met allele also contributes to impaired M1 plasticity (Kleim *et al.*, 2006; Cheeran *et al.*, 2008; McHughen *et al.*, 2010). However, this is not always a consistent finding (Li Voti *et al.*, 2011; Nakamura *et al.*, 2011; Witte *et al.*, 2012). In the present study, we examined M1 plasticity in the same subjects using three different plasticity-inducing protocols of similar duration. We found that M1 plasticity was evident in Val/Val subjects for all tasks, whereas the Met allele carriers only showed M1 plasticity after the simple ballistic task. We therefore suggest that BDNF is important for human M1 plasticity induction, but the magnitude of the effect is dependent on the intervention used.

Methodological factors assessing plasticity, specifically test intensity, are unlikely to significantly contribute to the findings in the present study. We used a test intensity that evoked a MEP amplitude of ~10% M-max (which is thought to adjust for some of the non-linearity properties within the spinal motor neuron pool, Lackmy & Marchand-Pauvert, 2010) compared with a more conventional 1 mV test intensity. In FDI, a TMS-evoked MEP amplitude of 1 mV and 10% M-max are both situated within the steep linear portion of the sigmoidal recruitment curve (plateau at ~45% of M-max), which is critical for detecting plastic changes in M1 (Devanne *et al.*, 1997). In addition, previous studies demonstrate that a large range of stimulus intensities (encompassing MEPs of 1 mV and 10% M-max) result in similar corticomotor excitability changes following PAS (Rosenkranz *et al.*, 2007b), simple ballistic training (Rogasch *et al.*, 2009) and complex visuomotor tracking (Perez *et al.*, 2004). Furthermore, no baseline difference in corticospinal output assessed by recruitment curve has been observed between the three different *BDNF* genotypes (Kleim *et al.*, 2006).

PAS is an experimental technique in humans that has been deliberately adapted from similar protocols used in brain slices and neuronal cultures, which demonstrate bidirectional spike-timing dependent plasticity (Dan & Poo, 2004; Caporale & Dan, 2008). The long-lasting (<60 mins) increase in MEP amplitude that is typically observed after PAS is thought to occur through LTP-like mechanisms (Stefan *et al.*, 2000; Stefan *et al.*, 2002) in cortical circuits (Di Lazzaro *et al.*, 2009a), although spinal circuits may also be affected (Meunier *et al.*, 2007). Two previous studies have investigated the effect of the *BDNF* polymorphism on PAS-induced plasticity. The initial study by Cheeran *et al.* (2008), performed on 9 Val/Val and 9 Met allele carriers, reported an increase in MEPs in the Val/Val group in the homotypic target (median nerve innervated abductor pollicis brevis) muscle after PAS that just failed to reach statistical significance ($P = 0.07$) and a significant increase in MEPs in the heterotopic (ulnar innervated) ADM muscle, which were not present in the Met allele carriers after PAS. In contrast, a similar study using a larger sample size (15 Val/Val and 15 Met allele carriers)

failed to show a MEP increase following PAS for both the Val/Val and Met allele carriers (Witte *et al.*, 2012). Our results provide support for a BDNF dependent effect on the change in MEPs after PAS, showing a significant increase in MEPs in Val/Val subjects but no change in Val/Met subjects (Figure 5.2). Furthermore, we have extended this observation by examining the 'dose' effect of BDNF on PAS-induced plasticity by comparing the responses in three different *BDNF* genotypes that are known to differ in their activity-dependent release of BDNF from cortical neurons (Egan *et al.*, 2003). With this comparison we show that there is no change in MEPs after PAS in *BDNF* Met/Met carriers, with similar responses in *BDNF* Val/Met individuals. We can therefore conclude that BDNF plays an important role in PAS-induced plasticity in humans, but the effect is not directly related to the putative reduction in activity-dependent BDNF release from cortical neurons in Met allele carriers.

Motor skill learning can be considered the acquisition of new patterns of muscle activation in time and space to improve performance of a motor task (Sanes & Donoghue, 2000). Motor learning consists of several phases, with the initial phases involving mechanisms of synaptic plasticity such as LTP and LTD (Karni *et al.*, 1998). This use-dependent plasticity plays a beneficial role in functional recovery from CNS injury and motor learning (Nudo *et al.*, 1996b). Several studies in M1 have shown that the mechanisms of motor skill learning all depend on protein synthesis, in which BDNF seems to play a major role (Kleim *et al.*, 2003; see Adkins *et al.*, 2006). LTP, LTD, and activity dependent increases in BDNF are all seen in M1, which is considered a crucial site for motor learning (Sanes & Donoghue, 2000; Klintsova *et al.*, 2004). Furthermore, LTP is BDNF dependent (Patterson *et al.*, 1996) and inhibition of BDNF in rat M1 disrupts motor reorganisation and impairs skilled motor performance (see Adkins *et al.*, 2006). In humans, several lines of evidence using TMS also provide strong support that motor learning induces plasticity in human M1 (e.g. Classen *et al.*, 1998; Muellbacher *et al.*, 2002). However, it has recently been shown with cervicomedullary stimulation that the relative contribution of cortical and spinal circuits to use-dependent

plasticity may depend on the task performed, with a significant contribution from spinal circuits during the simple ballistic task, whereas cortical circuits are thought to make a greater contribution during the complex visuomotor task (Giesebrecht *et al.*, 2012).

Along with experimentally induced plasticity, the *BDNF* polymorphism has been shown to dramatically influence use-dependent plasticity in human M1. For example, subjects with the *BDNF* (Val/Met) polymorphism exhibit less motor map reorganisation and reduced changes in M1 excitability following training on several motor tasks (Kleim *et al.*, 2006), although this deficit can be overcome with intense training over multiple days (McHughen *et al.*, 2011). Neuroimaging techniques also support reduced short-term plasticity in *BDNF* Val/Met subjects, with a greater reduction in brain activation volume in the Met allele carriers after index finger training (McHughen *et al.*, 2010). We sought to further explore these training-dependent effects by examining the change in MEPs after simple ballistic and complex visuomotor tracking movements in the three different *BDNF* genotypes. Interestingly, we found striking differences in the extent of use-dependent plasticity between the two motor tasks in *BDNF* Met allele carriers (Figure 5.4). For the simple ballistic task, there was an increase in MEPs in all subjects that was independent of *BDNF* genotype. In contrast, the complex visuomotor tracking task resulted in no change in MEPs for the *BDNF* Val/Met subjects and a reduction in MEPs for the *BDNF* Met/Met group. These divergent findings between tasks may result from the greater task demands during visuomotor tracking, which requires the integration of attentional, memory, visual and motor systems for accurate task performance. In contrast, the simple ballistic task relies less on sensory feedback and more on feedforward mechanisms for optimising the motor command to produce a rapid movement in the desired direction. This task related difference is in line with a recent report showing that spinal mechanisms make a significant contribution to use-dependent plasticity during the ballistic task compared with the visuomotor task (Giesebrecht *et al.*, 2012). As a result, cortical mechanisms are likely to make a greater contribution to use-dependent plasticity

during the complex visuomotor task than the ballistic task (Giesebrecht *et al.*, 2012). It is known from animal studies that complex motor tasks are more likely to modify the expression of BDNF in M1 (Klintsova *et al.*, 2004), and that activity-dependent increases in BDNF are observed in multiple brain regions and not restricted to the primary motor cortex (Ploughman *et al.*, 2005), suggesting that differences in activity dependent BDNF release from cortical neurons (Egan *et al.*, 2003) may contribute to the *BDNF* genotype differences in use-dependent plasticity during the complex motor task. However, while it is evident that BDNF promotes neuronal survival and growth in the cortex and spinal cord (see Vayman and Gomez-Pinilla, 2005), the influence of *BDNF* genotype on spinal mechanisms or spinal motoneurons in use-dependent plasticity remains unknown. Furthermore, it is unclear at this stage why the MEPs were reduced in the *BDNF* Met/Met subjects after complex visuomotor tracking. However, this is likely to reflect LTD-like processes in the involved synapses (Bütefisch *et al.*, 2000), with some studies showing that a shortage or blockade of BDNF increases susceptibility to LTD (Kinoshita *et al.*, 1999; Kumura *et al.*, 2000). Nonetheless, given that Met/Met subjects demonstrate the capacity for increased motor cortex plasticity after the ballistic training task, this effect is unlikely to be due to a lifetime of exposure to reduced BDNF secretion from cortical neurons in these subjects, but is more likely related to the task-specific requirements of complex visuomotor performance.

There is strong evidence that the neural pathways involved in motor learning and those activated with PAS involve overlapping and functionally important cortical circuits. Some studies have shown that a period of motor skill learning abolished the response to a subsequent PAS protocol that produced LTP-like responses when given alone (Ziemann *et al.*, 2004; Stefan *et al.*, 2006). Furthermore, when PAS is given prior to motor learning, this enhances motor performance for both simple (Jung & Ziemann, 2009) and complex motor tasks (Rajji *et al.*, 2011), providing strong evidence that similar mechanisms and neural circuits are involved in PAS and motor learning. However, our divergent findings between

different *BDNF* genotypes suggest that the association is likely to be a complex one. For example, we found a similar increase in MEP amplitude after all three interventions in Val/Val subjects, an increase in MEP amplitude only after the simple ballistic task in Val/Met and Met/Met subjects, and a decrease in MEP amplitude after the visuomotor task in Met/Met subjects. The different responses between PAS and motor training are likely to occur because the specific synapses targeted by PAS represent only a subset of the synaptic network that encodes the motor task. The reasons why these responses are different between *BDNF* genotypes are unknown, but may reflect a specific component of the behavioural intervention such as the repetition of movements, attention to the task, or the motivation to achieve (and actual achievement of) improved task performance. Interestingly, the differences in MEP amplitude between *BDNF* genotypes were abolished in the active state when subjects performed a low level voluntary contraction. This could be due to a greater contribution of spinal motor neurons to the MEP in an active muscle (Di Lazzaro *et al.*, 1998a; Thickbroom, 2011), or the reduction in TMS intensity necessary for the active state influencing the contribution of early and late I waves to the MEP during muscle contraction (Sakai *et al.*, 1997; Di Lazzaro *et al.*, 1998a).

Despite obvious differences in motor cortex plasticity between different *BDNF* genotypes, the functional significance of this remains unclear. The findings on motor behaviour from previous studies have been inconsistent, with one study showing reduced short-term (single session) motor performance (McHughen *et al.*, 2010), whereas most studies have found no difference in motor performance between different *BDNF* genotypes (Kleim *et al.*, 2006; Fritsch *et al.*, 2010; Li Voti *et al.*, 2011; McHughen *et al.*, 2011). One factor that we thought might contribute to these disparate findings is the type of task used to assess motor performance. However, we found no difference in short-term motor performance or learning between different *BDNF* genotypes in either the simple ballistic or the complex visuomotor tracking task (Figure 5.3 and Table 5.2). There was a small tendency for the Met/Met

subjects to have impaired motor performance, as shown by the reduced index finger acceleration, increased tracking error (Figure 5.3) and increased lag time (Table 5.2) at the end of training, but these effects were not large enough to reach statistical significance. Given that the *BDNF* polymorphism is known to be associated with cognitive deficits (Egan *et al.*, 2003) and anxiety-related behaviours (Hashimoto, 2007), it is possible that performance differences between *BDNF* genotypes are only observed during cognitively demanding tasks that act to heighten anxiety levels, such as in the driving based simulation used by McHughen et al. (2010). Furthermore, the reduced use-dependent plasticity in Met allele carriers may be more closely related to deficits in the consolidation and retention of motor skills that have been detected over multiple training sessions (Fritsch *et al.*, 2010; McHughen *et al.*, 2010), which are likely to be more relevant to situations involving the recovery of motor skills after neurological injury, such as in stroke (Siironen *et al.*, 2007).

In conclusion, we have used TMS to examine the role of BDNF in promoting experimentally-induced and use-dependent motor cortex plasticity in people with different *BDNF* genotypes. We found that the modulation of motor cortex excitability was strongly influenced by the *BDNF* polymorphism, but the effect was dependent on the intervention used. Although motor cortex excitability was increased after all tasks in *BDNF* Val/Val subjects, this increase was only observed after simple ballistic training of the index finger in *BDNF* Met allele carriers. Interestingly, we also found unique plasticity in the rare Met/Met subjects, who showed an increase in MEPs after the simple ballistic task, no change after PAS, and a reduction in MEPs after complex visuomotor tracking. However, these differences in use-dependent plasticity between *BDNF* genotypes did not affect short-term motor performance and learning for either task. This lack of effect on motor performance and learning, particularly in Met/Met subjects, may have occurred because the experiments were performed on healthy young subjects, who are likely to have multiple mechanisms at their disposable to overcome deficits in specific aspects of motor system function. Given that the *BDNF* Met allele (see Bath &

Lee, 2006) and deficits in BDNF levels (see Zuccato & Cattaneo, 2009) have been associated with altered susceptibility to some neurological and psychiatric disorders, future work will need to establish the effect of *BDNF* genotype on motor cortical plasticity during the performance of complex motor tasks in motor systems that have been compromised by disease or injury.

6. General Discussion

Over the last few decades, the introduction of TMS has allowed non-invasive and painless assessment of brain function in humans. More recently, the technique of TMS has been advanced to induce cortical reorganisation and plasticity. In particular, I have used the technique of PAS to experimentally-induce plasticity throughout my studies. Assessment of brain function following experimentally-induced and use-dependent plasticity is often aimed at improving treatment for a number of neurological injuries and diseases. However, as TMS is not specific enough in how it acts on populations of neurons, it is more likely to promote compensatory mechanisms rather than restore neuronal function following neurological injury or disease. For example, TMS has shown promising outcomes for functional motor recovery following stroke (see Khedr & Fetoh, 2010), although improvement between affected individuals is often varied (Nowak *et al.*, 2010). Functional improvement following neurological insult may, in part, be affected by a number of factors that influence the effectiveness of plasticity, many of which remain unknown. In this thesis, I focussed on factors that are likely to play a major role in contributing to the variability in plasticity in studies involving healthy subjects. Specifically, the role of physical activity, ageing, hand preference, task complexity, and *BDNF* genotype were assessed as there is strong evidence suggesting that these factors influence plasticity. However, how these factors influence M1 plasticity and motor learning in humans are poorly understood. Therefore, the studies within this thesis have been aimed at identifying and further understanding factors which can influence plasticity induction in human motor cortex. Understanding these multifactorial determinants can help to optimise therapeutic rehabilitation strategies following neurological injury or disease.

6.1. Aerobic exercise and motor cortex plasticity

In my first experimental study I examined whether a history of regular physical activity (primarily involving lower limb muscles such as running and cycling) can influence

neuroplasticity in M1 areas not involved in performing the exercise (i.e. a small hand muscle). Assessment of cortical excitability with TMS (input-output curve and test intensity) suggests that highly active individuals have increased motor cortex excitability and enhanced neuroplasticity following PAS compared with sedentary people. Therefore, this was the first study to demonstrate that participation in regular physical activity may offer global benefits to motor cortex function that enhances neuroplasticity. An important next step will be to functionally translate the PAS-induced findings in this thesis to determine whether people who participate in regular physical activity have enhanced motor learning and use-dependent plasticity in M1. In addition, exercise influence on M1 function in this thesis solely focussed on habitual physical activity assessed by questionnaire. Future studies need to address the type, intensity, and duration of exercise that is most beneficial to M1 plasticity. If the generalised neuroplastic enhancement within M1 by physical activity is confirmed in more detail with future studies, the therapeutic implications of this finding may be enormous. For example, studies indicate that motor cortex reorganisation in limbs immobilised by a lesion in the motor region (compromising the motor system) can occur over the weeks and months following injury, which correlates with functional recovery (see Nudo, 2006). Therefore, introducing physical activity participation in stroke rehabilitation during this period offers the potential to significantly improve motor function in affected limbs, with the extent of functional improvement dependent on the severity of the lesion. Furthermore, as shown in Chapter 2 with enhanced PAS-induced plasticity in highly active individuals, future neuroplasticity studies should consider including an assessment on the amount of physical activity performed by an individual.

6.2. Brain hemispheres, ageing, task complexity, and motor cortex plasticity

For my second experimental study I examined the extent of motor learning and use-dependent plasticity in M1 following simple ballistic thumb training of the right (dominant) and left (non-dominant) hands of young and old adults. It is commonly acknowledged that plasticity

in motor cortical circuits is reduced with advancing age (see Zimerman & Hummel, 2010). It is also evident that asymmetries are present in the motor system (Amunts *et al.*, 1996), with clear differences in motor performance between left and right hands (Kabbash *et al.*, 1993). However, in the few studies conducted in young adults, no hemispheric differences in the extent of motor cortex plasticity have been reported (Garry *et al.*, 2004; Ridding & Flavel, 2006; Gallasch *et al.*, 2009), although more sustained MEP facilitation is present for the left hand following motor training (Gallasch *et al.*, 2009). For Chapter 3, I selected a motor task involving ballistic thumb movement to induce use-dependent plasticity as TMS studies indicate that motor learning with ballistic training is, at least in part, dependent on adaptations in M1 (Muellbacher *et al.*, 2001; Muellbacher *et al.*, 2002; Carroll *et al.*, 2008)..

Short-term motor learning of the simple ballistic task was diminished in older adults compared with young adults, but only for the right (dominant) hand. This age-related decline in motor learning asymmetry may result from compensatory mechanisms of the cerebral motor system (such as increased recruitment of bilateral brain areas with movement) in older adults (Ward, 2006). This may lead to less cortical lateralisation in older adults, which result in similar motor learning capabilities for both hands in the elderly (Kalisch *et al.*, 2006). Therefore, future studies examining the extent of motor learning between young and old adults should consider an age-related decrease in hand dominance. For example, given the age-related reduction in cortical lateralisation, assessing the right hand of right-handed young and old adults is more likely to provide more meaningful information on any age-related changes in motor learning.

Following the simple motor task, MEPs increased for both hands in young and old adults. However, there was a greater increase in MEP amplitude of the right hemisphere (left non-dominant hand) compared with the left hemisphere (right dominant hand). The asymmetries between hemispheres could not simply be explained by increased motor learning, with motor

performance improvement greater for the right hand of young adults. These findings suggest that during simple ballistic thumb training there is greater strengthening of corticomotor circuits for control of the left compared with the right hand. This would lead to the assumption that use-dependent plasticity in M1 is more effective for the left hand (right hemisphere), despite MEP facilitation evident for both hands. However, a stronger correlation between motor training improvement and the change in MEP was present for the right compared with the left hand (Chapter 3). This suggests that changes in motor cortex excitability reflect a more functional role (magnitude of behavioural performance) in the right hand. Furthermore, a significant correlation between motor training improvement and the change in MEP of the right hand has previously been shown in older adults (Rogasch *et al.*, 2009). However, given that there is a weak (right hand) or no (left hand) correlation between short-term motor learning and MEP change, and subjects still learn the task, this would indicate that the change in MEP amplitude may not be the most suitable marker of use-dependent plasticity. It may be that other measures that assess consolidation and retention of motor skills over multiple training sessions are more appropriate indicators of use-dependent plasticity. Nonetheless, for future TMS studies using the change in MEP after motor training as an indicator of use-dependent plasticity in M1, it is recommended that the right hand be used, particularly when including an older population to assess any age-related changes in plasticity.

The extent of MEP facilitation following the simple motor task in Chapter 3 was not different between young and old adults. This finding was surprising, given that previous studies have identified an age-related decrease in M1 use-dependent plasticity (Sawaki *et al.*, 2003; Rogasch *et al.*, 2009). Differences in the subject population are likely to contribute to these disparate findings, given that the experimental techniques used between studies were similar. For example, it is generally accepted that physical activity levels decrease with progressing age (Ravussin & Bogardus, 1989). However, no differences in self-reported physical activity

levels between young and old adults were present in Chapter 3, and it is possible that this older population was more physically active than those of previous studies. This is important as I previously demonstrated in Chapter 2 that PAS-induced plasticity in M1 was greater in a physically active group. It is therefore possible that sustained exercise in older adults contribute to the similar extent of use-dependent plasticity in M1. Other factors specific to the subject population that may have contributed to similar MEP changes in young and old adults include the prior history of synaptic activity (see Ridding & Ziemann, 2010), attentional focus (McNevin *et al.*, 2000), and emotional state of subjects (Tormos *et al.*, 1997). Genetic factors are another possibility that may help explain the similar magnitude of MEP change in young and old adults, given their substantial contribution to the motor system plasticity response (Missitzi *et al.*, 2011). In particular, studies have shown that the *BDNF* polymorphism, which has a common incidence of 30-50% in the general population depending on ethnicity (Bath & Lee, 2006), alters plasticity in M1 (Kleim *et al.*, 2006; Cheeran *et al.*, 2008; Fritsch *et al.*, 2010; McHughen *et al.*, 2010). Therefore, it is possible that a misrepresentation of genetic factors, such as *BDNF* genotype, present in either the young or older population contribute to similar use-dependent plasticity.

Previous TMS studies examining M1 use-dependent plasticity in older adults, including Chapter 3, have all been restricted to simple motor tasks. Task difficulty is expected to influence the magnitude of M1 reorganisation and plasticity (Flament *et al.*, 1993; Rao *et al.*, 1993; Wexler *et al.*, 1997; McNevin *et al.*, 2000; Stefan *et al.*, 2004). Furthermore, complex tasks are likely to be more functionally relevant for activities of daily living and rehabilitation. For example, complex tasks often involve multimodal processes, such as the integration of attentional, memory, visual, and motor systems for optimal visuomotor tracking. This reliance on sensory feedback for optimal task performance is important for simple daily activities that become increasingly difficult with advancing age, such as tying shoelaces, and to further enable increases in function with rehabilitation. Therefore, use-

dependent plasticity in young and old adults following a single motor training session of complex visuomotor tracking was examined in Chapter 4 (my third experimental study).

The visuomotor tracking task was performed with reduced precision and accuracy in old compared with young adults, which is in support of previous studies using complex motor tasks (Light & Spirduso, 1990; Smith *et al.*, 1999a). Alterations in the central and peripheral nervous systems are thought to be responsible for these age-related changes in motor function. Despite diminished performance in older adults, the extent of motor learning (improvement in performance) with the visuomotor tracking task was similar between young and old adults. This finding was somewhat surprising as previous studies have demonstrated that an age-related decrease in motor learning becomes more pronounced with increasing task difficulty (Light & Spirduso, 1990; Smith *et al.*, 1999a). However, this is not a novel finding as other previous studies have also shown that short-term motor learning can remain similar in young and old adults (see Voelcker-Rehage, 2008; Brown *et al.*, 2009). One caveat in determining the extent of motor learning with the more complex visuomotor tracking task in young and older adults is the significantly better baseline performance in the young group. This may lead to a ceiling effect, which limits the potential for improvements with training in young adults. Selecting a complex motor task with comparable baseline performance levels in young and old adults would overcome this issue. However, this issue remains problematic as performance usually declines with advancing age (Grabiner & Enoka, 1995), which is likely to accentuate age-related baseline differences in motor performance.

Following a training period of the visuomotor tracking task, the extent of MEP facilitation and the modulation of intracortical inhibition were not different between young and old adults. This finding may be explained by the increased complexity and attentional demand of visuomotor tracking, which may require increased activation of corticospinal neurons that have direct projections to motor neurons in older adults. In contrast, this same task may be

relegated to less direct pathways in young subjects, which are not amenable to study with TMS. In addition, as previously indicated with the ballistic task in Chapter 3, similar MEP and SICI changes in young and old adults following complex visuomotor tracking training may result from factors specific to the subject population. These factors, present in either the young or older population, include the amount of physical activity performed (Chapter 2), prior history of synaptic activity (see Ridding & Ziemann, 2010), emotional state of subjects (Tormos *et al.*, 1997), and a misrepresentation of genetic factors (Missitzi *et al.*, 2011). Nonetheless, the similar extent of M1 plasticity between young and old adults following simple ballistic (Chapter 3) and complex visuomotor (Chapter 4) training suggests that a reduction in plasticity is not an obligatory consequence of the ageing process. Factors specific to the older population examined, such as lifestyle factors involving physical activity, are likely to contribute to this maintenance in use-dependent plasticity. Therefore, studies targeting lifestyle factors common with ageing (such as decreased physical activity) and identifying their impact on M1 plasticity maintenance has the potential to guide healthy ageing interventions aimed at promoting brain health across the life-span.

Despite the absence of an age-related reduction in plasticity following either ballistic movement or visuomotor tracking, it is recommended that future use-dependent studies in M1 use the more complex task for several reasons. Firstly, the task properties of visuomotor tracking have added functional relevance for activities of daily living and rehabilitation. Whereas the production of a rapid movement in the desired direction relies more on feedforward mechanisms, the motor command required for optimal visuomotor tracking relies more on sensory feedback (memory and vision). In most daily activities, sensory feedback is critical for optimal and accurate movements. For example, the motor commands to optimally execute the movements required for making a cup of tea rely heavily on sensory feedback (Land *et al.*, 1999). However, it is important to note that both feedback and feedforward processes are likely to be involved in optimal task performance (Desmurget & Grafton, 2000).

Secondly, the visuomotor tracking task primarily targets a cortical component. While it is evident that an increase in M1 activation is required to optimally execute a more complex task, it has also been demonstrated that changes in excitability at a spinal level following the simple ballistic task are not present after the complex visuomotor tracking task (Giesebrecht *et al.*, 2012). This finding suggests that cortical mechanisms are likely to provide a greater relative contribution to MEP facilitation during the complex visuomotor task compared with the simple ballistic task. Therefore, visuomotor tracking is considered a more functionally relevant task that has a strong cortical component compared with a ballistic movement.

6.4. BDNF genotype and motor cortex plasticity

In my fourth experimental study I examined whether a common single nucleotide polymorphism in one or both alleles of the *BDNF* gene was an important influence on neuroplasticity. There are inconsistent findings on the influence of *BDNF* genotype on M1 plasticity, which may be attributed to the different methods that have been applied to induce plasticity. To help distinguish these inconsistencies, I assessed three protocols known to induce M1 plasticity in a healthy young population. Specifically, experimentally-induced (PAS) and use-dependent (simple ballistic and complex visuomotor tracking) plasticity interventions were examined in people with different *BDNF* genotypes (Val/Val, Val/Met, and Met/Met) over different sessions (Chapter 5). Furthermore, the rare Met homozygote *BDNF* genotype (5-15% of the general population, Shimizu *et al.*, 2004) was purposely examined to determine whether the putative reduction in activity-dependent BDNF release from cortical neurons in Met allele carriers affects M1 plasticity in humans.

Motor performance and short-term learning of both motor tasks were similar between different *BDNF* genotypes. However, there was a tendency for motor performance to be impaired in Met/Met subjects, evident by the reduced index finger acceleration and increased visuomotor tracking error and lag time at the end of training. These effects are quite small,

and a larger population of subjects would be required to detect functional differences in these subjects. Additionally, other assessments of motor learning, involving consolidation and retention of motor skills, may detect behavioural deficits in Met/Met subjects, which should be examined in future studies. It is also possible that the young healthy population examined has other mechanisms available at their disposal to overcome deficits in specific aspects of motor system function resulting from the *BDNF* polymorphism. Therefore, functional deficiencies in Met allele carriers may become markedly evident when available mechanisms become limited, such as in motor systems compromised by disease or injury, and should also be examined in future studies.

There was a significant increase in MEP amplitudes following all plasticity interventions in Val/Val subjects. In contrast, Met allele carriers only showed increased MEPs following the simple ballistic motor task. This finding suggests that Met allele carriers have appropriate mechanisms in place for plasticity. However, plasticity in Met allele carriers, as assessed by changes in MEP amplitude, is not expressed under some circumstances. For example, there were no changes in MEPs following PAS for Met allele carriers (Val/Met and Met/Met genotypes), which also indicates that the extent of PAS-induced plasticity is not directly related to the putative reduction in activity-dependent BDNF release from cortical neurons in Met allele carriers. Interestingly, MEPs were reduced for the *BDNF* Met/Met genotype, but remained unchanged for the Val/Met group following the complex visuomotor tracking task. This effect in Met/Met subjects is more likely related to the task-specific requirements of complex visuomotor performance than a lifetime of exposure to reduced BDNF secretion from cortical neurons, given the increased motor cortex plasticity observed in these subjects after simple ballistic training. Once again, identifying the factors that can modulate plasticity induction in Met allele carriers is of potential functional and therapeutic importance. For example, rehabilitation following a neurological insult, such as stroke, may be tailored for identified Met allele carriers to include increased complex motor training sessions to optimise

M1 plasticity induction and motor learning. Furthermore, future experimentally-induced and use-dependent plasticity studies should consider the *BDNF* genotype of each individual, given the substantial influence of the *BDNF* polymorphism on M1 plasticity.

6.5. Limitations

As with all studies, those conducted in this thesis were subject to a number of limitations. Specifically, I will discuss the drawbacks of using TMS to assess plasticity, experimental design issues and, where possible, I will describe any course of action that can overcome these limitations.

6.5.1. TMS and motor cortex plasticity

In contrast with animal studies, it is not feasible in humans to directly examine mechanisms of synaptic modification using slice preparations (a synapse or neuronal circuit isolated from the intact nervous system). Therefore, TMS has been used as a non-invasive and painless tool to provide indirect evidence from the intact nervous system in humans. One distinct advantage of TMS over other neuroimaging techniques such as fMRI and MEG is its ability to assess excitatory and inhibitory processes of brain function. This makes TMS a unique tool in assessing the neurophysiological mechanisms of brain function following a physiological (learning) or pathological (injury or disease) event. However, as with all indirect techniques, studies that use TMS need to be interpreted with caution.

6.5.1.1. The MEP

The assessment of motor cortex function by the evoked MEP is one confounding factor of TMS. The MEP reflects the excitability of corticocortical and corticospinal neurons, but also reflects the excitability of spinal motor neurons responsible for the movement of a particular muscle (Rothwell *et al.*, 1999). Therefore, despite strong evidence suggesting that changes in MEPs induced by rTMS paradigms (Stefan *et al.*, 2000; Di Lazzaro *et al.*, 2009a, b) or motor training (Classen *et al.*, 1998; Muellbacher *et al.*, 2002) have a cortical component, changes in

spinal circuits cannot be excluded. One technique used to examine changes in spinal circuits on the MEP is to stimulate (with transcutaneous electrical or magnetic pulses) the descending pathways directly at the level of the brainstem. This form of stimulation elicits cervico-medullary evoked potentials (CMEPs), which, in humans, are believed to reflect excitation of the corticospinal pathways at the point of the pyramidal decussation (Ugawa *et al.*, 1991). There is evidence that plasticity can be induced at the level of the spinal cord (Taylor & Martin, 2009) and changes in CMEP excitability have been observed after motor training of a simple ballistic task, but not complex visuomotor tracking (Giesebrecht *et al.*, 2012). Therefore, altered spinal excitability after an appropriate intervention, as assessed by CMEPs, is likely to influence the change in MEP amplitude. However, studies that have assessed CMEPs are rare, most likely due to the feasibility and discomfort (pain) associated with eliciting CMEPs, as high stimulus intensities are often needed to activate these deep axons. As an alternative measure of cortical excitability, SICI (paired-pulse TMS) was also assessed in the majority of studies conducted in this thesis. There is strong evidence that SICI is cortical in origin. For example, cervical epidural recordings showed that when given alone, a subthreshold conditioning pulse did not evoke any recognisable descending activity (Di Lazzaro *et al.*, 1998b). However, when paired together as in SICI, the conditioning pulse reduced the amplitude of synaptically evoked corticospinal volleys (I-waves) and EMG responses evoked by the test pulse (Di Lazzaro *et al.*, 1998b). Therefore, any change in the amount of inhibition from SICI following an intervention likely reflects a functional change in the motor cortex.

6.5.1.2. MEP test size

In hand muscles, the TMS intensity that induces a MEP amplitude of ~1 mV before an intervention is used to assess the change in MEP after the intervention (Stefan *et al.*, 2000; Rosenkranz & Rothwell, 2006). This change in the 1 mV MEP amplitude is used as a marker of plastic change, which I have used for Chapters 2 (PAS), 3 (simple ballistic motor task), and

4 (complex visuomotor tracking task). However, recent information suggests that a MEP size set as a percentage of the maximal M-wave should be used to reduce variability in measures of cortical excitability as this adjusts for some of the non-linearity properties within the spinal motor neuron pool (Lackmy & Marchand-Pauvert, 2010). This method was adopted in Chapter 5. However, this is particularly important for ageing studies, as there is typically a decrease in the number of motor neurons (see Doherty *et al.*, 1993), which results in a reduced M-wave, as shown in Chapters 3 and 4. For these chapters, normalisation of MEPs to maximal M-waves were not reported as M-wave data was incomplete for older subjects. However, when MEPs were normalised to maximal M-waves for the two studies, the findings were unaltered (data not shown).

6.5.1.3. SICI

Paired-pulse TMS is increasingly used to examine changes in intracortical inhibition with motor learning. It is evident that SICI varies with the size of the test pulse (Chen *et al.*, 1998; Roshan *et al.*, 2003), with an evoked MEP of 10-30% maximal M-wave recommended for inter- and intra-individual comparisons (Lackmy & Marchand-Pauvert, 2010). If, as expected, the MEP amplitude increases following motor training, this may confound interpretation of SICI, given the influence of the test pulse size. However, the issue of whether or not to adjust the test pulse after motor training remains unresolved. I have assessed both unadjusted (Chapter 3) and adjusted (Chapter 4) test pulses after motor training in my studies. However, it is difficult to conclude from these studies whether it is most appropriate to adjust the test pulse or not to assess SICI, as the motor task performed was different between studies. A number of previous studies have logically adjusted the test pulse after motor training based on the finding that SICI varies depending on the MEP size (e.g. Perez *et al.*, 2004; Rosenkranz *et al.*, 2007a). However, it has also been shown that estimates of SICI are systematically affected by the intensity of the test pulse, regardless of excitability state (Zoghi *et al.*, 2003; Garry & Thomson, 2009). If this finding is correct, then adjusting

the intensity of the test pulse to match MEP size before motor training may essentially confound interpretation of SICI. Therefore, the issue of whether to adjust the test intensity after training when measuring SICI remains unresolved and warrants further investigation.

6.5.2. Mechanisms of human motor cortex plasticity

Another limitation is that markers of neuroplasticity in the human M1 are assumed to be due to LTP/LTD mechanisms, as these cannot be measured directly. However, there is strong evidence that the changes in MEP amplitude following PAS-induced and use-dependent plasticity in humans are similar to those in animal preparations, which are known to involve LTP and LTD mechanisms (see Hess & Donoghue, 1996; Rioult-Pedotti *et al.*, 2000). For example, changes in MEP amplitude have a rapid onset, are long lasting (outlasts manipulation), reversible (returns to baseline), and muscle (input) specific (see Classen *et al.*, 1998; Muellbacher *et al.*, 2001; Ziemann *et al.*, 2008). Additional evidence of LTP- and LTD-like mechanisms in the human M1 is the dependence on NMDA receptor activation (Bütefisch *et al.*, 2000; Stefan *et al.*, 2000; Wolters *et al.*, 2003) and downregulation of GABAergic inhibition (Ziemann *et al.*, 2001) to induce neuroplasticity. Therefore, despite it not being feasible to directly measure LTP/LTD mechanisms in humans, the overwhelming similarities to direct measures in animal studies provides strong evidence that PAS-induced and use-dependent plasticity are due to LTP- and/or LTD- like mechanisms.

6.5.3. Motor cortex excitability and motor learning in humans

It is commonly accepted that motor learning increases MEP amplitude (Muellbacher *et al.*, 2001; Ziemann *et al.*, 2001) and decreases SICI (Liepert *et al.*, 1998; Garry *et al.*, 2004; Perez *et al.*, 2004; Gallasch *et al.*, 2009). However, on an individual level, changes in motor cortex excitability do not typically reflect the magnitude of behavioural performance (Li Voti *et al.*, 2011). This has been observed throughout all use-dependent plasticity studies involving either simple ballistic (Chapters 3 and 5) or complex visuomotor tracking (Chapters 4 and 5)

tasks in this thesis. It is likely that factors influencing the plasticity response (see Ridding & Ziemann, 2010), but have no effect on short-term motor learning, confound the association between changes in motor cortex excitability and the extent of task improvement. In addition, all cortical excitability and inhibitory TMS measures throughout this thesis were performed after motor training. It may be possible that the MEP response varies considerably with time or in an activity specific manner, and that an association between motor cortex excitability change and behavioural improvement is present only throughout motor training during performance of the task.

6.5.4. Experimental design

The experimental design used in these studies may also play a part in the interpretation of the findings. In this thesis, a cross-sectional design was used for all studies. A cross-sectional design involves separate subject cohorts tested at one time point to determine if differences exist. For example, I have examined separate subject populations that vary based on several important factors (exercise, age, hand preference, genetics) that can influence motor cortex plasticity and motor learning in a single experimental session. However, it is possible that the groups tested have other confounding factors influencing motor cortex plasticity besides the one being investigated. This may include a number of lifestyle factors that contribute to the plasticity response, such as socioeconomic status, level of education, and nutritional status. To limit variables of lifestyle factors, a longitudinal design (test the same individuals of one cohort at least twice over time) could be implemented. However, a number of problems also exist in this design. For instance, it is very time consuming, results may later prove inadequate (development of new techniques and measurements), and is likely to involve subject attrition (either voluntary or involuntary, such as individuals sustaining neurological injury or disease). Therefore, given the resource and time-constraints associated with longitudinal designs, a cross-sectional design remained the most appropriate experimental approach for the studies in this thesis.

6.6. Future directions

Despite the mentioned study limitations, the novel findings from this thesis have advanced our understanding of the influences on human M1 plasticity induction. However, a full comprehension of M1 function and plasticity in the healthy and compromised motor systems, with the intention to optimise treatment following neurological injury or disease, remains incomplete and warrants further investigation.

A number of future studies stemming from those conducted in this thesis have been mentioned throughout this discussion. For example, it will be important to determine whether people who participate in regular physical activity have functional benefits in M1 (enhanced motor learning and use-dependent plasticity) and what lifestyle factors (such as physical activity levels) promote brain health across the life-span. However, it would be of particular interest to assess the interaction between multiple factors capable of influencing the effectiveness of M1 plasticity examined in this thesis. For example, does exercise play a beneficial role in M1 plasticity and learning in older adults, and is this influenced by different *BDNF* genotypes? If, as expected, the benefits of regular exercise evident in PAS-induced M1 plasticity are functionally translated, then it is more than likely that these benefits are most pronounced in the ageing population, given the substantial benefit of exercise on brain plasticity in the ageing hippocampus (Colcombe *et al.*, 2004; Kramer *et al.*, 2006). Furthermore, it might also be possible that reduced short-term M1 plasticity and motor learning in Met allele carriers may be alleviated in those participating in regular physical activity, given that exercise has demonstrated increased levels of BDNF in M1 (Ding *et al.*, 2004; Klintsova *et al.*, 2004). Identifying the impact of these factors on M1 plasticity from such a study could potentially help guide healthy ageing interventions and have significant therapeutic implications.

6.7. Concluding remarks

The findings in this thesis suggest that sustained regular exercise, hand preference, and *BDNF* genotype help explain the variable response to protocols that induce M1 plasticity in healthy adults. However, it is unclear why there was no age-related decrease in plasticity in the studies in this thesis, as has been commonly observed in previous studies. Further studies aimed at examining lifestyle factors common with ageing (such as decreased physical activity) and their impact on M1 plasticity may assist in understanding this phenomenon. In order to further understand factors that influence M1 plasticity in humans, future studies should assess subject characteristics based on physical activity levels, hand preference, and *BDNF* genotype as potential confounding factors in the response. Furthermore, there may be potential to capitalise on these factors to optimise M1 plasticity and recovery of motor function following neurological injury. For example, motor function in a limb that has been immobilised by a neurological insult could be improved with participation in physical activity, as this may offer a generalised neuroplastic enhancement within M1. In addition, as M1 plasticity is considerably influenced by the *BDNF* polymorphism, identifying the *BDNF* genotype of an individual who has had a neurological insult allows for modified rehabilitation, such as increasing the number of motor training sessions for Met allele carriers, to optimise M1 plasticity induction.

7. Appendices

7.1. Appendix I: Publications arising from thesis.

Cirillo J, Lavender A.P., Ridding M. C., & Semmler J. G. (2009) *Motor Cortex Plasticity Induced by Paired Associative Stimulation is Enhanced in Physically Active Individuals.* J Physiol 587, 5831-5842.

Cirillo J., Rogasch N. C., and Semmler J. G. (2010) *Hemispheric differences in use-dependent corticomotor plasticity in young and old adults.* Exp Brain Res 205, 57-68.

Semmler J.G., and **Cirillo J.** (2010) *Exercise can help rewire the brain: neuroplasticity and motor cortex function in physically active individuals.* Physiology News 81, 26-28.

Cirillo J., Todd G., and Semmler J. G (2011) *Corticomotor excitability and plasticity following complex visuomotor training in young and old adults.* Eur J Neurosci, 34, 1847-1856

Cirillo J., Hughes J., Ridding M.C., Thomas P.Q., Semmler J.G. (2012) *Differential modulation of motor cortex excitability in BDNF met allele carriers following experimentally-induced and use-dependent plasticity.* Submitted to Eur J Neurosci

7.2. Appendix II: Presentations and abstracts arising from thesis.

Title: Neuroplasticity and the human motor cortex 2010

Research Institute of National Rehabilitation Center for Persons with Disabilities,

Tokorozawa, Japan

Title: Neuroplasticity and the human motor cortex 2010

University of Tokyo, Tokyo, Japan

Title: Use-dependent motor cortex plasticity following complex visuomotor training in young and old adults 2010

29th International Congress of Clinical Neurophysiology Conference, Kobe, Japan

Title: Left-Right Differences in Cortico-motor Function with Advancing Age 2009

School of Molecular and Biomedical Sciences,

MBS Postgraduate Symposium, The University of Adelaide

Title: Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals 2009

29th Australian Neuroscience Meeting, Canberra, Australian Capital Territory, Australia.

Title: Influence of Exercise on Brain Function 2008

School of Molecular and Biomedical Sciences,

MBS Postgraduate Symposium, The University of Adelaide

Title: Exercise, Ageing and Motor Cortex Plasticity in Humans 2008

Discipline of Physiology,

Physiology Seminar Series, The University of Adelaide

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