FACTORS INFLUENCING THE INDUCTION OF NEUROPLASTIC CHANGES IN HUMAN MOTOR CORTEX

A thesis submitted for the Degree of

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by

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Abstract

The human primary motor cortex (M1) undergoes structural and functional change throughout life by a process known as neuroplasticity. Techniques which artificially induce neuroplastic changes are seen as potential adjunct therapies for neurological conditions reliant on neuroplasticity for recovery of function. Unfortunately, the reported improvements in function when these techniques have been used in combination with regular rehabilitation have so far been inconsistent. One reason attributed to this is the large variability in effectiveness of these techniques in inducing neuroplastic change. This thesis has investigated factors influencing the effectiveness and reproducibility of neuroplasticity induction in human M1 using several experimental paradigms.

The effectiveness and reproducibility of inducing neuroplasticity in human M1 using two variants of a paired associative stimulation (PAS) protocol was investigated in the first set of experiments (Chapter 2). Both protocols repeatedly paired a peripheral electrical stimulus to the median nerve of the left wrist with single-pulse transcranial magnetic stimulation (TMS) delivered 25 ms later to the contralateral M1. Neuroplastic changes were quantified by comparing the amplitude of the muscle evoked potential (MEP) recorded in abductor pollicis brevis (APB) muscle by suprathreshold TMS prior to and following PAS. With both protocols, neuroplasticity induction was more effective, and the responses across sessions more reproducible, if the experiments were performed in the afternoon compared to the morning.

Subsequent experiments confirmed the time of day modulation of PAS-induced neuroplasticity by repeatedly testing twenty-five subjects on two separate occasions,

once in the morning (8 am), and once in the evening (8 pm) (Chapter 3). Time of day was also shown to modulate GABAergic inhibition in M1. In a further set of experiments, a double-blind, placebo-controlled study demonstrated that artificially elevated circulating cortisol levels (with a single oral dose of hydrocortisone) inhibits PAS-induced neuroplasticity in the evening (8 pm), indicating that the time of day modulation of neuroplasticity induction with PAS is due, at least in part, to differences in circulating cortisol levels (Chapter 3).

The cortical circuits that are modulated by PAS have also been shown to be important in motor learning. Therefore, the final set of experiments, described in Chapter 4, investigated whether motor-training-related changes in motor performance (and cortical excitability) following a ballistic motor training task are also modulated by time of day. Twenty-two subjects repeatedly abducted their left thumb with maximal acceleration for thirty minutes during two experimental sessions (morning (8 am) and evening (8 pm)) on separate occasions. Motor training improved motor performance, and increased cortical excitability, however these changes were independent of time of day. It may be that the motor training task and/or outcome measures used were not sufficiently sensitive to detect a subtle time of day effect of motor training on motor performance. Alternatively, the normally functioning motor system may be able to compensate for changes in cortical excitability to maintain optimal motor performance.

These findings have important implications for therapies reliant on neuroplasticity for recovery of function, and indicate that rehabilitation may be most effective when circulating cortisol levels are low.

Declaration

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

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Aims and general introduction

The human nervous system reorganises the strength of connections between neurons throughout adult life. This reorganisation is termed neuroplasticity, and is an important process associated with learning, memory and recovery from neurological insult. In recent years, several experimental techniques have been developed to artificially induce neuroplastic change in human cortex. Ultimately, it is hoped that these techniques will aid in promoting recovery from various neurological insults such as stroke.

One problem associated with these techniques is the large variability in effectiveness for inducing neuroplastic change. My initial study for this thesis, detailed in Chapter 2, sought to identify and understand the factors contributing to this variability. Two variants of a "paired associative stimulation" (PAS) protocol were used to induce plastic change in human motor cortex. The protocols differed in terms of frequency of stimulation and duration of the intervention. A range of neurophysiological and experimental variables were assessed to determine whether they influenced the extent of neuroplasticity induction by PAS. Subjects were randomly divided into the two PAS protocol groups and each subject was tested with the same protocol on three separate occasions, with each session at least one week apart. None of the neurophysiological variables examined reliably predicted an individual's response to the intervention. However, with both PAS protocols the induction of neuroplastic change was more effective, and more reproducible, for experiments conducted in the afternoon compared with the morning.

Since different subjects participated in the morning and afternoon experiments, my second set of experiments, detailed in Chapter 3, sought to directly test the hypothesis that time of day influenced neuroplasticity induction in human motor cortex. Subjects were assessed on two separate occasions, separated by at least one week. One session was in the morning (8 am), the other in the evening (8 pm), with the order of the sessions randomised. Salivary cortisol concentration was also measured before and after PAS. Cortisol release is under circadian control, and cortisol is known to inhibit learning and memory. These experiments demonstrated that time of day affects neuroplasticity induction with PAS, with significant neuroplasticity induction observed in evening experiments, but not in the morning. Salivary cortisol levels were greater in the morning than the evening, however, there was no significant relationship between the amount of neuroplasticity induced and salivary cortisol concentration in this study.

A third double-blind, placebo-controlled study was conducted to investigate more conclusively whether cortisol levels influence neuroplasticity induction. This study is also presented in Chapter 3. Subjects attended two experimental sessions at 8 pm (when endogenous cortisol levels are low), separated by at least one week. Prior to receiving PAS, subjects received either a single oral dose of hydrocortisone (which is metabolised to cortisol) or a placebo. Salivary cortisol levels were higher, and neuroplasticity induction by PAS was less effective when subjects were given oral hydrocortisone. This experiment provided strong evidence that neuroplasticity induction in human motor cortex is, at least in part, modulated by circulating levels of cortisol.

PAS is believed to induce neuroplastic change by mechanisms that are known to be important in motor learning. Chapter 4 details the fourth set of experiments which

aimed to determine whether there was a functional correlate for the time of day effect on neuroplasticity induction revealed by PAS. Subjects performed a ballistic thumb abduction motor training task on two separate occasions, in the morning (8 am) and evening (8 pm), separated by at least one week. The order of the sessions was randomised. The motor training task improved motor performance (as measured by maximum thumb acceleration) and also increased cortical excitability (assessed by TMS), but the extent of performance improvement following training was not dependent upon time of day. It may have been that the motor training task was not sufficiently sensitive to detect a subtle time-of-day modulation of motor performance following motor training, or that the normally functioning motor system is able to compensate for changes in cortical excitability to maintain motor output.

These studies demonstrate that the induction of neuroplasticity in human M1 using PAS is dependent on time of day. Neuroplasticity induction is more effective, and the reproducibility of the induced effects is greater, if experiments are performed in the evening. This effect is, at least to some extent, modulated by circulating cortisol levels. A functional correlate for the time-of-day modulation of neuroplasticity induction has not been found - motor performance changes induced with a repetitive ballistic thumb training task are not modulated by time of day. These findings have important implications for therapies reliant on neuroplasticity for recovery of function, and indicate that most effective rehabilitation may occur when circulating cortisol levels are low.

1. Literature review

Humans are capable of an amazing array of movements, which allow us to perform all the necessary activities of daily living. Although we learn the fundamentals of these skills early in life, there is continual improvement in efficiency and effectiveness throughout life. Until recently it was believed that the cortical component of the adult motor system was stable and essentially incapable of change (Hallett, 2001). However, it is now known that the adult motor system is able to reorganise by a process referred to as neuroplasticity (Pons et al., 1988; Sanes and Donoghue, 2000). Neuroplasticity is defined by Donoghue and colleagues (1996) as "any enduring change in cortical properties (that are) either morphological or functional." These neuroplastic changes include synaptogenesis, alteration of synaptic efficacy and unmasking of hidden synapses. An example of neuroplasticity is the recovery in motor performance after stroke. Following the cerebrovascular event, cortical regions associated with the stroke undergo spontaneous recovery, and in the early stage this is largely due to resolution of oedema. However, it also seems likely that neuroplastic changes begin to occur which attempt to regain lost function (Hallett, 2001). However, in many cases, full recovery of motor function is not achieved. By artificially driving neuroplastic change in the sensorimotor cortex, it is hoped that recovery from such neurological conditions may be improved. Artificial modulation of neuroplasticity may also improve function in conditions where neuroplasticity is overactive, such as in various forms of dystonia.

Several techniques have been recently developed for the artificial induction of neuroplastic change in human sensorimotor cortex, and these have shown some promise in improving treatment outcomes following stroke (Hummel et al., 2005; Kim et al., 2006; McDonnell et

al., 2007a). Unfortunately the neuroplastic change induced with these paradigms is variable, limiting their effectiveness as a therapeutic tool. The experiments reported in this thesis have aimed to improve our understanding of the factors which influence the reliability and effectiveness of neuroplasticity induction in human motor cortex. The studies described in this thesis utilised an experimental paradigm that relies on electromagnetic stimulation of the nervous system to induce neuroplastic changes in motor cortex (Chapters 2 and 3), and in Chapter 4 neuroplasticity was induced by a behavioural motor training paradigm involving repeated ballistic movements of a digit.

In this introductory chapter, I will briefly describe the relevant anatomical and functional organisation of the motor cortex and corticospinal system, and discuss how the motor cortical organisation can be temporarily modified with different techniques used to induce neuroplastic changes. I will also review what is currently known about the factors influencing the induction of neuroplastic change with these techniques.

1.1. The human motor cortex

The cerebral cortex contains approximately 30 billion neurons and a similar number of glial cells (Mountcastle, 1978), and until the late nineteenth century, the accepted dogma was that these neurons functioned as one entity – that is, the brain was devoid of compartmentalisation. It wasn't until the separate pioneering work of Jackson and Penfield who challenged this view, by suggesting that the brain may in fact contain localised regions with separate function. The English neurologist John Hughlings Jackson observed in 1873 that epileptic seizures (in the motor area) progressed in an ordered sequence, starting in the periphery and progressing centrally (Jackson, 1873). He reasoned

that this phenomenon could only be explained if there existed compartmentalisation within the motor areas of the cortex.

These findings were in agreement with the later observations of the Canadian neurosurgeon Wilder Penfield. He used direct electrical stimulation of the exposed cortex of patients undergoing surgical treatment for epilepsy to construct a topographical map, or "homunculus". This map showed which particular region of the sensory cortex was responsible for a particular sensation (Penfield and Boldrey, 1937). A similar homunculus was later demonstrated for the motor cortex (Penfield and Welch, 1951). The motor responses are organised in a medial to lateral topography of the lower limb, upper limb, head and face (Sanes and Donoghue, 2000).

Although separate somatotopic regions in the motor cortex do exist, the organisation is not as discretely somatopically organised as the Penfield homunculus would suggest (Rizzolatti et al., 1998; Schieber, 2001; Schieber, 2002). Functional magnetic resonance imaging studies have demonstrated that finger and wrist movements activate multiple sites and have overlapping representations in primary motor cortex (M1) (Sanes et al., 1995). Also, neural populations have been shown to encode for different movement directions (Georgopoulos et al., 1986). These findings suggest that rather than distinct regions of the motor (and sensory) cortex being solely involved with one particular muscle or muscle group, it now appears that there are multiple, overlapping and converging subregions of the cortex subserving movements around particular joints (Lemon, 1993; Rizzolatti et al., 1998). This allows the cortex to be extremely flexible and adaptable, and provides a framework by which the cortex is able to be modified to optimise performance, particularly if one region is damaged.

The cellular architecture of the cortex has been well described, following Brodmann's initial investigations (Brodmann, 1909). The cerebral cortex can be broadly divided into 6 main horizontal layers (I-VI), numbered from the outer surface of the cortex. The different layers are distinguished by the different cell populations present within each layer. In addition, certain layers of the cortex are primarily involved with receiving input from other cortical regions, whereas others are associated with sending information to other regions. Neurons within the cerebral cortex can be broadly divided into two main cell types - the pyramidal or projection neurons, and the non-pyramidal or stellate interneurons. Pyramidal cells are the main output cells of the motor cortex. The pyramidal cells are primarily located in cortical layers III, V and VI, and the dendrites of the pyramidal cells have been shown to extend vertically and horizontally, with extensive networks in layers II-IV (Porter and Lemon, 1993). Layer V of the primary motor cortex contains giant pyramidal cells which are also known as "Betz cells". These cells are the only ones in this layer that terminate directly onto motor neurons in the ventral horn of the spinal cord. The dendrites are covered with spines which receive the majority of synaptic input, with some estimates of up to 60,000 spines on a single pyramidal cell (Cragg, 1975). The pyramidal neurons use the excitatory amino acid glutamate as their primary neurotransmitter (Streit, 1987).

The stellate cells of the motor cortex, which encompass the interneurons of the cortex, are divided into the spiny and non-spiny stellate cells and constitute approximately 25% of the neurons in the motor cortex. The dendrites of the stellate cells are organised radially, and do not extend outside the cortex. The spiny stellate cells are the most widespread

interneurons, and are primarily located in layer IV (Lund, 1984). The spiny stellate cells use glutamate as their neurotransmitter (Jones, 1981).

The non-spiny stellate cells are located in all layers of the motor cortex, and primarily use the the inhibitory neurotransmitter γ-aminobutyric acid (GABA) to form inhibitory synapses (Jones, 1981). GABAergic neurons are unevenly distributed across the cortical layers with the highest concentration in layers I and II (up to 100%), whereas levels as low as 15% are seen in other areas (Hendry and Jones, 1981).

1.1.1. The corticospinal system

Several descending fibre systems are known to influence spinal cord activity (Brodal, 1969). The largest of these is the pyramidal tract, the fibres of which originate in layer V of the pre-motor, motor and sensory cortices and travel through the medullary pyramids. The pyramidal tract contains over 1 million axons (Heffner and Masterton, 1975), with the vast majority (~ 75%) of these fibres crossing the midline at the junction of the medulla and spinal cord, where they continue down the spinal cord (Brinkman and Kuypers, 1973). Here the fibres enter the lateral column of the spinal cord white matter and descend to all levels of the spinal cord, where they are known as the lateral corticospinal tract. These fibres then synapse with motor neurons and interneurons in the ventral horn of the spinal cord which innervate the muscles of the limbs and trunk. Of the small proportion of the fibres that do not cross the midline, most descend in the ventromedial columns of the spinal cord and innervate the trunk muscles (Brodal, 1969).

Only a select few primates share the ability to independently and precisely control movements of their digits (Porter and Lemon, 1993). This unique ability is attributed to

the monosynaptic connection between the motor cortex and spinal motor neurons (Porter and Lemon, 1993). The corticospinal neurons with the direct, monosynaptic connection to spinal motor neurons are termed cortico-motoneuronal (CM) cells (Bernhard et al., 1953). This projection is the most important pathway involved in the cortical control of hand muscles for skilled, fractionated movements of the digits (Lemon, 1993). The operation of this pathway can be assessed non-invasively in humans using transcranial magnetic stimulation (TMS) (see section 1.2.2), and I have used TMS to induce neuroplastic change in motor cortex (Chapters 2 and 3) and to quantify these changes (Chapters 2, 3 and 4).

1.1.2. Physiological importance of GABA-mediated inhibition

The importance of GABAergic cortical neurons in modulating motor control has been known for some time. In this section I will review the physiology of GABA-mediated intracortical inhibition, with particular emphasis on its role in fine-tuning motor control and also in modulating neuroplasticity.

The effects of the neurotransmitter GABA are mediated by three receptor classes - $GABA_A$, $GABA_B$ and $GABA_C$. $GABA_A$ and $GABA_B$ receptors are the most widely distributed receptors in the central nervous system (Watanabe et al., 2002). $GABA_A$ receptors are the most abundant receptor class, and are found at most GABAergic synapses. These receptors form membrane channels which, when activated, lead to an increase in permeability of chloride (Cl^-) ions. The influx of Cl^- leads to membrane hyperpolarisation, resulting in an inhibitory postsynaptic potential (IPSP). Physiological, pharmacological and anatomical investigations of rat hippocampal slice preparations have demonstrated that the IPSP has a relatively brief time course - and consists of a fast (3-8 ms) and slow (30-70 ms) inhibitory response (Pearce, 1993).

The second type of GABA receptor is the $GABA_B$ receptor. This is a G-protein coupled receptor which mediates K^+ or Ca^{2+} conductance. Activation of the receptor increases K^+ or decreases Ca^{2+} conductance, and is associated with slower synaptic inhibition than the $GABA_A$-mediated inhibition (Kerr and Ong, 1995). The $GABA_B$-mediated inhibition has a slow onset, with a peak at approximately 100 ms, and decays over several hundred milliseconds (Solis and Nicoll, 1992). $GABA_B$ receptors are located on both the presynaptic as well as the postsynaptic side of the synapse.

The third receptor class is the $GABA_C$ receptor, which is insensitive to the $GABA_A$ receptor antagonist bicuculline, and the $GABA_B$ receptor agonist baclofen (Johnston et al., 1975). These receptors are coupled to a Cl^- selective ion channel.

Due to the generation of an IPSP, GABA hyperpolarises the postsynaptic neuron and makes it more difficult for the initial axon segment to reach the firing threshold for action potential generation (Krnjevic and Schwartz, 1967). This is an important process which allows fine tuning of motor commands by inhibiting unwanted movements. For example, primates injected with the GABA antagonist bicuculline showed marked disruption of task-related activity in pyramidal tract neurons and overflow of movement (Matsumura et al., 1992). In human primary motor cortex, GABAergic inhibition appears to be dynamically modulated to prevent unwanted movements. A selective “lifting off” of GABAergic inhibition of corticospinal neurons is associated with selective activation of the appropriate muscle for a particular movement (Ridding et al., 1995c). These findings were extended by Zoghi et al. (2003) who demonstrated that GABAergic inhibition is

differentially modulated for sub-populations of corticospinal neurons controlling selective fractionated contractions of hand muscles (Zoghi et al., 2003).

Several motor disorders characterised by difficulty isolating the appropriate movement, also show impaired GABAergic inhibition in motor cortex, such as Parkinson's Disease (Ridding et al., 1995a) and focal dystonia (Ridding et al., 1995b). The motor abnormalities associated with focal dystonia may well be multi-factorial (Hallett, 2006). A reduction in GABAergic inhibition seems likely to contribute to the impaired muscle activation, but it appears also to be important in neuroplasticity (see section 1.3.1.). Evidence in animal studies supports the view that a reduction in GABA-mediated inhibition facilitates synaptic potentiation and modulation of representational maps (Jacobs and Donoghue, 1991; Jones, 1993). In humans, long-term musical training results in improved motor performance, and this is also associated with structural and functional changes in the musician's brain (Munte et al., 2002). One example of this is a reduction in $GABA_A$ mediated intracortical inhibition in motor cortex (Nordstrom and Butler, 2002), and between motor cortex of one hemisphere and the opposite side (Ridding et al., 2000b). It has been proposed that the reduction in $GABA_A$ mediated inhibition is necessary for the induction of neuroplastic change (Ziemann et al., 2001). The reduced inhibitory tone may provide a mechanism by which a musician can improve motor performance by training-induced neuroplastic change. However, if the reduction in GABAergic inhibition is extreme, as in focal dystonia (Ridding et al., 1995b), the changes may result in a pathological enhancement of neuroplasticity (Quartarone et al., 2003).

Therefore, modulation of GABA-mediated inhibition in motor cortex is critical for the control of fine movements and also in the acquisition of new motor skills and memories. It

may also be important in the recovery from various neurological insults. In the experiments I described in Chapters 2 and 3, I quantified both $GABA_A$- and $GABA_B$-mediated intracortical inhibition, to assess whether these neurophysiological measures were important in modulating neuroplasticity induction in human motor cortex.

1.2. Non-invasive brain stimulation techniques for human motor cortex

The number of techniques available to investigate human cortical function have increased dramatically over the last few decades, providing a major boost to neuroscience research. Examples of these techniques include functional magnetic resonance imaging (fMRI), positron emission topography (PET) and magnetoencephalography (MEG), transcranial electrical stimulation (TES) and transcranial magnetic stimulation (TMS). Each technique uses a slightly different approach to investigate cortical function, and each has advantages and disadvantages over the others. For example, a PET scan quantifies metabolic (usually glucose) changes occurring in different brain regions, whilst fMRI identifies hemodynamic changes. Both scans provide excellent spatial resolution, but relatively poor temporal resolution. Conversely, MEG, which measures magnetic fields produced by electrical activity in the brain, has excellent temporal resolution. A limitation of these imaging techniques is that it is not possible to determine whether the changes being detected are due to inhibitory or excitatory phenomena, or to be certain of the functional class of neurons responsible for the imaged activity.

This drawback has been overcome with the development of non-invasive cortical stimulation techniques such as transcranial magnetic stimulation (TMS) (and to a lesser extent transcranial electrical stimulation (TES)). These provide excellent temporal resolution with relatively good spatial resolution, but importantly also identify the

functional class of the neurons contributing to the activity. In addition they provide a quantitative measure of neuronal excitability, and allow the investigator to study both excitatory and inhibitory processes.

In the next section I will discuss non-invasive stimulation of human motor cortex in more detail, since I used TMS during my experiments to provide information on the physiological properties of the neuronal population being stimulated.

1.2.1. Transcranial electric stimulation

Until relatively recently, the only method for stimulating the corticospinal pathway of humans was to directly stimulate the exposed cortex. Such opportunities are relatively rare, and were essentially restricted to access during neurosurgery. Non-invasive stimulation of human cortex through the scalp using transcranial electrical stimulation (TES) was a major advance in neurophysiology (Merton and Morton, 1980). The TES technique involves passing a large direct current between two electrodes on the scalp overlying the motor cortex. TES can activate the underlying corticospinal neurons, either at the neuronal cell body, at the axon hillock or first node of Ranvier (Day et al., 1987; Day et al., 1989). However, a major drawback of this method is that due to the high resistance of the scalp, skin and bone, a high stimulus intensity is required to activate the underlying cortical structures, resulting in direct activation of scalp nociceptive afferents and muscles, which produces significant pain and discomfort for the subject.

1.2.2. Transcranial magnetic stimulation

The limitations associated with TES were overcome by the development of transcranial magnetic stimulation (TMS) some five years later (Barker et al., 1985). The stimulator consists of a tightly-wound insulated wire coil, connected to a series of capacitors. When a large but brief electrical current is discharged through the coil (typically 5000A, with a 100 μs rise-time), a magnetic field is induced which will cause current to flow in conductive structures, such as nerve cells (Barker et al., 1985). Importantly, this magnetic field is unattenuated by biological tissue and stimulates the conductive structures as if they were being directly stimulated with electrodes. If the stimulus is of sufficient intensity, TMS induces multiple descending volleys of action potentials in corticospinal neurons (Rothwell, 1991; Burke et al., 1993). The descending volleys evoke excitatory post-synaptic potentials in α-motoneurons. With sufficient temporal and spatial summation, the threshold for action potential generation is reached in the motoneuron and a motor evoked potential (MEP) can be observed in the surface electromyogram (EMG) of the target muscle.

1.2.2.1. Use of TMS

As mentioned previously, TMS can be used to non-invasively, albeit indirectly, assess the excitability of corticospinal neurons activated by the TMS pulse. The excitability of the underlying structures can be assessed in a number of ways. For example, single-pulse TMS can delivered at various scalp positions using a coordinate system which is referenced to the vertex (Cohen et al., 1991b; Wassermann et al., 1992). The excitability of corticospinal projections can be indirectly, but quantitatively assessed by measuring either the peak-to-peak amplitude or the area of the MEP produced with a given TMS intensity at a given scalp site. This allows for the construction of a “map” of the TMS-

evoked responses in the muscle(s) of interest. The scalp site with the largest MEP response is often referred to as the 'optimal site'. Several factors are known to influence the stimulus response property of MEPs with single-pulse TMS, such as training (Beck et al., 2007; Rosenkranz et al., 2007), ageing (Oliviero et al., 2006; Muller-Dahlhaus et al., 2008; Tecchio et al., 2008) and also various neurological disorders such as stroke (Hallett, 2001), Parkinson's Disease (Lefaucheur, 2005; Morgante et al., 2006) and focal hand dystonia (Quartarone et al., 2003).

The excitability of corticospinal projections can also be assessed by a stimulus response curve, which is produced by stimulating M1 (at the 'optimal site') with a range of TMS intensities, and plotting the size of the MEP against TMS intensity. This input-output curve reflects a balance between inhibitory and excitatory inputs to the motor cortex and motor neuron pool (Devanne et al., 1997). The relationship between TMS intensity and MEP amplitude is sigmoidal in hand muscles (Devanne et al., 1997; Pitcher et al., 2003), and the slope of the steep portion of the curve is influenced by several factors including age (Pitcher et al., 2003) and training (Rosenkranz et al., 2007).

There is considerable inter-subject variability in the response to TMS at a given stimulus intensity. This variability is due to several factors including anatomical differences, electrode placement and other environmental stimuli (Carroll et al., 2001; Koski et al., 2005). Therefore, the TMS intensities used to construct a stimulus-response curve are usually expressed as a percentage of the threshold for producing a MEP. The TMS threshold is determined when the muscle is relaxed (resting motor threshold; RMT) or when it is being voluntarily contracted (active motor threshold; AMT). The RMT is thought to reflect the membrane excitability of corticospinal neurons and the interneurons

projecting on to these output neurons (Ziemann et al., 1996b; Chen et al., 1997b). The threshold is operationally defined as the minimum TMS intensity that will produce a MEP above a certain (arbitrary) amplitude in a given number of trials. There is considerable trial-to-trial variability in the amplitude (or area) of the MEP produced at a given intensity, therefore it is necessary to use multiple trials to provide a more accurate measure of threshold (Rossini et al., 1994). By increasing the number of trials used in threshold determination, Rossini et al. (1994) demonstrated that there was an improved reliability of the threshold measure. That study suggested that a criterion of 5 out of 10 MEPs above 50 μV be used, as this provided the most reliable measure of resting motor threshold determination. More recently, however, a study by Carroll et al. (2001) reported a high degree of correlation of resting threshold measures in subjects across three experimental sessions when measured with the 3 out of 5 MEPs above 50 μV criterion (Carroll et al., 2001).

Active motor threshold is assessed during voluntary muscle activation, usually at a low contraction level (eg 5% maximum voluntary contraction (MVC)). Since the voluntary contraction increases the excitability of the cortical and spinal neurons, a lower TMS intensity is required to produce a MEP in the active muscle, hence AMT is lower than RMT. In the present study I have assessed whether RMT or AMT influence neuroplasticity induction (Chapter 2).

1.2.2.2. Intracortical inhibition and facilitation

In the previous section (section 1.2.2.1.), I discussed how TMS can be used to assess the excitability of corticospinal projections using single-pulse TMS. However, by using paired-pulse TMS, the function of short-latency intracortical inhibitory (SICI) and

intracortical facilitatory (ICF) networks can also be assessed. In the following section I will discuss how these networks are assessed with TMS.

In 1993 Kujirai and colleagues were the first to describe a method for investigating the function of intracortical inhibitory and facilitatory circuits in the human motor cortex (Kujirai et al., 1993). In that study, two TMS pulses were delivered at various interstimulus intervals (ISIs) of between 1 and 15 ms. The first stimulus, which was termed the conditioning stimulus, is a weak stimulus intensity that is subthreshold for producing a MEP. The conditioning TMS pulse activates intracortical circuits which then modulate the response of the corticospinal neurons to the second, suprathreshold test stimulus. The effectiveness of the modulation of the response to the test stimulus by the conditioning stimulus is quantified by expressing the size of the MEP produced by the paired-pulse TMS as a percentage of the size of the MEP response to the test stimulus given alone. With short ISIs (1-6 ms) there is suppression of the test MEP by the conditioning stimulus, where as at longer ISIs (8-15 ms), the MEP is facilitated (Kujirai et al, 1993).

The inhibition of MEPs at short ISIs is generally attributed to intracortical $GABA_A$-mediated inhibition (Kujirai et al., 1993; Ziemann et al., 1996a; Florian et al., 2008). However, different mechanisms appear responsible for inhibition at different short ISIs (Hanajima et al., 1998; Hanajima et al., 2003). With an ISI of 1-2 ms, the inhibition is influenced by the refractory period of the target cells or collision of inhibitory interneuron impulses (Hanajima et al., 2003). These effects are not present with an ISI of 3-5 ms, and the inhibition at these intervals is influenced by administration of the $GABA_A$-receptor agonist lorazepam (Ziemann et al., 1996a; Di Lazzaro et al., 2000). Thus, Hanajima et al.

(2003) suggest that ISIs of between 3 and 5 ms are best used to investigate $GABA_A$-mediated intracortical inhibition. For this reason an ISI of 3 ms was used in the present study to assess SICI.

Paired-pulse TMS at short ISIs can produce facilitatory as well as inhibitory effects. If the strength of the conditioning stimulus is suprathreshold for producing a MEP, MEPs evoked by the second, subthreshold stimulus are facilitated by a conditioning stimulus at certain ISIs (Di Lazzaro et al., 1998a). This occurs because a single, suprathreshold TMS pulse produces multiple descending volleys. These descending volleys have a discharge rate of ~ 600 Hz, corresponding to an inter-volley interval of ~ 1.5 ms. Animal studies have shown that electrical cortical stimulation activates the pyramidal tract neurons directly and indirectly (Patton and Amassian, 1954). The initial volley directly activates corticospinal axons, whereas the later volleys activate the pyramidal cells indirectly via synaptic activation of interneurons. The initial wave was termed a direct or D-wave, where as the later waves were termed indirect or I-waves. Depending on the stimulus intensity, multiple I-waves can be produced, and are named in order of their latency (I_1-, I_2-, I_3- etc) (Day et al., 1989). Similar findings have also been reported in humans using TMS (Di Lazzaro et al., 1998a). When paired-pulse stimulation is given with ISIs at approximately I-wave intervals, the MEP is facilitated due to facilitatory interaction between I-waves (Ziemann et al., 1998). This MEP facilitation is referred to as short-interval intracortical facilitation (sICF).

The facilitation of MEPs can also occur at longer ISIs and is due to different mechanisms than those observed with shorter ISIs. The facilitation seen with longer ISIs (8-15 ms) is due to activation of glutamatergic interneurons by the conditioning stimulus which then

change the composition of the descending corticospinal volleys produced by the test stimulus (Kujirai et al., 1993; Ziemann et al., 1996c). Intracortical mechanisms responsible for SICI/ICF are not dependent on the strength of corticospinal projections to the individual muscle as relative stimulus intensities are similar for a variety of muscle groups (distal and proximal) tested (Chen et al., 1998).

$GABA_B$-mediated inhibition can also be assessed with single-pulse TMS. When a single-pulse TMS is delivered when the target muscle is tonically activated (approximately 15% MVC), a period of EMG silence is observed after the MEP. This period of EMG silence can last for up to 300 ms, and is termed the cortical silent period (Inghilleri et al., 1993). The cortical silent period duration is proportional to the stimulus intensity used, not the amplitude of the MEP (Ho et al., 1998) or the degree of muscle activation (Inghilleri et al., 1993). The inhibition in EMG activity following the MEP is due to inhibitory phenomena that originate both cortically and spinally. However, H-reflex testing (Fuhr et al., 1991) and direct epidural recordings (Chen et al., 1999) have demonstrated that the late part of the silent period is due mainly due to cortical mechanisms, as the spinal motor neuron pool excitability has returned to normal. In the early part of the cortical silent period, however, there is a reduction in spinal excitability. The inhibition that is mediated by $GABA_B$ receptors is believed to be important in modulating the inhibitory tone acting on corticospinal neurons, thus influencing the descending commands from the motor cortex, and is known to be abnormal in several diseases influencing motor control, such as stroke (Liepert et al., 2000) and focal hand dystonia (Quartarone et al., 2003).

1.2.2.3. Safety

The general consensus is that TMS produces transient changes in cortical activity without any prolonged effects. Indeed, studies investigating the health effects of single-pulse TMS (where repeated stimuli are delivered at low frequencies, generally around 0.2 Hz) have found no serious adverse effects (Cohen and Hallett, 1987; Bridgers, 1991). The most common side effect reported is a transient headache (~5%).

The recent development of repetitive TMS (rTMS), which can produce trains of TMS pulses at high frequency (> 10 Hz) and high intensity, has also led to an increased reporting of more serious side effects. The most serious side effect reported has been seizure induction, not only in people prone to seizures, but also in neurologically normal subjects (Pascual-Leone et al., 1992). These findings were later supported by Wassermann and colleagues (1996a) who reported that in several years of using rTMS, two seizures had been induced in neurologically normal subjects. These subjects received prolonged, high intensity rTMS, but importantly, the inter-train rTMS interval was short (less than 1 sec) (Wassermann et al., 1996a). This led to the formulation of international guidelines on safe use of rTMS (Wassermann, 1998). Other studies, however, which have investigated potential side-effects from rTMS have shown that high intensity and high frequency stimulation does not necessarily induce seizures (Yamada et al., 1995; Wassermann et al., 1996b; Anderson et al., 2006). In a study investigating the effect of prolonged high intensity stimulation, monkeys were exposed to 7000 transcranial magnetic stimuli at maximum stimulator output over a period of thirty days. The authors reported that the monkeys had no short- or long-term effects (Yamada et al., 1995). A more recent study in humans showed that almost 2.5 hours of rTMS per day (almost 13,000 magnetic pulses) for three days (at an intensity of up to 120% RMT) did not induce any serious adverse

health effects in healthy young men (Anderson et al., 2006). The most common side effect was headache (19%), although this was not significantly different from sham stimulation (17%). Thus it appears that single-pulse TMS is safe, but there is an increased risk of adverse effects with increasing stimulus intensity, frequency and duration.

The Transcranial Magnetic Stimulation Adult Safety Screen (TASS) was developed to alert investigators to any potential factors that may predispose subjects to adverse effects from TMS (Keel et al., 2001). A modified version of the TASS was completed by each subject who participated in the experiments that contributed to this thesis. A copy of the modified TASS is included in the Appendix.

1.3. Neuroplasticity

Plasticity is a fundamental property of the nervous system which allows it to continually adapt to optimise performance. Neuroplasticity is a use-dependent modification in central nervous system structure and function. Neuroplasticity is critical for learning and memory (Sanes and Donoghue 2000), but is also important for recovery from brain injury (Nudo et al. 1996). It was initially believed that plasticity only occurred in the developing nervous system, but it is now known that plasticity not only occurs, but is crucial to the normal function of the adult nervous system (Sanes and Donoghue, 2000). Understanding the neural substrates and mechanisms involved in neuroplasticity improves our understanding of the fundamental physiological basis of learning and memory. By artificially or experimentally inducing neuroplastic change, it may also be possible to develop more effective treatment regimes for various neurological disorders (Ridding and Rothwell, 2007). The purpose of the experiments I undertook for this thesis was to improve understanding of the factors which influence the ability to induce neuroplastic change in human motor cortex.

Plasticity has been demonstrated throughout the brain in various animal models. These include slice preparations of the rodent hippocampus (Bear and Abraham, 1996), visual cortex (Kirkwood et al., 1996) and sensory cortex (Finnerty et al., 1999). Plasticity has also been demonstrated *in vivo*, including the primate (Recanzone et al., 1993) and human auditory cortex (Jancke et al., 2001) and human motor cortex (Donoghue, 1995; Karni et al., 1995). The primary motor cortex (M1) is known to be involved in higher level function rather than simply executing the movement (Donoghue and Sanes, 1994), is a crucial site for motor learning (Karni et al., 1998), and is the region studied in the experiments described in this thesis.

1.3.1. Mechanisms of neuroplasticiy

Neuroplasticity is brought about by several mechanisms. Rapid changes are a result of unmasking of pre-existing, yet silent synaptic connections (Jacobs and Donoghue, 1991; Malinow et al., 2000) or post-synaptic modification of neuron excitability (Woody et al., 1991). These changes can be caused by several events including an increase in excitatory neurotransmitter release or a reduction (or removal) of tonic inhibition (Kaas, 1991; Chen et al., 2002). Studies on rodents have found that the administration of the GABA antagonist bicuculline results in both rapid changes in the cortical representation of M1 (Jacobs and Donoghue, 1991), and an increase in the strength of synaptic connections in M1 (Hess and Donoghue, 1994), indicating that GABAergic neurons play a critical role in modulating plasticity. Human studies support the animal experiments and have clearly demonstrated that a reduction of GABA-mediated inhibition facilitates neuroplastic change in primary motor cortex (Ziemann et al., 2001).

Another mechanism that is important in short-term reorganisation is a change in synaptic efficacy. Potentiation of synapses was first demonstrated in the rabbit hippocampus by Bliss and Lomo (1973). High-frequency stimulation of the perforant pathway to the dentate gyrus in the rabbit hippocampus resulted in an increase in the amplitude of the excitatory post-synaptic potentials (EPSPs) in the target neurons for minutes to hours following the cessation of the stimulation. This increase in synaptic efficacy is referred to as long-term potentiation (LTP), and requires high-frequency stimulation of excitatory afferents (Bliss and Lomo, 1973). A decrease in synaptic efficacy can also be induced with lower frequencies of stimulation, and is referred to as long-term depression (LTD) (Dudek and Bear, 1992).

Following the initial description of LTP and LTD by Bliss and Lomo (1973), much interest has centered on trying to better understand the mechanisms underlying the change in synaptic efficacy with high (and low) frequency stimulation. Much of this work has focussed on the CA1 region of the hippocampus, however LTP/LTD has been described in many brain regions. Activity-dependent potentiation of synapses is characterised by four main properties which include: cooperativity, input-specificity, and associativity of inputs (Bliss and Collingridge, 1993; Aroniadou and Keller, 1995), and involvement of GABA receptors (Davies et al., 1991; Bliss and Collingridge, 1993; Hess and Donoghue, 1994; Aroniadou and Keller, 1995). This form of potentiation can be broadly divided into potentiation that is either *N*-methyl-D-aspartate (NMDA) receptor-dependent or NMDA receptor-independent (Bliss and Collingridge, 1993). The NMDA receptor plays an important role in the induction of NMDA-receptor dependent potentiation due to its unique structural and functional characteristics (Bliss and Collingridge, 1993). Specifically, the NMDA receptor is a ligand-gated (glutamate) ion channel that is blocked by Mg^{2+} in a voltage-dependent manner. This characteristic of the NMDA receptor helps explain the properties of activity-dependent LTP.

Cooperativity refers to a threshold intensity for induction of LTP (Bliss and Collingridge, 1993), which is a function of not only the stimulus intensity, but also the pattern of stimulation (Bliss and Lomo, 1973). Thus, the level of depolarization of the NMDA receptor needs to reach a critical threshold level to expel the Mg^{2+} (Bliss and Collingridge, 1993). LTP is input-specific because it is only the inputs that are active along the pathway at the time of stimulation that are potentiated (Andersen et al., 1977). In this case, the pre-synaptic release of sufficient glutamate is necessary to activate enough NMDA receptors to

potentiate the synapse (Bliss and Collingridge, 1993). LTP can be associative since a weak input can be potentiated if it is active at the same time as a strong tetanic input to a separate but convergent input (Bi and Poo, 2001) or by concomitant activation of the pre- and post-synaptic cells (Buonomano and Merzenich, 1998). The strong input is required to expel the Mg^{2+} from the NMDA receptor, and thus allow it to respond to the weaker input. Associative plasticity is also referred to as Hebbian plasticity, since it is consistent with Hebb's postulate which proposed that synapses linking two cells would be strengthened if the two cells were active at the same time (Hebb, 1949).

Sustained, long-term changes in cortical reorganisation likely involves other mechanisms, in addition to LTP. These include axonal sprouting and regeneration and also alterations in the number, size and shape of synapses (Kaas, 1991; Chen et al., 2002). In order to prevent plasticity from becoming rampant, a stabilising mechanism exists that is termed "homeostatic plasticity" (Turrigiano and Nelson, 2004). The dynamic modulation of synaptic strength occurs through a process known as synaptic scaling, where by cortical neurons regulate their own firing rates (Turrigiano et al., 1998). Excitatory synaptic strength is scaled down when the firing rate of the neuron is increased and *vice versa*. This is achieved by modulating the glutaminergic synaptic current by altering the quantal amplitude of the α–amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor-mediated component of excitatory neurotransmission (Turrigiano and Nelson, 1998). The changes in synaptic strength associated with LTP and LTD occur much quicker than the changes associated with synaptic scaling. It is thought that this allows both mechanisms to function coincidentally, allowing the localised changes in synaptic strength to occur, but maintained within a safe window (Turrigiano and Nelson, 2000). This stabilising mechanism maintains neuronal activity within acceptable levels, and provides a

mechanism to dynamically adjust synaptic strength to maintain stability. Without this mechanism operating to modulate the activity of the neural circuits, activity-dependent plasticity such as LTP and LTD would be driven to extremes. In such situations, continued potentiation of synapses would result in an eventual saturation of their efficacy, whilst continued depression would result in quiescence (Turrigiano and Nelson, 2004).

1.3.2. Techniques for induction of neuroplasticity in humans

The ability to artificially induce neuroplastic change in human cortex offers neuroscientists the exciting prospect of being able to improve functional outcomes in conditions where reorganisation is important in recovery of function. In this section I will discuss the different techniques currently available to induce neuroplastic change in human cortex. These techniques can be broadly divided into three main categories: repetitive peripheral stimulation, repetitive cortical stimulation and repetitive combined peripheral and cortical stimulation.

Afferent input to M1 has been demonstrated to be a powerful modulator of cortical excitability. Studies in both humans and animals have demonstrated that when afferent input to the cortex is reduced, either by permanent deafferentation or amputation of a limb or digit, there is a dramatic expansion of the cortical representation of the surrounding, unaffected regions (Kaas et al., 1983; Merzenich et al., 1983a; Merzenich et al., 1983b; Merzenich et al., 1984; Cohen et al., 1991a). As a result of these findings, several studies have modulated cortical excitability by temporarily changing afferent input to the cortex. Two main experimental paradigms exist to induce these changes. The first technique involves temporary deafferentation following transient (~30 mins) forearm ischaemia (Brasil-Neto et al., 1992a; Ridding and Rothwell, 1995; Ziemann et al., 1998a; Ziemann et

al., 1998b). This reduction in afferent input to the motor cortex results in a rapid expansion of the cortical representational zone in muscles proximal to the forearm ischaemia. The induced changes, which manifest as an increase in MEP amplitude of the target muscle, are reliant on a rapid removal of GABA-mediated inhibition (Ziemann et al., 1998b), and are thought to be due to LTP-like mechanisms, since they are reliant on a rapid removal of GABA-mediated inhibition, are NMDA receptor dependent, and are relatively long lasting (> 1 hour) (Ziemann et al., 1998a; Ziemann et al., 1998b).

Changes in cortical excitability can also be induced by a second method, which involves increasing the sensory input to the sensorimotor cortex. This can involve repeated electrical stimulation of the afferent nerve (Ridding et al., 2000a; Kaelin-Lang et al., 2002) or motor point (Ridding and Uy, 2003; Pyndt and Ridding, 2004), or vibration of the muscle (Rosenkranz and Rothwell, 2003). All of these protocols lead to a change in afferent input to the motor cortex, and a corresponding change in cortical excitability that persists once the afferent stimulation ceases. However, the mechanism by which cortical excitability is modified appears to be different for the various stimulation protocols. It is thought that prolonged stimulation of the nerve or muscle vibration modifies $GABA_A$ mediated intracortical inhibitory circuits (Kaelin-Lang et al., 2002; Rosenkranz and Rothwell, 2003), whereas motor point stimulation increases the excitability of intracortical excitatory circuits, without a change in intracortical inhibition (Pyndt and Ridding, 2004).

Repetitive cortical stimulation can also induce LTP- and LTD-like changes in cortical excitability. These techniques involve repetitive TMS (rTMS), however the techniques differ in terms of the frequency, duration and intensity of stimulation. Depending on the frequency of stimulation, rTMS can either enhance or depress cortical excitability. The

increase in cortical excitability outlasts the period of stimulation (Chen et al., 1997a; Maeda et al., 2000). In general, low frequency stimulation (<1 Hz) depresses cortical excitability (Chen et al., 1997a), whereas high frequency stimulation (>5 Hz) enhances cortical excitability (Maeda et al., 2000). It has recently been shown that the number of TMS pulses is also important in determining the sign of the induced changes (Hamada et al., 2008). Previously reported short-term facilitatory effects with 10 Hz and 20 Hz rTMS (Pascual-Leone et al., 1994; Maeda et al., 2000) were reversed with a different number of pulses (Hamada et al., 2008). The mechanism(s) responsible for the induced changes in the cortex are not entirely clear. Modification of synaptic efficacy (through LTP- and LTD-like effects) appears a likely candidate mechanism (Hallett, 2000). This is because the induced effects outlast the period of stimulation, the effects induced obey the "homeostatic plasticity" principle (see section 1.3.1.) (Iyer et al., 2003), and finally, the increase in cortical excitability is associated with a reduction in $GABA_A$-mediated inhibition (Takano et al., 2004). However, there is also evidence to show that the after-effects of rTMS in primary motor cortex are abolished if cortical excitability is assessed when the muscles are activated (Touge et al., 2001). The authors concluded that this indicates that rTMS changes the level of excitability of the resting corticospinal system, rather than modulating synaptic efficacy.

High frequency rTMS can also be delivered in bursts of stimuli, which produce consistent, relatively long-lasting, and powerful effects on motor cortex excitability (again induced by LTP- and LTD-like effects). This type of stimulation is termed theta-burst stimulation or TBS (Huang et al., 2005). It involves the application of short bursts of high frequency (50 Hz) stimuli, repeated at 5 Hz (theta rhythm). By altering the temporal pattern of TBS, different effects can be induced in the motor cortex. Continuous trains of TBS reduce

cortical excitability, whilst intermittent trains increase cortical excitability (Huang et al., 2005). The duration of the induced effect differs for the two forms of TBS. Continuous TBS reduces cortical excitability for nearly 60 minutes following the intervention, whereas intermittent TBS increases cortical excitability for 15 minutes following the intervention (Huang et al., 2005). Since the changes in cortical excitability induced by continuous and intermittent TBS are relatively long-lasting and are NMDA-receptor dependent (Huang et al., 2007) the induced effects are thought to be due to LTP/LTD-like effects.

Another experimental paradigm for inducing persistent changes in cortical excitability is transcranial direct current stimulation (tDCS). A pair of saline-soaked surface electrodes are placed on the scalp, with the stimulation electrode (either cathode or anode) placed over the motor cortical representational field of the target muscle, and the other electrode placed over the contralateral orbit (Nitsche and Paulus, 2000). This form of stimulation can produce both decreases and increases in cortical excitability, with the direction of change in cortical excitability dependent on whether the stimulation is cathodal or anodal (Nitsche and Paulus, 2000). Cathodal stimulation results in a decrease in cortical excitability, whilst anodal stimulation has the opposite effect (Lang et al., 2004). The exact mechanism for these induced changes is still not clear, however it seems likely that the direct-current induces changes in resting membrane potential (Lang et al., 2004) that results in a change in NMDA-receptor activation (Liebetanz et al., 2002).

The third main technique for inducing neuroplastic change in M1 is by pairing a peripheral electrical stimulus with TMS to the contralateral motor cortex. Such a paradigm was first described by Stefan and colleagues (2000) and was termed paired associative stimulation (PAS). The 30-min PAS protocol induced persistent, yet reversible changes in cortical

excitability that remained for at least 30 minutes following the intervention. The peripheral electrical stimulus was delivered to the median nerve at the wrist, which is a mixed nerve, and provides motor innervation of the abductor pollicis brevis muscle (target muscle). A single-pulse suprathreshold TMS was delivered to the contralateral M1 at varying ISIs following the peripheral stimulus. With an ISI of 25 ms, focal increases in excitability were reported for APB corticospinal neurons for up to 30 minutes post-stimulation, but not in corticospinal neurons projecting to other muscles not innervated by the median nerve. An ISI of 25 ms is approximately the time taken for the afferent volley to reach M1, so that it arrives at M1 at the same time, or just before, the TMS depolarises the corticospinal neurons in M1. Longer ISIs (100 – 5000 ms) did not produce any changes in MEP amplitude following PAS. In an important follow-up experiment, Wolters et al. (2003) investigated the affect of various ISIs on PAS-induced neuroplasticity. The study confirmed that the maximum increase in cortical excitability following PAS occurred with an ISI of 25 ms. Importantly, the study also demonstrated that a shorter ISI of 10 ms produced significant inhibition of MEP amplitude. This is an important finding because it provides strong evidence that the induced effects of PAS require associativity of inputs, an important requirement of Hebbian plasticity. That is, LTP-like effects are induced if the effects of the peripheral stimulation precede the depolarisation of the post-synaptic cell by TMS. Conversely, if the depolarisation of the postysynaptic cell by TMS precedes the effect of the peripheral stimulation, LTD-like effects are induced (Wolters et al., 2003). The increase in APB MEP amplitude was likely due to changes occurring at a cortical level. This was supported by three lines of evidence. Firstly, F-waves were not modified by PAS (Stefan et al., 2000). Secondly, stimulation of the brainstem with TMS (which activates the descending pathways directly) was not affected by PAS (Stefan et al., 2000),

and thirdly, PAS increased the cortical silent period duration, which as previously discussed, is at least in part, mediated by cortical elements (Stefan et al., 2000).

The notion that the changes induced with PAS are entirely restricted to supraspinal, and indeed cortical sites was brought into question by a recent study by Meunier and colleagues (2007). The study investigated changes in H-reflex amplitude following PAS, and found that H-reflex amplitude was increased following PAS. H-reflex amplitude, rather than F-wave amplitude, is believed to more accurately reflect spinal motor neuron excitability (Eccles, 1955). The specific locus for the increase in spinal excitability following PAS was not established. Investigation of individual data revealed that in subjects where an increase in H-reflex amplitude was demonstrated, this was always associated with an increase in MEP amplitude. However, an increase in MEP amplitude following PAS was not always accompanied by an increase in H-reflex amplitude. The authors concluded that there was likely a cortical "origin" for the increase in H-reflex amplitude, possibly due to changes in pre-synaptic inhibition to the Ia afferents (Meunier et al., 2007). The pre-synaptic interneurons are known to receive cortical commands (Meunier, 1999). This proposed mechanism fits well with a recent finding which showed that the effects of PAS were enhanced if PAS was administered whilst the target muscle was undertaking a weak voluntary contraction (Kujirai et al., 2006a), since voluntary contraction is known to reduce presynaptic inhibition of Ia afferents onto motor neurons (Meunier and Pierrot-Deseilligny, 1989).

There is good evidence to suggest that the changes induced in M1 with PAS are due to LTP-like changes in synaptic efficacy. This is because the induced effects outlast the period of stimulation (Stefan et al., 2000), are blocked by the NMDA receptor antagonist

dextromethorphan (Stefan et al., 2002) and obey the Hebbian model of plasticity (Wolters et al., 2003), since LTP- and LTD-like changes can be induced depending on the ISI of the paired stimuli.

I chose to use PAS as the paradigm to investigate the factors influencing the reproducibility and effectiveness of neuroplasticity induction in M1. In the following section I will discuss the factors that influence the reproducibility and effectiveness of neuroplasticity induction in human M1.

1.3.3. Factors influencing the reproducibility and effectiveness of neuroplasticity induction in human M1

Despite the wide variety of experimental paradigms available to induce neuroplastic change in human motor cortex, a significant impediment to their incorporation into the clinical sphere has been the large variability in effectiveness of these techniques for inducing neuroplastic change. There is little available research on the factors influencing the ability to induce neuroplastic change in human cortex.

Several factors have been shown to influence a subject's response to TMS, and these can be broadly divided into extrinsic and intrinsic factors. Extrinsic factors are generally associated with subtle differences in experimental set-up. These include differences in coil position, electrode placement and other environmental stimuli (Carroll et al., 2001; Koski et al., 2005). Therefore, in order to minimise the effects of the extrinsic factors, the coil position needs to be continually monitored throughout experiments, and the electrodes should be positioned using a standardised set-up.

Even if the influence of extrinsic factors is minimised, the influence of intrinsic factors cannot be as easily controlled. There are several internal factors which can potentially contribute to the variability of neuroplasticity induction between individuals, or in the same individual tested on different occasions, and these include: fluctuations in anatomical and physiological variables, attention and hormone fluctuations (Koski et al., 2005).

Fratello and colleagues (2006) were the first to investigate intra- and inter-subject variability of PAS-induced neuroplasticity. Subjects were tested on two separate occasions, and the authors reported that the overall magnitude of the induced PAS effect was reproducible across sessions, however there was very high intra-individual variability. The high variability of the induced effects was not a result of session-to-session changes in resting motor threshold, as there was high reliability in resting motor threshold values across sessions (Fratello et al., 2006), which is in agreement with other findings (Maeda et al., 2002; Wolf et al., 2004).

The variability of responses associated with PAS is not limited to the previously mentioned factors influencing a subject's response to TMS. Several other factors may also be important, and these are described below.

Since the timing of the paired stimuli in PAS is critical to determine the direction and magnitude of the induced change, one factor that could conceivably contribute to inter-subject variability to PAS is a subject's height, more specifically the conduction time to the motor cortex. Thus, if the ISI is kept constant across subjects, slight differences in arm length, and hence conduction time will influence the precise timing of when the afferent volley reaches M1. In order to account for this variation, a PAS protocol was designed

which took this variation into account (Ziemann et al., 2004). Electroencephalography (EEG) recordings were used to determine the latency of each individual's median nerve somatosensory-evoked cortical potential (N_{20}). This latency was used to determine the ISI in each PAS protocol. However, even when individual differences in the latency of the afferent volley were taken into account, this PAS protocol is still associated with significant inter-individual variability (Bergmann et al., 2008).

Resting excitability of neuronal populations is constantly changing. Indeed, rhythmic activity has been recorded from several regions of the brain, and in a wide range of frequencies. The exact role of these oscillations in cortical activity is not well understood, but it may play a role in "binding" remote brain regions, thus providing a way of enhancing information transfer between the separate brain regions (Buzsaki and Draguhn, 2004). One of these rhythms is known as the theta rhythm (4-7 Hz). It has been shown that the ability to induce LTP in rat hippocampal slice preparations is dependent on the phase of the theta oscillation. LTP was induced on the positive phase of the theta cycle, but not on the negative phase (Huerta and Lisman, 1995). Thus, the ability to induce LTP-like changes in the cortex is influenced by internal oscillations of neuronal excitability. This will likely influence the trial-to-trial effectiveness of the PAS intervention, thus contributing to variability not only between subjects, but between sessions in the same subject.

The ability to learn a motor sequence is dependent on attentional resources (Hazeltine et al., 1997), and the ability to induce neuroplastic change in M1 is also modulated by attention (Stefan et al., 2004). In a study investigating the affect of attention on PAS-induced neuroplasticity, subjects whose attention was directed to the non-target hand, and

were not looking at the target muscle (APB), had significantly reduced PAS-induced neuroplasticity compared to when subjects were asked to look at, and pay attention to the stimulated hand (Stefan et al., 2004). Thus, in order to maximise the effectiveness of neuroplasticity induction, it is important to maintain a subject's attention to the stimulated hand. This was achieved in the Stefan et al. (2004) study, and also in the experiments I undertook for this thesis by asking the subjects to look at their hand throughout the intervention. The subjects' attention was also directed to the stimulated hand by asking them to count the number of low intensity (200% perceptual threshold) electrical stimuli given to the thumb of the target hand during PAS. The number of stimuli was kept low so as to keep the demand on working memory low, and the stimuli were delivered during the period between paired-PAS stimuli so as not to interfere with the induction of PAS effects in M1.

The induction of neuroplastic change is also influenced by voluntary muscle activity occurring before, during and after the intervention. The increase in cortical excitability induced by 20 s of theta burst stimulation is turned into cortical inhibition if it is *preceded* by a 5 minute isometric contraction of the thumb (~25 % MVC) (Gentner et al., 2008). If there is continuous voluntary activity *during* the intervention, the induced effects from the neuroplasticity protocol appear to be enhanced. For example, an excitatory PAS protocol produces a more robust increase in cortical excitability compared to that produced at rest (Kujirai et al., 2006a), whilst an inhibitory rTMS protocol produced a longer-lasting effect when applied during a weak voluntary contraction (Todd et al., 2009). If an isometric contraction of the target muscle *follows* a 1 Hz rTMS paradigm, the usual inhibitory effects are abolished (Touge et al., 2001). The reversal of the induced effect when voluntary activity precedes the stimulation paradigm has been proposed to be due to metaplasticity

(Gentner et al., 2008), where the prior synaptic activation affects the subsequent induction of neuroplastic change by the rTMS. Voluntary activation reduces intracortical inhibition (Ridding et al., 1995c), which may explain the increased efficacy of the effects induced with the stimulation paradigms during voluntary activation. Another possibility, suggested by Kujirai et al. (2006) is that subjects are more attentive to the task when they were contracting their muscles. This might be important as attentional direction has been shown to be important in modulating the extent of PAS-induced changes (Stefan et al., 2004). A voluntary contraction after the stimulation paradigm may disrupt the changes in synaptic efficacy induced by the neuroplasticity induction paradigms. This is supported by *in vivo* animal studies which have shown that spontaneous activity reverses activity-induced modifications of synaptic efficacy (Zhou et al., 2003). Therefore the activation state of subjects undergoing plasticity induction is critically important, and great care needs to be taken in controlling activation levels.

Considerable evidence exists that the ageing motor system is less plastic (Sawaki et al., 2003; Ward et al., 2008). In a recent study, it was shown that elderly subjects respond less effectively to PAS, and their results are more variable than young subjects (Muller-Dahlhaus et al., 2008). In an extension to that study, the age-related reduction in PAS-effectiveness was shown to be restricted to females only - there was no significant age-related decline in males (Tecchio et al., 2008). Therefore, variability of PAS-induced effects will be greater if there is a large spread in subject age, or if elderly subjects are predominantly used.

The elderly females assessed in the Tecchio et al. (2008) study were in menopause. Menstrual cycle hormones have been shown to be important in modulating cortical

excitability (Smith et al., 1999; Smith et al., 2002; Inghilleri et al., 2004). During the late follicular phase, when estradiol levels are high, and progesterone levels are low, rTMS-induced increases in cortical excitability were greater than during the early follicular phase, when estradiol levels (and progesterone) were low (Inghilleri et al., 2004). Thus if repeated measures are taken from the same pre-menopausal female subjects, differences in hormone levels associated with the different stages of the menstrual cycle are likely to influence neuroplasticity induction.

Several other neuromodulators have also been implicated in neuroplasticity, and changes in their levels between sessions, and between individuals, could conceivably contribute to the variability in neuroplasticity induction. Physical activity can increase levels of brain derived neurotrophic factor (BDNF), which has an important influence on neuronal plasticity. For example, BDNF knockout mice show impaired LTP induction in the CA1 region of the hippocampus (Korte et al., 1995). In addition, a short period of exercise enhances cognitive function in rats, and is associated with elevated levels of BDNF (Vaynman et al., 2004). Although no studies have yet examined the direct effect of exercise induced increases in BDNF on plasticity, recent studies showed that humans with a BDNF polymorphism (which prevents expression of BDNF) do not undergo experience-dependent plasticity in M1 following a repetitive motor training paradigm (Kleim et al., 2006), nor do they undergo neuroplastic changes induced with rTMS (Cheeran et al., 2008). It is possible then, that another source of interindividual variability of plasticity is genetic differences in the expression of neuromodulators which may influence plasticity, an example of this is BDNF.

Another physiological variable that has not been investigated to date, but is likely to contribute significantly to the variability associated with neuroplasticity induction is circadian variation in various hormone levels. In the first set of experiments I undertook (Chapter 2), I sought to minimise the between-trial effects of circadian variation in hormone levels by re-testing individual subjects at the same time of day, although some subjects were tested in the morning, and others in the afternoon. These data revealed a time-of-day effect on neuroplasticity induction that was investigated in more detail in Chapters 3 and 4. In the following section I will briefly discuss the physiology of circadian rhythms, and how these rhythms can modulate neuroplasticity.

1.4. Circadian rhythms

The change in light intensity associated with the continual rotation of the earth around its axis has provided a mechanism for organisms to adjust their activity to optimise performance and survival. This modulation of physiological activity within a roughly 24-hour period is known as a circadian rhythm. It was initially believed that these circadian variations were simply a passive response to the environment. However, in the 18th century the French astronomer Jean-Jacques d'Ortous de Mairan demonstrated that the heliotrope plant, which raises its leaves by day, and closes them at night, continued to undergo these circadian changes even if the plant was placed in a dark closet. This demonstrated that circadian rhythms are endogenously controlled by the organism.

Circadian rhythms in mammals are generated by an endogenous pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore and Eichler, 1972; Stephan and Zucker, 1972). The SCN are paired structures in the anterior hypothalamus, just above the optic chiasm, each containing approximately 10,000 neurons (Reppert and Weaver, 2001).

Destruction of the SCN results in a dramatic loss of rhythmicity (Moore and Eichler, 1972; Stephan and Zucker, 1972). When individual SCN cells are isolated, they maintain circadian rhythmicity (Welsh et al., 1995), indicating that rhythmicity is generated intracellularly. Much work has been undertaken to identify the molecular mechanism responsible for this intracellular rhythmicity, with much of this work carried out in *Drosophila*. It is clear now that the internal rhythmicity is generated by a gene expression cycle, with two interlocked feedback loops. These consist of an interacting positive and negative transcriptional-/translational-feedback loop (for review see Reppert and Weaver, 2001). The resultant phosphorylation and proteolysis of the expressed "clock" proteins are important in imparting the circadian rhythmicity to the SCN.

Despite the fact that the rhythmicity associated with the SCN is internally generated, experiments in which the normal light-dark cycle is deliberately modified have shown that the daily oscillations in physiological function do not occur with a strict 24 hour period. When humans are denied continual environmental input regarding light and dark cycles (such as being kept in constant dark), the rhythmicity of these circadian oscillations is extended. This "free-running" occurs with a period of approximately 25 hours. These periods are remarkably stable amongst species, but vary considerably between species, and can in fact be shorter or longer than 24 hours in different species (for review see Herzog, 2007).

Since these rhythms are not exactly 24 hours in length, there needs to be a mechanism to allow resetting and entrainment of the pacemaker, and the resulting circadian rhythm, to the local environmental time (Czeisler and Klerman, 1999). The most powerful environmental signal for resetting of the circadian rhythm is light (Czeisler, 1995). Several

pathways transmit photic information to the SCN, but the main pathway is known as the retinohypothalamic tract (RHT), which provides a direct projection from the retina to the SCN (Moore and Lenn, 1972). Other indirect, multisynaptic pathways are also present, including from the intergeniculate leaflet of the thalamus (Moore and Card, 1985).

The SCN is divided into two main functional zones - the ventrolateral and dorsomedial zone. The ventrolateral zone receives direct light-related input from the retina, whilst the output from the SCN occurs from the dorsomedial section (Abrahamson and Moore, 2001). Since individual neurons in the SCN have their own internal oscillators, intense interest has surrounded how SCN neurons maintain synchrony with other neurons both within the same section and between other sections of the SCN, yet the precise mechanism remains unclear. Many cells within the SCN contain GABA (Card and Moore, 1984), and both $GABA_A$ and $GABA_B$ receptors have been found within the SCN (Francois-Bellan et al., 1989). Administration of GABA, acting through the $GABA_A$ receptor, synchronises activity of SCN neurons (Liu and Reppert, 2000; Shirakawa et al., 2000). Thus GABA release appears to play a critical role in coupling neural activity in individual SCN neurons. Recent evidence indicates that the rhythmic release of GABA may itself be regulated by the phasic release of vasoactive intestinal peptide (VIP) (Itri et al., 2004).

It is well established that many other neurons exhibit oscillatory circadian behaviour, and these exist not only in the cortex, but also in the periphery. The oscillations of the separate, remote neurons are controlled by the SCN, since it is at the top of the hierarchical tree. The SCN exerts its control on the other pacemaker cells via both neuronal (Inouye and Kawamura, 1979) and humoral (Silver et al., 1996) routes. It is still not entirely clear how the neuronal signal is generated in the SCN to both control the oscillation of remote

oscillators, but also to influence the circadian release of hormones in other centres. However, since the SCN terminals contain not only GABA, but also glutamate (Hermes et al., 1996), and also a variety of neuropeptides including vasopressin (VP), VIP and somatostatin (Buijs et al., 2003), there exists the potential for a signalling framework with much variety and complexity.

1.4.1. Neuromodulators responsible for circadian effects on plasticity

Since there are extensive neuronal connections between the SCN, the pituitary and other brain regions important in hormonal control, it is hardly surprising that the circulating levels of many compounds exhibit circadian rhythmicity (Czeisler and Klerman, 1999). These include cortisol, melatonin and thyroid stimulating hormone. Smaller, yet significant circadian changes have also been reported in levels of dopamine, glutamate, GABA, prolactin, growth hormone and parathyroid hormone (Czeisler and Klerman, 1999; Castaneda et al., 2004). Several of these compounds act as neuromodulators. A neuromodulator is defined as any compound "that regulates or modifies electrical impulses flowing through neural tissues by enhancing, inhibiting, extending or shortening them" (Abejon and Reig, 2003).

One of the most widely studied neuromodulators is cortisol. Cortisol secretion occurs throughout the day, with the highest plasma concentration occurring in the early morning. This is followed by a reduction during the day, reaching a nadir in the evening. Although the ultimate control for cortisol release occurs in the SCN, the actual signal to release cortisol arises from the paraventricular nucleus, where corticotrophin-releasing hormone (CRH) regulates the release of adrenocorticotropin (ACTH) from the pituitary (Buijs et al., 2003). The change in circulating levels of cortisol throughout the day seems to be related

to a change in the pulse amplitude of cortisol release, rather than the frequency of its release (Van Cauter and Refetoff, 1985).

Elevated cortisol levels are known to impair LTP, and learning and memory. Injection of a corticosterone metabolite in rat dentate gyrus significantly impaired LTP induction (Dubrovsky et al., 1987). The most striking effect was an immediate reduction in post-synaptic spike activity, with a delayed reduction in EPSP amplitude. Investigations in humans have shown that both acute and chronic increases in cortisol levels impair learning and memory. A single oral dose of hydrocortisone, which elevated cortisol levels to a level similar to that seen in psychological or physiological stress, was shown to impair memory retrieval (de Quervain et al., 2000). Chronically elevated cortisol levels, as seen in Cushing's Disease, are also accompanied by significant learning and memory deficits (Grillon et al., 2004).

Melatonin is the principal hormone secreted from the pineal gland, and modulates several physiological processes. Melatonin levels are highest during the night, and lowest during the day (Weinberg et al., 1979). Administration of melatonin to rats inhibits LTP in hippocampal slice preparations (Soto-Moyano et al., 2006), and also has been shown to impair cognitive processing in humans (Rogers et al., 1998).

Several other studies have demonstrated that there is circadian variation in various aspects of the induction and consolidation of neuroplasticity, including signal transduction along the cyclic adenosine monophosphate (cAMP) transduction pathway, and mitogen-activated protein kinase (MAPK) phosphorylation (Eckel-Mahan et al., 2008). It appears that homeostatic mechanisms again play a role in maintaining a balance between the differing

effects of the various neuromodulators on neuroplasticity. A recent study measured blood samples from human subjects to assess levels of BDNF and cortisol. It was shown that the levels of these two neuromodulators, which have opposite effects on plasticity, have the same circadian rhythm. Therefore, high levels of BDNF are associated with high cortisol levels. Thus, it may be that the two neuromodulators might be physiologically co-regulated to help maintain homeostasis of plasticity (Begliuomini et al., 2008).

1.4.2. Sleep

The hormonal modulation of bodily functions is not simply due to the circadian output of the SCN. It is in fact a complex interaction between these circadian rhythms, light exposure, gender, age, neuroendocrine feedback mechanisms, and the timing of sleep and wakefulness (Czeisler and Klerman, 1999).

Sleep is not a uniform state, but is in fact a complicated process that consists of dynamic alteration between two different sleep states: rapid eye movement (REM) sleep and non-REM (NREM) sleep. The NREM stage of sleep can be further classified into four stages (1-4), with the final two stages (3 and 4) often referred together as slow-wave activity (SWA) sleep. This stage of sleep is characterised by high-amplitude oscillations in EEG activity in the delta frequency (0.5-4 Hz) range (Walker and Stickgold, 2006).

When the effects of sleep and nocturnal oscillations are dissociated, the level of several hormones have been shown to be modulated primarily by sleep, rather than night-time *per se* (Czeisler and Klerman, 1999). Some of the hormones most strongly modulated by sleep include growth hormone, prolactin and thyroid stimulating hormone, and to a lesser extent, cortisol (Czeisler and Klerman, 1999).

The profound changes in electrophysiology, neurochemistry and functional anatomy that are associated with these different stages of sleep, make the sleeping brain (and in fact the various stages of sleep) biologically distinct from the waking brain (Walker and Stickgold, 2006).

One major role attributed to sleep is its role in memory formation and retention. Humans trained to learn a motor sequence performed the sequence more accurately following a night of sleep, compared to an equivalent period of time spent awake (Walker et al., 2005). These performance changes were correlated to the period of time spent in NREM sleep. Functional imaging studies have shown that when human subjects are deprived sleep, there is an impairment in their ability to commit new experiences to memory, and this impairment is due to a reduction in hippocampal activation (Yoo et al., 2007).

The mechanism by which a lack of sleep impairs memory is becoming better understood. Cellular studies have demonstrated that sleep deprivation impairs LTP induction in rat hippocampal slice preparations (Davis et al., 2003), and is also associated with a generalised reduction in hippocampal neuron excitability (McDermott et al., 2003).

Recently, it has been proposed that sleep, in particular SWA during NREM sleep, may also play a role in synaptic homeostasis (Tononi and Cirelli, 2006). According to the theory, which has recently been supported by experiments on rats (Vyazovskiy et al., 2008), periods of wakefulness are associated with net synaptic potentiation, whereas sleep is associated with net synaptic depression. Thus, during the waking period, synapses become progressively more saturated with information. In order to prevent neuronal saturation,

synaptic depression (or downscaling) is proposed to occur during sleep to maintain homeostasis (Tononi and Cirelli, 2006).

The circadian system and sleep/wake cycles have profound effects on physiology. One of these is to modulate learning and memory. Despite the fact that M1 undergoes learning-related changes, no research has yet investigated whether the ability to undergo neuroplastic change in M1 is modulated by a circadian rhythm. The experiments I have undertaken, which are reported in this thesis in Chapters 2-4, address this gap in our knowledge of circadian physiology and neuroplasticity.

1.5. Functional correlates of neuroplasticity

In order for artificially induced neuroplasticity, using paradigms described in this thesis, to be useful in improving treatment outcomes for various neurological conditions, it is necessary to demonstrate that the circuits which are reorganised are functionally important. That is, while the corticospinal system is clearly important in skilled movements, do techniques such as PAS activate circuits that are important in motor learning? In the following section I will describe what is currently known about the motor cortical circuits that are activated by learning a motor task, and whether these are the same as those activated by the artificial neuroplasticity induction paradigms such as PAS and rTMS. In addition, I will review the functional changes that are observed when these circuits are artificially modified by techniques such as rTMS.

Motor learning is associated with improvements in motor performance. The neural substrates responsible for the usage-dependent changes caused by motor learning have been extensively investigated. Learning results in rapid functional reorganisation of

several cortical areas including pre-motor area (PMA) and supplementary motor area (SMA), as well as M1 (Sanes, 2003). These functional changes include modification of synaptic efficacy (LTP- and LTD-like mechanisms) and modulation of intracortical inhibition (Sanes, 2003). These changes have been reported in several species including rodents (Kleim et al., 1996), non-human primates (Nudo et al., 1996) and humans (Pascual-Leone et al., 1995; Classen et al., 1998; Muellbacher et al., 2001). Compelling evidence for the critical role of M1 in consolidating practice-related motor performance gains was shown by Muellbacher et al. (2002), where performance gains of a simple motor skill were reduced when rTMS was used to disrupt M1 processing for a brief period after the training was performed.

The performance improvements of a training task are associated with short-term (Karni et al., 1995) and long-term cortical changes (Karni et al., 1995; Pascual-Leone et al., 1995). These changes are likely to occur due to a variety of mechanisms including unmasking of hidden connections, the formation of new synaptic connections, and LTP- and LTD-like modification of existing synaptic connections.

Although motor learning and neuroplasticity induction paradigms both induce LTP-like changes in M1, it is conceivable (yet unlikely), that different circuits are activated by the two. Strong evidence that both paradigms activate the same structures in M1 was first demonstrated in rat M1 (Rioult-Pedotti et al., 1998; Rioult-Pedotti et al., 2000). Learning of a forelimb skill training task resulted in increased synaptic efficacy of the forelimb area of the trained M1, but not the untrained M1. Synaptic efficacy was quantified by measuring the amplitude of field potentials in forelimb M1 slice preparations of the sacrificed animals. Another important finding was that the amount of LTP that could be

induced (with tetanic stimulation of forelimb M1 cortical slice) in the trained M1 was less than the untrained M1, whereas the amount of LTD that could be induced was enhanced (Rioult-Pedotti et al., 2000). This finding is consistent with the homeostatic theory of plasticity induction, and provides strong evidence that similar pathways are being activated in the behavioural learning task and the experimental induction of LTP/LTD. Similar findings have been shown in humans. For example, Ziemann and colleagues (2004) showed that a motor training task resulted in motor learning in M1, and this prevented subsequent induction of LTP-like changes in M1 with a PAS paradigm, whereas LTD-like changes using a similar PAS paradigm were enhanced.

If motor learning increases cortical excitability due to LTP-like mechanisms, it would be expected that improvements in performance following a motor training task would be correlated with the changes in cortical excitability. Indeed, several studies have shown such a correlation (Pascual-Leone et al., 1995; Muellbacher et al., 2001; Ziemann et al., 2001; Garry et al., 2004). However, when the time course of changes in motor performance and cortical excitability were assessed, the association between the two variables was lost at 30 days – the performance improvements had remained, but cortical excitability had returned to normal (Muellbacher et al., 2001). This may indicate that the increase in cortical excitability is critical for the early part of motor learning, and that consolidation involves different mechanisms and sites.

Several studies have investigated whether artificially induced changes in cortical excitability may lead to changes in motor performance, with experiments performed on both neurologically normal and neurologically compromised subjects. The studies on neurologically normal subjects have produced mixed results. Some investigators have

demonstrated a change in motor performance of both the contralateral (Jancke et al., 2004) and ipsilateral hands (Kobayashi et al., 2004; Dafotakis et al., 2008) following rTMS, however several studies have shown no change (Muellbacher et al., 2000; Rossi et al., 2000). In any case, the effects on motor performance in neurologically normal subjects following artificial changes in cortical excitability appear to be subtle at best. This might indicate that the motor system is capable of compensation to any large changes in cortical excitability in order to maintain performance (Iyer et al., 2003).

One of the ultimate goals of investigating the artificial induction of neuroplasticity is to improve treatment outcomes in patients with brain injury or neurological diseases. It is therefore heartening that promising functional changes following neuroplasticity intervention in neurologically compromised patients have been reported. For example, cortical stimulation combined with motor training improves functional gains in chronic stroke patients more than rehabilitative training alone (Hummel et al., 2005; Kim et al., 2006). Since these changes were induced in chronic stroke patients, the improvements are unlikely to be due to spontaneous recovery of function, which can be expected in the acute phase following stroke. Functional improvements in (sub-acute) stroke patients have also been demonstrated with repeated afferent stimulation (McDonnell et al., 2007a). In patients with Parkinson's Disease, finger tapping speed was improved in the stimulated hand of patients following rTMS of the cerebellum, but not in the unstimulated hand or in control subjects (Sommer et al., 2002). One explanation for these results may be that changes in cortical excitability will manifest as functional gains more readily in conditions where the motor system is compromised (such as in stroke, or Parkinson's Disease). In these conditions, the aim of the intervention is to return cortical excitability back to normal levels, which appears then to also lead to a demonstrable improvement in motor function.

The final experimental chapter of my thesis aimed to further investigate the link between motor performance and changes in cortical excitability. More specifically, if similar circuits are activated by motor practice and artificial stimulation paradigms, then it follows that factors that influence the effectiveness of neuroplasticity induction with artificial stimulation paradigms (namely time of day), may also influence usage- or learning-dependent plasticity following motor practice, and the performance improvement with training. No studies to date have specifically examined this issue, however there is some evidence that performance of various motor tasks is dependent on time of day (Miller et al., 1992). These include force discrimination (Miller et al., 1992), muscle strength (Wyse et al., 1994) and a basic motor flicking task (Edwards et al., 2007). However, none of these studies specifically examined whether the *learning* of a motor task was influenced by time of day. Therefore, the final experimental chapter (Chapter 4) investigated whether changes in motor performance and cortical excitability induced by a repetitive motor training paradigm are influenced by time of day. I hypothesised that changes in motor performance and cortical excitability following a motor training task will be greater in the evening compared to the morning, and that these time of day changes would be associated with changes in circulating levels of cortisol. The results of this experiment are described in Chapter 4.

2. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation

2.1. Abstract

Several paired-associative stimulation (PAS) protocols induce neuroplastic changes in human motor cortex (M1). To understand better the inherent variability of responses to PAS, we investigated the effectiveness and reproducibility of two PAS paradigms, and neurophysiological and experimental variables that may influence this. Motor evoked potentials (MEPs) were elicited by transcranial magnetic stimulation (TMS) of right M1, and recorded from surface EMG of left abductor pollicis brevis (APB) and first dorsal interosseous before and after PAS. PAS consisted of electrical stimulation of left median nerve paired with TMS over right M1 25 ms later. Twenty subjects were given one of two PAS protocols: short (132 paired stimuli at 0.2 Hz) or long (90 paired stimuli at 0.05 Hz), and were re-tested with the same protocol on 3 separate occasions, with 11 subjects tested in the morning and 9 in the afternoon. Neurophysiological variables assessed included MEP amplitude, resting and active motor threshold, short-interval intracortical inhibition, intracortical facilitation and cortical silent period duration. The short PAS protocol produced greater APB MEP facilitation (51%) than the long protocol (11%), and this did not differ between sessions. The neurophysiological variables did not consistently predict responses to PAS. Both PAS protocols induced more APB MEP facilitation, and greater reproducibility between sessions, in experiments conducted in the afternoon. The mechanism for this is unclear, but circadian rhythms in hormones and neuromodulators known to influence neuroplasticity warrant investigation. Future studies involving PAS

should be conducted at a fixed time of day, preferably in the afternoon, to maximise neuroplasticity and reduce variability.

2.2. Introduction

The capacity of the nervous system to re-organise (plasticity) is of fundamental importance for learning and memory (Sanes and Donoghue 2000), and recovery from brain injury (Nudo et al., 1996). Several non-invasive techniques have been developed which induce cortical organisational changes that persist for minutes to hours beyond the intervention. In the motor cortex, these changes manifest as alterations in cortical excitability that can be readily quantified using transcranial magnetic stimulation (TMS). The experimental protocols employed in human subjects involve either peripheral nerve stimulation (Ziemann et al., 1998a; Ridding et al., 2001), cortical stimulation (Pascual-Leone et al., 1994), or a combination of peripheral and cortical stimulation (Stefan et al., 2000; Ridding and Taylor, 2001; Quartarone et al., 2003; Ridding and Flavel, 2006). The latter technique has been termed paired associative stimulation or PAS. PAS combines low-frequency, percutaneous electrical stimulation of a peripheral nerve with TMS over the contralateral motor cortex. When the timing between the peripheral and central stimuli is appropriate, PAS results in an increase in cortical excitability that persists for more than 30 minutes after stimulation, and is thought to be due to LTP-like mechanisms (Stefan et al., 2000).

The original PAS protocol developed by Stefan et al. (2000) has been subtly modified by subsequent groups, with the duration of PAS, frequency of stimulation, and stimulus intensity varying between protocols (Stefan et al., 2004; Ziemann et al., 2004; Morgante et al., 2006). One limitation on the use of PAS is that the changes in cortical excitability induced by the technique vary widely in a group of subjects. Attention has been shown to influence the effectiveness of PAS (Stefan et al. 2004), but there is little information on other sources of variation in the responses to PAS within a group of normal subjects, or

whether there are advantages in using one protocol over another in this regard. One aim of the present study was to assess whether the normal range of variation in neurophysiological parameters related to cortical excitability are useful predictors of the effectiveness of PAS. We also evaluated the reproducibility of cortical excitability changes induced by PAS in individual subjects by testing them on three occasions at the same time of day at least one week apart. Some subjects were tested in the morning, and others in the afternoon. We used two previously described variants of the PAS protocol in these studies to establish whether one had advantages over the other in terms of effectiveness and reproducibility. Preliminary results of this study have been reported (Sale et al., 2006).

2.3. Materials and Methods

Twenty subjects (14 female, 6 male; aged 18-34 yrs) participated in this study. All subjects were right handed (median LQ = 0.80, range 0.36 – 1.00) as assessed by the Oldfield handedness questionnaire (Oldfield, 1971), and had no history of specialised use of their hands. Subjects had no known neurological disorders, and had not previously been exposed to protocols used for cortical plasticity induction. Ten subjects were assigned to a "short" PAS protocol group (7 female, 3 male), and the other ten to a "long" PAS protocol group (7 female, 3 male) (details below). Each subject was tested with one PAS protocol on three separate occasions at the same time of day, separated by at least one week. For the short PAS protocol, 6 subjects were tested in the morning and 4 subjects in the afternoon. For the long PAS protocol, 5 subjects were tested in the morning and 5 in the afternoon. The PAS experiments in the morning commenced at approximately 10:10 am, and those in the afternoon at approximately 2:20 pm. All subjects gave written informed

consent prior to participation in the study, which was approved by the University of Adelaide Human Research Ethics Committee.

Subjects were seated comfortably in an experimental chair with their left shoulder abducted at approximately 45° to allow the arm and hand to rest in a manipulandum. The hand was positioned with the proximal phalanx of the left thumb in a metal ring attached to a load cell. The position of the load cell was adjusted to facilitate measurement of thumb abduction force. Thumb abduction force was displayed on an oscilloscope in front of the subject to provide visual feedback, and was also digitized online (2 kHz) via a CED 1401 interface (Cambridge Electronic Design) and stored on computer for offline analysis.

Surface electromyographic (EMG) recordings from the abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscle of the left hand were obtained using bipolar Ag-AgCl electrodes placed 2 cm apart. The EMG signals were amplified 1000 times, filtered (5 Hz – 500 Hz), digitized online (2 kHz/channel) via a CED 1401 interface, and stored on computer for offline analysis. The EMG signals of both muscles were displayed to the subject on an oscilloscope to assist them in maintaining EMG silence when required. During trials requiring EMG silence, trials containing prestimulus EMG activity were discarded.

The abduction force exerted by the left thumb during a maximum voluntary contraction (MVC) was measured at the beginning of each session. Subjects were aided in the task by visual feedback of thumb abduction force displayed on an oscilloscope. Three MVC trials were obtained for each session with at least 30 s rest given between each trial. The MVC was taken as the largest thumb abduction force produced in the three trials.

TMS was applied using a figure-of-eight coil (outer coil diameter 90mm) and two Magstim 200 magnetic stimulators, with the output directed through a 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. The coil was positioned at the optimal scalp position for eliciting a muscle evoked potential (MEP) in the relaxed left APB muscle. The optimal scalp position was marked for reference with a pen, and the coil position was continually checked throughout the experiment.

Electrical stimuli were applied to the median nerve at the wrist using a constant current stimulator (DS7 stimulator, Digitimer Co. Ltd., Hertfordshire, UK) with bipolar surface electrodes, separated by 30 mm, and with the cathode distal. Stimuli were square wave pulses with a pulse width of 200 μs.

2.3.1. "Short" and "Long" PAS protocols

Subjects received one of two paired associative stimulation (PAS) protocols, which were modified slightly from those initially described by Stefan and colleagues (2000). Both PAS protocols involved a series of paired stimuli: percutaneous electrical stimulation of the median nerve at the left wrist, and TMS delivered 25 ms later to the right motor cortex. The intensity of the peripheral electrical stimulus (approximately 300% of perceptual threshold) was sufficient to produce a small, but clearly visible motor response (M-wave) in APB. The amplitude of this M-wave was approximately 200 μV, and it was monitored throughout the PAS intervention to ensure a consistent level of peripheral nerve stimulation. The median nerve stimulus was followed 25 ms later by a suprathreshold TMS at the optimal scalp site for evoking MEPs in the left APB. The two PAS protocols

differed in terms of the number and frequency of the paired stimuli, and the TMS intensity. The 'short' protocol consisted of 132 paired stimuli applied at a frequency of 0.2 Hz over an 11-minute period. For this protocol, the TMS intensity was 130% resting motor threshold (RMT) (*cf.* Stefan et al., 2000). The 'long' protocol consisted of 90 paired stimuli applied at a frequency of 0.05 Hz for 30 minutes. In this protocol, the intensity of the TMS was sufficient to evoke a MEP in the relaxed APB of 0.5-1.0 mV (*cf.* Stefan et al., 2000), which in practice resulted in the use of a TMS intensity 130 ± 2% RMT.

The subject's attentional focus 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 during the PAS interventions. To quantify this, subjects received intermittent weak (200% perceptual threshold) electrical stimuli to their left thumb via ring electrodes (Stefan et al., 2004). Between 7 and 10 stimuli were applied at random intervals throughout each PAS protocol and subjects were instructed to count and remember the number of thumb stimuli they received. When a thumb stimulus was delivered, it occurred at the mid-point of the interval between successive paired stimuli in the PAS protocol. After the PAS protocol had concluded, subjects were asked to report the number of stimuli delivered to the thumb. An error score was calculated, which was the difference between the number of stimuli delivered to the thumb and the number reported by each subject.

2.3.2. Measures of cortical excitability

Several measures of motor cortical excitability were made prior to, and following, the PAS intervention. These included resting motor threshold (RMT), active motor threshold (AMT), MEP amplitude, cortical silent period (CSP) duration, and short-interval

intracortical inhibition (SICI) and intracortical facilitation (ICF). An approximate time-line for the sequence of these tests is shown in Fig. 2.1.

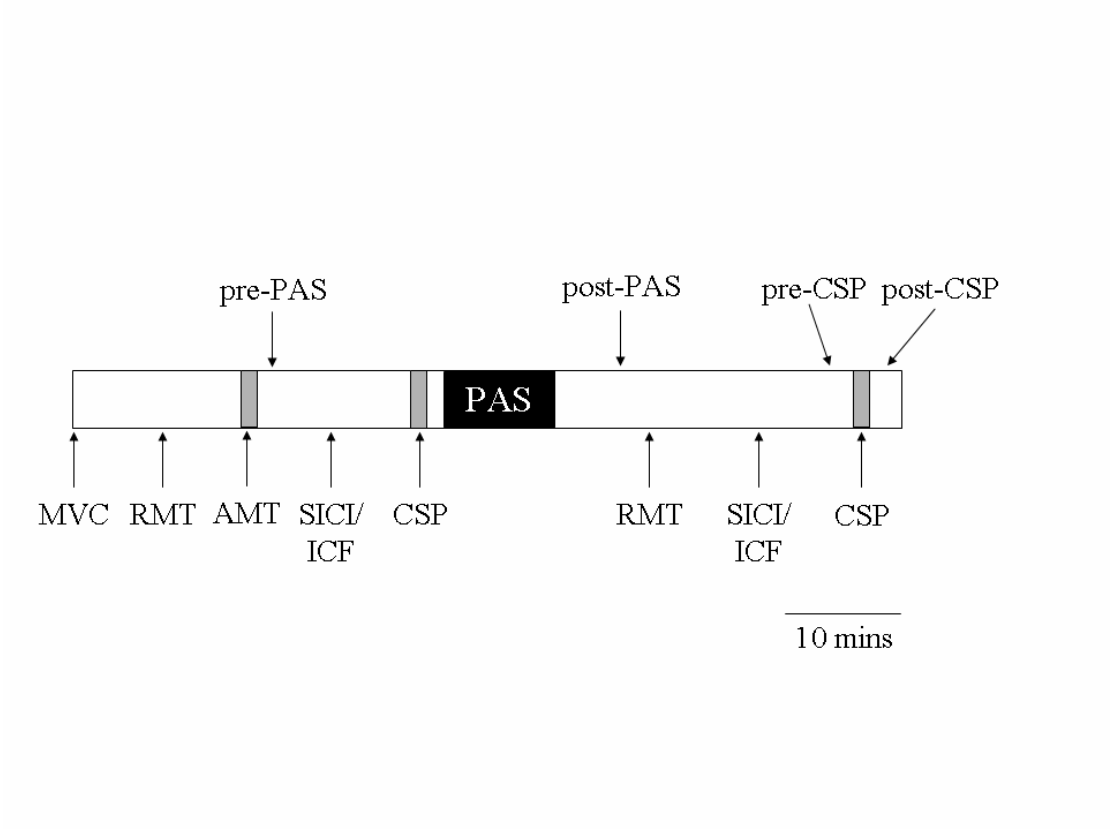

Figure 2.1
Diagrammatic representation of the testing protocol indicating the approximate relative timings for assessment of neurophysiological parameters before and after PAS. The grey bars indicate brief voluntary activation of the APB muscle. Measurements of the APB MEP were made at four time points: pre-PAS, post-PAS, pre-CSP and post-CSP. (MVC maximum voluntary contraction, RMT resting motor threshold, AMT active motor threshold, SICI/ICF short-interval intracortical inhibition/intracortical facilitation, CSP cortical silent period, PAS paired associative stimulation).

RMT was defined as the minimum TMS intensity required to evoke a MEP in the relaxed APB of at least 50 μV in 3 out of 5 consecutive trials. AMT was defined as the minimum stimulus intensity required to evoke a MEP in the APB muscle of at least 200 μV in 3 out of 5 consecutive trials during a weak voluntary thumb abduction (5% MVC). The stimulus intensity was expressed as a percentage of maximum stimulator output (% MSO).

To assess corticomotor excitability, mean MEP peak-to-peak amplitude in resting APB and FDI was assessed by measuring the peak-to-peak amplitude of the 10 individual trials then averaging them. Test TMS intensity (SI_{pre}) was determined prior to PAS and was set at an intensity sufficient to produce a MEP in APB of 0.5-1.0 mV. This TMS intensity was used to investigate resting MEP amplitude immediately prior to (pre-PAS), five minutes following PAS (post-PAS), and prior to (pre-CSP) and immediately following (post-CSP) the assessment of the cortical silent period. The times (relative to the end of PAS) at which MEP amplitudes were assessed for the three post-PAS measures were: post-PAS, 5.3 ± 0.2 min; pre-CSP, 20.3 ± 0.2 min and post-CSP, 22.6 ± 0.4 min. These data provide information about the time-course of the change in cortical excitability induced by PAS, and the effect of an intervening voluntary contraction (for CSP assessment) on the changes in cortical excitability induced by PAS.

Cortical silent period duration measurements were made during a low-level voluntary contraction of APB (15% MVC) before and after PAS (Fig. 2.1). Subjects were given visual feedback of thumb abduction force via an oscilloscope. TMS intensity was 130% RMT and 10 TMS were delivered at a frequency of 0.2 Hz. CSP duration was taken as the time from the TMS pulse until the resumption of pre-stimulus levels in the rectified EMG. CSP duration measurements were made off-line on individual trials and averaged for the 10 trials.

Paired-pulse TMS was used to assess SICI and ICF for APB using a protocol similar to that initially described by Kujirai et al. (1993). A subthreshold conditioning stimulus preceded a suprathreshold test stimulus by either 3 ms (SICI) or 10 ms (ICF). The intensity of the conditioning stimulus was 90% of AMT, and the test stimulus intensity

pre-PAS was SI_{pre}. The test stimulus intensity in paired-pulse trials was adjusted following PAS so that the test MEP amplitudes were equivalent pre- and post-PAS. Each data block consisted of 10 trials for each of 3 conditions; test stimulus alone, conditioning and test stimulus (ISI = 3 ms), and conditioning and test stimulus (ISI = 10 ms). The order of presentation of the three conditions was pseudorandomised throughout the trials. Stimuli were given every 5 seconds. The effect of the conditioning stimulus was quantified by expressing the conditioned MEP amplitude as a percentage of the unconditioned test MEP amplitude.

All data were found to have homogenous variance (Bartlett's test, $P > 0.05$). To determine the effect of the PAS protocols on MEP amplitude a four-way analysis of variance (ANOVA) was performed with within-subject factors of TREATMENT (two levels: pre-PAS and post-PAS), MUSCLE (two levels: APB and FDI) and SESSION (three levels: first session, second session, third session) and between-subject factor of PROTOCOL (two levels: short and long PAS protocols). Separate three-way repeated measures ANOVA assessed the effect of PAS on RMT, SICI, ICF and CSP for APB with within-subject factors TREATMENT (pre-PAS, post-PAS), SESSION (first session, second session, third session) and between-subject factor PROTOCOL (short, long). APB AMT was assessed only for the pre-PAS state, and two-way repeated measures ANOVA was used to assess the temporal reliability of AMT measures, with within-subject factor SESSION (first session, second session, third session) and between-subject factor PROTOCOL (short, long). APB MEP amplitude was measured at four time points: pre-PAS, post-PAS (5.3 ± 0.2 mins following PAS), pre-CSP (20.3 ± 0.2 mins following PAS) and post-CSP (22.6 ± 0.4 mins following PAS). The time course of APB MEP facilitation, and also the effect of voluntary activation (between pre-CSP and post-CSP) on APB MEP

amplitude following PAS were assessed with a three-way repeated measures ANOVA with within-subject factors of TIME COURSE (four levels: pre-PAS, post-PAS, pre-CSP, post-CSP) and SESSION (first session, second session, third session) and between-subject factor of PROTOCOL (short, long).

To assess the reproducibility of all the neurophysiological measures across the three experimental sessions, the intra-class correlation coefficient (ICC) was calculated (Shrout and Fleiss, 1979).

A multiple regression analysis was used to examine the relationship between the neurophysiological measures assessed prior to PAS and the amount of APB MEP facilitation induced by each PAS protocol. RMT, SICI, ICF and CSP were correlated with the percentage change in APB MEP amplitude following PAS (post-PAS APB MEP amplitude / pre-PAS APB MEP amplitude x 100) in each experiment. The strength of the relationship was quantified by the coefficient of determination (r^2).

Each subject was re-tested at the same time of day in each session, and experiments were undertaken either in the morning beginning at 10:10 am (11 subjects) or the afternoon beginning at 2:20 pm (9 subjects). In order to assess the effect of time of day on APB MEP facilitation seen with each PAS protocol a four-way repeated-measures ANOVA was performed with dependent variable APB MEP amplitude and within-subject factors of TREATMENT (pre-PAS, post-PAS) and SESSION (first, second, third), and between-subject factors of PROTOCOL (short, long) and TIME OF DAY (am, pm).

For all analyses $P < 0.05$ was chosen as the significance level, and unless stated otherwise, all group data are given as mean ± SEM. Fisher's PLSD post-hoc tests were performed as appropriate

2.4. Results

All subjects completed the three experimental sessions. TMS intensity used for test MEPs (SI_{pre}) was not significantly different between the short and long PAS protocols (66.8 ± 2.6% MSO *vs*. 72.2 ± 2.4% MSO, respectively), corresponding to 126 ± 2 % RMT vs. 130 ± 2 % RMT. Intensity of peripheral nerve stimulation during PAS was 6.4 ± 0.5 mA for the short protocol and 6.0 ± 0.6 mA for the long protocol, an insignificant difference. TMS intensity during PAS was not significantly different for the two protocols. For the short protocol, TMS intensity was 68.4 ± 1.9 % MSO (130% RMT) and 72.2 ± 2.4 % MSO (this intensity produced a MEP of 0.5-1 mV, which corresponded to 130 ± 2% RMT) for the long protocol. Pre-PAS APB MEP amplitude was not significantly different between protocols (short = 0.88 ± 0.04 mV, long = 0.92 ± 0.06 mV; $P > 0.05$). The TMS intensity of the test stimulus used for the paired-pulse trials following PAS was 66.0 ± 2.7 % MSO for the short protocol, and 72.0 ± 2.4 % MSO for the long protocol, an insignificant difference. These results show that the stimulus intensities used for PAS and to assess MEP amplitude were very similar for the subjects receiving the short and long PAS protocols.

The group data for RMT, AMT, SICI, ICF and CSP are summarised in Table 2.1. There was no significant effect of SESSION for any of the measures of cortical excitability, and therefore the results were pooled across sessions. There was also no significant difference between the short and long PAS protocols for any of these variables. RMT, SICI and ICF

were unchanged following PAS ($F_{1,34}$ = 0.6053, P > 0.05; $F_{1,28}$ = 0.062, P > 0.05; $F_{1,36}$ = 0.0005, P > 0.05 respectively). AMT was not re-assessed following PAS. The duration of the CSP increased significantly following PAS (TREATMENT $F_{1,36}$ = 30.964, P < 0.05). The interaction of TREATMENT x PROTOCOL was not significant ($F_{1,116}$ = 0.828, P > 0.05), indicating both protocols had a similar effect on CSP. ICCs for these neurophysiological variables are also shown in Table 2.1. All measures, except for ICF, were highly reproducible across sessions.

	Short protocol		Long protocol		All subjects	
	Pre-PAS	Post-PAS	Pre-PAS	Post-PAS	Pre-PAS	Post-PAS
RMT (% MSO)	52.4 ± 1.5	52.6 ± 1.5	55.2 ± 1.4	55.5 ± 1.4	53.8 ± 1.0	54.08 ± 1.04
r_{ICC}	*0.97*	*0.98*	*0.93*	*0.96*	0.95	*0.96*
AMT (% MSO)	41.9 ± 1.6	-	42.9 ± 1.4	-	42.4 ± 1.0	-
r_{ICC}	*0.95*		*0.94*		*0.94*	
SICI (% test alone)	48.9 ± 6.0	53.7 ± 8.4	48.4 ± 5.9	39.9 ± 5.2	48.7 ± 4.2	46.3 ± 4.8
r_{ICC}	*0.80*	*0.78*	*0.90*	*0.89*	*0.84*	*0.83*
ICF (% test alone)	123.5 ± 10.4	123.9 ± 12.3	139.0 ±	137.4 ± 13.3	131.3 ± 7.7	130.7 ± 9.0
r_{ICC}	*0.20*	*0.23*	11.4	*0.69*	*0.28*	*0.51*
			0.30			
CSP duration (ms)	171.8 ± 3.7	180.0 ± 4.1	170.7 ± 4.1	186.5 ± 4.7	171.2 ± 2.7	183.3 ± 3.1*
r_{ICC}	*0.92*	*0.78*	*0.87*	*0.87*	*0.84*	*0.87*

Table 2.1
Neurophysiological variables before and after PAS. r_{ICC} intra-class coefficient; * $P < 0.05$ versus Pre-PAS (ANOVA)

PAS induced focal changes in MEP amplitude of the target APB muscle. The changes in MEP amplitude following application of the two PAS protocols are shown in Figure 2.2. In these representative subjects, APB MEP amplitude increased following both PAS protocols, although the increase was greater with the short protocol. FDI amplitude was unchanged following PAS with either protocol. For the group data, repeated-measures ANOVA indicated that the effect of PAS was not significantly different between sessions ($F_{2,34} = 0.544$, $P > 0.05$), thus data were pooled across sessions. The effect of PAS on the

two muscles investigated was significantly different, as indicated by a significant MUSCLE x TREATMENT interaction ($F_{1,34}$ = 9.864, P < 0.01). This was due to a significant increase in MEP amplitude in APB following PAS (Fisher, P < 0.05), while there was no significant change in FDI MEP amplitude (Fisher, P > 0.05: see Figures 2.2 and 2.3)

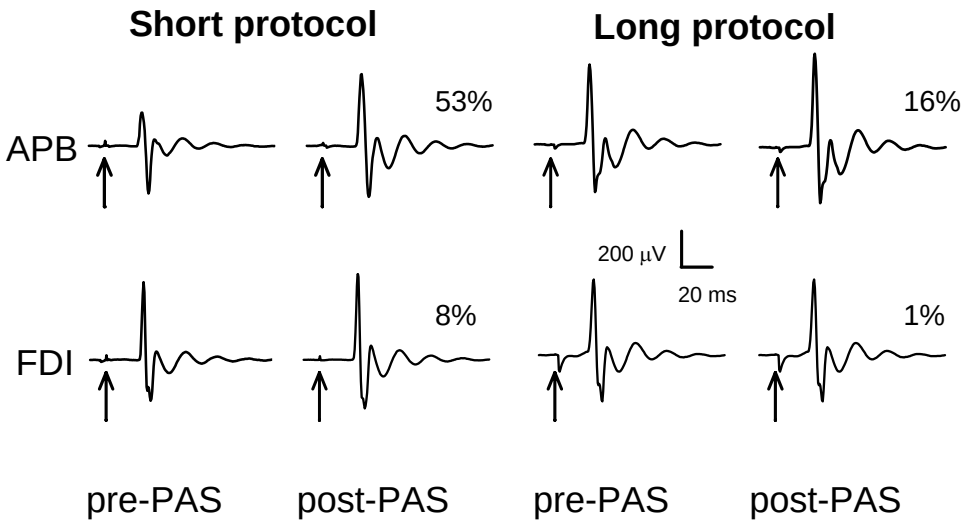

Figure 2.2
MEPs from left APB and FDI in two representative subjects before (pre-PAS) and after (post-PAS) paired associative stimulation (PAS). One subject received the "short" PAS protocol and the other received the "long" protocol. Each MEP is an average of ten responses to TMS in the resting muscle. Timing of TMS is indicated by the arrow. There was greater APB MEP facilitation following the short protocol than the long protocol. PAS had little effect on the amplitude of MEPs in the non-target FDI muscle. Numbers indicate the percent increase in MEP amplitude after PAS.

Since PAS selectively facilitated responses in APB, a two-way ANOVA (TREATMENT, PROTOCOL) was used to determine whether there was a difference in the extent of APB MEP facilitation between the two PAS protocols. There was a significant TREATMENT x PROTOCOL interaction ($F_{1,34} = 5.017$, $P < 0.05$), indicating a difference in the amount of MEP facilitation seen with the two protocols. *Post-hoc* analysis revealed that the short protocol induced a significant facilitation of APB MEP amplitude of approximately 51% (Fisher, $P < 0.05$). The long protocol induced an 11% increase in APB MEP amplitude, however this increase failed to reach statistical significance (Fisher, $P > 0.05$). These data are summarised in Figure 2.3.

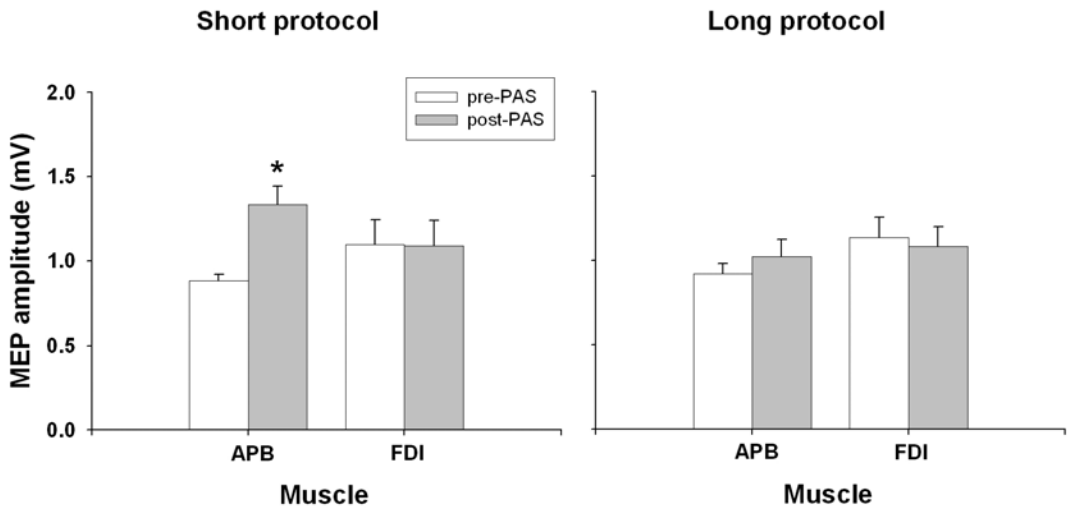

Figure 2.3
Group (mean ± SEM) MEP amplitude data for APB and FDI before and after the two PAS protocols. A different group of ten subjects were tested with each protocol, and each subject was tested on three occasions. On average the short protocol induced a 51% increase in APB MEP amplitude (asterisk, Fisher $P < 0.05$), whereas the long protocol induced a non-significant 11% increase in APB MEP amplitude. Neither PAS protocol induced a significant change in FDI MEP amplitude.

APB MEP amplitude was tested at four separate time points: before PAS (pre-PAS), and at three time points following PAS (post-PAS, pre-CSP and post-CSP), and these data are summarised in Figure 2.4. There was no effect of SESSION on APB MEP amplitude ($F_{2,108} = 0.083$, $P > 0.05$), and therefore the data were pooled for SESSION. There was a significant effect of TIME COURSE ($F_{3,229} = 4.079$, $P < 0.01$) and PROTOCOL ($F_{1,229} = 4.225$, $P < 0.05$) on APB MEP amplitude. *Post-hoc* tests revealed significant APB MEP facilitation at all three time points following PAS with the short protocol (Fisher, $P < 0.05$). There was no significant difference in the amount of APB MEP facilitation at any of the three time points following PAS (post-PAS *vs* pre-CSP *vs* post-CSP) (Fisher, $P > 0.05$). This indicates that the voluntary contraction performed for the CSP measurement

did not significantly affect the amount of MEP facilitation seen in APB (Fisher, $P < 0.05$), and the PAS effect lasted at least 23 min. There was no significant facilitation of APB MEP amplitude at any time-point following PAS with the long protocol.

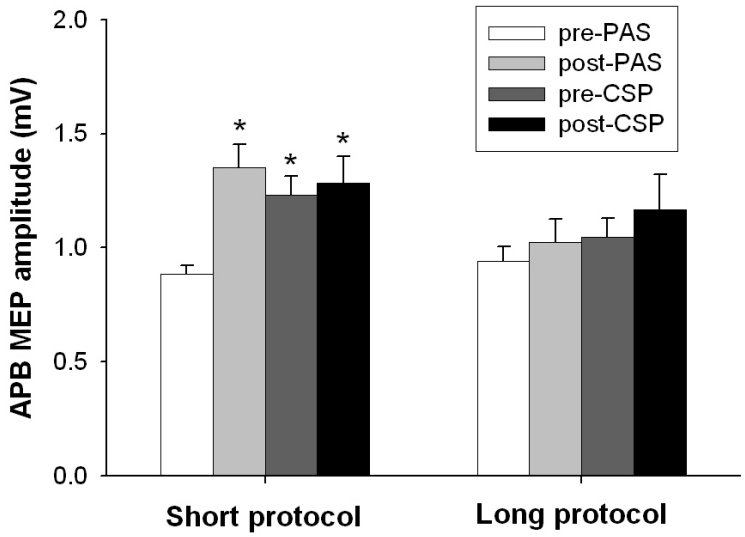

Figure 2.4
The time course of APB MEP facilitation following the two PAS protocols (short and long). A different group of ten subjects were tested with each protocol, and each subject was tested on three occasions. Mean SEM APB MEP amplitude was measured at four time points during the experiment: prior to PAS intervention (pre-PAS), 5 min following PAS (post-PAS), approximately 20 min following PAS (pre-CSP), approximately 23 min following PAS, after voluntary activation of APB (post-CSP). With the short protocol, APB MEP amplitude was significantly facilitated at all time points following PAS (asterisks, Fisher; P < 0.05 vs pre-PAS), and APB MEP facilitation after PAS was not affected by an intervening voluntary activation of APB (pre-CSP vs. post-CSP, P > 0.05). There was no significant change in APB MEP amplitude at any time point following PAS with the long protocol.

There was no significant effect of PROTOCOL ($F_{1,54} = 2.904$, $P > 0.05$), nor SESSION ($F_{2,54} = 2.833$, $P > 0.05$) on attention-related error scores (number of attention stimuli missed during PAS). The mean error score for the short protocol was 0.6 ± 0.2, and the long protocol was 0.3 ± 0.1. The maximum error score reported by a single subject was 3 (subjects received between 7 and 10 attentional stimuli). In 39 experiments (out of a total of 60) subjects correctly reported the number of attentional stimuli they received. Linear

regression analysis indicated that there was no significant correlation between APB MEP facilitation induced by PAS and attention-related errors ($r^2 = 0.002$, $P > 0.05$).

The overall amount of APB MEP facilitation following PAS was greater for the subjects receiving the short protocol (Fig. 2.3). The effectiveness of PAS was not influenced by session in either the short and long protocol groups (Fig. 2.5A). There was, however, considerable variability in the response to PAS for the same subject across the three sessions (Fig 2.5B). ICC for all subjects (pooled for PAS protocols) across all sessions was $r_{icc} = 0.55$ ($P < 0.05$). When the data were separated by protocol, the ICC for MEP facilitation in the long protocol was $r_{icc} = 0.68$ ($P < 0.05$), which indicated a strong correlation across sessions in the amount of MEP facilitation. It should be remembered, however, that the long protocol did not induce significant MEP facilitation (Figs. 2.3, 2.5A). For the short protocol, the ICC was $r_{icc} = 0.29$ ($P > 0.05$). This indicates a higher degree of variability in individual responses to PAS across sessions using the short protocol.

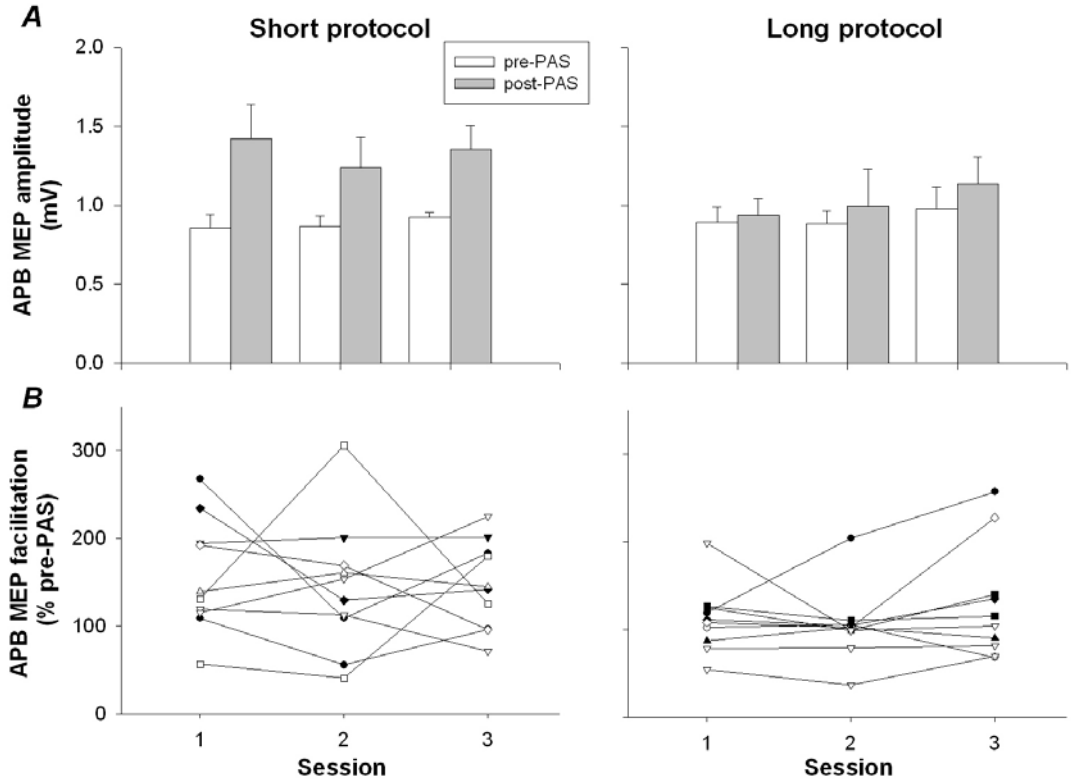

Figure 2.5

Variability of PAS induced facilitation following the two protocols (short and long) across three experimental sessions. The group data are shown in the top panels (A) with average APB MEP amplitude before PAS (white bars) and after PAS (grey bars) for the three sessions. The data are pooled for subjects tested in the morning and afternoon. There was no significant effect of session on APB MEP amplitude with either protocol (ANOVA; $P > 0.05$). Individual subject responses to PAS in the three sessions are shown in the lower panels (B) as the percentage change in APB MEP amplitude, with data from each subject in a protocol group having a unique symbol. Open symbols indicate morning experiments, and filled symbols indicate afternoon experiments. It can be seen that there is a large amount of variability both between subjects, and within subjects across sessions, that is more prominent for the short protocol.

To assess whether there was a physiological basis for this individual variation in PAS effectiveness, a multiple regression analysis was performed. This analysis included each of the baseline variables (RMT, AMT, SICI, ICF and CSP) and the extent of APB MEP facilitation post-PAS for each session. Data from the short and long protocols were analysed separately. Data for RMT, SICI and CSP duration are shown in Figure 2.6. For the five physiological variables examined, the only baseline measure significantly correlated with the extent of APB MEP facilitation was CSP duration with the long protocol. This showed a significant ($r^2 = 0.24$, $P < 0.01$) negative relationship, such that

subjects with longer pre-PAS CSP duration tended to show less APB MEP facilitation following PAS with the long protocol. There was no significant relationship between CSP duration and APB MEP facilitation with the short PAS protocol.

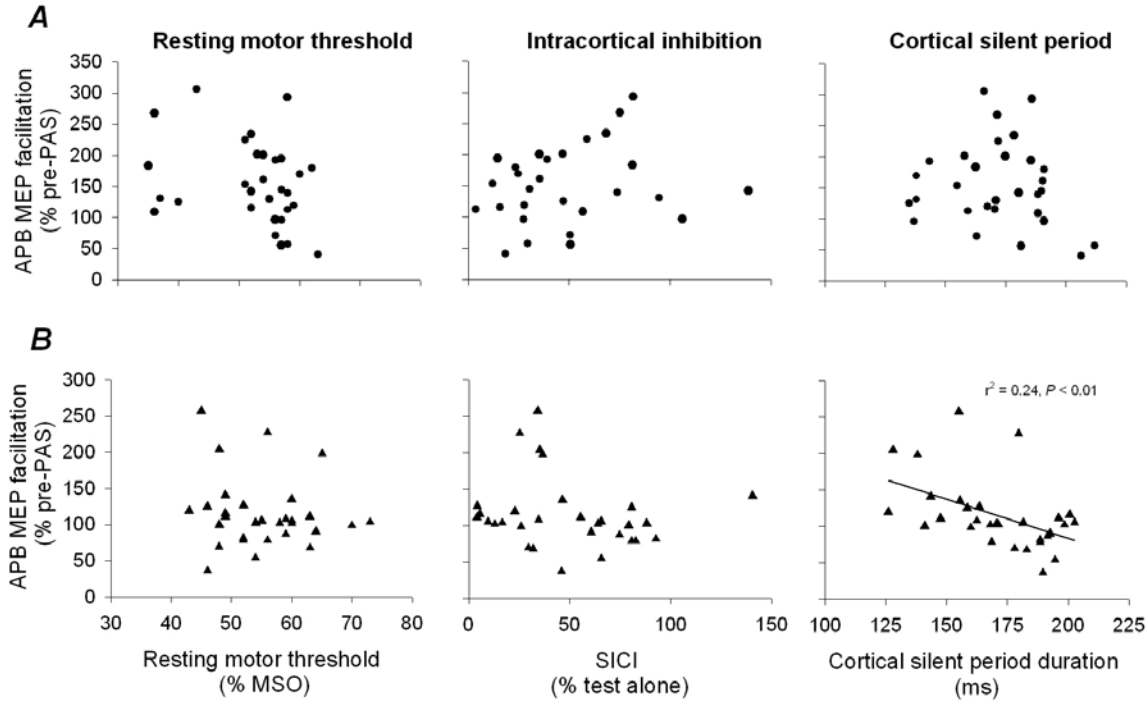

Figure 2.6
Relationship between three neurophysiological measures of cortical excitability and the amount of APB MEP facilitation following PAS. Top panels (A, circles) show data obtained from ten subjects, re-tested three times with the short protocol. Lower panels (B, triangles) show data obtained from ten subjects, re-tested three times with the long protocol. The neurophysiological measures of resting motor threshold, short-interval intracortical inhibition and cortical silent period duration are plotted against the extent of APB MEP facilitation following PAS. Significant linear regression fit is shown for CSP duration and APB MEP facilitation with the long PAS protocol.

Four-way ANOVA on the APB MEP data revealed that the time of day at which the experiment was performed influenced the APB response to PAS (Fig. 2.7). There was a significant effect of TREATMENT ($F_{1,96}$ = 13.622, P < 0.001), indicating that PAS facilitated the APB MEP. There was a TREATMENT x TIME OF DAY interaction ($F_{1,96}$ = 4.565, P < 0.05), with PAS inducing significant MEP facilitation (49%) in the afternoon, but no significant MEP facilitation (16%) when PAS was applied in the morning (Fig. 2.7). The short PAS protocol produced more MEP facilitation than the long protocol ($F_{1,96}$ =

5.71, $P < 0.02$), and this was consistent for morning and afternoon experiments (PAS PROTOCOL x TREATMENT x TIME OF DAY interaction: $F_{1,96} = 0.087$, $P > 0.05$). For the short protocol, APB MEP amplitudes in the morning were 0.88 ± 0.05 mV (pre-PAS) and 1.22 ± 0.12 mV (post-PAS), and in the afternoon were 0.89 ± 0.06 mV (pre-PAS) and 1.52 ± 0.19 mV (post-PAS). For the long protocol, APB MEP amplitudes in the morning were 0.91 ± 0.10 mV (pre-PAS) and 0.82 ± 0.08 mV (post-PAS), and in the afternoon were 0.93 ± 0.07 mV (pre-PAS) and 1.23 ± 0.20 mV (post-PAS). PAS effectiveness was not influenced by SESSION ($F_{2,96} = 0.533$, $P > 0.05$).

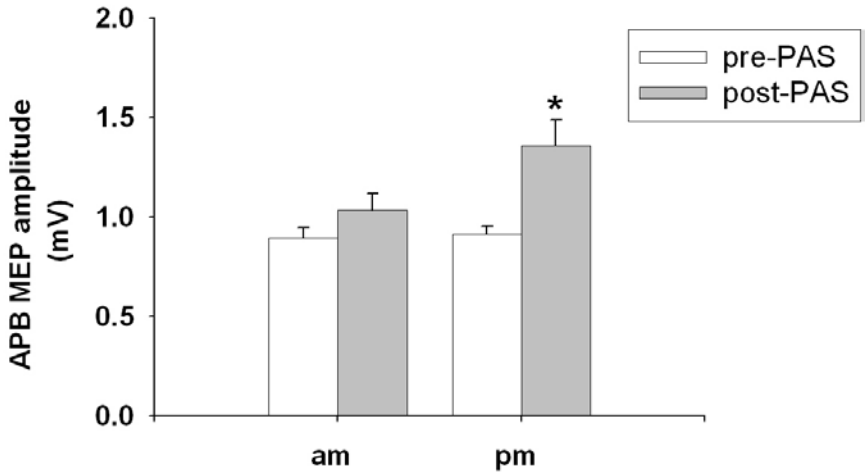

Figure 2.7
Effect of time of day on response to PAS. Data are from all subjects across all three testing sessions in either the morning (am) or afternoon (pm) collapsed across testing protocols. APB MEP amplitudes (mean ± SEM) are shown before (white bars) and after (grey bars) PAS. Post-PAS APB MEP amplitudes in the afternoon were significantly greater than pre-PAS APB MEP amplitude in the afternoon (asterisk; Fisher, $P < 0.05$). Post-PAS APB MEP amplitude in the afternoon was also significantly greater than post-PAS APB MEP amplitude in the morning (asterisk; Fisher, $P < 0.05$).

Since time of day influenced the response to PAS, the ICCs for MEP facilitation were calculated for subjects tested in the morning and afternoon. ICC was higher for the subjects tested in the afternoon ($r_{icc} = 0.71$; $P < 0.02$, n = 9) than the morning ($r_{icc} = 0.39$; $P = 0.17$, n = 11), indicating that the amount of MEP facilitation seen for each subject was more consistent between sessions for experiments conducted in the afternoon. Afternoon

experiments were more reliable regardless of the PAS protocol used. For the short protocol the ICC was r_{icc} = 0.011 (P > 0.05, n = 6) in the morning and r_{icc} = 0.812 (P < 0.05, n = 4) in the afternoon. The long protocol had an ICC of r_{icc} = 0.296 (P > 0.05, n = 5) in the morning, and r_{icc} = 0.513 (P < 0.05, n = 5) in the afternoon.

2.5. Discussion

The purpose of this study was twofold; first, to compare the efficacy and variability of two PAS protocols by repeatedly testing the same subjects, and second, to identify neurophysiological variables or experimental factors which might influence a subject's response to PAS. The results demonstrate a significant difference in effectiveness of the two PAS protocols, with the short protocol inducing an increase in MEP amplitude of approximately 51% and the long protocol inducing a small and non-significant increase in MEP amplitude of approximately 11%. A further novel finding of this study is that both PAS protocols induced significantly more MEP facilitation when applied in the afternoon, and also that the MEP facilitation was more consistent across sessions for experiments conducted in the afternoon. The neurophysiological parameters tested in the present study were unable to predict an individual subject's response to PAS consistently, although there was a weak negative correlation between silent period duration and facilitation for the long PAS protocol.

2.5.1. Differing effectiveness of short and long PAS protocols

The current study compared the effectiveness of two previously-employed PAS protocols. Stefan et al. (2004) have previously reported that both of these protocols produce "qualitatively similar effects" and result in significant target muscle MEP facilitation. In

the present study, these findings have been supported to some degree in that the short protocol resulted in significant MEP facilitation restricted to the target muscle (Fig. 2.3). However, while the long protocol did produce a small MEP facilitation in APB, it did not reach a significant level. Previous studies using the "long" protocol have reported variable MEP facilitation of the target muscle. The Stefan et al. studies (2000 & 2004) reported a 77% and ~ 65% increase in MEP amplitude respectively, whilst the study by Ridding and Flavel (2006) reported a 25% increase in MEP amplitude for the left upper limb, and a 35% increase in MEP amplitude for the right upper limb. A possible explanation for the differences between the present study and the previous studies may lie in the time of day at which subjects were tested (not reported by Stefan et al., 2000; Stefan et al., 2004). We have shown that time of day influences the response to PAS (Fig. 2.7), and in the present study the long PAS protocol given in the afternoon produced a 32% facilitation of the APB MEP. If subjects were tested predominantly in the afternoon in previous studies, then greater MEP facilitation would be expected, irrespective of the PAS protocol used. Another possible explanation for differences with previous studies may lie in subject selection. Subjects who had not previously participated in PAS experiments were selected for the present study. In contrast, Stefan et al. (2004) used subjects who had previously shown MEP facilitation following PAS. The significant ICC values in the present study for repeated tests on the same subjects show that there is a degree of reproducibility in the response to PAS that could lead to bias if subjects are selected on the basis of their response to an initial experimental session. Thus the reduced effectiveness of the long PAS protocol in the present study compared to previous reports could be due to the subject selection criteria. The Stefan et al. (2000 & 2004) studies examined the dominant hand, whereas the present study used the non-dominant hand. We do not consider this to be an

important difference, as Ridding & Flavel (2006) recently found no significant difference in the effectiveness of a "long" PAS protocol between hemispheres.

2.5.2. Reproducibility of PAS effects

The amount of MEP facilitation induced by PAS differs widely between subjects, and also between sessions in the same subject (Fig. 2.5B; Fratello et al. 2006). Both internal and external factors may contribute to this variability (Koski et al., 2005). Internal variability is associated with attention, hormonal fluctuations and other anatomical or physiological variables which may influence a subject's response to TMS. External factors which can contribute to the variability include coil position, electrode placement and other environmental stimuli.

In a study to examine the influence of attention on PAS-induced MEP facilitation, Stefan et al. (2004) demonstrated that MEP facilitation is maximised when a subject looks at, and attends to, the target muscle. This demonstrated that attention is important in modifying the response to PAS. In the present study, all subjects were instructed to look at, and focus their attention on, the target muscle. The fact that there were minimal error scores throughout both PAS protocols indicates that subjects successfully focused their attention on the stimulated hand and suggests that attention was not likely to be a major contributor to the variability in responses to PAS in the present study.

A possible source of variation in the response to PAS is changes in the levels of hormones during the menstrual cycle, as this can influence cortical excitability (Smith et al., 1999). Fourteen of our subjects were female, and since experiments were conducted approximately one week apart without reference to phase of the menstrual cycle, hormone

levels would differ between sessions. There have been no studies of the influence of menstrual cycle on PAS effectiveness. Since there were equal numbers of females in both protocols, it is unlikely that menstrual cycle-related changes in hormones would account for the differences we reported between PAS protocols.

Variability in responses to single TMS pulses are attributed to fluctuations in both cortical and spinal excitability (Rossini et al., 1991; Burke et al., 1995; Ellaway et al., 1998; Funase et al., 1999; Carroll et al., 2001; Darling et al., 2006). As is customary, in the present study the MEP was averaged over a number of stimuli to obtain a reliable estimate of corticomotor excitability pre- and post-PAS. Ten stimuli were used for the average as there is little improvement in the reliability of the MEP amplitude assessment by using more stimuli (Brasil-Neto et al., 1992b).

Despite the widespread use of PAS, including several studies which have used it to repeatedly test the same subjects (Ziemann et al., 2004; Stefan et al., 2006), there are few quantitative data on the reproducibility of PAS with repeated testing. A recent study by Fratello et al. (2006) was the first to assess the reliability, and quantify the within-subject variability of PAS across multiple experimental sessions. These authors demonstrated that there was a consistent overall level of MEP facilitation for a group of subjects between two PAS experimental sessions, and this conclusion was confirmed and extended in the present study for three sessions (Fig. 2.5A). These observations are important because they show it is not necessary to use naïve subjects to reliably quantify the effectiveness of PAS. This facilitates the use of PAS in a cross-over experimental design to test the effectiveness of a specific intervention (e.g., a drug, or a training regimen) on cortical plasticity.

Fratello et al. (2006) reported poor reproducibility of PAS-induced MEP facilitation within the same subjects across two experimental sessions, with an ICC near zero. Their PAS protocol differed from those used in the present study, and produced a 32-36% increase in APB MEP. For the short protocol in the present study (51% increase in MEP size), the ICC was higher (r_{icc} = 0.29) than that reported by Fratello et al. (2006), but also not significant. For the long protocol, the ICC was significant (r_{icc} = 0.68), indicating that the effect of PAS was reliable for individuals, however the importance of this finding is lessened by the fact that the long protocol did not produce significant MEP facilitation (~11%; Figs. 2.4 & 2.5A). The overall conclusion from the two studies is that intra-individual reliability in the response to PAS is low when the PAS protocol produces significant MEP facilitation. The important new observation in the present study is that individual responses to PAS were much more consistent for experiments conducted in the afternoon (r_{icc} = 0.71; $P < 0.02$) compared with the morning (r_{icc} = 0.39; P = 0.17), and irrespective of the PAS protocol used.

2.5.3. Factors influencing the effectiveness of PAS

A second aim of the present study was to examine whether baseline neurophysiological measures were associated with a subject's response to PAS. It has been proposed that reduced SICI may facilitate induction of cortical neuroplasticity (Ilic et al., 2004). In support of this Quartarone and colleagues (2003) demonstrated that subjects with focal dystonia, and reduced resting SICI, exhibited an exaggerated response to PAS. In the present study in normal subjects, there was no correlation between pre-PAS SICI and the extent of PAS-induced MEP facilitation (Fig. 2.6). Similar results were reported recently by Ridding and Flavel (2006). The range of variation in SICI in normal subjects is quite

large (Fig. 2.6), yet does not seem to be an important determinant of the susceptibility to cortical excitability changes induced by PAS.

Stefan et al. (2002) demonstrated a transient reduction of $GABA_A$-mediated SICI *during* PAS. However, it has also been shown with several PAS protocols that SICI is unchanged *following* PAS at a time when MEPs are facilitated (Ridding and Taylor, 2001; Stefan et al., 2002). This suggests that although modulation of SICI during PAS may play a role in the induction of neuroplasticity, the lasting excitability change seen following PAS is not associated with ongoing changes in SICI.

There was no significant change in ICF following PAS, which supports previous findings using a similar protocol (Kujirai et al., 2006b). ICF is due to activation of glutamatergic pathways (Liepert et al., 1997; Ilic et al., 2002). In the present study we found no correlation between baseline ICF and the effectiveness of PAS, and this relationship has not been examined previously. We conclude that the baseline level of ICF does not influence the effectiveness of PAS, nor is modulation of ICF important for the induction of neuroplastic changes associated with PAS.

We also found no correlation between either baseline RMT or AMT and the effectiveness of PAS. There was a weak negative correlation between cortical silent period duration and PAS effectiveness, with the long protocol but not the short protocol (Fig. 2.6). Given the lack of significant MEP facilitation with the long protocol the importance of this finding is unclear, but it may warrant further investigation. Overall, the neurophysiological variables investigated in the present study were poor predictors of the extent of corticomotor excitability changes induced by PAS.

We found a significant increase in CSP duration following PAS, which confirms the finding of Stefan et al. (2000). This indicates that the synaptic re-organisation associated with PAS increases the effectiveness of inhibitory circuits mediating the CSP (probably $GABA_B$ mediated; Werhahn et al., 1999) which are activated by TMS during voluntary contraction. We also demonstrated that the MEP facilitation induced by PAS was unaffected by a brief voluntary contraction performed during the cortical silent period assessment (Fig 2.4). This indicates that the changes in the intracortical circuits which have been re-organised in the resting state by PAS are relatively robust, and are able to withstand voluntary activation. PAS-induced changes in corticomotor excitability are more robust than those produced by1 Hz rTMS, as the latter are abolished after voluntary contraction (Touge et al., 2001).

2.5.4. Circadian effects on cortical neuroplasticity

PAS was more effective in subjects tested in the afternoon than in the morning (Fig. 2.7), and responses to PAS over three sessions were more reproducible in the group of subjects tested in the afternoon. The reason for this time of day effect is not clear, however endogenous circadian rhythms could conceivably contribute. The available evidence suggests that PAS induces motor cortical plasticity by LTP-like mechanisms (Stefan et al., 2000; Stefan et al., 2002; Wolters et al., 2003), and LTP in the hippocampus is modulated by a circadian rhythm (Raghavan et al., 1999; Chaudhury et al., 2005). Endogenous rhythms for melatonin and cortisol warrant consideration, as melatonin (Collins and Davies, 1997; El-Sherif et al., 2003) and a corticosterone metabolite (Dubrovsky et al., 1985) modify LTP in hippocampus, and high levels of cortisol impair memory in humans (Newcomer et al., 1999; de Quervain et al., 2000).

During the times that our experiments were undertaken (daytime, commencing around 10:10 am or 2:20 pm), plasma melatonin concentrations are negligible (McIntyre et al., 1987), and thus a change in melatonin concentration is unlikely to account for the present findings. Plasma cortisol concentrations rise shortly after awakening, and then progressively decline during the day (Ranjit et al., 2005). Therefore, an endogenous cortisol rhythm (with higher cortisol levels in the morning inhibiting LTP) could contribute to our findings. Also, the rise in cortisol concentrations after waking is brief and variable (Ranjit et al., 2005). This may help explain why there is more variability in the response to PAS when it is applied in the morning, and why the response is larger and more consistent when PAS is applied in the afternoon. A limitation of the present study is that individual subjects were not re-tested in both the morning and afternoon sessions. Further studies are warranted to address this limitation and also to investigate more directly the mechanisms responsible for the time of day influence on PAS effectiveness by assessing endogenous neurohormone levels when the experiments are conducted, and manipulating these levels by drug administration.

In conclusion, the present study has demonstrated that both effectiveness and reproducibility of PAS is improved when experiments are conducted in the afternoon. Future studies involving PAS should be conducted at a fixed time of day, preferably in the afternoon, to maximise the neuroplastic effect and reduce variability. PAS is being investigated as an augmentation to neuro-rehabilitation therapies designed to promote beneficial neuroplastic changes following brain injury such as stroke (Uy et al., 2003). Our findings indicate that PAS should be applied in the afternoon for optimal effects on cortical neuroplasticity and presumably its potential therapeutic benefit.

3. Cortisol inhibits neuroplasticity induction in human motor cortex

3.1. Abstract

We investigated whether plasticity of human motor cortex (M1) is influenced by time of day, and whether changes in circulating levels of cortisol contribute to this effect. Neuroplasticity was induced using paired associative stimulation (PAS), involving electrical stimulation of left median nerve, paired with transcranial magnetic stimulation over right M1 25 ms later (90 pairs at 0.05 Hz). Surface EMG was recorded from left abductor pollicis brevis (APB) and first dorsal interosseous (FDI). Cortisol levels were assessed from saliva. Time-of-day modulation of PAS effectiveness was assessed in 25 subjects who were tested twice, at 8 am and 8 pm on separate days. In a second double-blind study, 17 subjects were tested with PAS at 8 pm on two occasions following administration of oral hydrocortisone (24 mg) or placebo. The motor evoked potential (MEP) in resting APB increased significantly after PAS in the evening (when endogenous cortisol levels were low), but not in the morning. Oral hydrocortisone prevented facilitation of the APB MEP following PAS, and in the drug study mean salivary cortisol levels were negatively associated with PAS effectiveness. The $GABA_B$-mediated cortical silent period for APB was longer in the morning than evening, and lengthened by PAS and oral hydrocortisone. We conclude that neuroplasticity in human M1 and $GABA_B$-dependent intracortical inhibitory systems are influenced by time of day and modified by circulating levels of cortisol.

3.2. Introduction

Experimental techniques that induce neuroplastic changes in human motor cortex (M1) allow investigation of conditions thought to involve aberrant neuroplasticity, including

focal dystonia (Quartarone et al., 2003) and Parkinson's Disease (Morgante et al., 2006). Encouraging evidence suggests induction of M1 neuroplasticity with such techniques improves treatment outcomes in chronic stroke patients (Uy et al., 2003; Kim et al., 2006; McDonnell et al., 2007a). Several techniques can induce neuroplastic changes in humans that persist for minutes to hours after intervention, including cortical stimulation (Pascual-Leone et al., 1994; Berardelli et al., 1998), peripheral stimulation (Ridding et al., 2001; Charlton et al., 2003), and combined cortical and peripheral nerve stimulation (Stefan et al., 2000; Ridding and Taylor, 2001; Ridding and Flavel, 2006). The latter technique, termed paired associative stimulation (PAS), comprises electrical stimulation of a peripheral nerve followed by transcranial magnetic stimulation (TMS) of contralateral M1. With a 25-ms interstimulus interval between paired stimuli, 30 minutes of PAS induces an increase in corticomotor excitability in the target muscle that lasts for 30 minutes or more (Stefan et al., 2000). Since TMS activates pyramidal tract neurons indirectly (Day et al., 1987), and the afferent volley from the peripheral stimulus is known to alter corticospinal neuron excitability (Mariorenzi et al., 1991), it is thought that near-synchronous arrival of the two inputs with PAS induces Hebbian-like changes in synaptic efficacy, through a long-term potentiation (LTP)-like process (Stefan et al., 2000).

The variability in responses to PAS (Fratello et al., 2006; Sale et al., 2007) limits its usefulness for investigating pathophysiological changes in the brain, and as an adjunct to rehabilitative therapy. We recently found, *inter alia*, that the effectiveness and reproducibility of PAS-induced neuroplasticity was influenced by time of day of the PAS intervention (Sale et al., 2007), suggesting PAS-induced neuroplasticity may be subject to circadian modulation. However, the experimental design did not allow direct testing of this hypothesis as a different group of subjects participated in the morning and afternoon

experiments. The first aim of the present study was to provide a direct test of a time-of-day influence on M1 neuroplasticity by testing each subject with PAS at 8 am and 8 pm on separate days. The results support the hypothesis that neuroplasticity induction with PAS is greater in the evening compared to the morning.

Cortisol is a neuromodulator that could contribute to a time-of-day variation in PAS effectiveness. Plasma cortisol concentration is highest in the morning, immediately upon awakening, and declines to reach a nadir ~14 hours after waking (Ranjit et al., 2005). Neuroplastic changes induced by PAS are due to LTP-like mechanisms, and LTP is inhibited by a cortisol metabolite in rats (Dubrovsky et al., 1985). We measured salivary cortisol levels in the morning and evening to determine whether circadian changes in endogenous cortisol levels are associated with altered effectiveness of PAS. In a second experiment, subjects were tested at 8 pm (low endogenous cortisol levels), and circulating cortisol levels were manipulated by administration of a single oral dose of hydrocortisone (or placebo, on separate days). The results support the hypothesis that elevated circulating cortisol levels inhibit PAS-induced neuroplastic change in human M1.

3.3. Materials and Methods

3.3.1. Subjects

All subjects gave written informed consent prior to participation in the study, which was approved by the University of Adelaide Human Research Ethics Committee.

3.3.2. Experimental arrangement

Subjects were seated comfortably in an experimental chair with their left shoulder abducted at ~45° to allow the arm and hand to rest on a manipulandum. The hand was positioned such that the proximal phalanx of the left thumb rested in a metal ring attached to a load cell. The position of the load cell was adjusted so that it measured thumb abduction force. Thumb abduction force was displayed on an oscilloscope in front of the subject to provide visual feedback, and was filtered (low-pass at 50 Hz) and digitized online (2 kHz) via a CED 1401 interface (Cambridge Electronic Design), and stored on computer for offline analysis.

Surface electromyographic (EMG) recordings from abductor pollicis brevis (APB) and first dorsal interosseous (FDI) muscle of the left hand were obtained using bipolar Ag-AgCl electrodes in a belly-tendon montage. EMG signals were amplified 1000 times, filtered (5 Hz – 500 Hz), digitized online (2 kHz/channel) via a CED 1401 interface, and stored on computer for offline analysis. EMG signals from both muscles were displayed for the subject on an oscilloscope to help them maintain EMG silence when required. During trials requiring EMG silence, trials containing prestimulus EMG activity were discarded.

3.3.3. Maximum voluntary contractions

The abduction force exerted by the left thumb during a maximum voluntary contraction (MVC) was measured at the beginning of each session. Subjects were aided in the task by visual feedback of thumb abduction force displayed on an oscilloscope. Three trials were obtained in each session with at least 30 s rest given between each trial. The MVC force was defined as the largest thumb abduction force observed in the 3 trials.

3.3.4. Transcranial magnetic stimulation (TMS) and electrical stimulation of median nerve.

All subjects completed a TMS safety screen (Keel et al., 2001), and were excluded if there was a family history of epilepsy, were taking any neuroactive drugs or had undergone neurosurgery. Monophasic TMS was applied through a figure-of-eight coil (outer diameter of each wing 90 mm) and a Bistim module (Magstim, Whitland, Dyfed, UK) which connected two Magstim 200 magnetic stimulators (Magstim, Whitland, Dyfed, UK). This allowed the output of both machines to be directed through the same coil. The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane at the optimal scalp site to evoke a MEP in the relaxed APB muscle of the left hand. With this coil placement, current flow was induced in a posterior to anterior direction in the brain. The optimal scalp position was marked with a pen, and the coil was held throughout the experiment by hand, with the position continually checked throughout the experiment. Electrical stimuli were applied to the median nerve at the wrist using a constant current stimulator (DS7 stimulator, Digitimer Co. Ltd., Hertfordshire, UK) with bipolar surface electrodes separated by 30 mm, and with the cathode proximal. Stimuli were square waves with a pulse width of 200μs.

3.3.5. Paired associative stimulation (PAS)

The PAS protocol used in the present study has been described previously (Sale et al., 2007). PAS involves a series of paired peripheral and cortical stimuli. An electrical stimulus was delivered to the median nerve of the left wrist at an intensity sufficient to produce a small motor response in APB (approximately 300% of perceptual threshold). The electrical stimulus was followed 25 ms later by suprathreshold TMS over the hand area of the right motor cortex. TMS intensity was established prior to PAS, and produced a MEP in resting APB of 0.5-1.0 mV (this intensity is termed SI_{pre}). For PAS, ninety paired peripheral and cortical stimuli were delivered at a frequency of 0.05 Hz (duration 30 mins).

The subject's attentional focus is an important factor influencing PAS effectiveness (Stefan et al., 2004). Therefore, subjects were instructed to direct their attention onto the stimulated (left) hand during the PAS intervention. To quantify this, subjects received intermittent weak (200% perceptual threshold) electrical stimuli to their left thumb via ring electrodes (Stefan et al., 2004). Between 7 and 10 stimuli were applied at random intervals throughout the 30-min PAS session, and subjects were instructed to count and remember the number of thumb stimuli they received. When a thumb stimulus was delivered, it occurred at the mid-point of the interval between successive paired stimuli in the PAS protocol. After the PAS protocol had concluded, subjects were asked to report the number of stimuli delivered to the thumb. An error score was calculated, which was the difference between the number of stimuli delivered to the thumb and the number reported by the subject.

3.3.6. Experiment 1: Effect of time of day and endogenous cortisol levels on PAS effectiveness

Twenty five subjects (11 M, 14 F; aged 20-48 years; mean 27 ± 2 years) participated in this study. Each subject was tested on two separate occasions, once in the morning (8 am) and once in the evening (8 pm). The order of the two sessions was randomised and the sessions were separated by at least one week. During the morning (am) session, PAS commenced at 8:33 am ± 2 mins, and in the evening (pm) session, PAS commenced at 8:15 pm ± 2 mins. Subjects had been awake 126 ± 6 mins prior to the commencement of PAS in the morning experiments, and 766 ± 13 mins in the evening experiments. Subjects were instructed to have a light meal prior to both experiments. The last meal was consumed 98 ± 6 minutes prior to the commencement of PAS in the morning experiments, and 107 ± 5 minutes in the evening experiments, an insignificant difference (unpaired *t*-test).

3.3.7. Single and paired-pulse TMS measures of motor cortex excitability

Several measures of motor cortex excitability were made prior to and following the PAS intervention during each experimental session (see Figure 3.1A for time-line of experimental assessments).

Resting motor threshold (RMT) was defined as the minimum stimulus intensity required to evoke a MEP in relaxed APB of > 50 μV in 3 out of 5 consecutive trials (Carroll et al., 2001). Active motor threshold (AMT) was defined as the minimum stimulus intensity required to evoke a MEP of > 200 μV in the active (5% MVC) APB in 3 out of 5 consecutive trials. The stimulus intensity was expressed as a percentage of maximum stimulator output (% MSO). RMT was assessed prior to and following PAS in all

experiments. AMT was assessed prior to PAS only in Experiment 1 (see below). All TMS pulses were delivered using the output of the Bistim module, which reduces the output by ~10% compared with a single Magstim and leads to higher threshold values as %MSO.

Mean peak-to-peak amplitude of the APB and FDI MEP at rest was calculated by averaging the individual peak-to-peak amplitudes of MEPs elicited by 20 TMS (0.2 Hz, intensity SI_{pre}) delivered immediately prior to (pre-PAS), and five minutes following PAS (post-PAS).

We assessed cortical inhibition as GABAergic systems influence LTP in rat motor cortex (Hess and Donoghue, 1994) and plasticity in human M1 (Butefisch et al., 2000; McDonnell et al., 2007b). The cortical silent period (CSP) duration for APB was assessed with a TMS intensity of 130% RMT, and a contraction strength of 15% MVC. Subjects were given visual feedback of their force level via an oscilloscope. Ten TMS were delivered at a frequency of 0.2 Hz. The duration of the APB CSP was measured off-line from the rectified EMG traces for each trial, and averaged for the 10 trials. CSP duration was defined as the time from TMS onset until the resumption of continuous EMG at pre-stimulus levels.

Short-interval intracortical inhibition (SICI) was assessed using a protocol similar to that described previously (Kujirai et al., 1993). Paired-pulse TMS was delivered at an interstimulus interval (ISI) of 3ms. The first (conditioning) stimulus was subthreshold for producing a MEP in active APB, and activates intracortical inhibitory circuits that reduce the size of the MEP elicited by the suprathreshold test stimulus delivered 3 ms later. Four conditioning intensities were used: 60%, 70%, 80% and 90% AMT. The test stimulus

intensity was set at SI_{pre} prior to PAS. Since the extent of SICI may be influenced by the size of the test MEP, the test TMS intensity was adjusted post-PAS to match the amplitude of the test MEP with that seen pre-PAS. This TMS intensity was termed SI_{post}. The effect of conditioning TMS intensity on MEP amplitude was assessed in separate blocks. Each block consisted of 20 trials (0.2 s^{-1}), which included 10 paired stimuli and 10 test alone stimuli in pseudorandom order. The order of testing blocks containing each conditioning intensity was randomised. The size of the conditioned MEP was expressed as a percentage of the unconditioned MEP in each block to quantify the effectiveness of SICI.

3.3.8. Salivary cortisol assay

Saliva samples were collected from each subject prior to commencement of PAS (pre-PAS), and at the end of each experiment (post-PAS). Saliva was frozen at -20 deg C until assayed. On the day of assay the saliva samples were thawed and centrifuged. Twenty-five μL of saliva was assayed in duplicate for cortisol by ELISA (HS-Cortisol; Salimetrics, LLC, State College, PA, U.S.A.).

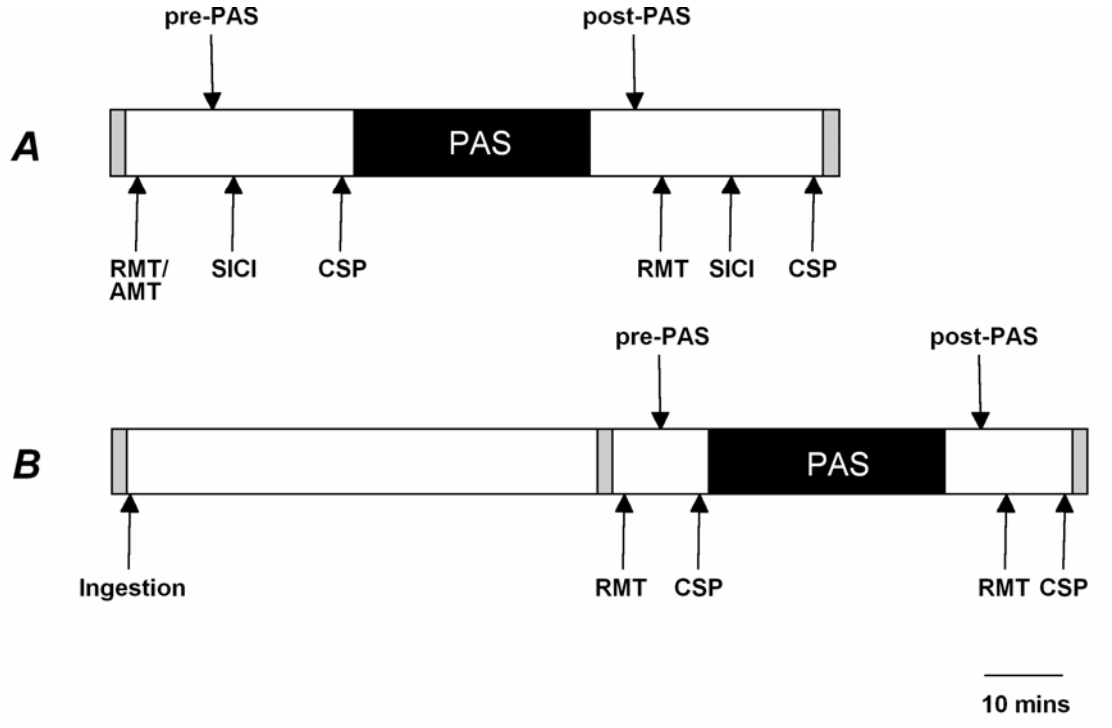

Figure 3.1
Schematic representation of the testing protocol indicating approximate relative timings for assessment of neurophysiological parameters before and after PAS in experiment 1 (A) and experiment 2 (B). The grey bars indicate when saliva samples were collected. Measurements of APB MEP amplitude were made at two time points: pre-PAS and post-PAS. Ingestion, oral dose of hydrocortisone or placebo.

3.3.9. Experiment 2: Exogenous cortisol and PAS effectiveness

Seventeen subjects (8 M, 9 F; aged 19-35 years; mean 24 ± 1 years) participated in a randomised, double-blind, placebo-controlled study to determine whether a single oral dose of hydrocortisone modulates PAS effectiveness (referred to hereinafter as the exogenous cortisol study). One subject complained of a mild headache (hydrocortisone session), but completed the experiment. One additional subject was tested but withdrew due to feeling unwell (in placebo session).

Subjects were tested at 8 pm (when endogenous cortisol is low) on 2 occasions separated by at least one week. Subjects received a gelatin-cased capsule containing either

hydrocortisone (Hysone 24 mg; Alphapharm, Australia) or starch (order randomised for session 1 and 2) which was consumed with a glass of low-fat milk 60 minutes prior to the commencement of the experiment. Subjects were instructed to fast for three hours prior to the commencement of the experiment. The last meal was consumed 195 ± 18 minutes prior to the commencement of PAS in the placebo experiments, and 199 ± 41 minutes in the cortisol experiments, an insignificant difference (unpaired *t*-test). PAS commenced at 8:09 pm ± 2 mins in the placebo session and 8:08 pm ± 1 min in the cortisol session. Subjects had been awake for 738 ± 27 mins in the placebo session, and 737 ± 32 mins in the cortisol session, an insignificant difference (unpaired *t*-test). Saliva samples were taken prior to ingestion of medication (pre-ingestion), prior to PAS (pre-PAS) and following PAS (post-PAS), and later assayed for salivary cortisol concentration.

The experimental procedures and protocol for the exogenous cortisol study were as for experiment 1, except that AMT and SICI were not assessed (see Figure 3.1B for time-line of experimental procedures).

3.3.10. Statistical analysis

3.3.10.1. Experiment 1

Three-way repeated measures analysis of variance (ANOVA) was performed on MEP amplitude data from APB and FDI with within-subject factors INTERVENTION (two levels: pre-PAS and post-PAS), TIME OF DAY (two levels: am and pm) and between-subject factor GENDER (two levels: male and female) to determine the effect of PAS and time of day on the extent of MEP facilitation. Two-way repeated measures ANOVA assessed the effect of INTERVENTION and TIME OF DAY on APB resting motor threshold and cortical silent period. One-way repeated measures ANOVA assessed the

effect of TIME OF DAY on APB active motor threshold. Three-way repeated measures ANOVA with within-subject factors of INTERVENTION, TIME OF DAY and CONDITIONING INTENSITY (four levels: 60%AMT, 70%AMT, 80%AMT and 90%AMT) assessed the effect of these factors on SICI.

3.3.10.2. Experiment 2

Three-way repeated measures ANOVA was performed on MEP amplitude data from APB and FDI with within-subject factors INTERVENTION (two levels: pre-PAS and post-PAS) and MEDICATION (two levels: Hydrocortisone and placebo) and GENDER (two levels: male and female) to determine the effect of PAS and medication on the extent of MEP facilitation. Two-way repeated measures ANOVA assessed the effect of INTERVENTION and MEDICATION on APB resting motor threshold and cortical silent period.

Linear regression analysis was used on data from experiments 1 and 2 to examine the relationship between salivary cortisol concentration and both APB MEP facilitation (post-PAS MEP amplitude/pre-PAS MEP amplitude) and the pre-PAS cortical silent period duration. The salivary cortisol concentration data were log-transformed to improve homoschedasticity. The strength of the relationship was quantified by the coefficient of determination (r^2).

For all analyses $P < 0.05$ was chosen as the significance level, and unless stated otherwise, all group data are reported as mean ± SEM. Fisher's PLSD post-hoc tests were performed as appropriate.

3.4. Results

3.4.1. Experiment 1: Effect of time of day and endogenous cortisol levels on PAS effectiveness

TMS intensity used during PAS (SI_{pre}) was not significantly different in the endogenous cortisol experiments between the morning and evening sessions (76 ± 3% MSO *vs*. 74 ± 3% MSO, respectively), corresponding to 127 ± 3 % RMT *vs*. 127 ± 3 % RMT. Intensity of peripheral nerve stimulation during PAS was 6.5 ± 0.5 mA for the morning session and 5.8 ± 0.5 mA for the evening session, an insignificant difference (paired *t*-test).

ANOVA revealed no effect of TIME OF DAY ($F_{1,24} = 2.14$) nor INTERVENTION ($F_{1,24} = 1.65$) on resting motor threshold in the endogenous cortisol experiments. In the morning, RMT was 60 ± 2% MSO pre-PAS and 59 ± 2% MSO post-PAS. In the evening, RMT was 59 ± 2% MSO pre-PAS and 57 ± 3% MSO post-PAS. There was also no significant effect of TIME OF DAY on active motor threshold ($F_{1,24} = 3.14$). AMT was only assessed prior to PAS. In the morning AMT was 48 ± 2 % MSO, and in the evening 46 ± 2 % MSO.

The extent of MEP facilitation induced in APB by PAS was larger in the evening sessions than in the morning, and this effect was consistent across GENDER ($F_{1,23} = 0.40$), thus the data were pooled for males and females. The group data on PAS effectiveness for the 25 subjects are summarised in Fig. 3.2. On average APB MEP amplitude increased by 15% in morning experiments, and by 41% in evening experiments (Fig. 3.2A). There was a significant effect of TIME OF DAY ($F_{1,24}= 7.67$, $P = 0.011$) and INTERVENTION ($F_{1,24} = 9.84$, $P = 0.005$) on APB MEP amplitude. There was also a TIME OF DAY x INTERVENTION interaction ($F_{1,24} = 4.45$, $P = 0.045$) indicating the effectiveness of PAS

was influenced by the time of day. *Post-hoc* analysis revealed that there was significant APB MEP facilitation in the evening (Fisher, $P < 0.001$), whereas there was no significant change in APB MEP amplitude following PAS in the morning (Fisher). APB MEP amplitude prior to PAS was not significantly different between sessions (a.m., 0.76 ± 0.07 mV; p.m., 0.78 ± 0.06 mV). APB MEP amplitude following PAS (post-PAS) was significantly greater in the evening compared to the morning ($P < 0.001$) (Fig. 3.2A). As expected, PAS did not result in significant facilitation of MEPs evoked in the FDI muscle (INTERVENTION, $F_{1,24} = 0.95$). The FDI MEP was 3% smaller after PAS in the morning, and 16% larger after PAS in the evening (Fig. 3.2B). There was no significant effect of TIME OF DAY on FDI MEP amplitude ($F_{1,24} = 0.76$).

Time of day did not influence attention-related error scores indicating that the subjects were attending to the task equally well in the morning and evening experiments. The mean error score in the morning session was 0.24 ± 0.10, and in the evening session 0.28 ± 0.09 ($F_{1,24} = 0.07$).

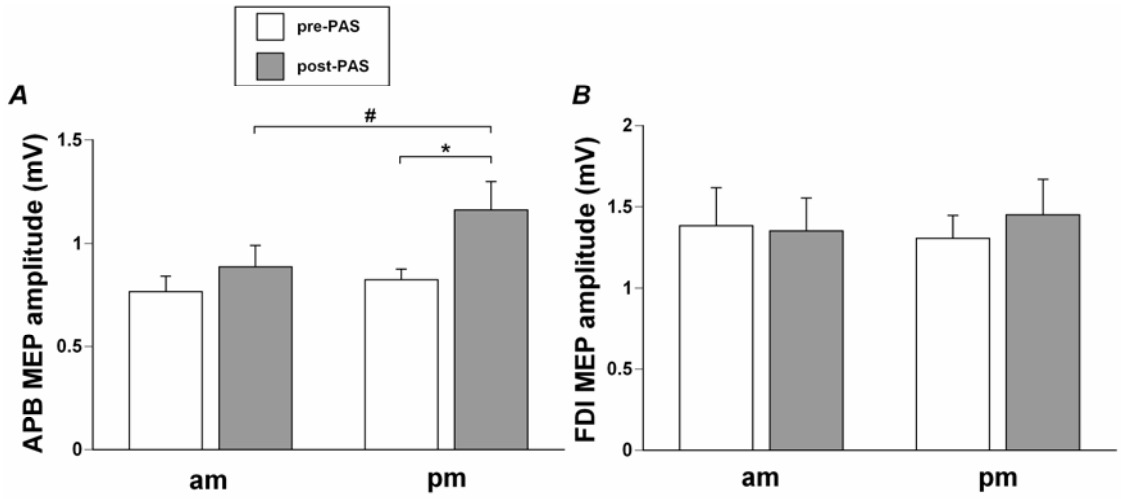

Figure 3.2

Group (mean ± SEM) MEP amplitude for APB (A) and FDI (B) before (pre-PAS) and after (post-PAS) paired associative stimulation. Data are from morning experiments (am) on the left, and evening (pm) experiments on the right. APB MEP amplitude was significantly larger post-PAS

than pre-PAS in the evening session (* P < 0.001), but not in the morning. APB MEP amplitude post-PAS was significantly greater in the evening than the morning (# P < 0.001). FDI MEP amplitude (B) was not affected by PAS or time of day.

Cortical silent period data from Expt. 1 are summarised in Fig. 3.3. Both TIME OF DAY ($F_{1,24}$ = 9.04, *P* = 0.006) and INTERVENTION ($F_{1,24}$ = 27.47, *P* < 0.001) influenced cortical silent period duration. The interaction term was not significant ($F_{1,24}$ = 0.28), indicating that PAS had similar effects on the cortical silent period in morning and evening experiments. Cortical silent period duration was significantly shorter in the evening (pre-PAS, 183 ± 5 ms) compared to the morning (pre-PAS, 193 ± 7 ms) (*P* = 0.016), and the duration of the silent period increased by ~6% following PAS (pre-PAS, 188 ± 4 ms *vs.* post-PAS 199 ± 4 ms (*P* = 0.002).

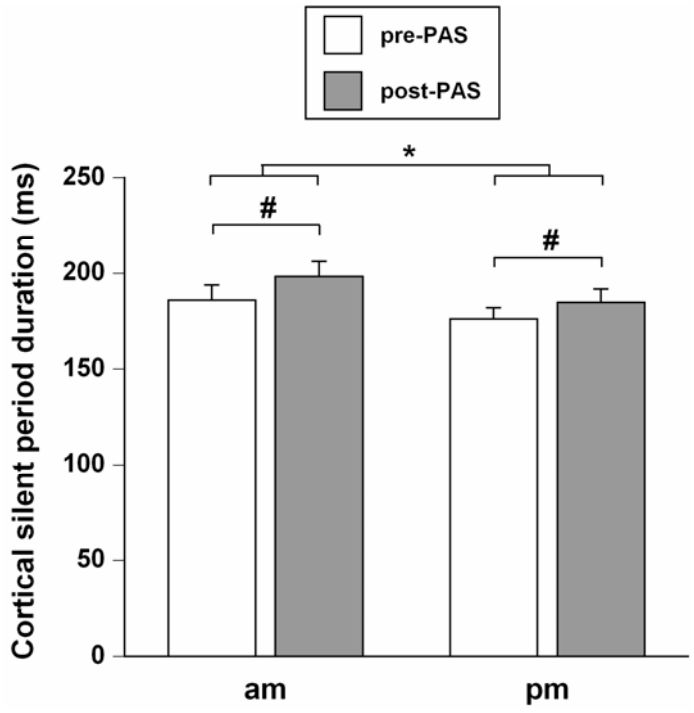

Figure 3.3

Group (mean ± SEM) data of cortical silent period duration before (pre-PAS) and after (post-PAS) paired associative stimulation. Data from morning experiments (am) are on the left, and evening (pm) experiments are on the right. Cortical silent period duration was significantly longer in the morning (* P = 0.006) than in the evening. There was a significant increase in cortical silent period duration after PAS in both the morning and evening sessions (# P = 0.002).

PAS did not significantly alter SICI (INTERVENTION, $F_{1,72} = 0.30$; see Figure 3.4), and the overall effectiveness of SICI was similar in the morning and evening (TIME OF DAY, $F_{1,72} = 1.29$). As expected, SICI was a function of conditioning TMS intensity ($F_{3,72} = 25.76$, $P < 0.001$). *Post-hoc* analysis revealed that MEP suppression with a conditioning intensity of 60% AMT was significantly less than MEP suppression using all other conditioning intensities (Fisher, $P < 0.001$), and that MEP suppression with a conditioning intensity of 70% AMT was significantly less than MEP suppression using conditioning intensities of 80% AMT and 90% AMT (Fisher, $P = 0.001$). Conditioning at 60% AMT did not suppress the MEP (paired *t*-test), while higher conditioning intensities produced significant inhibition of the MEP (paired *t*-tests, $P < 0.001$). The APB MEP amplitude in single-pulse trials did not differ pre- and post-PAS (INTERVENTION, $F_{1,72} = 4.17$), due to a small reduction of test TMS intensity post-PAS (76% MSO pre-PAS vs. 74% MSO post-PAS). There was also no significant effect of TIME OF DAY ($F_{1,72} = 0.01$) on MEP amplitude in single-pulse trials. This indicates that test MEP amplitudes were well matched throughout the assessment of SICI.

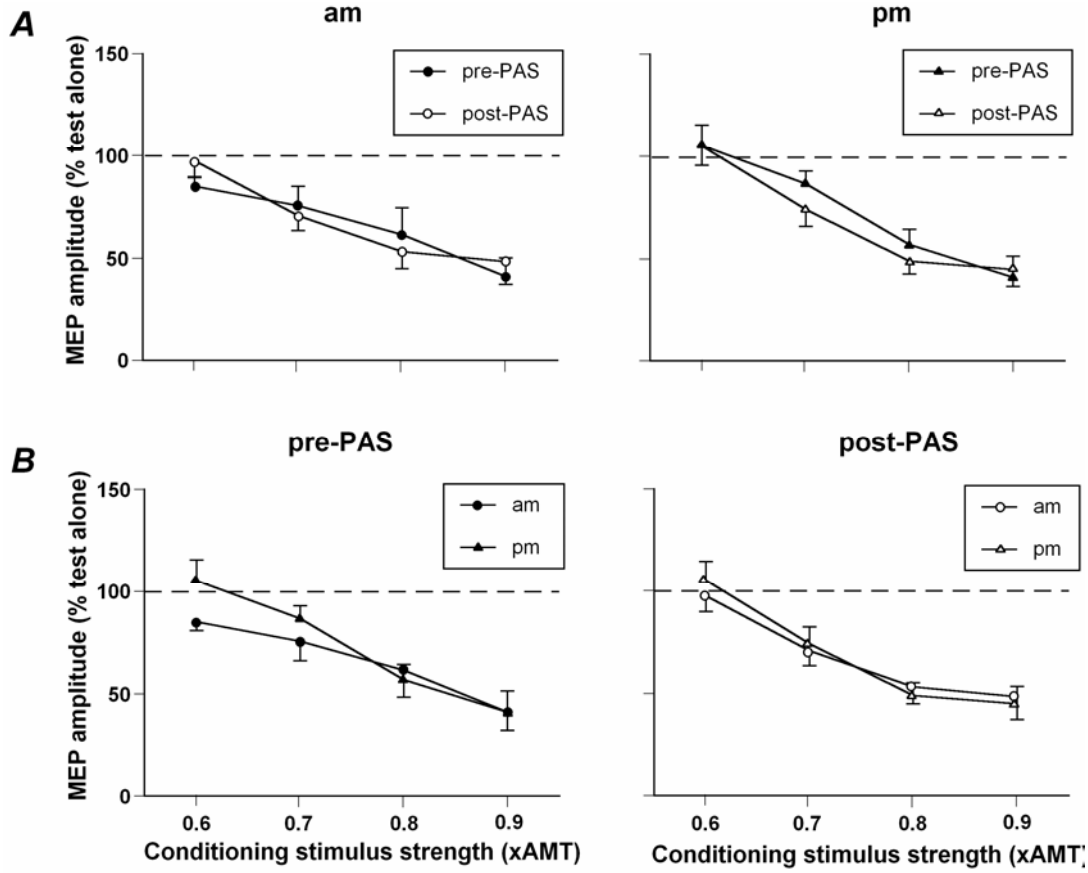

Figure 3.4

Influence of time of day (A) and PAS (B) on SICI. Group (mean ± SEM) SICI data from 20 subjects assessed in the morning (am; circles) and evening (pm; triangles), before (pre-PAS; filled symbols) and after (post-PAS; open symbols) paired associative stimulation. SICI was assessed with paired-pulse TMS using four different conditioning TMS intensities (60-90% AMT), with an interstimulus interval of 3 ms. SICI was quantified as percentage of MEP amplitude obtained in conditioned trials compared with test-alone trials. SICI was unchanged after PAS both in the A.M. and PM.. The overall level of SICI did not differ between A.M. and P.M. sessions.

3.4.2. Experiment 2: Exogenous cortisol and PAS effectiveness

TMS intensity used during PAS (SI_{pre}) was not significantly different between the placebo and cortisol sessions (75 ± 3% MSO *vs*. 75 ± 3% MSO, respectively), corresponding to 123 ± 2 % RMT *vs*. 123 ± 3 % RMT. The intensity of peripheral nerve stimulation during PAS was 6.7 ± 0.6 mA for the placebo session and 6.5 ± 0.5 mA for the cortisol session, an insignificant difference (paired *t*-test).

ANOVA revealed no significant effect of MEDICATION ($F_{1,16}$ = 0.48) nor INTERVENTION ($F_{1,16}$ = 0.01) on resting motor threshold. In the placebo session, RMT was 61 ± 2% MSO pre-PAS and 61 ± 2% MSO post-PAS. In the cortisol session, RMT was 61 ± 2% MSO pre-PAS and 60 ± 2% MSO post-PAS.

There was significant facilitation of APB MEP amplitude following PAS in the placebo session, but not in the session when subjects received oral hydrocortisone (Fig. 3.5A). This effect was consistent across GENDER ($F_{1,15}$ = 0.21), and thus data were pooled for males and females. Two-way repeated measures ANOVA indicated no significant effect of MEDICATION ($F_{1,16}$ = 0.64) or INTERVENTION ($F_{1,16}$ = 3.28), but there was a significant MEDICATION x INTERVENTION interaction ($F_{1,16}$ = 9.78, P = 0.007), indicating the effect of PAS on MEP amplitude was influenced by medication. APB MEP amplitude increased significantly by 28% in the placebo session following PAS (Fisher, P = 0.039), yet with oral hydrocortisone APB MEP amplitude decreased (non-significantly) by 6% (Fisher). FDI MEP amplitude (Fig 3.5B) was not influenced by MEDICATION ($F_{1,16}$ = 3.77) nor INTERVENTION ($F_{1,16}$ = 1.98), and the interaction term was not significant ($F_{1,16}$ = 0.35)

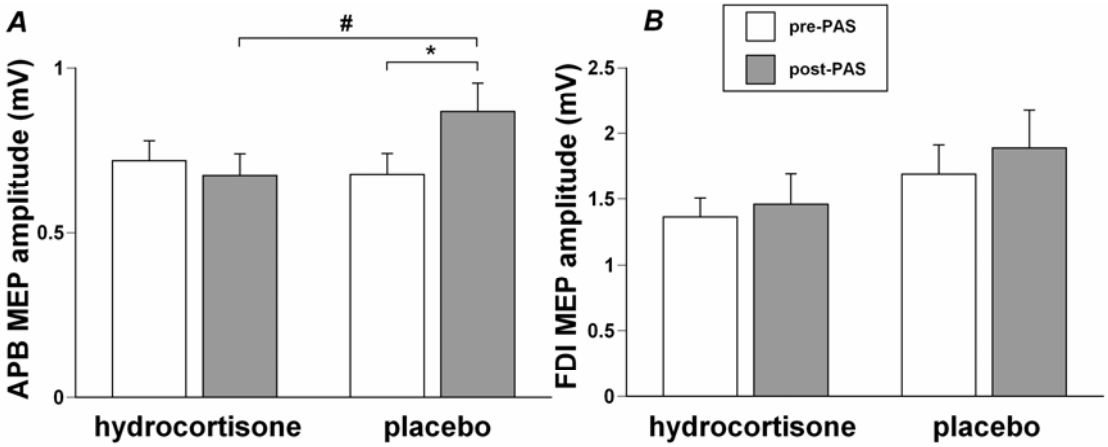

Figure 3.5

Group (mean ± SEM) MEP amplitude for APB (A) and FDI (B) before (pre-PAS) and after (post-PAS) paired associative stimulation. Data from the hydrocortisone (cortisol) administration session are on the left, and data from the placebo session are on the right. APB MEP amplitude was significantly larger post-PAS than pre-PAS in the placebo session (* P = 0.007), but not in the cortisol session. APB MEP amplitude post-PAS was significantly greater in the placebo than in the cortisol session (# P = 0.039). FDI MEP amplitude (B) was not affected by PAS or medication.

Cortical silent period data from Expt. 2 are summarised in Fig. 3.6. Both MEDICATION ($F_{1,16}$ = 5.16, P = 0.037) and INTERVENTION ($F_{1,16}$ = 216.37, P < 0.001) influenced cortical silent period duration. The interaction term was not significant ($F_{1,16}$ = 2.28), indicating that PAS had similar effects on the cortical silent period in hydrocortisone and placebo sessions. Cortical silent period duration was significantly shorter in the placebo (pre-PAS, 193 ± 5 ms) compared to the hydrocortisone session (pre-PAS, 206 ± 3 ms) (P < 0.001), and the duration of the silent period increased by ~10% following PAS (pre-PAS, 199 ± 3 ms *vs*. post-PAS 218 ± 3 ms (P < 0.001).

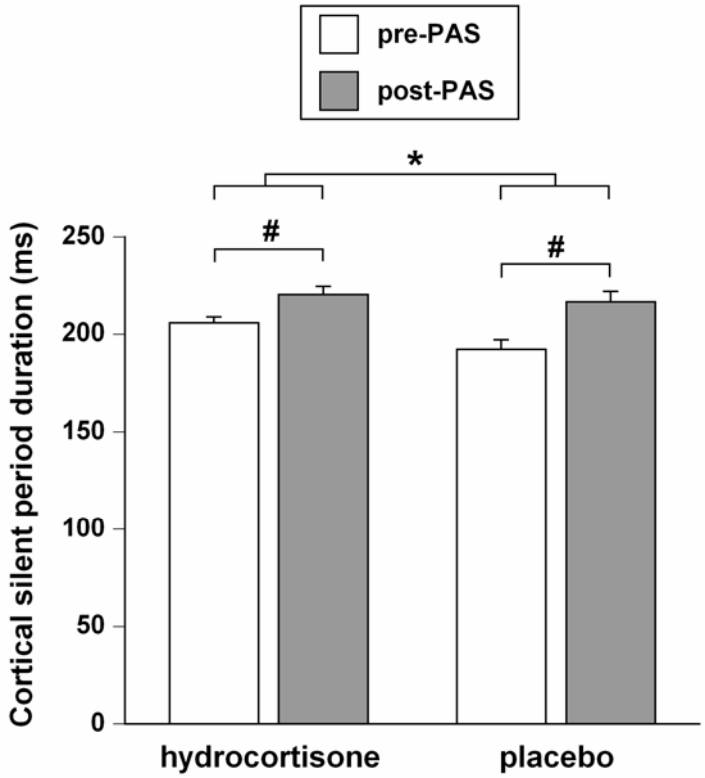

Figure 3.6

Group (mean ± SEM) data of cortical silent period duration before (pre-PAS) and after (post-PAS) paired associative stimulation. Data from the hydrocortisone (cortisol) administration group are on the left, and data from the placebo group are on the right. Cortical silent period duration was significantly longer in the cortisol (* $P < 0.001$) than in the placebo group. There was a significant increase in cortical silent period duration after PAS in both sessions (# $P < 0.001$).

3.4.3. Salivary cortisol levels and relationships with PAS effectiveness and cortical silent period

Saliva samples obtained during the experiments were assayed for salivary cortisol concentration. For experiment 1, saliva samples were taken prior to (pre-PAS) and following PAS (post-PAS). There was a significant effect of TIME OF DAY ($F_{1,24}$ = 86.90, $P < 0.001$) and INTERVENTION ($F_{1,24}$ = 10.72, P = 0.003) on salivary cortisol concentration (Fig. 3.7A). There was also a significant interaction of TIME OF DAY x INTERVENTION ($F_{1,24}$ = 5.57, P = 0.027). *Post-hoc* analysis revealed that salivary cortisol concentration prior to PAS in the morning session was higher than at all other

times of saliva sampling ($P < 0.001$). Salivary cortisol concentration following PAS (post-PAS) in the morning session was greater than salivary cortisol concentration in the evening sessions ($P < 0.001$; Fig. 3.7A).

For experiment 2, saliva samples were taken prior to ingestion of the medication (pre-ingestion) as well as prior to (pre-PAS) and following PAS (post-PAS). There was a significant effect of TIME (3 levels: pre-ingestion, pre-PAS, post-PAS) ($F_{2,32} = 5.07$, $P = 0.012$) and MEDICATION ($F_{1,32} = 11.46$, $P = 0.004$) on salivary cortisol levels (Fig. 3.7B). There was also a significant TIME x MEDICATION ($F_{1,32} = 4.78$, $P = 0.015$) interaction. *Post-hoc* analysis revealed that oral hydrocortisone administration elevated salivary cortisol levels, with both pre-PAS and post-PAS cortisol concentrations significantly higher than pre-ingestion levels ($P < 0.001$). There was no significant difference in salivary cortisol concentration during the placebo experiments.

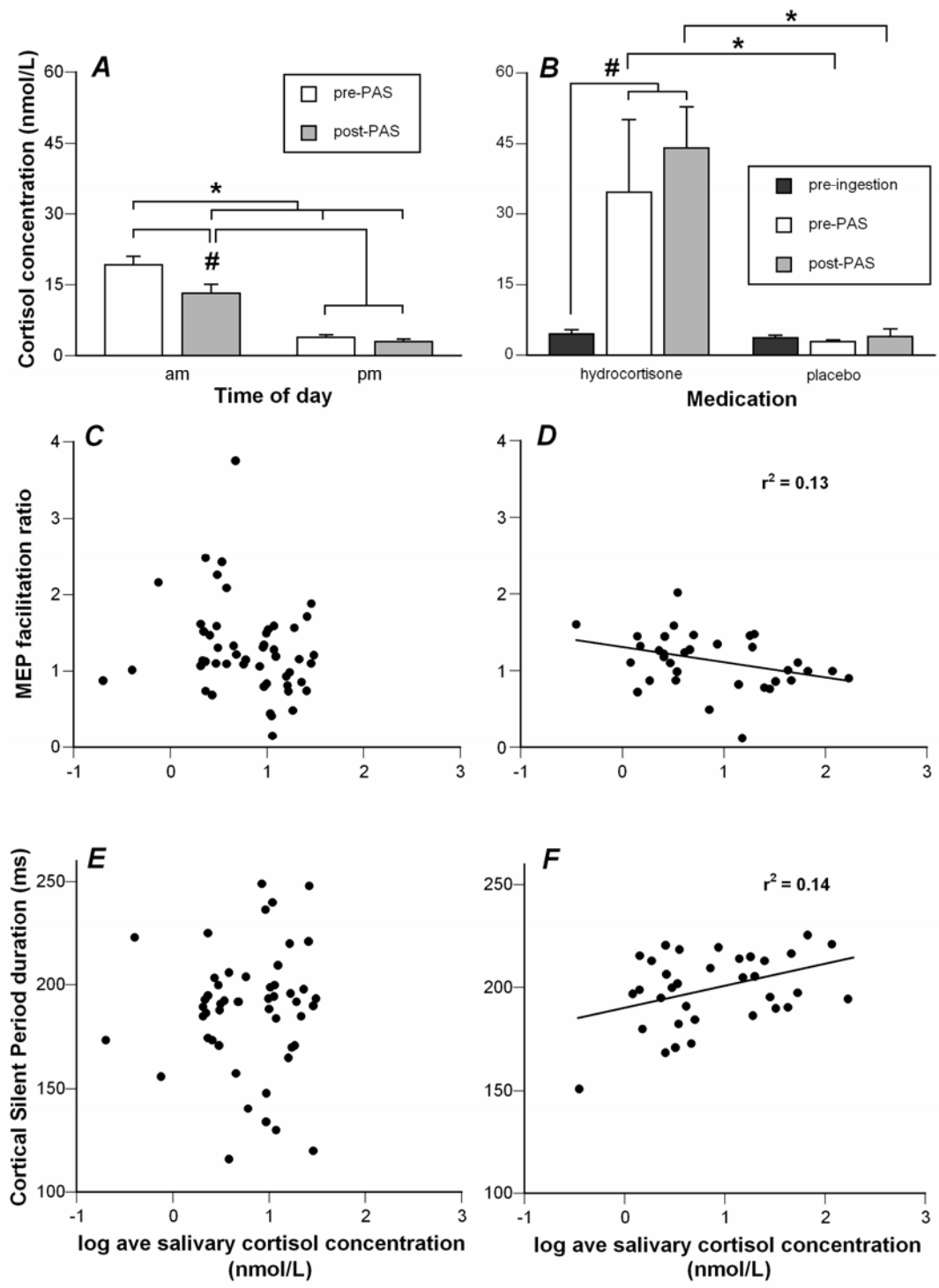

Figure 3.7

Salivary cortisol concentration (A,B) and the relationship between salivary cortisol concentration and APB MEP facilitation after PAS (C,D) and cortical silent period duration (E,F). Data from study 1 (endogenous cortisol) are on the left, and data from study 2 (hydrocortisone administration) are on the right. Pre-PAS salivary cortisol concentration in the morning was significantly greater than all other samples (A) (*P < 0.001). Post-PAS salivary cortisol concentration in the morning was significantly greater than both P.M. samples, but less than the pre-PAS A.M. sample (#P < 0.001). B, Exogenous cortisol administration significantly elevated salivary cortisol concentration in the evening compared with placebo (*P < 0.001). C, Linear regression analysis revealed a nonsignificant relationship (r^2 = 0.04) between the log of average salivary cortisol concentration

and APB MEP facilitation ratio (post-PAS MEP amplitude/pre-PAS MEP amplitude) in study 1 (endogenous cortisol). D, Linear regression analysis revealed a significant negative relationship (r^2 = 0.13, P = 0.039) between the log of average salivary cortisol concentration and APB MEP facilitation ratio in study 2 (hydrocortisone administration). E, Nonsignificant relationship (r^2 = 0.002) between the log of average salivary cortisol concentration and pre-PAS cortical silent period duration in study 1. F, Significant relationship (r^2 = 0.14, P = 0.028) between the log of average salivary cortisol concentration and pre-PAS cortical silent period duration in study 2.

Since salivary cortisol concentration changed over the time it took to deliver PAS (Fig. 3.7A), the pre-PAS and post-PAS salivary cortisol concentrations were averaged to provide a value that reflects the mean circulating cortisol level *during* PAS. This was used in the linear regression analysis of the association between salivary cortisol level and MEP facilitation induced by PAS (Fig. 3.7C, D), and cortical silent period duration (Fig. 3.7E, F). For experiment 1, there was no significant relationship between the (log) average salivary cortisol concentration and the extent of APB MEP facilitation (r^2 = 0.04; Fig 3.7C). In contrast, with exogenous cortisol administration in experiment 2, there was a weak but significant negative relationship between the average salivary cortisol concentration and the extent of APB MEP facilitation following PAS (r^2 = 0.13, *P* = 0.039; Fig 3.7D). There was greater facilitation of the APB MEP following PAS when salivary cortisol concentration was low. There was no significant relationship between average salivary cortisol concentration and pre-PAS cortical silent period duration in experiment 1 (r^2 = 0.002; Fig 3.7E). However, in experiment 2, there was a significant positive relationship between salivary cortisol concentration and pre-PAS cortical silent period duration (r^2 = 0.14, *P* = 0.028; Fig 3.7F). There was no significant relationship between the pre-PAS CSP values and MEP facilitation ratio for either experiment 1 (r^2 = 0.02, n = 50) or experiment 2 (r^2 = 0.08, n = 34) (data not shown).

3.5. Discussion

The principal novel findings from this study are that MEP facilitation following PAS was influenced by time of day and oral hydrocortisone administration. There was significant MEP facilitation in the target muscle following PAS in the evening. However, during periods of high circulating cortisol levels (in the morning or with oral hydrocortisone in the evening), there was no significant MEP facilitation. Additionally, the time of day, PAS, and circulating cortisol levels influenced CSP duration.

3.5.1. M1 neuroplasticity is influenced by time of day

We recently reported that PAS was more effective in experiments conducted in the afternoon than the morning (Sale et al., 2007), however different subjects were tested at those times. The present study extends that result by showing, in the same group of subjects, that PAS effectiveness is greater in the evening than the morning.

PAS induces M1 plasticity by LTP-like mechanisms (Stefan et al., 2000, 2002; Wolters et al., 2003, Ziemann et al., 2004). M1 is considered the primary site of corticomotor excitability change induced by PAS (Stefan et al., 2000; Wolters et al., 2003; Muller et al., 2007), but we have not excluded effects on remote sites such as spinal cord or ipsilateral primary sensory cortex (Murakami et al., 2008). PAS-induced changes in spinal excitability have been reported (Meunier et al., 2007), although others find no change (Stefan et al., 2000; Wolters et al., 2003).

It has been proposed that wakefulness favours LTP-like processes and leads to net synaptic potentiation, whereas sleep favours LTD-like processes and leads to homeostatic synaptic depression (Tononi and Cirelli, 2006). According to this theory, prolonged wakefulness

should make induction of LTP-like plasticity more difficult, as LTP can be saturated by prior LTP-like learning (Rioult-Pedotti et al., 2000; Whitlock et al., 2006). LTP in rat cerebral cortex is induced more readily 1-60 min after sleep than after several hours of wakefulness (Vyazovskiy et al., 2008). In contrast, we found PAS more effective in the evening, when subjects had been awake 766 ± 13 min, compared with the morning when they had been awake 126 ± 6 min. The reasons for this discrepancy are not clear, but our results suggest other factors, such as cortisol levels, influence LTP-like neuroplasticity.

3.5.2. Cortisol and M1 neuroplasticity

Time-of-day modulation of PAS effectiveness may be due to circadian changes in neuromodulators. LTP in hippocampus exhibits a circadian rhythm (Raghavan et al., 1999; Chaudhury et al., 2005). Cortisol is a candidate neuromodulator as it has a circadian rhythm, and a corticosterone metabolite impairs LTP in rat hippocampus (Dubrovsky et al., 1985). Acute (Newcomer et al., 1999; de Quervain et al., 2000) and chronic (Starkman et al., 1992) elevation of plasma cortisol have been associated with memory impairment in humans.

Plasma cortisol concentration rises quickly after awakening in the morning, and is low during the afternoon and evening (Ranjit et al., 2005). PAS effectiveness is enhanced in the evening (Fig. 3.2) when endogenous cortisol is low. Effects of PAS in the evening are blocked by a single oral dose of hydrocortisone (Fig. 3.5), which is metabolised to cortisol in the body.

Cortisol has wide-ranging effects on physiological processes, and we are unable to determine whether impairment of neuroplasticity by hydrocortisone is directly mediated by

cortisol or indirectly by effects on other neuromodulator(s). Cortisol may also delay the time-course of PAS effects, rather than reducing them. Future studies could address this by assessing MEP facilitation at multiple time-points after PAS.

Cortisol is rapidly metabolised upon release (Edwards et al., 2001), and fluctuations in cortisol levels *during* Experiment 1 (Fig. 3.7A) may contribute to the lack of association between salivary cortisol levels and PAS effectiveness. Higher levels of cortisol in Experiment 2 (Fig. 3.7B) may be more effective in suppressing neuroplasticity, and contribute to the significant negative association between salivary cortisol and neuroplasticity in Experiment 2. A single oral dose of hydrocortisone impairs learning and memory in humans (de Quervain et al., 2000; Hsu et al., 2003), and salivary cortisol levels in Expt. 2 are in the range reported by de Quervain et al. (2000).

Although circulating cortisol levels appear to influence PAS-induced neuroplasticity, the weak correlation between these variables indicates a contribution from other factors. Since Experiment 1 was performed at two different times of day, circadian modulation of other (non-measured) neuromodulators may have influenced results, including dopamine (Castaneda et al., 2004) and melatonin (Collins and Davies, 1997; El-Sherif et al., 2003), both of which influence LTP (Collins and Davies, 1997; Kusuki et al., 1997; El-Sherif et al., 2003). Since both sessions of Experiment 2 were in the evening, between-session circadian variation in other neuromodulators would have less influence on PAS effectiveness in Expt. 2 than in Expt. 1.

Subjects participated in two experimental sessions, separated by at least one week. Between-session changes in factors known to modulate cortical excitability, including

physical activity (Cotman and Engesser-Cesar, 2002), may have influenced the results. Changes in menstrual cycle hormones also influence cortical excitability (Smith et al., 1999), however we found no gender differences in PAS-induced neuroplasticity. Menopausal women aged >50 years are less responsive to PAS (Tecchio et al., 2008), however that study reported no differences between *young* men and women, consistent with the present study.

3.5.3. Modulation of intracortical inhibition

GABAergic inhibitory tone modulates LTP-like plasticity, and we considered whether changes in M1 inhibitory tone influenced PAS effectiveness. Down-regulation of $GABA_A$-mediated inhibition promotes LTP induction in rat motor cortex slices (Hess & Donoghue, 1994) and use-dependent neuroplasticity induction in human M1 (Butefisch et al., 2000). Additionally, the $GABA_B$ receptor agonist baclofen suppresses LTP-like plasticity in human M1 (McDonnell et al., 2007b). $GABA_B$ inhibitory tone in M1, as assessed by CSP, was higher in morning sessions, and with evening hydrocortisone administration. This is when PAS was less effective, suggesting $GABA_B$ inhibitory tone may modulate PAS effectiveness, consistent with findings obtained with baclofen (McDonnell et al., 2007b). However, the absence of a significant relationship between pre-PAS CSP duration and MEP facilitation ratio in Experiment 1 and Experiment 2 argues against a direct effect of physiological variation in $GABA_B$ inhibitory tone on PAS effectiveness.

A prolonged CSP following PAS confirms previous results (Stefan et al., 2000; Quartarone et al., 2003; Sale et al., 2007). The CSP is believed to have both a spinal and ($GABA_B$ receptor-mediated) cortical component (Werhahn et al., 1999). Spinal mechanisms

contribute to EMG suppression in the first part of the silent period (< 100 ms), as H-reflexes are suppressed (Fuhr et al., 1991; Uncini et al., 1993). The later part of the CSP, when motoneuron excitability is at pre-stimulus levels, is attributed to cortical inhibitory mechanisms (Inghilleri et al., 1993; Chen et al., 1999). The increase in CSP duration following PAS and with high cortisol levels (Figs. 3.3 & 3.5) is therefore most likely due to changes in cortical inhibition. CSP duration was increased following PAS, regardless of whether the MEP was facilitated. This is not surprising as the inhibitory processes responsible for the CSP are relatively independent of the extent of activation of corticospinal descending projection to motoneurons by TMS (Ho et al., 1998), and the CSP was assessed during voluntary activation whereas PAS-induced MEP facilitation was assessed at rest.

SICI is believed to reflect $GABA_A$ inhibition (Ilic et al., 2002; Ziemann, 2003). There was no change in SICI following PAS, which supports previous findings (Ridding and Taylor, 2001; Stefan et al., 2002; Quartarone et al., 2003; Kujirai et al., 2006b). Kujirai et al. (2006) found reduced SICI following PAS using a coil orientation that preferentially activated late I-waves which are more susceptible to intracortical inhibition (Nakamura et al., 1997; Di Lazzaro et al., 1998b; Hanajima et al., 1998). Therefore, it's possible that PAS may produce subtle changes in SICI not detected with the coil orientation we used.

SICI was not influenced by time of day (or cortisol levels) in Experiment 1, and does not appear to modulate PAS effectiveness. Circadian variation in GABA release has been demonstrated in the mammalian brain (Castaneda et al., 2004; Gompf and Allen, 2004) , and $GABA_A$-induced chloride currents are enhanced by metabolites of steroid hormones (Majewska et al., 1986). Our assessment of SICI may not be sufficiently sensitive to detect small time-of-day changes in SICI (see above). Alternatively, perhaps there is little

modulation of GABA release (following PAS or at different times of day) onto $GABA_A$ receptors in the M1 circuitry acted upon by paired-pulse TMS.

In conclusion, neuroplasticity induction in human M1 with PAS was more effective in the evening, when endogenous cortisol levels were low. Hydrocortisone administration in the evening raised circulating cortisol levels and impaired M1 neuroplasticity. PAS-induced LTP-like plasticity engages neural circuits involved in motor learning in humans (Ziemann et al., 2004; Stefan et al., 2006). Our findings have important implications for rehabilitative therapies utilising neuroplastic change in human M1 to promote functional recovery (Uy et al., 2003; Kim et al., 2006; McDonnell et al., 2007a), as they suggest that plasticity induction, and presumably any therapeutic benefit, would be enhanced when circulating cortisol levels are low.

4. Time of day does not modulate improvements in motor performance following a repetitive ballistic motor training task

4.1. Introduction

In recent years several techniques have been developed to induce neuroplastic change in human cortex. These techniques either involve direct modulation of cortical networks (Pascual-Leone et al., 1994; Berardelli et al., 1998; Huang et al., 2005), a combination of both peripheral and cortical stimulation (Stefan et al., 2000; Ridding and Taylor, 2001; Ridding and Flavel, 2006), or repetitive peripheral stimulation (Ridding et al., 2001; Charlton et al., 2003). The neuroplastic changes induced with these protocols produce a change in cortical excitability, which can be assessed non-invasively by single-pulse transcranial magnetic stimulation (TMS).

There is considerable interest in applying these techniques as adjunct therapies in rehabilitation, thus potentially improving rehabilitation outcomes for conditions reliant on neuroplasticity for recovery of function. Unfortunately, one limitation of these techniques, which has thus far limited their clinical use, is the large variability in effectiveness of inducing neuroplastic change (Fratello et al., 2006; Sale et al., 2007). In Chapter 2 I showed that time of day influences the effectiveness and reproducibility of neuroplasticity induction in human primary motor cortex (M1) (Sale et al., 2007). Using the technique of paired associative stimulation (PAS) to induce plasticity within the human motor cortex (Stefan et al., 2000), neuroplasticity induction was shown to be more effective and reproducible when experiments were performed in the afternoon compared with the morning (Sale et al., 2007). The time of day modulation of neuroplasticity induction is due, at least in part, to changes in circulating cortisol levels (Sale et al., 2008).

The cortical circuits modulated by PAS are also important for motor learning (Ziemann et al., 2004). A repeated maximum thumb abduction training task resulted in motor learning (indicated by an increase in peak acceleration) and this prevented a subsequent increase in cortical excitability following PAS (Ziemann et al., 2004). Since neuroplasticity induction by PAS, in cortical circuits involved in motor learning is modulated by time of day (Sale et al., 2007; Sale et al., 2008), the purpose of the present study was to determine whether motor learning and training-induced changes in cortical excitability were likewise modulated by time of day. I hypothesise that improvements in motor learning, and training-induced neuroplasticity, will be greater in the evening compared with the morning, and these differences will be associated with changes in circulating cortisol levels.

4.2. Materials and Methods

4.2.1. Subjects

Twenty-two right-handed subjects (aged 19-37 years; 10 females) participated in the study, which was approved by the University of Adelaide Human Research Ethics Committee. All subjects had no known history of neurological conditions, and gave written informed consent to participate in the study. Each subject participated in two separate experimental sessions, once in the morning (8am) and once in the evening (8pm). The experimental sessions were separated by at least one week, and the order was randomised. Eleven subjects began with the morning session, and eleven began with the evening session.

4.2.2. Recording

Surface electromyographic (EMG) recordings were made from left abductor pollicis brevis (APB) muscle. EMG signals were amplified (x1000), filtered (20-500 Hz) and digitized

(2000 Hz) using a CED 1401 interface (Cambridge Electronic Design) and stored on computer for off-line analysis.

Acceleration recordings were made from the left thumb using a dual-axis accelerometer (ADXL311 Analog Devices Inc, MA, USA). The accelerometer was affixed to a splint which was taped to the second phalanx of the thumb, with its axes aligned to record abduction and flexion about the joint. Acceleration signals were amplified (x3), filtered (low pass 50 Hz) and digitized (2000 Hz) using a CED 1401 interface and stored on a computer for off-line analysis. Subjects were provided with visual feedback about abduction and flexion acceleration for each trial, and were encouraged to minimise flexion movements throughout the experiment. Flexion data were not further analysed.

4.2.3. Motor Training (MT) task

The MT task was based on one previously described (Ziemann et al., 2004; Ridding and Flavel, 2006) involving rapid abduction of the thumb. The left hand was placed in a device that constrained the left forearm in mid-range supination and maintained the wrist in ~45 degrees extension. The left shoulder was kept in a neutral position (slight external rotation) and the elbow was flexed at ~90 degrees. The device allowed movement of the thumb in all planes. The MT task consisted of repeated abduction of the left thumb at maximal acceleration, paced by an auditory tone (1000 Hz tone, 100 ms duration) once every 2 seconds. Subjects were provided with performance feedback which consisted of visual display of maximum thumb acceleration on a computer screen for each trial, as well as verbal encouragement throughout the MT task.

4.2.4. Quantification of training-induced changes

4.2.4.1. Maximum thumb abduction acceleration

To quantify the effect of the MT task on motor performance, ten trials of maximal left thumb abduction acceleration were obtained prior to and following the MT task. In order for subjects to familiarise themselves with the required task they were allowed a few (<5) practice trials of the thumb abduction task at the start of the first experimental session only.

4.2.4.2. Transcranial magnetic stimulation (TMS)

Cortical excitability changes associated with the MT task were quantified using TMS. All subjects completed a TMS safety screen (Keel et al., 2001), and were excluded if there was a family history of epilepsy, were taking any neuroactive drugs or had undergone neurosurgery. Monophasic TMS was applied through a figure-of-eight coil (outer diameter of each wing 90mm) which was connected to a Magstim 200 magnetic stimulator (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 at the optimal scalp site to evoke a motor evoked potential (MEP) in the relaxed APB muscle of the left hand. With this coil placement, current flow was induced in a posterior to anterior direction in the brain. The optimal scalp position was marked with a pen, and the coil was held by hand, with the position continually checked during TMS. Prior to MT, the TMS intensity required to produce MEPs with a peak-to-peak amplitude of between 0.5-1.0 mV was determined (SI_{pre}). Ten MEPs using SI_{pre} were obtained prior to MT (0.2 Hz), and then again following MT. The cortical excitability data were obtained immediately prior to the maximum thumb abduction acceleration data for both pre- and post-MT.

4.2.5. Experimental protocol

Motor performance was assessed by measuring maximum left thumb abduction acceleration prior to (pre-MT) and five minutes following MT (post-MT). Thumb acceleration data were obtained immediately after MEP trials. Subjects were given strong verbal and visual encouragement to perform their best possible thumb accelerations. There was no restriction on the frequency of thumb acceleration trials during the pre-MT and post-MT assessment periods.

Maximum thumb acceleration data were collected during the MT task and later analysed for rate of improvement in thumb acceleration and coefficient of variation of thumb acceleration during training (see below).

Cortical excitability was assessed by measuring mean peak-to-peak amplitude of the APB MEP at rest. It was calculated by averaging the individual peak-to-peak amplitudes of MEPs elicited by 10 TMS (0.2 s^{-1}, intensity SI_{pre}) delivered immediately prior to (pre-MT), and five minutes following MT (post-MT).

4.2.6. Salivary cortisol assay

Saliva samples were collected from each subject prior to commencement of MT, and at the end of each experiment. Saliva was frozen at -20C until assayed. On the day of assay the saliva samples were thawed and centrifuged. Twenty-five µL of saliva was assayed in duplicate for cortisol by ELISA (HS-Cortisol; Salimetrics, LLC, State College, PA, U.S.A.).

4.2.7. Statistical analysis

Separate three-way repeated measures analyses of variance (ANOVAs) were performed on thumb acceleration and APB MEP amplitude with within-subject factors INTERVENTION (two levels: pre-MT and post-MT) and TIME OF DAY (two levels: am and pm) and SESSION (two levels: first and second).

To investigate changes in thumb acceleration *during* the MT task, data were divided into six separate five minute epochs (0-5 mins, 5-10 mins, 10-15 mins, 15-20 mins, 20-25 mins, 25-30 mins). Mean APB acceleration and the coefficient of variation (CV) of APB acceleration were calculated for the separate epochs. Separate two-way repeated measures ANOVAs were performed on the mean thumb acceleration and CV data with within-subject factors EPOCH (six levels: 0-5 mins, 5-10 mins, 10-15 mins, 15-20 mins, 20-25 mins, 25-30 mins) and TIME OF DAY.

A two-way repeated measures ANOVA was performed on salivary cortisol concentration with within-subject factors INTERVENTION and TIME OF DAY.

Linear regression analysis was used to assess a relationship between salivary cortisol concentration and changes in motor performance with training (max. acceleration post-MT/max. acceleration pre-MT), and change in cortical excitability (APB MEP facilitation: post-MT MEP amplitude/pre-MT MEP amplitude). The salivary cortisol concentration data were log-transformed to improve homoschedasticity. The strength of the relationship was quantified by the coefficient of determination (r^2).

For all analyses $P < 0.05$ was chosen as the significance level, and unless stated otherwise, all group data are reported as mean ± SEM. Bonferroni *post-hoc* tests for multiple comparisons were performed as appropriate.

4.3. Results

All subjects participated in all experiments, and no adverse effects were noted.

4.3.1. Motor performance and motor training

Three-way repeated measures ANOVA revealed no effect of SESSION ($F_{1,40} = 2.957$) on maximum thumb acceleration (and no significant SESSION x INTERVENTION interaction ($F_{1,40} = 1.214$)) so data were pooled for session. A two-way ANOVA revealed MT significantly increased maximum thumb acceleration by 28% ($F_{1,21} = 22.61$, $P < 0.001$; see Figure 4.1), but MT was not influenced by TIME OF DAY ($F_{1,21} = 0.284$). In addition, there was no significant interaction between INTERVENTION and TIME OF DAY ($F_{1,21} = 1.543$) indicating the improvement in thumb acceleration following training was independent of time of day.

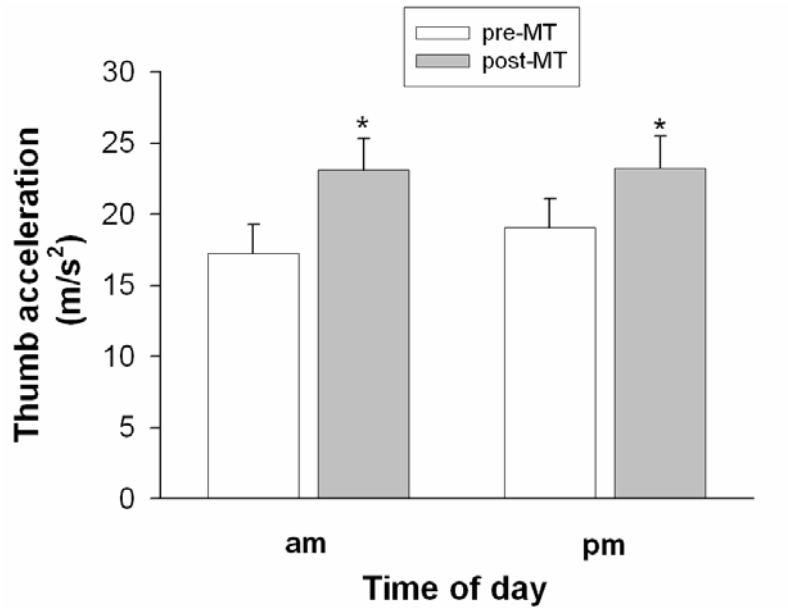

Figure 4.1
Improvement in maximum thumb acceleration after motor training is not influenced by time of day. Average (n = 10) maximum left APB abduction acceleration values (mean ± SEM) recorded from 22 subjects who participated in two experimental sessions: morning (am) or evening (pm) on separate occasions. Average maximum thumb acceleration values are shown before (pre-MT) and after (post-MT) motor training. Maximum acceleration increased significantly following motor training (*; P < 0.001), and this effect was independent of time of day.

Mean maximum acceleration values during the MT task are shown in figure 4.2A. Two-way repeated measures ANOVA revealed a significant effect of EPOCH ($F_{5,105}$ = 13.01, *P* < 0.001). *Post-hoc* analysis revealed that mean maximum acceleration during the 0-5 minute epoch was significantly less than at the 10-15 minute (*P* = 0.003), 15-20 minute (*P* < 0.001), 20-25 minute (*P* < 0.001) and 25-30 minute epochs (*P* < 0.001). Mean maximum acceleration during the 5-10 minute epoch was significantly less than during the 15-20 minute (*P* < 0.001), 20-25 minute (*P* < 0.001) and 25-30 minute (*P* < 0.001) epochs. Mean maximum acceleration during the 10-15 minute epoch was significantly less than during the 20-25 minute (*P* = 0.002) epoch. Motor performance during the MT task was not influenced by TIME OF DAY ($F_{1,105}$ = 0.885). The interaction term EPOCHxTIME OF DAY was not significant ($F_{5,105}$ = 1.322), indicating that the improvement in performance during the MT task was independent of time of day.

The variability of maximum thumb acceleration data during the MT task is shown in Figure 4.2B. Two-way repeated measures ANOVA revealed a significant effect of EPOCH on the coefficient of variation of maximum thumb acceleration data during the MT task ($F_{5,105} = 7.915$, $P < 0.001$). Post-hoc analysis revealed that the CV of acceleration during the 0-5 minute epoch was significantly greater than during the 15-20 minute ($P < 0.020$), 20-25 minute ($P < 0.001$) and 25-30 minute epochs ($P < 0.001$). The CV of acceleration for the 5-10 minute epoch was significantly greater than during the 25-30 minute epoch ($P < 0.001$). The CV of acceleration for the 10-15 minute epoch was significantly greater than the 20-25 minute ($P = 0.019$) and 25-30 minute epochs ($P = 0.004$). The CV of acceleration during the MT task was not influenced by TIME OF DAY ($F_{1,105} = 0.273$). The interaction term EPOCHxTIME OF DAY was not significant ($F_{5,105} = 0.200$), indicating that the reduction in CV during the MT task was independent of time of day.

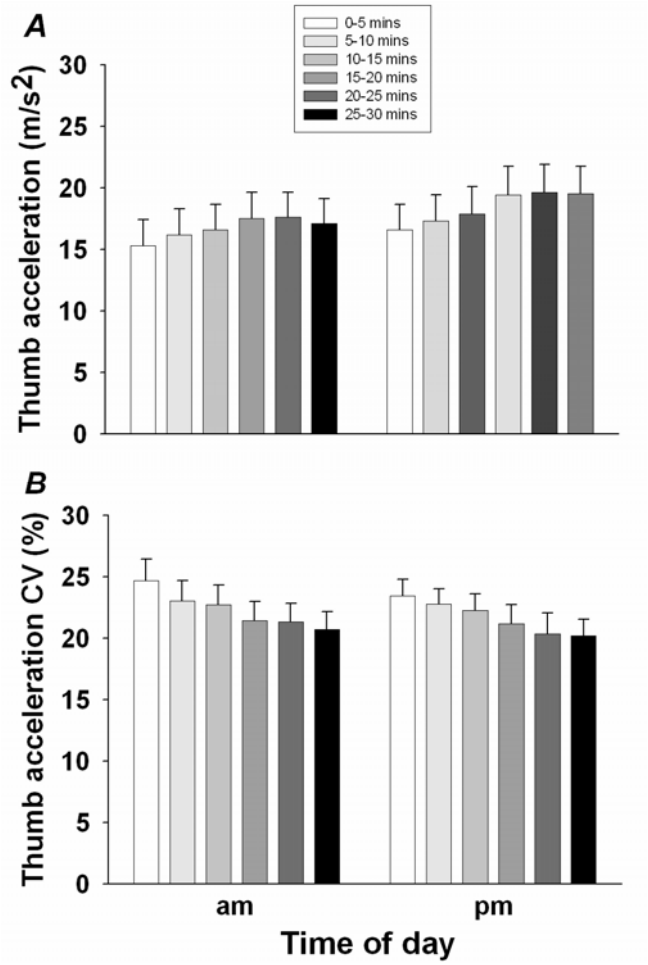

Figure 4.2

Time of day does not influence maximum left thumb acceleration nor the coefficient of variation of maximal thumb acceleration during a motor training task. Average APB peak abduction acceleration (A) and coefficient of variation (B) values (mean ± SEM) recorded from 22 subjects during a motor training task on two separate occasions: morning (am) and evening (pm). Data are divided into six 5-minute epochs for the motor training task (0-5 mins, 5-10 mins, 10-15 mins, 15-20 mins, 20-25 mins, 25-30 mins). Acceleration increased significantly during MT, for both morning and evening sessions. The trial-to-trial variability of acceleration (CV) reduced significantly during MT, and this effect was similar in morning and evening sessions.

4.3.2. Cortical excitability and motor training

The stimulus intensity used to evoke test MEPs was 5% higher in the evening (73.6 ± 2.8 %MSO) compared with the morning (70.4 ± 2.4 %MSO) (paired t-test, P = 0.037). Despite a time-of-day difference in test TMS intensity, pre-MT APB MEP amplitudes were not significantly different between groups (am = 0.65 ± 0.07 mV; pm = 0.67 ± 0.07 mV; paired t-test).

A three-way ANOVA on the APB MEP amplitude data revealed no significant effect of SESSION ($F_{1,40}$ = 0.008), thus results were pooled for session. A subsequent two-way ANOVA revealed a significant effect of motor training on MEP amplitude (INTERVENTION effect: $F_{1,21}$ = 4.463, P = 0.047). APB MEP amplitude increased significantly by 23% following MT (Figure 4.3). There was no effect of TIME OF DAY on MEP amplitude ($F_{1,21}$ = 0.165). In addition, there was no significant interaction between INTERVENTION and TIME OF DAY ($F_{1,21}$ = 0.086) indicating the increase in MEP amplitude following MT was independent of time of day.

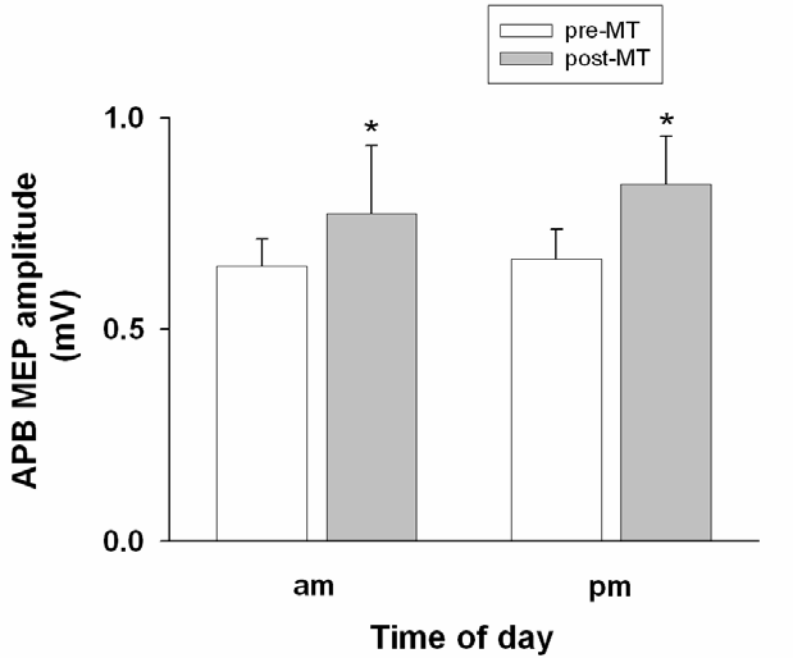

Figure 4.3

Increases in APB MEP amplitude after motor training are not influenced by time of day. Average (n = 10) APB MEP amplitude (mean ± SEM) recorded from 22 subjects who participated in two experimental sessions: morning (am) and evening (pm) on separate occasions. Average APB MEP amplitudes are shown before (pre-MT) and after (post-MT) motor training. APB MEP amplitude increased significantly following motor training (*; P = 0.047), and this effect was independent of time of day.

4.3.3. Neuroplasticity, motor performance and salivary cortisol concentration

Linear regression analysis between the changes in motor performance (acceleration) with training, and the extent of APB MEP facilitation associated with training showed no significant relationship ($r^2 < 0.001$). These results are shown in Figure 4.4.

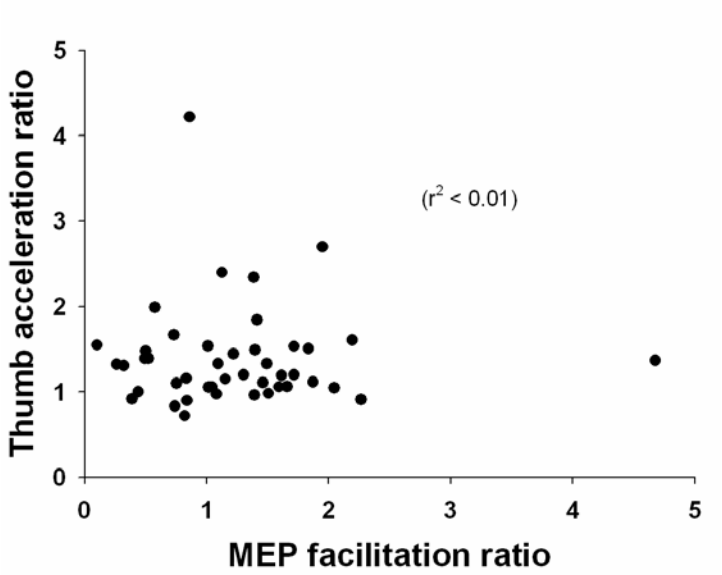

Figure 4.4
Relationship between motor performance improvement and cortical excitability changes following MT. Linear regression analysis revealed a non-significant relationship ($r^2 < 0.01$) between acceleration ratio (max. acceleration post-MT/max. acceleration pre-MT) and MEP facilitation ratio (MEP amplitude post-MT/MEP amplitude pre-MT). Data include morning and evening experiments.

Salivary cortisol concentration was significantly greater in the morning compared with evening experiments ($F_{1,21} = 52.633$, $P < 0.001$). Additionally, salivary cortisol concentration was significantly less following MT ($F_{1,21} = 8.737$, $P = 0.008$). The interaction term TIME OF DAY x INTERVENTION was also significant ($F_{1,21} = 7.477$, $P = 0.012$. *Post-hoc* analysis revealed a significant reduction in salivary cortisol concentration following MT in the morning but not the evening experiments ($P = 0.001$). Salivary cortisol concentration data are shown in Figure 4.5.

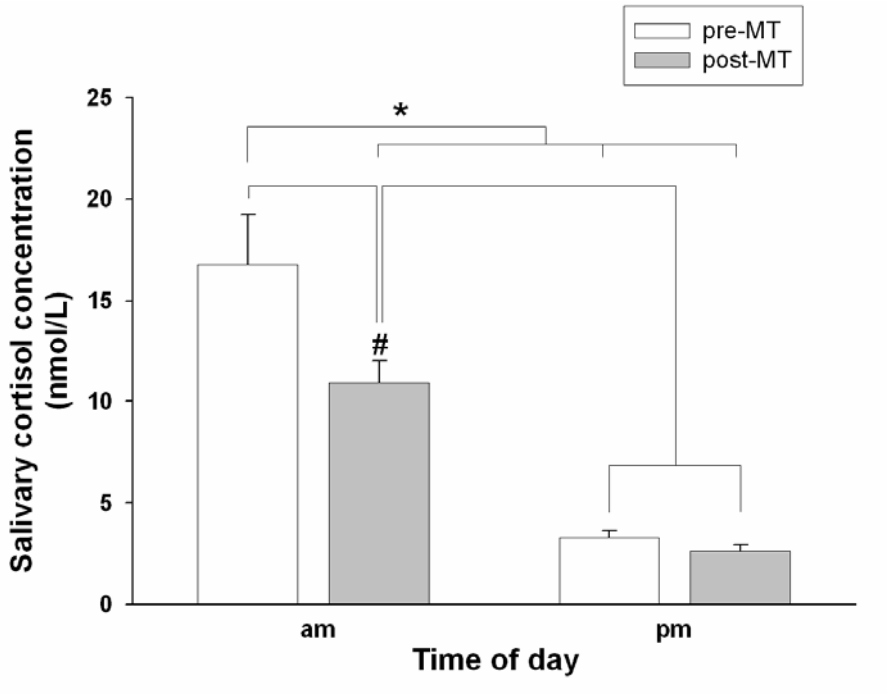

Figure 4.5
Salivary cortisol concentration is higher in the morning than the evening. Pre-MT salivary cortisol concentration in the morning was significantly greater than all other samples (*; $P < 0.001$). Post-MT salivary cortisol concentration in the morning was significantly greater than both pm samples, but significantly less than pre-MT morning concentration (#; $P = 0.008$).

Since salivary cortisol concentration significantly reduced over the time it took to perform the MT task in the morning, the pre-MT and post-MT salivary cortisol concentrations were averaged to provide a value that reflects the mean circulating cortisol level *during* MT. This was used in the linear regression analysis of the association between salivary cortisol concentration and both motor performance and the extent of APB MEP facilitation. There was no significant relationship between motor performance and the (log) of average salivary cortisol concentration ($r^2 = 0.005$; Figure 4.6A), nor the extent of APB MEP facilitation and the (log) of average salivary cortisol concentration ($r^2 = 0.016$; Figure 4.6B).

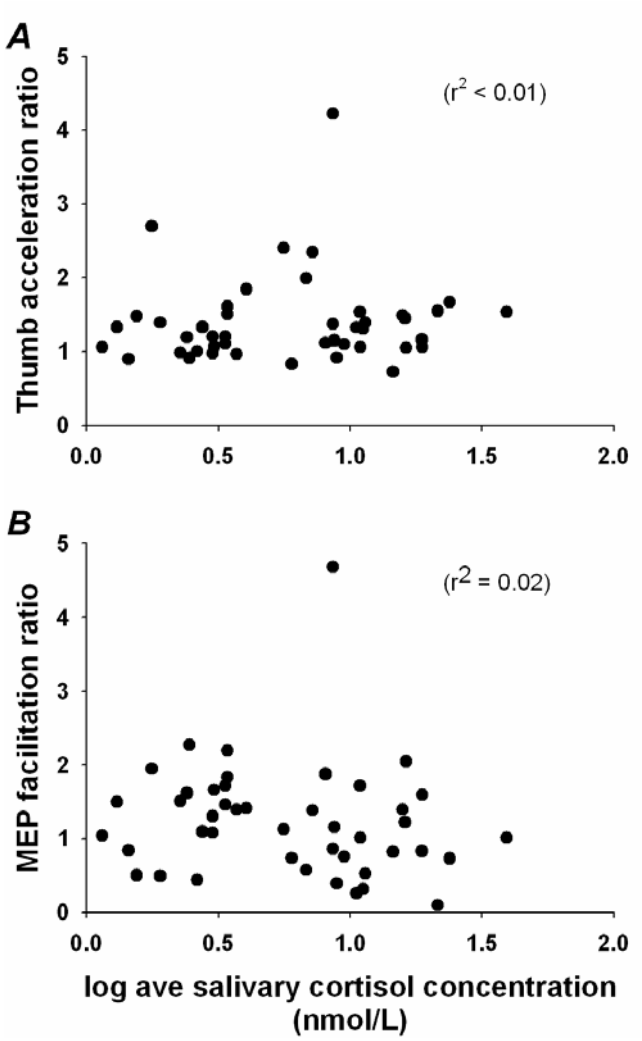

Figure 4.6
Relationship between motor performance (A), cortical excitability (B) and salivary cortisol concentration. Linear regression analysis revealed a non-significant ($r^2 < 0.01$) relationship between acceleration ratio (max. acceleration post-MT/max. acceleration pre-MT) and the log of average salivary cortisol concentration (A). Linear regression analysis revealed a non-significant ($r^2 = 0.02$) relationship between MEP facilitation ratio (MEP amplitude post-MT/MEP amplitude pre-MT) and the log of average salivary cortisol concentration (B). Data include morning and evening experiments.

4.4. Discussion

The principal finding from the present study was that MT induces changes in motor performance and cortical excitability, but these changes are not modulated by time of day. Repeated maximal left thumb abduction for thirty minutes increased motor performance (indicated by an increase in maximal acceleration of thumb abduction) and induced an increase in motor cortical excitability (indicated by an increase in MEP amplitude).

However, the magnitude of the changes in both motor performance and cortical excitability were similar in the morning and evening sessions.

Several previous studies have investigated changes in motor performance and cortical excitability following a motor training task (Pascual-Leone et al., 1995; Muellbacher et al., 2001; Ziemann et al., 2004; Ridding and Flavel, 2006). The magnitude of the reported improvements in motor performance and cortical excitability in the present study are largely in agreement with the results of these studies. The present study sought to expand on these previous findings by investigating whether time of day influenced improvements in motor performance and cortical excitability following a repetitive motor training task.

4.4.1. Does time of day modulate changes in motor performance and cortical excitability following a motor training task?

One mechanism thought responsible for the increase in cortical excitability following motor training is a strengthening of synaptic connections by long-term potentiation or LTP (Sanes and Donoghue, 2000). Supporting evidence in favour of this has come from human (Ziemann et al., 2004) and animal (Rioult-Pedotti et al., 1998; Rioult-Pedotti et al., 2000) studies in which motor learning modified subsequent induction of LTP-like plasticity. The increase in cortical excitability in the human study was achieved using PAS (Stefan et al., 2000). The increase in cortical excitability associated with PAS induces LTP-like changes in M1 (Stefan et al., 2000; Stefan et al., 2002; Ziemann et al., 2004; Wolters et al., 2005).

We have previously demonstrated that the magnitude of neuroplastic change induced in M1 with PAS is dependent on the time of day (Sale et al., 2007). Induction of neuroplastic change was more effective, and the variability of responses was reduced, if experiments

were performed in the afternoon (Sale et al., 2007) or evening (Sale et al., 2008) compared with the morning. Since LTP-like plasticity is more easily induced in the evening with PAS, and LTP-like mechanisms are thought important for learning-induced cortical plasticity (Ziemann et al., 2004), the purpose of the present study was to determine whether time of day modulates changes in motor performance (and cortical excitability) following motor training. More specifically, I hypothesised that motor training-related changes in motor performance and cortical excitability would be greater in the evening. Performance of several motor tasks have been shown to be dependent on time of day (Miller et al., 1992). For example, force discrimination (Miller et al., 1992), muscle strength (Wyse et al., 1994), and performance of a basic motor flicking task (Edwards et al., 2007) are all influenced by time of day. However, none of these studies have examined whether the *learning* of these tasks was influenced by time of day. My results suggest that training-related improvements in motor performance (Figure 4.1) and cortical excitability (Figure 4.3) following motor training are not influenced by the time of day.

Although no time of day differences in motor performance were reported following completion of the MT task, perhaps the rate of improvement in performance *during* the MT task was different in the morning and evening experiments. That is, although the magnitude of the improvement in motor performance was similar in morning and evening experiments, the improvement in performance in the evening experiments may have occurred more rapidly. McDonnell and Ridding (2006) demonstrated that there were differences in the rate, rather than the overall magnitude of improvement in a grooved pegboard task following afferent stimulation (McDonnell and Ridding, 2006). Their finding indicates that modulation of the rate of improvement in a training task, rather than the magnitude of the overall improvement is possible. Analysis of the thumb acceleration

data during the MT task revealed that motor performance improved significantly during the MT task, however the time-course of improvement in performance was similar in the morning and evening experiments (Figure 4.2A).

One factor that could have potentially influenced the results is subject attention, which has been shown to be important in modulating the effectiveness of neuroplasticity induction with PAS (Stefan et al., 2004). Neuroplasticity induction of the target muscle was most effective if the subject was paying attention to, and looking at, the target muscle. Conversely, if the subject was distracted and not looking at the target muscle, neuroplasticity induction was reduced (Stefan et al., 2004). In the present study, subjects received constant verbal encouragement to maintain their maximum effort, and were aided in this by visual feedback of trial-to-trial performance. If subjects were not consistently attending to the training task, it would be likely that there would be greater trial-to-trial variability in thumb acceleration. We report no time-of-day changes in trial-to-trial variability of maximum thumb abduction as measured by the coefficient of variation of thumb acceleration (Figure 4.2B), and thus consider it unlikely that (a lack of) attention influenced the results.

Another possible explanation for the lack of modulation of MT by time of day is that the training task and/or outcome measures used may not have been sufficiently sensitive to allow detection of subtle time of day changes in motor performance. Although the training task used in the present study has been shown to effectively induce motor performance and cortical excitability changes, it is well known that different tasks engage corticomotoneuronal (CM) cells (which are indirectly activated with TMS) in M1 to a varying degree (Muir and Lemon, 1983; Flament et al., 1993). A precision grip task has

been shown to more effectively activate CM cells in monkey motor cortex than a power grip task (Muir and Lemon, 1983). Also, MEPs in first dorsal interosseous (FDI) muscle following TMS are known to be larger during a precision grip compared with a power grip (Datta et al., 1989) or simple index finger abduction task (Flament et al., 1993). Also, repetitive MT task using a precision grip have been demonstrated to induce persistent changes in cortical excitability (Muellbacher et al., 2001; Muellbacher et al., 2002; Garry et al., 2004). Jensen et al. (2005) extended these findings by comparing cortical reorganisation following repeated skill and strength training sessions, and showed that there was a significant increase in MEP excitability with skill but not strength training (Jensen et al., 2005). Therefore, the use of a training task involving a precision grip (such as a grooved pegboard task), which more effectively engages CM cells, may have been more sensitive to detect time of day fluctuations in neuroplasticity induction following MT.

4.4.2. What is the functional relevance of cortical excitability changes induced following a motor training task?

It is now well established that motor training induces changes at the cortical level. For example, a repetitive sequential finger training task, which improves motor performance (evidenced by reduced errors) results in an expansion of motor cortical maps of the practicing muscles (Pascual-Leone et al., 1995). Using a repetitive ballistic pinch training task, Muellbacher et al. (2001) also demonstrated an improvement in motor performance following the training task, and showed that the improvement in performance was associated with changes in cortical excitability. Several other authors have demonstrated such a relationship (Pascual-Leone et al., 1995; Ziemann et al., 2001; Garry et al., 2004). Since training-related changes in cortical excitability appear related to changes in motor

performance, several studies have investigated whether an experimentally-induced increase in cortical excitability will also improve motor performance. Indeed, some recent studies have demonstrated a change in motor performance of the contralateral (Jancke et al., 2004), and ipsilateral hands (Kobayashi et al., 2004; Dafotakis et al., 2008) following rTMS. However, there have also been reports that an increase in cortical excitability does not produce a change in motor performance (Muellbacher et al., 2000; Rossi et al., 2000). It is also still unclear if there is a direct causal link between changes in cortical excitability and motor performance. When the time course of changes in these two variables was assessed following motor training, Muellbacher et al. (2001) reported that whilst performance improvements remained 30 days after training, motor cortical excitability had returned to baseline. One possible explanation may be that the neuroplastic change induced in the motor cortex, as evidenced by an increase in cortical excitability, may represent an early stage of motor learning (Muellbacher et al., 2002), and that consolidation of that memory occurs in another area of the cortex. It appears, therefore, that changes in cortical excitability may induce changes in motor performance, but these changes are subtle in neurologically normal subjects.

The inability to reliably demonstrate changes in motor performance following modulation of motor cortical excitability in neurologically normal subjects may be because a normally functioning motor system can adapt to large changes in cortical excitability to maintain performance (Iyer et al., 2003). If, however, the motor system is damaged, such as in stroke or Parkinson's Disease, cortical excitability and motor performance are abnormal. For these conditions in which the motor system is compromised, experimentally induced changes in cortical excitability may manifest as functional gains more readily. There is

some encouraging early experimental evidence to support this theory. Several groups have now demonstrated that cortical stimulation combined with motor training improves functional gains in chronic stroke patients more than rehabilitative training alone (Hummel et al., 2005; Kim et al., 2006). Similar findings have recently been demonstrated in sub-acute stroke patients with afferent stimulation (McDonnell et al., 2007a). Similar improvements in motor performance have also been demonstrated in Parkinson's Disease patients, with an improvement in finger tapping speed in the stimulated hand following rTMS of the cerebellum (Sommer et al., 2002). No improvements in finger tapping speed were reported in the unstimulated hand or in control subjects. It may be that subtle time of day differences in motor performance exist following a motor training task, but in a normally functioning motor system, these changes are essentially undetectable. Future studies should endeavour to investigate whether performance improvements in a damaged motor system are influenced by time of day.

One curious finding of the present study is that the stimulus intensity used to evoke the pre-MT MEPs was significantly different between morning and evening experiments. This finding is not supported by previous studies investigating time of day changes in cortical excitability (Strutton et al., 2003; Sale et al., 2007; Sale et al., 2008). Since the criterion for determining the test stimulus intensity was broad (evoking a test MEP of between 0.5-1.0 mV) and the absolute difference in stimulus intensity was so small (~3% MSO), it appears unlikely that this finding indicates a time of day modulation of resting cortical excitability.

4.4.3. Relationship between salivary cortisol levels, motor performance and cortical excitability changes following MT

I assessed salivary cortisol concentration prior to and following MT in both morning and evening sessions because experimentally elevated circulating cortisol levels have been shown to inhibit neuroplasticity induction in human M1 (Sale et al., 2008). In addition, the morning peak in cortisol levels is associated with reduced neuroplasticity induction with PAS (Sale et al., 2008). In the present study, as expected, salivary cortisol levels were significantly higher in the morning compared to the evening experiments (Figure 4.5). However, linear regression analysis revealed no significant relationship between the (log of) average salivary cortisol concentration and the change in motor performance (Figure 4.6A) nor cortical excitability (Figure 4.6B) following MT. This finding indicates that changes in circulating cortisol concentration in this range do not modulate motor performance and cortical excitability changes following MT. One reason for this may be that the morning rise in cortisol concentration was not high enough to inhibit improvements in motor performance (and cortical excitability) following MT. In retrospect this is perhaps not an unexpected finding. Although I have previously demonstrated time of day modulation of neuroplasticity induction, there was no significant correlation between salivary cortisol levels and the amount of neuroplasticity induced with PAS (Sale et al., 2008). A significant relationship was only reported when cortisol levels were artificially elevated with oral hydrocortisone administration (Sale et al., 2008). Further studies which artificially elevate cortisol levels are therefore indicated to investigate whether elevated circulating cortisol levels (eg as induced physiologically by stress) modulate motor performance and neuroplasticity changes induced following motor training.

Another possible explanation for the lack of association between salivary cortisol concentration and the outcome measures assessed following MT is that other (non-measured) neuromodulators which are also under circadian control may have influenced performance improvements following the MT task. These include dopamine (Castaneda et al., 2004) and melatonin (Collins and Davies, 1997; El-Sherif et al., 2003), both of which influence LTP induction (Collins and Davies, 1997; Kusuki et al., 1997; El-Sherif et al., 2003).

In conclusion, a repetitive ballistic MT task results in an improvement in motor performance and increased motor cortical excitability that outlasts the training period. However, these changes are not influenced by the time of day, nor physiological variation in salivary cortisol concentration between experiments. These findings do not support the view that the motor performance improvements following MT are a functional correlate for the neuroplastic changes induced with PAS. The discrepancy may be because the ballistic MT task does not sufficiently engage M1, or that a normally functioning motor system is able to adapt to subtle changes in cortical excitability and maintain optimal motor performance.

5. Summary and concluding remarks

Over the last few years there has been much interest in the use of experimental paradigms utilising electromagnetic stimulation of neurons to induce neuroplastic change in human cortex. These changes are manifest as an increase (or decrease) in cortical excitability, with the ultimate aim of improving treatment outcomes for various neurological disorders. Several studies involving patients with various neurological conditions such as stroke have already demonstrated that an experimentally-induced increase in cortical excitability can lead to functional improvements. However these studies have not demonstrated consistent or substantial functional improvements. One reason attributed to the poor outcome is that the extent of neuroplastic change induced in human subjects by the stimulation paradigms is variable. The studies described within this thesis have investigated factors that influence the effectiveness and reproducibility of neuroplasticity induction in human primary motor cortex. A better understanding of these factors can be expected to ultimately lead to an improvement in treatment outcomes for neurological conditions which rely on neuroplasticity for recovery of function.

5.1. Effectiveness and reproducibility of paired associative stimulation

Several techniques have recently been developed which induce neuroplastic change in human motor cortex. For the experiments I undertook in Chapters 2 and 3, I chose to use a protocol known as paired associative stimulation (PAS) (Stefan et al., 2000). A number of variants of the initial protocol have been developed, yet no study had compared the effectiveness and reproducibility of any of these variants.

The first series of experiments I undertook, described in Chapter 2, compared the effectiveness and reproducibility of two previously used PAS protocols (a "short" and "long" PAS protocol). The study also examined whether the normal range of variation in several neurophysiological and experimental parameters may be useful predictors of the effectiveness of the two PAS protocols. The neurophysiological variables that were assessed included resting and active motor thresholds, and the effectiveness of intracortical inhibitory and facilitatory networks. In addition, time-of-day differences in PAS-effectiveness were also assessed.

Subjects received either the "short" or "long" PAS protocol on three separate occasions, separated by at least one week. The neuroplastic changes induced with the "short" protocol were greater than those induced with the "long" protocol. However, despite the "short" protocol being more effective, it was also less reproducible than the "long" protocol across the three experimental sessions.

There was no clear evidence that any of the neurophysiological variables that were assessed could be used to predict an individual's response to either PAS protocol. This was a somewhat surprising result, as previous studies have demonstrated that a reduction in intracortical inhibition (either with long-term musical training (Rosenkranz et al., 2007) or a reduction in sensory feedback (Ziemann et al., 2001)) can facilitate plastic changes in M1. However, a novel finding from this study was that time of day was an important factor in mediating the effectiveness and reproducibility of both PAS protocols. Neuroplasticity induction was more effective, and the induced responses more reproducible, if the experiments were performed in the afternoon (~1 pm) compared to the

morning (~8 am). This was the first study to demonstrate that the induction of neuroplasticity in human motor cortex was influenced by time of day.

5.2. Time of day and cortisol

The time of day modulation of neuroplasticity induction in M1 reported in Chapter 2 warranted further investigation. Since different subjects participated in the morning and afternoon experiments, differences in response to PAS *between* subjects may have contributed to the time of day finding. Therefore, the next experiment, which is described in Chapter 3, aimed to directly test whether neuroplasticity induction in human motor cortex is modulated by time of day. Subjects were tested twice, once in the morning (8 am) and once in the evening (8 pm) on separate occasions, separated by at least one week. In addition to quantifying neuroplastic change in the morning and evening experiments, the time of day variation in $GABA_A$-mediated intracortical inhibition, $GABA_B$-mediated cortical silent period duration and salivary cortisol concentration were also assessed. GABA and cortisol have been shown previously to influence neuroplasticity, and since they are under circadian control, it was hypothesised that time of day changes in the levels of these compounds might influence the effectiveness of PAS-induced neuroplasticity.

These experiments confirmed that neuroplasticity induction in human motor cortex is modulated by time of day, with enhanced neuroplasticity induction in the evening compared with the morning. This effect was not due to changes in $GABA_A$-mediated intracortical inhibition since the amount of short-latency intracortical inhibition was not modulated by time of day. The duration of the $GABA_B$-mediated cortical silent period was longer in the morning than the evening, and longer after PAS, which suggests that cortical inhibitory networks are modulated by time of day. However, since a direct relationship

between the duration of the cortical silent period and the amount of neuroplasticity induced with PAS was not demonstrated, I was unable to demonstrate a direct effect of $GABA_B$ inhibitory tone on PAS effectiveness.

Salivary cortisol levels were higher in the morning compared to the evening, however there was no significant correlation between endogenous salivary cortisol concentration and the magnitude of neuroplasticity induced following PAS. The large variability in PAS effectiveness, and the lack of a correlation between salivary cortisol concentration and the magnitude of neuroplasticity induced following PAS, is likely due to circadian variation in other (non-assessed) neuromodulators.

This study demonstrated conclusively that time of day influences neuroplasticity induction in human motor cortex. However, since there was no significant correlation between the levels of neuromodulators that were measured and the magnitude of neuroplasticity induced, a mechanistic explanation of the effect remained to be determined.

5.3. Cortisol administration

A further set of experiments was therefore undertaken to more directly investigate the role of circulating cortisol levels in modulating neuroplasticity induction. These experiments are also described in Chapter 3. Subjects were orally administered hydrocortisone or a placebo in the evening (when circulating cortisol levels are low) on two separate occasions in a double-blind study. Hydrocortisone administration significantly elevated circulating cortisol levels, and inhibited neuroplasticity induction following PAS. Elevated cortisol levels also increased the duration of the cortical silent period. Importantly, there was a significant negative relationship between salivary cortisol concentration and the magnitude

of neuroplasticity induced in M1. High levels of salivary cortisol were associated with low amounts of neuroplasticity induced in M1. A similar negative relationship also existed between cortical silent period duration and the magnitude of neuroplasticity induced in M1. These results provide strong evidence that elevated cortisol levels inhibit neuroplasticity induction in human motor cortex.

The experiments described in Chapter 3 demonstrate that neuroplasticity induction in human M1 is modulated by time of day, and that circulating cortisol levels are at least in part responsible for this effect. Due to time constraints it was not possible to investigate in detail the response to PAS at several different times of day, and thus conclusively establish that PAS-induced neuroplasticity is under circadian control. Future studies are therefore required to establish whether there is an optimal time of day to induce neuroplastic change in M1.

Clearly, however, other factors also influence neuroplasticity induction in M1, as evidenced by the relatively weak correlation between salivary cortisol concentration and neuroplasticity induction (Chapter 3). Further studies should investigate whether other neuromodulators also influence PAS-induced neuroplasticity. These include melatonin and serotonin, both of which are under circadian control, and have been shown to influence neuroplastic changes in various animal models. In addition, there is increasing evidence that genetic factors (in particular BDNF) play a pivotal role in determining an individual's response to neuroplasticity induction paradigms (Kleim et al., 2006; Cheeran et al., 2008). It would appear then, that even if an optimal paradigm (delivered at an optimal time of day) is established to induce neuroplastic change in M1, a proportion of individuals will be genetically predisposed to be unresponsive to artificial induction of neuroplasticity in M1.

Although this thesis has contributed to our understanding of factors that influence the effectiveness of neuroplasticity induction in human M1, it has not investigated the factors influencing the duration of these induced effects. The field is therefore, unfortunately, still some way off from reproducing the enduring changes in synaptic efficacy demonstrated in animal models. Future studies which improve understanding of the mechanisms contributing to the induction of sustained neuroplastic changes in human cortex would appear necessary to maximise potential functional improvements in subjects with neurological conditions reliant on neuroplasticity for recovery of function.

5.4. Is there a functional correlate to changes in cortical excitability in M1?

The final set of experiments, which are described in Chapter 4, were designed to investigate whether time of day modulates changes in motor performance and cortical excitability following a motor training task. It has been previously demonstrated that a motor training task improves motor performance and increases M1 cortical excitability, and the circuits that are modulated by the training task are also modulated by PAS (Ziemann et al., 2004). Therefore, it would seem likely that any factors that modulate PAS-induced increases in cortical excitability will also influence a motor-training-induced increase in motor performance and cortical excitability. In order to investigate potential time of day modulation of training-related changes in motor performance and cortical excitability, subjects undertook a motor training task on two separate occasions (8 am and 8 pm). The task involved repeated maximal left thumb abduction movements every 2 seconds for 30 minutes. This training task induces robust improvements in performance and cortical excitability that are likely due to neuroplastic changes in M1 (Muellbacher et al., 2001).

There was a significant improvement in motor performance, evidenced by an increase in maximum thumb acceleration, and a significant increase in cortical excitability following the training task. However, the training-related changes were not modulated by time of day. It is not entirely clear why time of day did not modulate motor performance and cortical excitability changes following the motor training task. It may be that the training task or outcome measures used were not sufficiently sensitive or complex enough to allow detection of a subtle time of day effect. Another possible explanation for this result is that the motor system, when functioning normally, is able to compensate for large changes in cortical excitability to maintain performance. However, this may not be the case when the motor system is compromised, such as following a stroke. When the stroke affects the motor region, cortical excitability in the affected hemisphere and motor performance in the contralateral limb is reduced. By enhancing cortical excitability with electromagnetic stimulation, functional improvements in stroke patients have been demonstrated (Hummel et al., 2005; Kim et al., 2006). Thus it appears that when attempts are made to normalise compromised cortical excitability, there will also likely be a normalisation in motor performance. It would therefore be interesting to see whether time of day changes in motor performance are present in stroke patients, which would have obvious clinical and therapeutic implications.

5.5. Concluding remarks

This thesis has demonstrated that changes in M1 cortical excitability induced with PAS are modulated by time of day, with larger and more reliable increases in cortical excitability induced in the evening compared with the morning. This effect is, at least to some extent, mediated by circadian variation in circulating cortisol levels. Time of day changes in

motor performance or cortical excitability following a motor training task were not demonstrated, however, despite PAS and motor training interacting with the same cortical circuits. Associative stimulation paradigms have been used in the past as an adjunct to physiotherapy and motor training to improve rehabilitation outcomes for stroke survivors. Therefore, the findings of the present study have potential clinical implications, and indicate that the most effective and reliable increases in cortical excitability, and presumably motor performance, can be achieved if the stimulation paradigm used to induce neuroplasticity (and associated motor training) is performed in the evening compared to the morning.

6. Appendices

6.1. Appendix I: Transcranial Magnetic Stimulation[†] (TMS) Adult Safety Screen

Name:
Date:
Age:

Please answer the following:

Have you ever:

Had an adverse reaction to TMS?	❍Yes	❍No
Had a seizure?	❍Yes	❍No
Had an electroencephalogram (EEG)?	❍Yes	❍No
Had a stroke?	❍Yes	❍No
Had a serious head injury (include neurosurgery)?	❍Yes	❍No
Had any other brain-related condition?	❍Yes	❍No
Had any illness that caused brain injury?	❍Yes	❍No
Do you have any metal in your head (outside the mouth) such as shrapnel, surgical clips, or fragments from welding or metalwork?	❍Yes	❍No
Do you have any implanted devices such as cardiac pacemakers, cochlear implant, medical pumps, or intracardiac lines?	❍Yes	❍No
Do you suffer from frequent or severe headaches?	❍Yes	❍No
Are you taking any medications?	❍Yes	❍No
Are you pregnant, or is it possible that you may be pregnant?	❍Yes	❍No
Does anyone in your family have epilepsy?	❍Yes	❍No
Do you need further explanation of TMS and its associated risks?	❍Yes	❍No

Participant signature:

Experimenter name: **Signature:**

*If you answered **yes** to any of the above, please provide details (use reverse if necessary):*

_____

_____

[†] For use with single-pulse TMS, paired-pulse TMS, or repetitive TMS.

6.2. Appendix II: Publications arising from this thesis

Sale MV, Ridding MC, Nordstrom MA. Cortisol inhibits neuroplasticity induction in human motor cortex. J Neurosci 2008;28:8285-8293.

Sale MV, Ridding MC, Nordstrom MA. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 2007;181:615-626.

6.3. Appendix III: Presentations and abstracts arising from this thesis

Sale MV, Ridding MC, Nordstrom MA (2008) Cortisol inhibits neuroplasticity induction in human motor cortex. *Australian Society for Medical Research annual meeting 2008 (SA),* pp.26

Sale MV, Ridding MC, Nordstrom MA (2007) Circadian modulation of associative plasticity and intracortical inhibition in human motor cortex. *Program no 82.4 2007 Abstract Viewer/Itinerary Planner. Washington DC. Society for Neuroscience 2007. Online*

Sale MV, Ridding MC, Nordstrom MA (2006) Variability of two paired associative stimulation (PAS) protocols used to induce neuroplastic changes in human motor cortex. *Proceedings of the Australasian Winter Conference on Brain Research* 24:10.5

7. Bibliography

Abejon D, Reig E. Is pulsed radiofrequency a neuromodulation technique? Neuromodulation 2003;6:1-3.

Abrahamson EE, Moore RY. Suprachiasmatic nucleus in the mouse: retinal innervation, intrinsic organization and efferent projections. Brain Res 2001;916:172-191.

Andersen P, Sundberg SH, Sveen O, Wigstrom H. Specific long-lasting potentiation of synaptic transmission in hippocampal slices. Nature 1977;266:736-737.

Anderson B, Mishory A, Nahas Z, Borckardt JJ, Yamanaka K, Rastogi K, George MS. Tolerability and safety of high daily doses of repetitive transcranial magnetic stimulation in healthy young men. J Ect 2006;22:49-53.

Aroniadou VA, Keller A. Mechanisms of LTP induction in rat motor cortex in vitro. Cereb Cortex 1995;5:353-362.

Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet 1985;1:1106-1107.

Bear MF, Abraham WC. Long-term depression in hippocampus. Annu Rev Neurosci 1996;19:437-462.

Beck S, Taube W, Gruber M, Amtage F, Gollhofer A, Schubert M. Task-specific changes in motor evoked potentials of lower limb muscles after different training interventions. Brain Res 2007;1179:51-60.

Begliuomini S, Lenzi E, Ninni F, Casarosa E, Merlini S, Pluchino N, Valentino V, Luisi S, Luisi M, Genazzani AR. Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. J Endocrinol 2008;197:429-435.

Berardelli A, Inghilleri M, Rothwell JC, Romeo S, Curra A, Gilio F, Modugno N, Manfredi M. Facilitation of muscle evoked responses after repetitive cortical stimulation in man. Exp Brain Res 1998;122:79-84.

Bergmann TO, Molle M, Marshall L, Kaya-Yildiz L, Born J, Roman Siebner H. A local signature of LTP- and LTD-like plasticity in human NREM sleep. Eur J Neurosci 2008;27:2241-2249.

Bernhard CG, Bohm E, Petersen I. New investigations on the pyramidal system in Macaca mulatta. Experientia 1953;9:111-112.

Bi G, Poo M. Synaptic modification by correlated activity: Hebb's postulate revisited. Annu Rev Neurosci 2001;24:139-166.

Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31-39.

Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973;232:331-356.

Brasil-Neto JP, Cohen LG, Pascual-Leone A, Jabir FK, Wall RT, Hallett M. Rapid reversible modulation of human motor outputs after transient deafferentation of the forearm: a study with transcranial magnetic stimulation. Neurology 1992a;42:1302-1306.

Brasil-Neto JP, McShane LM, Fuhr P, Hallett M, Cohen LG. Topographic mapping of the human motor cortex with magnetic stimulation: factors affecting accuracy and reproducibility. Electroencephalogr Clin Neurophysiol 1992b;85:9-16.

Bridgers SL. The safety of transcranial magnetic stimulation reconsidered: evidence regarding cognitive and other cerebral effects. Electroencephalogr Clin Neurophysiol Suppl 1991;43:170-179.

Brinkman J, Kuypers HG. Cerebral control of contralateral and ipsilateral arm, hand and finger movements in the split-brain rhesus monkey. Brain 1973;96:653-674.

Brodal A (1969) Neurological Anatomy in Relation to Clinical Medicine. Oxford University Press, New York

Brodmann K (1909) Vergleichende Lokalisationslehre der Grosshirnrinde ihren Prinzipien dargestellt auf Grund des Zellenbaues. Barth, Leipzig

Buijs RM, van Eden CG, Goncharuk VD, Kalsbeek A. The biological clock tunes the organs of the body: timing by hormones and the autonomic nervous system. J Endocrinol 2003;177:17-26.

Buonomano DV, Merzenich MM. Cortical plasticity: from synapses to maps. Annu Rev Neurosci 1998;21:149-186.

Burke D, Hicks R, Gandevia SC, Stephen J, Woodforth I, Crawford M. Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. J Physiol 1993;470:383-393.

Burke D, Hicks R, Stephen J, Woodforth I, Crawford M. Trial-to-trial variability of corticospinal volleys in human subjects. Electroencephalogr Clin Neurophysiol 1995;97:231-237.

Butefisch CM, Davis BC, Wise SP, Sawaki L, Kopylev L, Classen J, Cohen LG. Mechanisms of use-dependent plasticity in the human motor cortex. Proc Natl Acad Sci U S A 2000;97:3661-3665.

Buzsaki G, Draguhn A. Neuronal oscillations in cortical networks. Science 2004;304:1926-1929.

Card JP, Moore RY. The suprachiasmatic nucleus of the golden hamster: immunohistochemical analysis of cell and fiber distribution. Neuroscience 1984;13:415-431.

Carroll TJ, Riek S, Carson RG. Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. J Neurosci Methods 2001;112:193-202.

Castaneda TR, de Prado BM, Prieto D, Mora F. Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J Pineal Res 2004;36:177-185.

Charlton CS, Ridding MC, Thompson PD, Miles TS. Prolonged peripheral nerve stimulation induces persistent changes in excitability of human motor cortex. J Neurol Sci 2003;208:79-85.

Chaudhury D, Wang LM, Colwell CS. Circadian regulation of hippocampal long-term potentiation. J Biol Rhythms 2005;20:225-236.

Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC. A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. J Physiol 2008;586:5717-5725.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, Cohen LG. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997a;48:1398-1403.

Chen R, Cohen LG, Hallett M. Nervous system reorganization following injury. Neuroscience 2002;111:761-773.

Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res 1999;128:539-542.

Chen R, Samii A, Canos M, Wassermann EM, Hallett M. Effects of phenytoin on cortical excitability in humans. Neurology 1997b;49:881-883.

Chen R, Tam A, Butefisch C, Corwell B, Ziemann U, Rothwell JC, Cohen LG. Intracortical inhibition and facilitation in different representations of the human motor cortex. J Neurophysiol 1998;80:2870-2881.

Classen J, Liepert J, Wise SP, Hallett M, Cohen LG. Rapid plasticity of human cortical movement representation induced by practice. J Neurophysiol 1998;79:1117-1123.

Cohen LG, Bandinelli S, Findley TW, Hallett M. Motor reorganization after upper limb amputation in man. A study with focal magnetic stimulation. Brain 1991a;114 (Pt 1B):615-627.

Cohen LG, Bandinelli S, Topka HR, Fuhr P, Roth BJ, Hallett M. Topographic maps of human motor cortex in normal and pathological conditions: mirror movements, amputations and spinal cord injuries. Electroencephalogr Clin Neurophysiol Suppl 1991b;43:36-50.

Cohen LG, Hallett M. Cortical stimulation does not cause short-term changes in the electroencephalogram. Ann Neurol 1987;21:512-513.

Collins DR, Davies SN. Melatonin blocks the induction of long-term potentiation in an N-methyl-D-aspartate independent manner. Brain Res 1997;767:162-165.

Cotman CW, Engesser-Cesar C. Exercise enhances and protects brain function. Exerc Sport Sci Rev 2002;30:75-79.

Cragg BG. The density of synapses and neurons in normal, mentally defective ageing human brains. Brain 1975;98:81-90.

Czeisler CA. The effect of light on the human circadian pacemaker. Ciba Found Symp 1995;183:254-290; discussion 290-302.

Czeisler CA, Klerman EB. Circadian and sleep-dependent regulation of hormone release in humans. Recent Prog Horm Res 1999;54:97-130; discussion 130-132.

Dafotakis M, Grefkes C, Wang L, Fink GR, Nowak DA. The effects of 1 Hz rTMS over the hand area of M1 on movement kinematics of the ipsilateral hand. J Neural Transm 2008;115:1269-1274.

Darling WG, Wolf SL, Butler AJ. Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. Exp Brain Res 2006;174:376-385.

Datta AK, Harrison LM, Stephens JA. Task-dependent changes in the size of response to magnetic brain stimulation in human first dorsal interosseous muscle. J Physiol 1989;418:13-23.

Davies CH, Starkey SJ, Pozza MF, Collingridge GL. GABA autoreceptors regulate the induction of LTP. Nature 1991;349:609-611.

Davis CJ, Harding JW, Wright JW. REM sleep deprivation-induced deficits in the latency-to-peak induction and maintenance of long-term potentiation within the CA1 region of the hippocampus. Brain Res 2003;973:293-297.

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD. Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol 1989;412:449-473.

Day BL, Thompson PD, Dick JP, Nakashima K, Marsden CD. Different sites of action of electrical and magnetic stimulation of the human brain. Neurosci Lett 1987;75:101-106.

de Quervain DJ, Roozendaal B, Nitsch RM, McGaugh JL, Hock C. Acute cortisone administration impairs retrieval of long-term declarative memory in humans. Nat Neurosci 2000;3:313-314.

Devanne H, Lavoie BA, Capaday C. Input-output properties and gain changes in the human corticospinal pathway. Exp Brain Res 1997;114:329-338.

Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, Rothwell JC. Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. Clin Neurophysiol 2000;111:794-799.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 1998a;508 (Pt 2):625-633.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC. Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. Exp Brain Res 1998b;119:265-268.

Donoghue JP. Plasticity of adult sensorimotor representations. Curr Opin Neurobiol 1995;5:749-754.

Donoghue JP, Hess G, Sanes JN (1996) Substrates and mechanisms for learning in the motor cortex. In: Bloedel JR, Ebner TJ, Wise SP (eds) Acquisition and mechanisms for learning in the motor cortex. MIT Press, Cambridge (MA), pp 363-386

Donoghue JP, Sanes JN. Motor areas of the cerebral cortex. J Clin Neurophysiol 1994;11:382-396.

Dubrovsky B, Williams D, Kraulis I. Effects of corticosterone and 5 alpha-dihydrocorticosterone on brain excitability in the rat. J Neurosci Res 1985;14:117-128.

Dubrovsky BO, Liquornik MS, Noble P, Gijsbers K. Effects of 5 alpha-dihydrocorticosterone on evoked responses and long-term potentiation. Brain Res Bull 1987;19:635-638.

Dudek SM, Bear MF. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci U S A 1992;89:4363-4367.

Eccles JC. The central action of antidromic impulses in motor nerve fibres. Pflugers Arch 1955;260:385-415.

Eckel-Mahan KL, Phan T, Han S, Wang H, Chan GC, Scheiner ZS, Storm DR. Circadian oscillation of hippocampal MAPK activity and cAMP: implications for memory persistence. Nat Neurosci 2008;

Edwards B, Waterhouse J, Reilly T. The effects of circadian rhythmicity and time-awake on a simple motor task. Chronobiol Int 2007;24:1109-1124.

Edwards S, Clow A, Evans P, Hucklebridge F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. Life Sci 2001;68:2093-2103.

Ellaway PH, Davey NJ, Maskill DW, Rawlinson SR, Lewis HS, Anissimova NP. Variability in the amplitude of skeletal muscle responses to magnetic stimulation of the motor cortex in man. Electroencephalogr Clin Neurophysiol 1998;109:104-113.

El-Sherif Y, Tesoriero J, Hogan MV, Wieraszko A. Melatonin regulates neuronal plasticity in the hippocampus. J Neurosci Res 2003;72:454-460.

Finnerty GT, Roberts LS, Connors BW. Sensory experience modifies the short-term dynamics of neocortical synapses. Nature 1999;400:367-371.

Flament D, Goldsmith P, Buckley CJ, Lemon RN. Task dependence of responses in first dorsal interosseous muscle to magnetic brain stimulation in man. J Physiol 1993;464:361-378.

Florian J, Muller-Dahlhaus M, Liu Y, Ziemann U. Inhibitory circuits and the nature of their interactions in the human motor cortex a pharmacological TMS study. J Physiol 2008;586:495-514.

Francois-Bellan AM, Segu L, Hery M. Regulation by estradiol of GABAA and GABAB binding sites in the diencephalon of the rat: an autoradiographic study. Brain Res 1989;503:144-147.

Fratello F, Veniero D, Curcio G, Ferrara M, Marzano C, Moroni F, Pellicciari MC, Bertini M, Rossini PM, De Gennaro L. Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. Clin Neurophysiol 2006;117:2667-2674.

Fuhr P, Agostino R, Hallett M. Spinal motor neuron excitability during the silent period after cortical stimulation. Electroencephalogr Clin Neurophysiol 1991;81:257-262.

Funase K, Miles TS, Gooden BR. Trial-to-trial fluctuations in H-reflexes and motor evoked potentials in human wrist flexor. Neurosci Lett 1999;271:25-28.

Garry MI, Kamen G, Nordstrom MA. Hemispheric differences in the relationship between corticomotor excitability changes following a fine-motor task and motor learning. J Neurophysiol 2004;91:1570-1578.

Gentner R, Wankerl K, Reinsberger C, Zeller D, Classen J. Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. Cereb Cortex 2008;18:2046-2053.

Georgopoulos AP, Schwartz AB, Kettner RE. Neuronal population coding of movement direction. Science 1986;233:1416-1419.

Gompf HS, Allen CN. GABAergic synapses of the suprachiasmatic nucleus exhibit a diurnal rhythm of short-term synaptic plasticity. Eur J Neurosci 2004;19:2791-2798.

Grillon C, Smith K, Haynos A, Nieman LK. Deficits in hippocampus-mediated Pavlovian conditioning in endogenous hypercortisolism. Biol Psychiatry 2004;56:837-843.

Hallett M. Transcranial magnetic stimulation and the human brain. Nature 2000;406:147-150.

Hallett M. Plasticity of the human motor cortex and recovery from stroke. Brain Res Brain Res Rev 2001;36:169-174.

Hallett M. Pathophysiology of writer's cramp. Hum Mov Sci 2006;25:454-463.

Hamada M, Terao Y, Hanajima R, Shirota Y, Nakatani-Enomoto S, Furubayashi T, Matsumoto H, Ugawa Y. Bidirectional long-term motor cortical plasticity and metaplasticity induced by quadripulse transcranial magnetic stimulation. J Physiol 2008;586:3927-3947.

Hanajima R, Furubayashi T, Iwata NK, Shiio Y, Okabe S, Kanazawa I, Ugawa Y. Further evidence to support different mechanisms underlying intracortical inhibition of the motor cortex. Exp Brain Res 2003;151:427-434.

Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I. Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. J Physiol 1998;509 (Pt 2):607-618.

Hazeltine E, Grafton ST, Ivry R. Attention and stimulus characteristics determine the locus of motor-sequence encoding. A PET study. Brain 1997;120 (Pt 1):123-140.

Hebb DO (1949) The Organization of Behaviour. John Wiley & Sons, New York

Heffner R, Masterton B. Variation in form of the pyramidal tract and its relationship to digital dexterity. Brain Behav Evol 1975;12:161-200.

Hendry SH, Jones EG. Sizes and distributions of intrinsic neurons incorporating tritiated GABA in monkey sensory-motor cortex. J Neurosci 1981;1:390-408.

Hermes ML, Coderre EM, Buijs RM, Renaud LP. GABA and glutamate mediate rapid neurotransmission from suprachiasmatic nucleus to hypothalamic paraventricular nucleus in rat. J Physiol 1996;496 (Pt 3):749-757.

Herzog ED. Neurons and networks in daily rhythms. Nat Rev Neurosci 2007;8:790-802.

Hess G, Donoghue JP. Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J Neurophysiol 1994;71:2543-2547.

Ho KH, Nithi K, Mills KR. Covariation between human intrinsic hand muscles of the silent periods and compound muscle action potentials evoked by magnetic brain stimulation: evidence for common inhibitory connections. Exp Brain Res 1998;122:433-440.

Hsu FC, Garside MJ, Massey AE, McAllister-Williams RH. Effects of a single dose of cortisol on the neural correlates of episodic memory and error processing in healthy volunteers. Psychopharmacology (Berl) 2003;167:431-442.

Huang YZ, Chen RS, Rothwell JC, Wen HY. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol 2007;118:1028-1032.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. Theta burst stimulation of the human motor cortex. Neuron 2005;45:201-206.

Huerta PT, Lisman JE. Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. Neuron 1995;15:1053-1063.

Hummel F, Celnik P, Giraux P, Floel A, Wu WH, Gerloff C, Cohen LG. Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke. Brain 2005;128:490-499.

Ilic TV, Jung P, Ziemann U. Subtle hemispheric asymmetry of motor cortical inhibitory tone. Clin Neurophysiol 2004;115:330-340.

Ilic TV, Meintzschel F, Cleff U, Ruge D, Kessler KR, Ziemann U. Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. J Physiol 2002;545:153-167.

Inghilleri M, Berardelli A, Cruccu G, Manfredi M. Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. J Physiol 1993;466:521-534.

Inghilleri M, Conte A, Curra A, Frasca V, Lorenzano C, Berardelli A. Ovarian hormones and cortical excitability. An rTMS study in humans. Clin Neurophysiol 2004;115:1063-1068.

Inouye ST, Kawamura H. Persistence of circadian rhythmicity in a mammalian hypothalamic "island" containing the suprachiasmatic nucleus. Proc Natl Acad Sci U S A 1979;76:5962-5966.

Itri J, Michel S, Waschek JA, Colwell CS. Circadian rhythm in inhibitory synaptic transmission in the mouse suprachiasmatic nucleus. J Neurophysiol 2004;92:311-319.

Iyer MB, Schleper N, Wassermann EM. Priming stimulation enhances the depressant effect of low-frequency repetitive transcranial magnetic stimulation. J Neurosci 2003;23:10867-10872.

Jackson JH (1873) On the localization of movements in the cerebral hemisphere. In: West Riding Lunatic Asylum Reports

Jacobs KM, Donoghue JP. Reshaping the cortical motor map by unmasking latent intracortical connections. Science 1991;251:944-947.

Jancke L, Gaab N, Wustenberg T, Scheich H, Heinze HJ. Short-term functional plasticity in the human auditory cortex: an fMRI study. Brain Res Cogn Brain Res 2001;12:479-485.

Jancke L, Steinmetz H, Benilow S, Ziemann U. Slowing fastest finger movements of the dominant hand with low-frequency rTMS of the hand area of the primary motor cortex. Exp Brain Res 2004;155:196-203.

Jensen JL, Marstrand PC, Nielsen JB. Motor skill training and strength training are associated with different plastic changes in the central nervous system. J Appl Physiol 2005;99:1558-1568.

Johnston GA, Curtis DR, Beart PM, Game CJ, McCulloch RM, Twitchin B. Cis- and trans-4-aminocrotonic acid as GABA analogues of restricted conformation. J Neurochem 1975;24:157-160.

Jones EG (1981) Anatomy of cerebral cortex: columnar input-output organization. In: Schmitt FO, Worden FG, Adelman G, Dennis SG (eds) The organization of the cerebral cortex. MIT Press, Cambridge (MA)

Jones EG. GABAergic neurons and their role in cortical plasticity in primates. Cereb Cortex 1993;3:361-372.

Kaas JH. Plasticity of sensory and motor maps in adult mammals. Annu Rev Neurosci 1991;14:137-167.

Kaas JH, Merzenich MM, Killackey HP. The reorganization of somatosensory cortex following peripheral nerve damage in adult and developing mammals. Annu Rev Neurosci 1983;6:325-356.

Kaelin-Lang A, Luft AR, Sawaki L, Burstein AH, Sohn YH, Cohen LG. Modulation of human corticomotor excitability by somatosensory input. J Physiol 2002;540:623-633.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. Nature 1995;377:155-158.

Karni A, Meyer G, Rey-Hipolito C, Jezzard P, Adams MM, Turner R, Ungerleider LG. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proc Natl Acad Sci U S A 1998;95:861-868.

Keel JC, Smith MJ, Wassermann EM. A safety screening questionnaire for transcranial magnetic stimulation. Clin Neurophysiol 2001;112:720.

Kerr DI, Ong J. GABAB receptors. Pharmacol Ther 1995;67:187-246.

Kim YH, You SH, Ko MH, Park JW, Lee KH, Jang SH, Yoo WK, Hallett M. Repetitive transcranial magnetic stimulation-induced corticomotor excitability and associated motor skill acquisition in chronic stroke. Stroke 2006;37:1471-1476.

Kirkwood A, Rioult MC, Bear MF. Experience-dependent modification of synaptic plasticity in visual cortex. Nature 1996;381:526-528.

Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC. BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. Nat Neurosci 2006;9:735-737.

Kleim JA, Lussnig E, Schwarz ER, Comery TA, Greenough WT. Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. J Neurosci 1996;16:4529-4535.

Kobayashi M, Hutchinson S, Theoret H, Schlaug G, Pascual-Leone A. Repetitive TMS of the motor cortex improves ipsilateral sequential simple finger movements. Neurology 2004;62:91-98.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. Proc Natl Acad Sci U S A 1995;92:8856-8860.

Koski L, Schrader LM, Wu AD, Stern JM. Normative data on changes in transcranial magnetic stimulation measures over a ten hour period. Clin Neurophysiol 2005;116:2099-2109.

Krnjevic K, Schwartz S. The action of gamma-aminobutyric acid on cortical neurones. Exp Brain Res 1967;3:320-336.

Kujirai K, Kujirai T, Sinkjaer T, Rothwell JC. Associative plasticity in human motor cortex during voluntary muscle contraction. J Neurophysiol 2006a;96:1337-1346.

Kujirai K, Kujirai T, Sinkjaer T, Rothwell JC. Associative Plasticity In Human Motor Cortex Under Voluntary Muscle Contraction. J Neurophysiol 2006b;

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD. Corticocortical inhibition in human motor cortex. J Physiol 1993;471:501-519.

Kusuki T, Imahori Y, Ueda S, Inokuchi K. Dopaminergic modulation of LTP induction in the dentate gyrus of intact brain. Neuroreport 1997;8:2037-2040.

Lang N, Nitsche MA, Paulus W, Rothwell JC, Lemon RN. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. Exp Brain Res 2004;156:439-443.

Lefaucheur JP. Motor cortex dysfunction revealed by cortical excitability studies in Parkinson's disease: influence of antiparkinsonian treatment and cortical stimulation. Clin Neurophysiol 2005;116:244-253.

Lemon RN. The G. L. Brown Prize Lecture. Cortical control of the primate hand. Exp Physiol 1993;78:263-301.

Liebetanz D, Nitsche MA, Tergau F, Paulus W. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. Brain 2002;125:2238-2247.

Liepert J, Schwenkreis P, Tegenthoff M, Malin JP. The glutamate antagonist riluzole suppresses intracortical facilitation. J Neural Transm 1997;104:1207-1214.

Liepert J, Storch P, Fritsch A, Weiller C. Motor cortex disinhibition in acute stroke. Clin Neurophysiol 2000;111:671-676.

Liu C, Reppert SM. GABA synchronizes clock cells within the suprachiasmatic circadian clock. Neuron 2000;25:123-128.

Lund JS (1984) Spiny stellate neurons. In: Peters A, Jones EG (eds) Cerebral cortex: Cellular components of the cerebral cortex, vol 1. Plenum Press, New York, pp 255-308.

Maeda F, Gangitano M, Thall M, Pascual-Leone A. Inter- and intra-individual variability of paired-pulse curves with transcranial magnetic stimulation (TMS). Clin Neurophysiol 2002;113:376-382.

Maeda F, Keenan JP, Tormos JM, Topka H, Pascual-Leone A. Modulation of corticospinal excitability by repetitive transcranial magnetic stimulation. Clin Neurophysiol 2000;111:800-805.

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 1986;232:1004-1007.

Malinow R, Mainen ZF, Hayashi Y. LTP mechanisms: from silence to four-lane traffic. Curr Opin Neurobiol 2000;10:352-357.

Mariorenzi R, Zarola F, Caramia MD, Paradiso C, Rossini PM. Non-invasive evaluation of central motor tract excitability changes following peripheral nerve stimulation in healthy humans. Electroencephalogr Clin Neurophysiol 1991;81:90-101.

Matsumura M, Sawaguchi T, Kubota K. GABAergic inhibition of neuronal activity in the primate motor and premotor cortex during voluntary movement. J Neurophysiol 1992;68:692-702.

McDermott CM, LaHoste GJ, Chen C, Musto A, Bazan NG, Magee JC. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci 2003;23:9687-9695.

McDonnell MN, Hillier SL, Miles TS, Thompson PD, Ridding MC. Influence of Combined Afferent Stimulation and Task-Specific Training Following Stroke: A Pilot Randomized Controlled Trial. Neurorehabil Neural Repair 2007a;

McDonnell MN, Orekhov Y, Ziemann U. Suppression of LTP-like plasticity in human motor cortex by the GABA(B) receptor agonist baclofen. Exp Brain Res 2007b;180:181-186.

McDonnell MN, Ridding MC. Afferent stimulation facilitates performance on a novel motor task. Exp Brain Res 2006;170:109-115.

McIntyre IM, Norman TR, Burrows GD, Armstrong SM. Melatonin rhythm in human plasma and saliva. J Pineal Res 1987;4:177-183.

Merton PA, Morton HB. Stimulation of the cerebral cortex in the intact human subject. Nature 1980;285:227.

Merzenich MM, Kaas JH, Wall J, Nelson RJ, Sur M, Felleman D. Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. Neuroscience 1983a;8:33-55.

Merzenich MM, Kaas JH, Wall JT, Sur M, Nelson RJ, Felleman DJ. Progression of change following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys. Neuroscience 1983b;10:639-665.

Merzenich MM, Nelson RJ, Stryker MP, Cynader MS, Schoppmann A, Zook JM. Somatosensory cortical map changes following digit amputation in adult monkeys. J Comp Neurol 1984;224:591-605.

Meunier S. Modulation by corticospinal volleys of presynaptic inhibition to Ia afferents in man. J Physiol Paris 1999;93:387-394.

Meunier S, Pierrot-Deseilligny E. Gating of the afferent volley of the monosynaptic stretch reflex during movement in man. J Physiol 1989;419:753-763.

Meunier S, Russmann H, Simonetta-Moreau M, Hallett M. Changes in Spinal Excitability After PAS. J Neurophysiol 2007;97:3131-3135.

Miller LS, Lombardo TW, Fowler SC. Time of day effects on a human force discrimination task. Physiol Behav 1992;52:839-841.

Moore RY, Card JP. Visual pathways and the entrainment of circadian rhythms. Ann N Y Acad Sci 1985;453:123-133.

Moore RY, Eichler VB. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. Brain Res 1972;42:201-206.

Moore RY, Lenn NJ. A retinohypothalamic projection in the rat. J Comp Neurol 1972;146:1-14.

Morgante F, Espay AJ, Gunraj C, Lang AE, Chen R. Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias. Brain 2006;129:1059-1069.

Mountcastle VB (1978) An organizing principle for cerebral function. In: Edelman GM, Mountcastle VB (eds) The mindful brain. MIT Press, Cambridge (MA), pp 7-50.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. Exp Brain Res 2001;136:431-438.

Muellbacher W, Ziemann U, Boroojerdi B, Hallett M. Effects of low-frequency transcranial magnetic stimulation on motor excitability and basic motor behavior. Clin Neurophysiol 2000;111:1002-1007.

Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, Boroojerdi B, Poewe W, Hallett M. Early consolidation in human primary motor cortex. Nature 2002;415:640-644.

Muir RB, Lemon RN. Corticospinal neurons with a special role in precision grip. Brain Res 1983;261:312-316.

Muller JF, Orekhov Y, Liu Y, Ziemann U. Homeostatic plasticity in human motor cortex demonstrated by two consecutive sessions of paired associative stimulation. Eur J Neurosci 2007;25:3461-3468.

Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res 2008;187:467-475.

Munte TF, Altenmuller E, Jancke L. The musician's brain as a model of neuroplasticity. Nat Rev Neurosci 2002;3:473-478.

Murakami T, Sakuma K, Nomura T, Uemura Y, Hashimoto I, Nakashima K. Changes in somatosensory-evoked potentials and high-frequency oscillations after paired-associative stimulation. Exp Brain Res 2008;184:339-347.

Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. J Physiol 1997;498 (Pt 3):817-823.

Newcomer JW, Selke G, Melson AK, Hershey T, Craft S, Richards K, Alderson AL. Decreased memory performance in healthy humans induced by stress-level cortisol treatment. Arch Gen Psychiatry 1999;56:527-533.

Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527 Pt 3:633-639.

Nordstrom MA, Butler SL. Reduced intracortical inhibition and facilitation of corticospinal neurons in musicians. Exp Brain Res 2002;144:336-342.

Nudo RJ, Wise BM, SiFuentes F, Milliken GW. Neural substrates for the effects of rehabilitative training on motor recovery after ischemic infarct. Science 1996;272:1791-1794.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9:97-113.

Oliviero A, Profice P, Tonali PA, Pilato F, Saturno E, Dileone M, Ranieri F, Di Lazzaro V. Effects of aging on motor cortex excitability. Neurosci Res 2006;55:74-77.

Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 1995;74:1037-1045.

Pascual-Leone A, Valls-Sole J, Brasil-Neto JP, Cohen LG, Hallett M. Seizure induction and transcranial magnetic stimulation. Lancet 1992;339:997.

Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994;117 (Pt 4):847-858.

Patton HD, Amassian VE. Single- and multiple-unit analysis of cortical stage of pyramidal tract activation. J Neurophysiol 1954;17:345-363.

Pearce RA. Physiological evidence for two distinct GABAA responses in rat hippocampus. Neuron 1993;10:189-200.

Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studies by electrical stimulation. Brain 1937;60:389-443.

Penfield W, Welch K. The supplementary motor area of the cerebral cortex; a clinical and experimental study. AMA Arch Neurol Psychiatry 1951;66:289-317.

Pitcher JB, Ogston KM, Miles TS. Age and sex differences in human motor cortex input-output characteristics. J Physiol 2003;546:605-613.

Pons TP, Garraghty PE, Mishkin M. Lesion-induced plasticity in the second somatosensory cortex of adult macaques. Proc Natl Acad Sci U S A 1988;85:5279-5281.

Porter R, Lemon RN (1993) Corticospinal Function & Voluntary Movement. Clarendon Press, Oxford

Pyndt HS, Ridding MC. Modification of the human motor cortex by associative stimulation. Exp Brain Res 2004;159:123-128.

Quartarone A, Bagnato S, Rizzo V, Siebner HR, Dattola V, Scalfari A, Morgante F, Battaglia F, Romano M, Girlanda P. Abnormal associative plasticity of the human motor cortex in writer's cramp. Brain 2003;126:2586-2596.

Raghavan AV, Horowitz JM, Fuller CA. Diurnal modulation of long-term potentiation in the hamster hippocampal slice. Brain Res 1999;833:311-314.

Ranjit N, Young EA, Raghunathan TE, Kaplan GA. Modeling cortisol rhythms in a population-based study. Psychoneuroendocrinology 2005;30:615-624.

Recanzone GH, Schreiner CE, Merzenich MM. Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. J Neurosci 1993;13:87-103.

Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 2001;63:647-676.

Ridding MC, Brouwer B, Miles TS, Pitcher JB, Thompson PD. Changes in muscle responses to stimulation of the motor cortex induced by peripheral nerve stimulation in human subjects. Exp Brain Res 2000a;131:135-143.

Ridding MC, Brouwer B, Nordstrom MA. Reduced interhemispheric inhibition in musicians. Exp Brain Res 2000b;133:249-253.

Ridding MC, Flavel SC. Induction of plasticity in the dominant and non-dominant motor cortices of humans. Exp Brain Res 2006;171:551-557.

Ridding MC, Inzelberg R, Rothwell JC. Changes in excitability of motor cortical circuitry in patients with Parkinson's disease. Ann Neurol 1995a;37:181-188.

Ridding MC, McKay DR, Thompson PD, Miles TS. Changes in corticomotor representations induced by prolonged peripheral nerve stimulation in humans. Clin Neurophysiol 2001;112:1461-1469.

Ridding MC, Rothwell JC. Reorganisation in human motor cortex. Can J Physiol Pharmacol 1995;73:218-222.

Ridding MC, Rothwell JC. Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 2007;8:559-567.

Ridding MC, Sheean G, Rothwell JC, Inzelberg R, Kujirai T. Changes in the balance between motor cortical excitation and inhibition in focal, task specific dystonia. J Neurol Neurosurg Psychiatry 1995b;59:493-498.

Ridding MC, Taylor JL. Mechanisms of motor-evoked potential facilitation following prolonged dual peripheral and central stimulation in humans. J Physiol 2001;537:623-631.

Ridding MC, Taylor JL, Rothwell JC. The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 1995c;487 (Pt 2):541-548.

Ridding MC, Uy J. Changes in motor cortical excitability induced by paired associative stimulation. Clin Neurophysiol 2003;114:1437-1444.

Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. Science 2000;290:533-536.

Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP. Strengthening of horizontal cortical connections following skill learning. Nat Neurosci 1998;1:230-234.

Rizzolatti G, Luppino G, Matelli M. The organization of the cortical motor system: new concepts. Electroencephalogr Clin Neurophysiol 1998;106:283-296.

Rogers NL, Phan O, Kennaway DJ, Dawson D. Effect of daytime oral melatonin administration on neurobehavioral performance in humans. J Pineal Res 1998;25:47-53.

Rosenkranz K, Rothwell JC. Differential effect of muscle vibration on intracortical inhibitory circuits in humans. J Physiol 2003;551:649-660.

Rosenkranz K, Williamon A, Rothwell JC. Motorcortical excitability and synaptic plasticity is enhanced in professional musicians. J Neurosci 2007;27:5200-5206.

Rossi S, Pasqualetti P, Rossini PM, Feige B, Ulivelli M, Glocker FX, Battistini N, Lucking CH, Kristeva-Feige R. Effects of repetitive transcranial magnetic stimulation on movement-related cortical activity in humans. Cereb Cortex 2000;10:802-808.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 1994;91:79-92.

Rossini PM, Desiato MT, Lavaroni F, Caramia MD. Brain excitability and electroencephalographic activation: non-invasive evaluation in healthy humans via transcranial magnetic stimulation. Brain Res 1991;567:111-119.

Rothwell JC. Physiological studies of electric and magnetic stimulation of the human brain. Electroencephalogr Clin Neurophysiol Suppl 1991;43:29-35.

Sale MV, Ridding MC, Nordstrom MA (2006) Variability of two paired associative stimulation (PAS) protocols used to induce neuroplastic changes in human motor cortex. In: Proc Australasian Wint Conf Brain Res, vol 24, p 10.15

Sale MV, Ridding MC, Nordstrom MA. Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 2007;181:615-626.

Sale MV, Ridding MC, Nordstrom MA. Cortisol inhibits neuroplasticity induction in human motor cortex. J Neurosci 2008;28:8285-8293.

Sanes JN. Neocortical mechanisms in motor learning. Curr Opin Neurobiol 2003;13:225-231.

Sanes JN, Donoghue JP. Plasticity and primary motor cortex. Annu Rev Neurosci 2000;23:393-415.

Sanes JN, Donoghue JP, Thangaraj V, Edelman RR, Warach S. Shared neural substrates controlling hand movements in human motor cortex. Science 1995;268:1775-1777.

Sawaki L, Yaseen Z, Kopylev L, Cohen LG. Age-dependent changes in the ability to encode a novel elementary motor memory. Ann Neurol 2003;53:521-524.

Schieber MH. Constraints on somatotopic organization in the primary motor cortex. J Neurophysiol 2001;86:2125-2143.

Schieber MH. Motor cortex and the distributed anatomy of finger movements. Adv Exp Med Biol 2002;508:411-416.

Shirakawa T, Honma S, Katsuno Y, Oguchi H, Honma KI. Synchronization of circadian firing rhythms in cultured rat suprachiasmatic neurons. Eur J Neurosci 2000;12:2833-2838.

Silver R, LeSauter J, Tresco PA, Lehman MN. A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature 1996;382:810-813.

Smith MJ, Adams LF, Schmidt PJ, Rubinow DR, Wassermann EM. Effects of ovarian hormones on human cortical excitability. Ann Neurol 2002;51:599-603.

Smith MJ, Keel JC, Greenberg BD, Adams LF, Schmidt PJ, Rubinow DA, Wassermann EM. Menstrual cycle effects on cortical excitability. Neurology 1999;53:2069-.

Solis JM, Nicoll RA. Pharmacological characterization of GABAB-mediated responses in the CA1 region of the rat hippocampal slice. J Neurosci 1992;12:3466-3472.

Sommer M, Kamm T, Tergau F, Ulm G, Paulus W. Repetitive paired-pulse transcranial magnetic stimulation affects corticospinal excitability and finger tapping in Parkinson's disease. Clin Neurophysiol 2002;113:944-950.

Soto-Moyano R, Burgos H, Flores F, Valladares L, Sierralta W, Fernandez V, Perez H, Hernandez P, Hernandez A. Melatonin administration impairs visuo-spatial performance and inhibits neocortical long-term potentiation in rats. Pharmacol Biochem Behav 2006;85:408-414.

Starkman MN, Gebarski SS, Berent S, Schteingart DE. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. Biol Psychiatry 1992;32:756-765.

Stefan K, Kunesch E, Benecke R, Cohen LG, Classen J. Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. J Physiol 2002;543:699-708.

Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. Brain 2000;123 Pt 3:572-584.

Stefan K, Wycislo M, Classen J. Modulation of associative human motor cortical plasticity by attention. J Neurophysiol 2004;92:66-72.

Stefan K, Wycislo M, Gentner R, Schramm A, Naumann M, Reiners K, Classen J. Temporary occlusion of associative motor cortical plasticity by prior dynamic motor training. Cereb Cortex 2006;16:376-385.

Stephan FK, Zucker I. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci U S A 1972;69:1583-1586.

Streit P (1987) Glutamate and aspartate as transmitter candidates for systems of the cerebral cortex. In: Jones EG, Peters A (eds) Cerebral cortex: Functional properties of cortical cells, vol 2. Plenum Press, New York, pp 119-143

Strutton PH, Catley M, Davey NJ. Stability of corticospinal excitability and grip force in intrinsic hand muscles in man over a 24-h period. Physiol Behav 2003;79:679-682.

Takano B, Drzezga A, Peller M, Sax I, Schwaiger M, Lee L, Siebner HR. Short-term modulation of regional excitability and blood flow in human motor cortex following rapid-rate transcranial magnetic stimulation. Neuroimage 2004;23:849-859.

Tecchio F, Zappasodi F, Pasqualetti P, Gennaro LD, Pellicciari MC, Ercolani M, Squitti R, Rossini PM. Age dependence of primary motor cortex plasticity induced by paired associative stimulation. Clin Neurophysiol 2008;

Todd G, Rogasch NC, Flavel SC, Ridding MC. Voluntary movement and repetitive transcranial magnetic stimulation over human motor cortex. J Appl Physiol 2009;

Tononi G, Cirelli C. Sleep function and synaptic homeostasis. Sleep Med Rev 2006;10:49-62.

Touge T, Gerschlager W, Brown P, Rothwell JC. Are the after-effects of low-frequency rTMS on motor cortex excitability due to changes in the efficacy of cortical synapses? Clin Neurophysiol 2001;112:2138-2145.

Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB. Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 1998;391:892-896.

Turrigiano GG, Nelson SB. Thinking globally, acting locally: AMPA receptor turnover and synaptic strength. Neuron 1998;21:933-935.

Turrigiano GG, Nelson SB. Hebb and homeostasis in neuronal plasticity. Curr Opin Neurobiol 2000;10:358-364.

Turrigiano GG, Nelson SB. Homeostatic plasticity in the developing nervous system. Nat Rev Neurosci 2004;5:97-107.

Uncini A, Treviso M, Di Muzio A, Simone P, Pullman S. Physiological basis of voluntary activity inhibition induced by transcranial cortical stimulation. Electroencephalogr Clin Neurophysiol 1993;89:211-220.

Uy J, Ridding MC, Hillier S, Thompson PD, Miles TS. Does induction of plastic change in motor cortex improve leg function after stroke? Neurology 2003;61:982-984.

Van Cauter E, Refetoff S. Multifactorial control of the 24-hour secretory profiles of pituitary hormones. J Endocrinol Invest 1985;8:381-391.

Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 2004;20:2580-2590.

Vyazovskiy VV, Cirelli C, Pfister-Genskow M, Faraguna U, Tononi G. Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. Nat Neurosci 2008;11:200-208.

Walker MP, Stickgold R. Sleep, memory, and plasticity. Annu Rev Psychol 2006;57:139-166.

Walker MP, Stickgold R, Alsop D, Gaab N, Schlaug G. Sleep-dependent motor memory plasticity in the human brain. Neuroscience 2005;133:911-917.

Ward NS, Swayne OB, Newton JM. Age-dependent changes in the neural correlates of force modulation: an fMRI study. Neurobiol Aging 2008;29:1434-1446.

Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. Electroencephalogr Clin Neurophysiol 1998;108:1-16.

Wassermann EM, Cohen LG, Flitman SS, Chen R, Hallett M. Seizures in healthy people with repeated "safe" trains of transcranial magnetic stimuli. Lancet 1996a;347:825-826.

Wassermann EM, Grafman J, Berry C, Hollnagel C, Wild K, Clark K, Hallett M. Use and safety of a new repetitive transcranial magnetic stimulator. Electroencephalogr Clin Neurophysiol 1996b;101:412-417.

Wassermann EM, McShane LM, Hallett M, Cohen LG. Noninvasive mapping of muscle representations in human motor cortex. Electroencephalogr Clin Neurophysiol 1992;85:1-8.

Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol 2002;213:1-47.

Weinberg U, D'Eletto RD, Weitzman ED, Erlich S, Hollander CS. Circulating melatonin in man: episodic secretion throughout the light-dark cycle. J Clin Endocrinol Metab 1979;48:114-118.

Welsh DK, Logothetis DE, Meister M, Reppert SM. Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. Neuron 1995;14:697-706.

Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. J Physiol 1999;517 (Pt 2):591-597.

Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning induces long-term potentiation in the hippocampus. Science 2006;313:1093-1097.

Wolf SL, Butler AJ, Campana GI, Parris TA, Struys DM, Weinstein SR, Weiss P. Intra-subject reliability of parameters contributing to maps generated by transcranial magnetic stimulation in able-bodied adults. Clin Neurophysiol 2004;115:1740-1747.

Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, Benecke R, Classen J. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. J Neurophysiol 2003;89:2339-2345.

Wolters A, Schmidt A, Schramm A, Zeller D, Naumann M, Kunesch E, Benecke R, Reiners K, Classen J. Timing-dependent plasticity in human primary somatosensory cortex. J Physiol 2005;565:1039-1052.

Woody CD, Gruen E, Birt D. Changes in membrane currents during Pavlovian conditioning of single cortical neurons. Brain Res 1991;539:76-84.

Wyse JP, Mercer TH, Gleeson NP. Time-of-day dependence of isokinetic leg strength and associated interday variability. Br J Sports Med 1994;28:167-170.

Yamada H, Tamaki T, Wakano K, Mikami A, Transfeldt EE. Effect of transcranial magnetic stimulation on cerebral function in a monkey model. Electroencephalogr Clin Neurophysiol 1995;97:140-144.

Yoo SS, Hu PT, Gujar N, Jolesz FA, Walker MP. A deficit in the ability to form new human memories without sleep. Nat Neurosci 2007;10:385-392.

Zhou Q, Tao HW, Poo MM. Reversal and stabilization of synaptic modifications in a developing visual system. Science 2003;300:1953-1957.

Ziemann U. Pharmacology of TMS. Suppl Clin Neurophysiol 2003;56:226-231.

Ziemann U, Corwell B, Cohen LG. Modulation of plasticity in human motor cortex after forearm ischemic nerve block. J Neurosci 1998a;18:1115-1123.

Ziemann U, Hallett M, Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. J Neurosci 1998b;18:7000-7007.

Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J Neurosci 2004;24:1666-1672.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res 1996a;109:127-135.

Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol 1996b;40:367-378.

Ziemann U, Muellbacher W, Hallett M, Cohen LG. Modulation of practice-dependent plasticity in human motor cortex. Brain 2001;124:1171-1181.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 1996c;496 (Pt 3):873-881.

Zoghi M, Pearce SL, Nordstrom MA. Differential modulation of intracortical inhibition in human motor cortex during selective activation of an intrinsic hand muscle. J Physiol 2003;550:933-946.